An investigation into the link between vitamin D status, erectile dysfunction and cardiovascular risk factors in ageing men in New Zealand

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ABSTRACT

Background

Cardiovascular disease (CVD) is the leading cause of death worldwide, particularly amongst ageing males. Prevention and/or early identification and effective intervention are essential in the fight against CVD. Erectile dysfunction (ED) is a prevalent and multi-factorial condition that is now accepted to be an early marker of subclinical CVD: the common denominator is endothelial dysfunction. Both the enzymatic capability for bioactivation of vitamin D and the vitamin D receptor (VDR) are expressed in endothelial cells and vitamin D may play a role in endothelial function. Vitamin D deficiency (serum 25-hydroxyvitamin D (25(OH)D) concentrations <50 nmol/L) is a worldwide pandemic and serum 25(OH)D levels <75 nmol/L may result in metabolic and vascular deterioration leading to endothelial dysfunction, ED and CVD. Assessment of erectile function can be used to identify otherwise asymptomatic men at high risk of developing clinical CVD, at a time when effective intervention may prevent, delay or reverse its progression. Vitamin D status may be associated with ED and CVD risk and could help improve erectile function and vascular health.

Objectives

The aim of this research was to investigate the postulated link between vitamin D status, ED, and CVD risk factors. The objectives were (1) to assess the prevalence of ED (using the 5-item International Index of Erectile Function (IIEF-5)) and its associated sociodemographic, lifestyle, and medical correlates in New Zealand (NZ) men aged 40-70 years; (2) to investigate the relationship between vitamin D status (serum 25(OH)D concentration), ED and other CVD risk factors in men aged 40-70 years living in the Manawatu region of NZ; and (3) to examine the impact of common VDR gene (*VDR*) polymorphisms on this relationship.

Method

Two thousand men aged 40-70 years were randomly selected from the NZ Electoral Roll and sent an anonymous postal survey designed to assess the prevalence of ED and its sociodemographic, lifestyle, and medical risk factors. Six hundred men aged 40-70 years living in the Manawatu region were randomly selected from the NZ Electoral Roll and invited to participate in an observational study designed to provide a comprehensive health profile of self-reported healthy men and investigate the relationship between vitamin D status, ED, and a range of CVD risk factors. Eligible participants (n=100) completed a comprehensive health assessment including a medical history, anthropometric and cardiovascular assessment, fasting blood sample, computer-based questionnaire, a submaximal fitness test and a handgrip

strength test. Blood samples were assessed for four common *VDR* polymorphisms (rs11568820 (*Cdx*2), rs10735810 (*Fok*I), rs1544410 (*Bsm*I) and rs731236 (*Taq*I)) using polymerase chain reaction-high resolution amplicon melt (PCR-HRM) analysis.

Results

The survey showed 38.4% of respondents presented with ED (IIEF-5 ≤21). Older age, non-European ethnicity and current smoking were significant independent predictors of an increased risk of ED, while a high household income and regular vigorous physical activity (PA) were deemed protective. The observational study showed 30 men presented with ED and a further 37 men had <75 nmol/L 25(OH)D. There was a weak positive correlation between IIEF-5 scores and 25(OH)D levels (r_s=0.238, p=0.017). Men with <75 nmol/L had lower IIEF-5 scores compared to men with ≥75 nmol/L 25(OH)D (22(7) vs. 24(3), p=0.001). Men with ED had lower 25(OH)D levels compared to men without ED (74.5(34) vs. 84.5(24), p=0.062). Every 1 nmol/L of 25(OH)D predicted a 2% decrease in the age-adjusted risk of ED (age-adjusted OR=0.98 [0.96-1.00], p=0.046). The PCR-HRM analysis showed that the *Cdx*2, *Fok*I and *Bsm*I polymorphisms were all significantly associated with an adverse cardiovascular risk profile. The *Cdx*2 G allele was associated with lower IIEF-5 scores compared to the A allele (23(4) vs. 24(2), p=0.008) and the GA and GG genotypes were predictors of an increased age-adjusted risk of ED (age-adjusted OR=18.78 [1.98-178.60], p=0.011 and 8.53 [1.00-72.73], p=0.050 respectively). However, *Cdx*2 was not found to modify the age-adjusted association between 25(OH)D levels and ED (multi-adjusted OR=0.97 [0.95-1.00], p=0.032).

Conclusions

These results suggest that over a third of NZ men aged 40-70 years suffer from ED and it is associated with sociodemographic, lifestyle and medical factors simliar to CVD. Low serum 25(OH)D is associated with the presence and severity of ED in a self-reported healthy population. Common *VDR* polymorphisms are also associated with ED; however, they do not modify the association between serum 25(OH)D and ED. A randomised placebo-controlled human intervention trial is warranted to investigate whether improving vitamin D status in men with vitamin D deficiency and ED ameliorates symptoms and reduces the risk of CVD.

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1α-hydroxylase	25-hydroxyvitamin D 1-alpha-hydroxylase
24-hydroxylase	1,25 dihydroxyvitamin D 24-hydroxylase
$1,25(OH)_2D_3$	1,25-hydroxyvitamin D_3 (calcitriol)
25(OH)D	25-hydroxyvitamin D
95% CI	95% confidence interval
χ^2	Chi-squared
A:G	Android-to-gynoid fat ratio
AIx@HR75	Augmentation index adjusted to a heart rate of 75 bpm
AP@HR75	Augmentation pressure adjusted to a heart rate of 75 bpm
ANOVA	One-way analysis of variance
ANZSCO	Australian and New Zealand Standard Classification of Occupations
AUC	Area under curve
BACH	Boston Area Community Health Survey
BF%	Body fat percentage
BMI	Body Mass Index
BMSFI	Brief Male Sexual Function Inventory
BP	Blood pressure
BPH	Benign prostatic hyperplasia
CATI	Computer Assisted Telephone Interview
CC	Corpus cavernosum
CHD	Coronary heart disease
CVD	Cardiovascular disease
CVOD	Corporal veno-occlusive dysfunction
DBP	Diastolic blood pressure
DE	Delayed ejaculation
DM	Diabetes mellitus
DNA	Deoxyribonucleic acid
ED	Erectile dysfunction
EDV	End-diastolic velocity
EMAS	European Male Ageing Study
EPIC-PAQ	European Prospective Investigation into Cancer and Nutrition Physical Activity Questionnaire
FAMAS	Florey Adelaide Male Ageing Study

FFQ	Food Frequency Questionnaire
FPG	Fasting plasma glucose
FPI	Fasting plasma insulin
FT	Free testosterone
GOSS	Global Online Sexuality Survey
GSSAB	Global Study of Sexual Attitudes and Behaviours
HDL-c	High-density lipoprotein cholesterol
HOMA1	Homeostatic Model Assessment Index 1
HPFS	Health Professionals Follow-Up Study
HR	Heart rate
HRM	High resolution amplicon melt
ICSM	International Consultation in Sexual Medicine
IHD	Ischemic heart disease
IIEF	International Index of Erectile Function
IIEF-5	5-item International Index of Erectile Function
IQR	Interquartile range
IR	Insulin resistance
LD	Linkage disequilibrium
LDL-c	Low-density lipoprotein cholesterol
MAF	Minor allele frequency
MALES	Multinational Men's Attitudes to Life Events and Sexuality
MATeS	Men in Australia Telephone Survey
MetS	Metabolic syndrome
MI	Myocardial infarction
MMAS	Massachusetts Male Aging Study
mRNA	Messenger ribonucleic acid
MSAM-7	Multinational Survey of the Aging Male
NHANES	National Health and Nutrition Examination Survey
NHSLS	National Health and Social Life Survey
NPT	Nocturnal penile tumescence
NZANS	New Zealand Adult Nutrition Survey
OR	Odds ratio
PA	Physical activity
PCa	Prostate cancer

PCAW	Prostate Cancer Awareness Week
PCR	Polymerase chain reaction
PDE_5	Phosphodiesterase type 5
PDS	Penile Doppler sonography
PE	Premature ejaculation
PHQ-6	9-item Patient Health Questionnaire
PP	Pulse pressure
PSA	Prostate specific antigen
PSV	Peak systolic velocity
PTH	Parathyroid hormone
PTSD	Post-traumatic stress disorder
PVD	Peripheral vascular disease
PWA	Pulse Wave Analysis
PWV	Pulse Wave Velocity
RCT	Randomised controlled trial
RFLP	Restriction fragment length polymorphisms
RR	Relative risk
r_s	Spearman's rho
RXR	Retinoid-X receptor
SBP	Systolic blood pressure
SD	Standard deviation
SHBG	Sex hormone binding globulin
SHIM	Sexual Health Inventory for Men
SMC	Smooth muscle cell
SNP	Single nucleotide polymorphism
T2DM	Type 2 diabetes mellitus
TC	Total cholesterol
TG	Triglyceride
TT	Total testosterone
Tukey's HSD	Tukey's honest significant difference
UTR	Untranslated region
VDR	Vitamin D receptor
VDR	Vitamin D receptor gene
VDRE	Vitamin D response element

VO_2peak	Maximal oxygen consumption
WAMHS	Western Australia Men's Health Study
WC	Waist circumference
Well-LaD	Wellness, Lifestyle and Diet
WHO	World Health Organization
WHR	Waist-to-hip ratio
WHtR	Waist-to-height ratio
WSP	World Standard Population

CHAPTER 1

GENERAL INTRODUCTION, AIMS AND OBJECTIVES

1.0 INTRODUCTION

Erectile dysfunction (ED) is the persistent inability to attain and maintain an erection adequate for satisfactory sexual performance [1]. It is a common condition estimated to affect over 150 million men worldwide [2]; however, reported prevalence rates vary widely from 8-70% due to differences in the selected population, age range, adopted definition and assessment methodology [3-7]. The landmark 1987-1989 Massachusetts Male Aging Study (MMAS) [3] was the first large-scale population-based study (n=1709) to investigate the prevalence of ED and reported that 52% of Caucasian men in Massachusetts aged 40-70 years experienced some degree of ED. The most recent multinational population-based study, the 2010-2011 Global Online Sexuality Survey (GOSS) [8], found 37.7% of English-speaking internet users in the USA (n=2022) over 18 years of age suffered from some degree of ED, with 11% experiencing moderate-severe ED. Furthermore, the Global Study of Sexual Attitudes and Behaviours (GSSAB) investigated the population-based prevalence of ED in 29 countries (n=13618), including New Zealand (NZ, n=250), and found a 10% prevalence of moderate-severe ED in men aged 40-80 years [9] with 25% prevalence reported in NZ men [10]. However, the study focussed on sexual attitudes and behaviours, not ED per se and neither the prevalence of overall ED (including mild ED) nor the associated risk factors in NZ were reported. The small sample size, use of random-digit dialling and allowance for respondent substitution means these results are unlikely to be representative of the NZ population. The use of a structured telephone interview with a standardised questionnaire lacked anonymity and may have led to unreliable responses to sensitive questions. The use of two unvalidated questions to assess ED is incongruent with the multi-dimensional definition of ED and did not collect information on severity. Reliable data on the prevalence and severity of ED in NZ are therefore currently lacking.

ED is a multi-factorial and complex health issue caused by organic (anatomic, neurogenic, endocrinologic, vasculogenic) and/or psychogenic factors and often presents with comorbid conditions including hypertension, ischemic heart disease, stroke, peripheral arterial disease, and type 2 diabetes mellitus (T2DM) [3, 11-13]. Indeed, ED is now recognised as a sentinel marker of CVD in many men [14]. The Second Princeton Consensus on sexual dysfunction and cardiac risk concluded “a man with ED and no cardiac symptoms is a cardiac (or vascular) patient until proven otherwise” [15]. The early detection and correct treatment of ED are therefore essential for reducing cardiac risk. Although strongly associated with age [3], ED is not an inevitable result of ageing and its most commonly reported risk factors (hypertension, dyslipidaemia, atherosclerosis, diabetes mellitus, smoking, low physical activity (PA) and

obesity) are shared with cardiovascular disease (CVD) and are amenable to change [16]. However, although these risk factors are commonly accepted, research shows that their significance and relative importance differs between populations [4, 17, 18]. This highlights the need for population-based data on sociodemographic, lifestyle and medical correlates in NZ.

Current treatment options for ED include; oral therapies (phosphodiesterase type 5 (PDE_5) inhibitors or apomorphine), intraurethral or intracavernosal alprostadil, vacuum devices, surgical insertion of penile prostheses and psychosocial counselling [19-21]. However, epidemiological evidence supports a role for modifiable lifestyle factors (diet [22], obesity [23-26], exercise [23, 24, 27-29], smoking [30-34], alcohol consumption [35-39], stress, anxiety and depression [40, 41]) in the development of ED, particularly among men without comorbidities [35]. A lifestyle-first, rather than a pharmacology-first, approach is needed to address the underlying cause of ED, rather than merely treating the symptoms. Vasculogenic ED offers identification of men at risk of CVD at an early and potentially reversible stage of disease progression, allowing targeted intervention. Emerging research findings recommend that following a Mediterranean diet [22, 42], weight reduction [43, 44], increasing PA [45-48] and smoking cessation [49, 50] may help to ameliorate symptoms; however, the evidence is not yet scientifically compelling. Further research is needed to elucidate the effect of dietary and lifestyle changes on ED symptoms.

Vitamin D has historically been granted little scientific attention due to the assumption that accessibility to sunlight exposure and subsequent synthesis in the skin, will result in sufficient vitamin D in most people. However, over the past decade it has become apparent that, based on current recommended levels (a serum 25-hydroxyvitamin D concentration (25(OH)D) ≥50 nmol/L (20 ng/mL)) [51, 52], a significant number of people, especially in sub-tropical climates such as NZ, have insufficient vitamin D [53-58]. Considered a worldwide pandemic [59], the burgeoning level of vitamin D insufficiency has been linked to public health messages over the past two decades regarding the risk of skin cancer associated with sun exposure, together with an increasingly sedentary indoor lifestyle and increasing rates of obesity [60, 61]. It has led to a surge in interest in vitamin D and the discovery of its plethora of roles in human health. Advances in our knowledge of the various functions of vitamin D have indicated that the current recommendations, while sufficient for bone health, may be insufficient to support optimal long term health and that levels currently accepted as adequate (50-75 nmol/L (20-30 ng/mL) [62] may be associated with an increased risk of chronic disease. Epidemiological evidence now suggests that levels <75 nmol/L (30 ng/mL) are associated with metabolic and vascular deterioration leading to diabetes [63-65] and CVD [66-68], indicating that these cut-

offs need to be reconsidered. Published evidence supporting cardiometabolic benefits of supplementation remains inconsistent and does not demonstrate causality [51].

Cardiovascular tissues, including endothelial cells [69, 70], vascular smooth muscle cells [71, 72] and cardiomyocytes [73-75] express both the vitamin D receptor (VDR) and 25-hydroxyvitamin D 1-α-hydroxylase (1-α-hydroxylase, a cytochrome P450 enzyme encoded by the *CYP27B1* gene) which regulates the synthesis of calcitriol (1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$)), the main active metabolite of vitamin D [76]. This strongly supports a role for vitamin D in cardiovascular health. Several biologically plausible mechanisms have been proposed; however, recent research suggests that vitamin D is essential to the health of endothelial cells. Murine studies have shown that vitamin D insufficiency results in impaired vasodilation resulting from deficient production of two essential factors, nitric oxide and endothelium-derived hyperpolarising factor [77]. Nitric oxide is a potent inducer of smooth muscle cell relaxation and vasodilation. It plays a pivotal role in the regulation of the endothelium-dependent processes maintaining vascular wall homeostasis [78]. A vitamin D "micro-endocrine system" appears to exist in endothelial cells [70, 79]. Studies have demonstrated an association between vitamin D status and inflammatory conditions [80-82], suggesting it may regulate the expression of pro- and anti-inflammatory cytokines or have anti-inflammatory and/or antioxidant properties, possibly via a direct anti-oxidant role in scavenging free radicals before damage ensues [83].

Emerging evidence from human trials supports the role for vitamin D in endothelial health. In a cross–sectional study of Koreans with type 2 diabetes mellitus (T2DM) [84], serum 25(OH)D concentration was associated with arterial stiffness (measured by pulse wave velocity (PWV)). In a randomised, double-blind, placebo controlled trial involving 57 overweight African-Americans, 60,000 IU of vitamin D_3 per month over 16 weeks raised mean serum 25(OH)D levels from 34.3±2.2 to 100.9±6.6 nmol/l in the treatment group and improved endothelial function (measured by brachial artery flow-mediated dilation (FMD)), compared to the placebo group [85]. The integrity of endothelial cells is fundamental to cardiovascular health and endothelial dysfunction is associated with a range of adverse cardiovascular outcomes. Furthermore, variation in the gene encoding the VDR protein (*VDR*) may affect an individual's serum 25(OH)D level, their susceptibility to vitamin D insufficiency and their response to supplementation [86]. Therefore, it is possible that *VDR* polymorphisms may affect the relationship between vitamin D status and disease phenotypes, including ED as an early marker of CVD.

2.1 STUDY AIMS AND OBJECTIVES

The aim of the thesis is to investigate the postulated link between vitamin D, ED and CVD.

Specific objectives are:

- To assess the prevalence of ED (self-reported using the 5-item International Index of Erectile Function (IIEF-5) and the single-item self-report tool) and sociodemographic, lifestyle and medical correlates in men aged 40-70 years living in NZ using a nationwide postal survey.
- To investigate the relationship between vitamin D status (serum 25(OH)D concentration), ED (self-reported using the IIEF-5 and the single-item self-report tool) and CVD risk factors in men aged 40-70 years living in the Manawatu region of NZ using a cross-sectional observational study.
- To examine the effect of common polymorphisms of the VDR gene on the relationship between vitamin D status (serum 25(OH)D concentration), ED (IIEF-5 score) and CVD risk factors using real-time polymerase chain reaction- high resolution amplicon melt analysis (PCR-HRM) of DNA samples.

3.0 STUDY HYPOTHESES

Three research hypotheses will be investigated in this thesis:

Hypothesis 1: *ED is prevalent in ageing men in NZ and associated with sociodemographic, lifestyle and medical factors.*

Hypothesis 2: *Vitamin D status is a significant predictor of ED and CVD risk factors in ageing men and will remain so when other potential determinants of CVD are controlled for.*

Hypothesis 3: *The relationship between vitamin D status and ED is affected by common polymorphisms of the VDR gene.*

4.0 IMPLICATIONS

The proposed hypothesis is novel, particularly in making a link between vitamin D, ED and CVD risk. The outcomes of this research will 1) raise awareness of the prevalence of ED in NZ and its sociodemographic, lifestyle and medical correlates, highlighting important risk factors to support the early identification of ED within the community; 2) determine the association between vitamin D insufficiency, ED and CVD risk factors, identifying vitamin D insufficiency as a potential target in the treatment of ED and subclinical CVD; and 3) demonstrate the effect of common polymorphisms in the gene encoding the VDR protein on these relationships, emphasising the importance of its consideration in future vitamin D research studies. The implication is that improving vitamin D levels via nutritional and/or lifestyle intervention in men with hypovitaminosis D and ED could have positive effects on male sexual function with the added benefit of reduced risk and/or slowed progression of CVD. A widely accessible and acceptable nutritional and/or lifestyle intervention, vitamin D may be a novel, cheap, safe and effective alternative or adjunct treatment for ED, a major health problem worldwide for which current conventional treatment is often costly, ineffective or not well-tolerated. Although the debate over recommended vitamin D cut-offs continues, there are no reported toxicity effects or detrimental nutrient-nutrient interactions of vitamin D from natural dietary sources, supplementation (4,000 – 10,000 IU/day) [87-90] or safe sun exposure [91]. Furthermore, there have been no adverse effects reported amongst populations with high levels of 25(OH)D due to occupational or recreational sun exposure (e.g., 163 nmol/L (65 ng/mL) in lifeguards [92] and 115 nmol/L (46 ng/mL) in nomadic African tribes [93]). Supplementation or sensible sun exposure advice to increase vitamin D intake may be a safe and effective way to improve sexual function and reduce the risk of chronic disease. A randomised controlled trial to investigate this would be warranted.

5.0 STRUCTURE OF THE THESIS

This thesis is divided into 3 sections with 8 chapters each culminating in a list of references. Following the introduction (Chapter 1), each section (Section A, B and C) begins with a review of the literature pertinent to the content of that section (Chapter 2, 4 and 6) followed by a chapter presenting the methods and the results of the relevant research studies (Chapter 3, 5 and 7). As each study is presented as a manuscript for future publication it is inevitable that some degree of repetition exists. For abstracts see Appendix 1. The first study presents data from a nationwide survey designed to identify the prevalence of ED and its association with potential contributing sociodemographic, lifestyle and medical factors in NZ men aged 40-70 years (Chapter 3). The second study presents data on the vitamin D status, erectile function and cardiovascular health of self-reported "healthy" ageing men and demonstrates relationships between these factors after controlling for confounders (Chapter 5). The third study presents data on the frequency of four common *VDR* polymorphisms and demonstrates their relationship with vitamin D status, erectile function and cardiovascular risk factors (Chapter 7). The research process is shown in Figure 1.1. This thesis concludes with a discussion to draw together the results, highlighting key methodological considerations, implications of the results and suggestions for future research (Chapter 8).

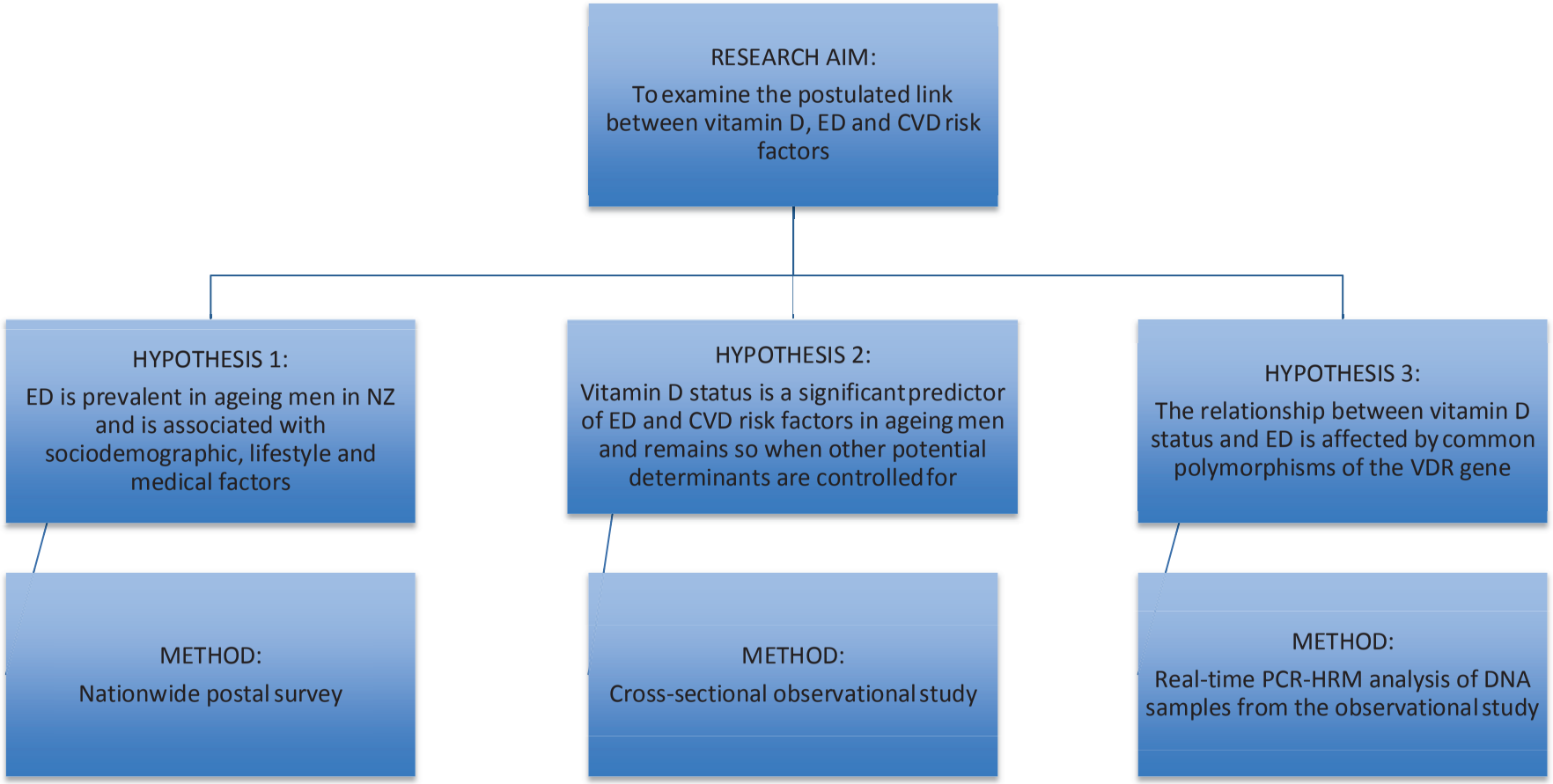

Figure 1.1. The research process followed in the various studies described in this thesis. CVD, cardiovascular disease; ED, erectile dysfunction; NZ, New Zealand; PCR-HRM, polymerase chain reaction-high resolution amplicon melt analysis; VDR, vitamin D receptor.

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CHAPTER 2

LITERATURE REVIEW - ERECTILE DYSFUNCTION AND ITS USE AS AN EARLY MARKER OF CARDIOVASCULAR DISEASE

1.0 INTRODUCTION

Erectile dysfunction (ED) is a prevalent condition worldwide that mainly affects men over 40 years of age [1]. It has serious implications on quality of life [2-4]. Although less than 25% of men appear to seek medical care for the condition [5-8], ED may be indicative of underlying diseases [10, 11] and have lifestyle antecendents amenable to change [10, 12-14]. It can be caused by organic and/or psychogenic factors [11]. Erections are a multifactorial process involving a complex interaction between the sensory, motor, neurological, hormonal, and vascular systems that is influenced by psychosocial, cultural and interpersonal elements [15]. Associated risk factors include ageing; co-morbidities (diabetes mellitus (DM), hypertension, hyperlipidaemia, obesity, cardiovascular disease (CVD)); lower urinary tract symptoms (LUTS); medication and drugs; trauma or surgery affecting the vascular system or nerve function in the spine or pelvis; psychosocial stress and depression; and lifestyle factors (smoking, physical inactivity and alcohol intake) [16]. In addition to its negative implications on quality of life for both men and their partners, organic ED is now widely accepted to be synonymous with endothelial dysfunction and a forewarning of systemic vascular disease [10]. This review will 1) provide an introduction to ED including penile anatomy and histology, erectile physiology, assessment and diagnosis and treatment options; 2) critically review the epidemiology of ED and its risk factors; and 3) examine its use as an early marker of ED. The early assessment, appropriate diagnosis and effective treatment of ED through complementary measures including nutritional and lifestyle intervention may help ameliorate the symptoms and slow, halt, or reverse the development of CVD.

2.1 BACKGROUND

2.2 Definition

ED is defined as the persistent inability to attain and maintain a penile erection adequate for sexual performance [11, 17]. This definition includes three elements of ED: 1) the inability to achieve an erection, 2) the inability to maintain an erection, and 3) the inability to attain satisfactory sexual performance. Although prior definitions existed and others have since been developed [1, 18-21], this definition is the most widely accepted. A clinical diagnosis of ED generally requires symptoms of at least 3-months duration, except in cases of trauma or injury [22]. Although other forms of sexual dysfunction in men occur including sexual desire, orgasm and pain disorders [22], this review will focus on ED.

2.3 Historical perspective

Historically, ED was once regarded as a punishment for adultery or the effect of witchcraft and thus strong grounds for marriage dissolution [23]. In fact the results of the 2010-2011 Global Online Sexuality Survey (GOSS) show that 48% of Middle Eastern men still believe that ED is caused by black magic [24]. However, the 1982 discovery of the sustained pro-erectile effect of vasodilation medication (*Papaverine*) when injected into the penile blood vessels by Virag [25] was a crucial turning point signifying a shift of focus from mysticism and subjective psychosocial observations to objective physiological scientific research. Since the release of oral erectogenic medications improvements have been made in public awareness of ED, with increasing levels of acceptance and candour about male health issues, aided by media coverage and the availability of safe and effective treatments. However, although awareness of ED has increased and social stigma has decreased over recent years, studies have suggested that less than half of men with the disorder seek treatment [16, 17, 26] and less than a quarter are treated [5-8, 27, 28]. Many men assume that ED is an inevitable part of ageing.

2.4 Sexual activity and ageing

Improved healthcare has led to increased human life expectancy and overall quality of life in the middle aged and elderly. The view of ED as a natural part of ageing is a common misconception, closely linked to the pervasive stereotype of an asexual old age [29]. Research strongly supports that sexual interest and activity persists into older age [30-32]. The results of the large 2001-2002 Global Study of Sexual Attitudes and Behaviours (GSSAB) [26] across 29 countries support the continuing desire for sexual activity in older age with more than 80% of men aged 40-80 years having had sexual intercourse in the past year. Men reporting very good to excellent health appear more likely to be both interested in sex and sexually active [33]. Although ageing is often associated with a decrease in all domains of sexual function (desire, erection, ejaculation and orgasm), a Swedish study [34] found that 46% of men aged 70-80 years continued to report sexual intercourse and orgasm at least monthly. Furthermore, research has suggested that interest in sex amongst ageing men has increased [33], most likely with changing expectations following the discovery of effective pharmacological interventions.

2.5 Attitudes, beliefs and behaviours

Desire for sex is usually necessary before men seek treatment yet most men with ED, especially younger men, do not seek treatment [35]. Furthermore, a substantial delay often exists between the manifestation of symptoms and help-seeking behaviour. Men often wait one [36] to three and a half years [17] from onset of symptoms before seeking help but generally only present for help once ED symptoms have worsened. Results from the GSSAB [26] showed that the main reasons for this were the belief that ED was a normal part of ageing, that it was not very serious, and that men were uncomfortable talking to their doctor about it. Other suggested reasons include anxiety about the safety of treatment options and the belief that ED is caused by stress or simply indicates the need for a healthier lifestyle [37]. Research has shown that older men may feel it is inappropriate to discuss sexual activity "at their age" [38], show resignation to the condition and not want to consult a doctor for treatment [39]. Embarrassment is one of the main reasons men do not seek treatment [40, 41]. Men generally prefer a discussion regarding sexual health to be initiated by their medical practitioner [42]; however, many believe their doctors are also embarrassed to discuss this subject. Indeed, health professionals may not raise the issue due to the possibility of causing offence [38]. Therefore, although there has been a decrease in the stigma and silence surrounding male sexual health issues, this is yet to be fully supported by the facilitation of comfortable discussions regarding sexual activity and function with medical professionals.

Men from most countries agree that ED is a source of great sadness for them and their partners, that they are not too old for sex, and that it is important for them to know they have the capability to perform sexually [40]. However, there are cultural differences in attitudes and beliefs, possibly as a result of differences in education, income, religion and the regulations governing the advertising of ED and ED medications. For example, men in France, Italy and Spain are more likely to believe that erection problems are psychological rather than physical and to feel that speaking face-to-face about their erection is impossible, whilst men in the USA and UK are more likely to feel strongly about getting treatment and more likely to accept pharmacological intervention [40]. Also, in Malaysia, both Malaysian and Chinese men often blame their partners and are concerned that ED will lead to their partner straying, whilst Indian men believe ED is due to fate [42]. Such beliefs need to be challenged as men need to understand that although ED is associated with ageing, it is not an inevitable result of ageing and may be related to serious underlying changes in vascular health and a valuable first sign of undetected CVD [10].

2.6 Impact of erectile dysfunction

2.6.1 Social impact

A satisfactory sex life is an important component of adult human relationships and ED can have a significant negative impact on quality of life for both the man and his sexual partner [43]. It can affect physical, emotional and psychological health with wider ranging implications for quality of life and life satisfaction due to the inability to maintain a satisfying sexual relationship [2]. ED can become a source of shame and embarrassment for some men. It can result in fear, loss of self-esteem and self-confidence [2, 4, 44]. Furthermore, sexual dysfunction is associated with mental health issues such as depression, anger and anxiety, and social health issues including drug and alcohol abuse [1, 3, 11, 41], Indeed, ED is strongly linked to male depression and has been shown to correlate with unhappiness [3]. It can dominate a man's thoughts and thus impact on all areas of his life. In addition to the psychological implications of ED, it can signal serious underlying and undiagnosed pathologies including diabetes, hypertension, CVD, peripheral vascular disease, neurologic and endocrine disorders [15]. This highlights the need to routinely ask questions regarding sexual function during medical assessment [45]. The diagnosis of ED offers an opportunity for early identification and treatment of underlying comorbidities. Furthermore, it is likely that ED may prove more of a motivating factor to make lifestyle changes than the long-term threat of chronic disease.

2.6.2 Economic impact

The cost of ED to the healthcare system remains unknown. Sexual function has, for many years, not been deemed a 'health' issue and thus lacked focused research and funding, with the exception of commercial pharmacological treatments. Available pharmacological treatment options are expensive and costs vary between countries, depending on subsidisation policies. In 2013, the cost of Viagra® (Pfizer, NY, USA) was $68 NZD for 4x25mg tablets. The recommended starting dose for most men is 50 mg [46], at a cost of $34 NZD per sexual encounter. The introduction of subsidised oral therapy into the public healthcare system is projected to incur a significant cost due to the high prevalence rate [47, 48] and on this basis most countries have rejected subsidisation. The New Zealand (NZ) government agency Pharmac does not currently offer subsidies for ED medications. However, treatment rates may be significantly lower than indicated by the prevalence rate, as most men do not seek treatment [5-8, 27, 28]. Furthermore, the effective pharmacological treatment of ED can dramatically improve quality of life for men and their partners, making it economically efficient [49, 50]. Current patents are due to expire and the market will open to lower-priced generic competition, increasing the economic availability of pharmacological interventions. However,

currently available pharmacological treatments can cause side effects and may be contraindicated or ineffective in some men [46, 51, 52]. Even when they are effective at treating the symptoms, pharmacological treatments do not address the cause of the disorder. This ensures repeat prescriptions and supports a lucrative market worth over 5 billion USD per annum in 2010 [53]. Due to the lack of subsidisation and subsequent monitoring of consumption in NZ, there is no information available on the value of this market in NZ. The wealth of this market internationally ensures that the focus remains on treatment rather than prevention. Research needs to focus on more widely acceptable, accessible and cost-effective nutritional and lifestyle interventions to prevent and/or treat the cause(s) of this disorder.

2.7 Penile anatomy and histology

Erections are a largely vascular event. The penis consists of a central urethra, surrounded by three erectile bodies (Figure 2.1): two lateral corpus cavernosa (CC) and the ventral corpus spongiosum [17]. The corpus spongiosum protects the urethra, encircling it and expanding to form a flattened cone at the head (the glans penis). These cavernous cylindrical tissues are bound by strong fibrous tissue (tunica albuginea), covered in a continuous layer of thin, loose integument and attached to the pubic bone via the fundiform and suspensory ligaments [54].

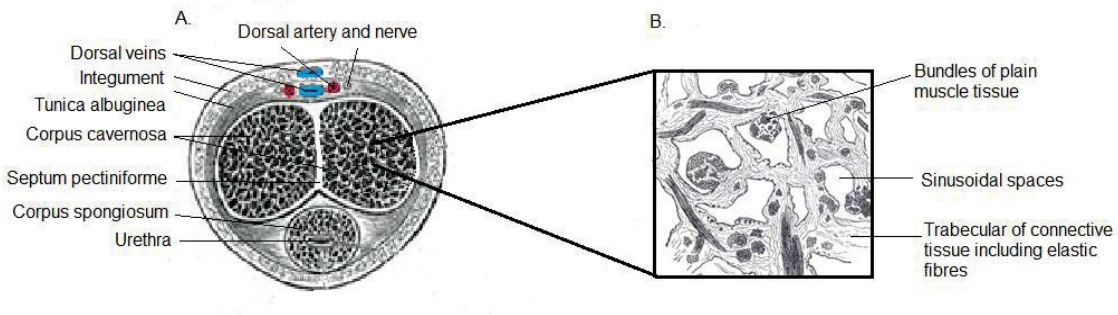

Figure 2.1. Transverse sections of (A) the human penis and (B) the corpus cavernosum in a flaccid state (adapted from Lewis [9])

The CC are vascular structures that provide rigidity to support tumescence. They are highly specialised sponge-like structures, subdivided into compartments by copious bands and cords composed of white fibrous tissue, elastic fibres and plain muscular fibres (trabeculae) containing a multitude of nerves and arteries (Figure 2.1) [54]. The trabeculae are stronger and larger around the circumference of the penis, and thicker behind the CC. The sinusoidal vessels are transversely orientated and larger in the centre than at the circumference. This means the penis can remain strong around the circumference, whilst leaving space for blood accumulation in the centre.

Vascular smooth muscle cells (SMC) make up around 40-50% of the cross-sectional area of the CC [55]. The remaining tissue is an extracellular matrix composed mainly of collagen (type I, III and IV) and elastin, providing a framework to support tumescence [56, 57]. The sinusoidal vessels, similar to veins, are lined with endothelial cells. Endothelial cells and neurons play an essential role in the regulation of SMC tone [58]. Control of the erectile process is determined by a complex balance of factors influencing the contraction and relaxation of SMC in the CC.

2.8 Erectile physiology

Penile tumescence occurs as a result of sexual stimulation and desire, transmitted to the penis via nervous impulses and chemical transmitters. This culminates in the relaxation of SMC and resultant dilation of penile blood vessels which allows blood to enter the sinusoidal vessels within the CC via the dorsal and cavernous arteries at systolic and diastolic pressure [17]. The penis swells resulting in tumescence. Pressure must be maintained for sufficient time to support penetration and ejaculation. The increased volume and pressure activates the corporo-veno-occlusive mechanism, effectively compressing the venous drainage so that blood is held in the CC until ejaculation or the end of sexual stimulation [17]. At this point, the blood exits via a series of blood vessels converging in the deep dorsal vein or the subtunical venular plexus at the root of the penis. This results in a loss of pressure and detumescence.

Achieving and maintaining an erection is a complex physiological process. It requires the ability to feel sexual desire and respond to sexual stimulation, correct penile anatomy and good endocrinological, neurogenic and vasculogenic health. Disruption of normal function at any stage in the erection process can have a drastic effect on a man's ability to function sexually. The erectile process is coordinated by a complex series of events involving psychosomatic, neuronal and vascular pathways and thus mediated by both central and peripheral pathways [58]. The following is a brief overview of some of the mediators involved.

2.8.1 Central control

Knowledge of the complex mechanisms involved in the central control (spinal and supraspinal pathways) of erections is increasing through data obtained mainly from experimental animal trials; however, the details of central regulation remain unclear [58]. Erectile events usually commence with sexual desire, involving the stimulation of arousal centres in the central brain through the processing and amalgamation of sensory stimuli (visual, tactile, olfactory and imaginative) [58]. Many areas of the brain are involved. For example, electrical or chemical stimulation of the medial preoptic area [59], hypothalamic paraventricular nucleus [60] or hippocampus [61] has been shown to induce erection in rats. The arousal signals generated are

transferred in a coordinated manner from the brain to the penis, a process mediated by a spinal network linking primary afferent neurons [58]. This network appears to receive and integrate both supraspinal and peripheral signals to either elicit, modulate or inhibit an erection [62].

Stimulation of non-adrenergic non-cholinergic (NANC) and adrenergic receptors appears to be responsible for central control of erections [63, 64]. Hormones and neurotransmitters play an important role in the maintenance of erection; however, the exact relationship between the blood supply and nerve supply is unknown. Very briefly, some of the centrally acting compounds shown to induce erections include dopamine and apomorphine (a nonselective dopamine receptor agonist) [63-65], oxytocin [66-68], adrenocorticotropic hormones (ACTH) and related peptides α-melanocyte-stimulating hormones (α-MSH) [58, 69], nitric oxide (NO) [70-74], and excitatory amino acids including L-glutamate [59] and N-methyl-D-aspartate (NMDA) [75, 76]. Sex hormones, particularly testosterone, are essential to the initiation and maintenance of male desire and libido [77] and androgens may be important mediators of erectile function; however, androgen deprivation does not always cause ED [78] and their role in sexual function is complex. Some of the centrally acting compounds shown to inhibit the erectile response include serotonin (5-hydroxytryptamine, 5-HT), gamma-aminobutyric acid (GABA) [79], cannabinoid receptor agonists [80], opiate agonists [81], and prolactin [78, 82, 83]. Serotonin is involved in both spinal and supraspinal erectile physiology through sympathetic and parasympathetic mechanisms [66] and appears to mainly inhibit male sexual behaviour [82], however it has an ability to either enhance or depress sexual function depending on the site of action and the 5-HT receptor involved [84], highlighting again the complexity involved in the regulation of erection.

Of the above factors, NO is considered the key central mediator of penile erection, modulating sexual behaviour within the central nervous system (CNS) and affecting tumescence [70-74]. Its importance as a physiological mediator is highlighted by the increase in NO in the hypothalamic paraventricular nucleus during both non-contact erections and sexual intercourse in rats [60, 72]. Furthermore, administration of the pro-erectile mediator NMDA in rats results in increased NO metabolites in the paraventricular nucleus [76], a response prevented by injection of NO synthase (NOS) inhibitors [71, 75, 85], and reinstated by simultaneous administration of the NO substrate L-arginine [71]. NO may also mediate the action of ACTH, α-MSH and serotonin antagonists ($5\text{-}HT_{2c}$) in inducing erections [71]. The complex interaction of the factors involved in the generation, propagation and transmission of nervous impulses and the intracellular transduction signals involved are unclear. Manipulation

of these mediators may offer benefits as therapeutic interventions but further research is needed. However, it is clear that spinal cord integrity and the preservation of normal sensory, hormonal and neurotransmitter function are essential for tumescence.

2.8.2 Peripheral control

Peripheral control of the erectile process is determined by the balance of a number of vasoactive substances and pathways influencing the contraction and relaxation of SMC in the CC of the penis. The SMC are adaptable and can alter their tone in order to support erectile function [86]. This is possible through their composition with interdigitating filaments of myosin and actin that are irregularly aligned and orientated in multiple directions creating a sliding mechanism of contraction [87]. The vasoactive substances involved induce changes in smooth muscle tone by altering pharmacomechanical coupling and/or electromechanical coupling to alter membrane potential, ultimately affecting intracellular Ca^{2+} levels and/or Ca^{2+} sensitivity [88]. At its most basic, contractile signals lead to increased Ca^{2+} levels triggering vasoconstriction whilst relaxant signals lead to reduced Ca^{2+} levels causing vasodilation.

Intracellular Ca^{2+} concentration and the action of the regulatory molecule calmodulin are essential to SMC tone. Myosin in the smooth muscle fibres is activated by Ca^{2+}-dependent calmodulin and phosphorylated by myosin light chain kinase (MLCK), resulting in cross-bridge movement along actin filaments. Myosin light chain phosphate (MLCP), a protein kinase C-potentiated inhibitor protein, dephosphorylates myosin to inactivate this movement [86]. The regulation of smooth muscle contractility can occur via either Ca^{2+}-dependent and/or Ca^{2+}-independent pathways through the stimulation of intracellular Ca^{2+} or inhibition of MLCP respectively. For example, the activity of MLCP can be affected by the Rho/Rho kinase (RhoA/ROK) pathway [89]. Agonists bind to G-protein-coupled receptors activating Rho proteins (GTPases) which subsequently activates ROK which phosphorylates MLCP [89]. The RhoA/ROK pathway affects MLCP and plays a role in the regulation of SMC contraction in genital tissue; however, the relative importance of this is uncertain.

Amongst the many factors implicated in the peripheral control of the erectile process, the L-arginine-nitric oxide-cyclic guanosine monophosphate (L-arginine-NO-cGMP) pathway is considered to be the key pathway [58]. NO is recognised as the primary neurotransmitter responsible for SMC vasodilation in the CC [90]. Early work with nitrovasodilators (e.g., nitroglycerin) demonstrated their ability to relax vascular tissues by increasing soluble guanylyl cyclase (sGC) and cGMP levels in tissue [91]. The importance of endothelial cells and their ability to produce endothelium-derived relaxing factor (EDRF) was discovered [92] and this was soon proven to be NO [93]. Sexual stimulation and the transmission of desire appear to

decrease adrenergic tone, increase NANC parasympathetic activity and stimulate endothelium-dependent relaxation in the CC by activating the L-arginine-NO-cGMP pathway [58]. In the presence of the limiting substrate L-arginine and oxygen, the Ca^{2+}/calmodulin dependent enzymes neuronal nitric oxide synthase (nNOS) and endothelial nitric oxide synthase (eNOS) convert L-arginine to NO and citrulline (Figure 2.2) in the autonomic nerve terminals and the endothelial cells respectively. Once released, NO from both sources permeates plasma membranes and enters the SMC in the CC and arteries. NO activates sGC causing an increase in cGMP concentration and resulting in activation of cGMP-dependent protein kinase (cGKI or protein kinase G) [94]. In contrast, other vasodilator agonists (e.g., vasoactive intestinal polypeptide (VIP)) control the erectile process via activation of G-protein-coupled-receptors, stimulating plasma membrane associated adenylyl cyclase and increasing the level of cyclic adenosine monophosphate (cAMP), resulting in activation of cAMP-dependent protein kinase (cAK or protein kinase A) [90, 94]. Both cGKI and cAK work by inhibiting Ca^{2+} release from storage, or stimulating Ca^{2+} recycling [95]. cGMP and/or cGKI may also be involved in the inhibition of ROK and the stimulation of MLCP activity via phosphorylation to inactivate contraction [96].

In addition to the pharmacomechanical coupling mentioned above, electromechanical coupling mechanisms alter the membrane potential. The effect of vasoconstrictors is in part mediated by activation of voltage-gated L-type Ca^{2+} channels by phosphatidylinositol 3,4,5-trisphosphate (PIP_3) [97] resulting in depolarisation and influx of extracellular Ca^{2+}. In contrast, the effect of vasodilators and NO is in part mediated by activation of K^+ channels (i.e. Ca^{2+}-activated maxi-K^+ channel (BK_{Ca})) and/or ATP-sensitive K+ channels by cGKI and cAK, resulting in hyperpolarisation and efflux of K^+ inactivating L-type Ca^{2+} channels and inhibiting Ca^{2+} influx [98]. NO has also been shown to directly activate BK_{Ca} channels in SMC of rabbit aorta suggesting mediation of membrane hyperpolarisation independent of cGKI and cAK [99]. Irrespective of the mechanism involved, NO results in the relaxation of penile tissues, increasing arterial blood flow and allowing distension of the sinusoidal spaces to support tumescence. Thus NO is an important factor in both central and peripheral mediation of the erectile process.

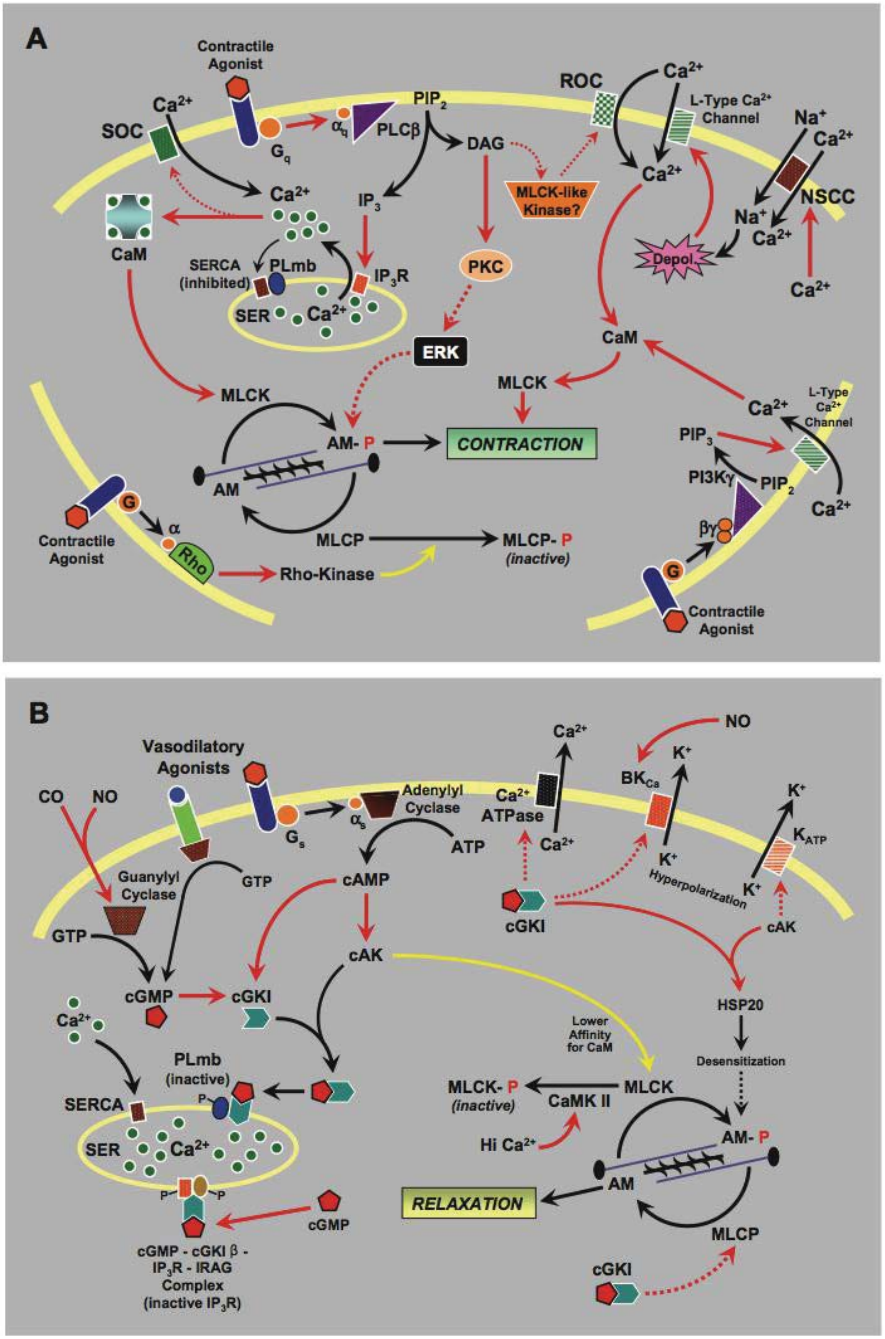

Figure 2.2. Signal transduction pathways regulating smooth muscle contraction (A) and relaxation (B). Red arrows denote association/binding/activation; yellow arrows, inhibition; dashed arrows, indirect/assumed interactions; AM, actin-myosin contractile apparatus; BK_{Ca}, calcium-activated maxi- K^+ channel; CaM, calmodulin; CaMK, calmodulin- dependent kinase; cAK, cAMP-dependent protein kinase; cGK, cGMP-dependent protein kinase; CO, carbon monoxide; DAG, 1,2-diacylglycerol; IP_3, inositol 1,4,5-trisphosphate; IP3R, IP3 receptor; IRAG, IP3R-associated cGK substrate; K_{ATP}, ATP-dependent K^+ channel; MLCP, myosin light chain phosphatase; MLCK, myosin light chain kinase; NANC, non-adrenergic non-cholinergic; NO, nitric oxide; O_2, oxygen; PI3K, phosphoinositide 3-kinase; PIP_2, phosphatidylinositol 4,5-bisphosphate; PIP_3, phosphatidylinositol 3,4,5- trisphosphate; PKC, protein kinase C; PLCb, phospholipase C beta; PLmb, phospholamban; ROC, receptor-operated channel; SER, smooth endoplasmic reticulum; SERCA, SER calcium ATPase. (Reprinted from *Journal of Sexual Medicine*, 7, Gratzke C, Angulo J, Chitaley K, Dai YT, Kim NN, Paick JS, Simonsen U U, et al., Anatomy, physiology, and pathophysiology of erectile dysfunction, 445-475, Copyright (2010), with permission from Elsevier) [58].

Ejaculation or loss of sexual stimulation results in sympathetic discharge and release of the vasodilatory agonist noradrenaline and cGMP is metabolised into GMP via phosphodiesterase type 5 (PDE_5). PDE_5 is preferentially expressed in the CC tissue. This causes SMC contraction and vasoconstriction, releasing blood from the CC leading to detumescence [100]. As shown in Figure 2.2, vasoconstrictor agonists (e.g., noradrenaline (NE), endothelin-1 (ET-1), angiotensin II (AT-II), prostaglandin F_{2a} (PGF_{2a}) and thromboxane A_2 (TxA_2)) bind to their respective receptors stimulating membrane-bound phospholipase C beta (PLC-β). This hydrolyses phosphatidylinositol 4,5-bisphosphate (PIP_2), releasing inositol trisphosphate (IP_3) and 1,2-diacylglycerol (DAG). IP_3 binds to specific receptors on the smooth endoplasmic reticulum (SER) which act as a Ca^{2+}-activated Ca^{2+} channel, increasing sensitivity to Ca^{2+} and facilitating Ca^{2+} induced release of Ca^{2+} from intracellular stores [101], resulting in activation of the actin-myosin contractile apparatus and smooth muscle contraction. Dissociation of vasoconstrictor agonists results in recycling of Ca^{2+} by the SER Ca^{2+}-ATPase pump, terminating the signal and restoring intracellular Ca^{2+} levels. In contrast, DAG, a secondary messenger, directly activates protein kinase C (PKC), regulating ion channels and phosphorylating substances to cause contraction. It may also mediate Ca^{2+}-independent contraction. Hydrolysis of DAG by lipases terminates DAG/PKC signaling [102]. It is evident that the mechanisms involved in the development and maintenance of an erection are highly complex and disruption at any point in the erectile process can lead to dysfunction. Normal vascular (endothelial and SCM) function is essential to erectile physiology.

2.9 Assessment and diagnosis

The aetiology of ED is generally multifactorial, with both organic and psychogenic factors often affecting the quality of an erection [103]. The recommendations of the International Consultation in Sexual Medicine (ICSM) for the clinical evaluation of sexual dysfunction classified ED into three categories based on aetiology: Type I psychogenic, Type II organic, or Type III mixed [104]. Approximately 80% of ED cases in ageing men are thought to be primarily organic in aetiology [11] and organic ED will be the focus of this thesis. Organic ED cases can be categorised into vasculogenic, neurogenic, endocrinological, anatomical and pharmacological. Vasculogenic ED is caused by vasculopathy leading to impaired SMC relaxation within the CC tissue, inadequate blood to the sinusoidal vessels and poor compression of the subtunical penile veins, causing corporal veno-occlusive dysfunction (CVOD) or venous leak [105]. It is reported to account for over 75% of cases of organic ED, although vasculogenic and neurogenic ED often present together [105]. It is important to thoroughly assess and diagnose ED, and to determine the aetiology in order to expose any underlying concomitant pathologies

and optimise the efficacy of treatment. As organic and psychogenic aetiologies are not mutually exclusive, it is important to highlight that assessment, diagnosis and treatment should address the possibility of both aetiologies.

2.9.1 Clinical setting

The accurate clinical evaluation of men with ED is controversial and continually evolving [106, 107]. Prior to the introduction of sildenafil in 1998, evaluation assessment commonly followed the "goal-directed" approach, tailoring evaluation to patient treatment goals and physical and mental condition. This remains the most common assessment method as it avoids costly and invasive testing. In contrast, "cause-directed" assessment focuses more strongly on extensive evaluation to determine the aetiology and best treatment option. With the availability of safe and effective oral medications, there is debate over whether such extensive evaluation is necessary. Indeed the recommendations of the ICSM [104] state that while assessment of sexual, medical, and psychosocial history is mandatory, both physical examination and laboratory testing are highly recommended.

Firstly, symptoms must be assessed. There are various self-reporting tools available [19, 108-110], including validated questionnaires, which can be used both to facilitate a conversation regarding sexual function and to evaluate the severity of symptoms. These will be discussed further in Section 2.8.2. Health professionals need to overcome their own embarrassment, be sensitive to potential patient embarrassment, and understand the physical medical aetiology and the psychological, behavioural and partner issues involved. Sensitivity to age, culture, religion, attitudes and beliefs regarding sexual function is essential to put patients at ease and ensure thorough assessment and diagnosis and appropriate support, education and treatment.

In some cases, the non-invasive assessment of symptoms and history of risk factors including comorbidities, medications and lifestyle factors may be sufficient to support first line treatment with oral medications. If medication is not effective, more costly and invasive subsequent evaluation may be justified [106]. An additional physical examination may be sufficient to identify some underlying issues including urogenital abnormalities, physical scarring and trauma, nerve damage, obesity, hypertension and atrial fibrillation [107]. However, the diagnosis of ED offers a unique opportunity for the early identification of underlying potentially life-threatening medical issues. There appears to be general acknowledgement that the following measures are advisable in a clinical setting (Table 2.1): a detailed medical, sexual and psychosocial history, a focused general and local urogenital examination and baseline laboratory assessments [107, 111]. Further diagnostic testing and urological examination (e.g., Nocturnal Penile Tumescence (NPT) testing and penile Doppler

sonography (PDS) (see Appendix 2)) can help to distinguish the aetiology of ED as psychosociological, vascular, neurological, hormonal or anatomical, or a combination of these [112].

Table 2.1. Recommendations for the assessment of erectile dysfunction (adapted from Ghanem [107] and Grant [111])

Stage 1: History	
A detailed medical, sexual and psychosocial history	
Stage 2: General physical examination	
Blood pressure, heart rate and heart rhythm	Rule out obvious abdominal abnormalities
Male secondary sex characters	Vibratory sensation
Gynecomastia	Waist circumference
Peripheral pulses	Scarring from surgery or trauma
Stage 3: Local physical examination	
Penis size, lesions, scars, fibrosis, and position of meatus	Digital rectal examination for prostate and seminal vesicles
Testicular size and consistency	Bulbocavernosus reflex*
Stage 4: Laboratory testing	
Fasting blood sugar	Full blood count*
Lipid profile	Renal function*
Hormone profile (Testosterone, prolactin, sex hormone binding globulin (SHBG)*, luteinising hormone (LH)*)	Liver function*
Prostate Specific Antigen (PSA)*	Electrolytes*
Thyroid hormones*	

*Optional

2.9.2 Research setting

There is no universally recognised gold standard method for assessing ED in research settings. ED is commonly assessed through self-reporting of symptoms using validated questionnaires such as the single question global subjective self-assessment [108] (Table 2.2) or the multi-item Brief Male Sexual Function Inventory (BMSFI [11 items, 3 in the erectile function domain]) [109], the International Index of Erectile Function (IIEF [15 items, 6 in the erectile function domain]) [19] or the abbreviated International Index of Erectile Function (IIEF-5 [5 items, 4 in the erectile function domain]) [110] (Table 2.3). The available tools differ in sensitivity and specificity; therefore, data gathered using these methods cannot be reliably compared.

Table 2.2. The single question global subjective self-assessment [1, 113]. Current erectile function is self-reported as one of 4 categories.

Erectile dysfunction (sometimes called impotence) means being unable to get and keep an erection that is rigid enough for satisfactory sexual activity	
Not impotent	*Always* able to get and keep an erection good enough for sexual intercourse
Minimally impotent	*Usually* able to get and keep an erection good enough for sexual intercourse
Moderately impotent	*Sometimes* able to get and keep an erection good enough for sexual intercourse
Completely impotent	*Never* able to get and keep an erection good enough for sexual intercourse

Table 2.3. The abbreviated 5-item International Index of Erectile Function (IIEF-5 (5 items, 4 in the erectile function domain)) [110]. The composite score for erectile function over the past 4 weeks ranges from 5-25 with ED generally defined as ≤21 (Severe (5-7), moderate (8-11), mild to moderate (12-16), mild (17-21)), no ED (22-25)).

1. How do you rate your confidence that you could get and keep an erection?				
1 Very low	2 Low	3 Moderate	4 High	5 Very high
2. When you had erections with sexual stimulation, how often were your erections hard enough for penetration?				
1 Almost never or never	2 Much less than half the time	3 About half the time	4 Much more than half the time	5 Almost always or always
3. During sexual intercourse, how often were you able to maintain your erection after you had penetrated (entered) your partner?				
1 Almost never or never	2 Much less than half the time	3 About half the time	4 Much more than half the time	5 Almost always or always
4. During sexual intercourse how difficult was it to maintain your erection to the completion of intercourse?				
1 Extremely difficult	2 Very difficult	3 Difficult	4 Slightly difficult	5 Not difficult
5. When you attempted sexual intercourse, how often was it satisfactory for you?				
1 Almost never or never	2 Much less than half the time	3 About half the time	4 Much more than half the time	5 Almost always or always

The landmark study into sexual function in ageing men, the longitudinal 1987-1997 Massachusetts Male Aging Study (MMAS), used the single question global subjective self-assessment tool to evaluate ED allowing classification into four categories of ED: not impotent, minimally impotent, moderately impotent or completely impotent (Table 2.2). It has been argued that this limits its comparability to recent research using more sensitive multi-item well-validated tools [114-116]. However the MMAS question was later validated against both the BMSFI [109] and the IIEF [19] and was found to compare favourably ($r=0.71-0.78$, $p<0.001$), reporting similar prevalence and risk factor associations with similar levels incomplete responses (MMAS 9%, BMSFI 8%, IIEF 18%) [108]. It was also clinically validated against the gold standard urological examination using a subsample of men from the MMAS (n=137 men, 55-85 years) and was found to accurately predict clinically diagnosed ED if an individual self-reported moderate, or complete ED (Spearman's rho (r_s)=0.80, area under the curve (AUC)=0.888) [117]. A simple single-item self-assessment of ED appears to be a practical tool for the assessment of prevalence in large population-based studies where detailed clinical measurement is impractical. The MMAS question, an adaptation of this or a similar simple self-assessment question have been used throughout the world and are the most widely used tools in published studies [7, 118-130].

In contrast to the single-item question, the IIEF-5 (Table 2.3), also called the Sexual Health Inventory for Men (SHIM), offers greater sensitivity to measure the severity of ED while remaining practical in a research setting [110]. The IIEF-5 was validated against clinically diagnosed ED (1036 ED, 116 controls) and was found to be highly sensitive (98%) and specific (88%) in correctly identifying ED. It contains 5-items written in simple English and uses a basic scoring system that supports a score for ED severity ranging from 5-25 with simple established cut-off points for consistent categorisation (ED ≤21 (Severe (5-7), moderate (8-11), mild to moderate (12-16), mild (17-21)), and no ED (22-25) [110]. The IIEF-5 is widely considered the best method available [5, 24, 114, 115, 131-136].

Erectile function can be assessed physiologically; however, this is often impractical and researchers commonly rely on self-reporting. Large epidemiological surveys investigating the prevalence and incidence of ED require a short self-reporting tool that can accurately evaluate ED symptoms, while clinical studies require a tool that will assess symptoms in response to treatment. Self-reporting is acknowledged to be fraught with issues surrounding reliability, accuracy and precision. However, as only a man and his partner have access to information about real-life sexual function, it is best assessed via self-reporting. The multidimensional nature of sexual dysfunction means that even a composite score generated from multi-item validated inventories such as the IIEF-5 will not be sufficiently sensitive and specific to truly represent the severity. Ideally studies investigating ED would include a combination of self-reporting and clinical assessment.

2.10 Treatment and prevention

The successful treatment of ED relies upon the accurate assessment and understanding of the aetiology; joint informed decision-making between the health professional, the patient and their partner based on their sexual life goals; and an appropriate combination of medical, psychosexual and lifestyle intervention [137-139] to treat both the symptoms and the underlying causes. To maximise efficacy, it is essential to fully educate both parties about the aetiology and its implications, the treatment options, their mode of action, and possible side effects.

2.10.1 Medical intervention

Guidelines outlining the appropriate management of ED are available but there is no uniform consensus on the recommendations. A step-wise progression of ED therapeutic intervention has been suggested [140] based on ease of administration, reversibility, invasiveness and cost with priority given to patient and partner preference. The majority of available treatment

options focus on improving blood flow to the penis. First-line therapy (oral erectogenic drugs, vacuum constriction devices and psychosexual therapy) can be used alone or in combination. Second-line therapy (intracavernosal injections, transurethral alprostadil pellets) and third-line therapy (penile surgical prosthesis) should only be considered for patients who either do not respond to, or show adverse effects from, first-line therapies. For additional information on these treatment options see Appendix 2.

Advances in pharmacological, mechanical and surgical treatment of ED have enabled the re-establishment of erectile function in most men, although their efficacy is dependent upon their appropriate use and patient motivation. However, these treatments offer short-term improvement of ED symptoms and do not address the specific etiological issues underlying the dysfunction. Addressing these, prior to or in combination with direct therapies should be considered a part of good clinical practice (e.g., the concomitant treatment of comorbidities such as CVD and the reassessment of current medications). Careful assessment of potential drug interactions is necessary when treating ED as medications can both cause ED and affect the efficacy of treatment [141]. Psychosexual counselling may be effective for men with identified psychosocial issues (e.g., relationship issues) and those who are reluctant to take pharmacological treatment or who wish to reduce reliance on drugs. Lifestyle intervention should be considered including weight reduction, increasing physical activity (PA) and smoking cessation. A patient centred holistic approach towards the treatment of ED is needed, including the incorporation of management of the symptoms, and the risk factors and comorbidities [142].

2.10.2 Lifestyle and dietary intervention

Lifestyle modifications may be beneficial and all men presenting with ED should be educated about the importance of making appropriate lifestyle changes. In particular, there is strong evidence to support losing weight [143-148], increasing PA [143, 149] and ceasing smoking [150, 151] in the treatment of ED. Furthermore, moderate consumption of alcohol [152] and caffeine [153] and increasing dietary intake of fruit, vegetable, nuts, whole grains and unsaturated fats [154, 155] may be beneficial, although further research is needed. Additionally, the efficacy of alternative treatments such as traditional herbal remedies and nutritional supplements (see Appendix 2) is an area ripe for further investigation. A recent review [156] found limited evidence supporting the efficacy of many of these alternative treatments and the available evidence was from *in vitro* and animal studies. As products containing these alternative remedies are already being sold for the treatment of ED, it is important that their safety and efficacy profiles are established through human intervention

studies. This includes a number of polyphenols (e.g., resveratrol, quercetin and kraussianones) which may be beneficial as ED treatments [156] and could explain the protective effects of increased consumption of fruit and vegetables [154, 155]. It is clear that ED is an area with significant potential for lifestyle and dietary intervention in the treatment and prevention of ED.

3.1 EPIDEMIOLOGY

3.2 Prevalence

Research into sexual function began in the 1940s. Kinsey and colleagues [157] conducted the first epidemiological study of sexual behaviour in the USA and found 1% prevalence of ED in men <30 years, 3% in men aged 30-45 years, 7% in men aged 45-55, 14% in men aged 55-65 years and 25% in men over 65 years of age. This suggested a strong relationship between ED and ageing; however, the sample size in older age groups was small, limiting the significance of these results. Research in the field was greatly extended in the 1980s with the development of modern epidemiological methods [22, 158, 159]. The 1987-1997 MMAS provided important cross-sectional and longitudinal data on male sexual health and ageing in Massachusetts USA (n=1709, 40-70 years, baseline data collection 1987-1989, follow-up 1995-1997) [1]. It was the first population-based study to investigate the prevalence, incidence and medical and lifestyle correlates of ED in ageing men. The overall prevalence of ED in men aged 40-70 years was approximately 52%. By 80 years of age it was suggested that only 25% of men would be able to achieve normal erections. The results showed approximately 40% prevalence in men in their 40s, 50% in their 50s, 60% in their 60s and 70% in their 70s [1], supporting a strong association between ED and ageing.

The MMAS figures are widely used in ED research and are often extended to other populations; however, each community has unique characteristics that will affect the prevalence of ED and while the MMAS is representative of the Massachusetts population, the lack of sociocultural diversity (96% Caucasian, predominately highly educated) limits its applicability to other populations. The MMAS also had no direct measure of ED, instead a post-hoc variable for subjective global self-assessment (see Table 2.2) based on the aforementioned definition of ED was generated using a clinical convenience sample [113] and applied retrospectively. Since the MMAS, numerous epidemiological studies have been conducted (Table 2.4). Published data generally represent population, community or clinical-based studies. Population [3, 5, 26, 27, 119, 120, 124, 127, 129, 160-162] and community-based [7, 28, 114, 118, 122, 125, 126, 131-133, 163-165] studies rely on self-reporting using surveys and can provide valuable information about the prevalence of ED in the population or community

group sampled. Clinical studies tend to investigate the prevalence of ED in a specific sample population of patients with a particular risk factor increasing their likelihood of ED. The prevalence of ED has been investigated in many countries and Table 2.4 presents a selection of studies conducted after the introduction of the 1993 National Institutes of Health Consensus Panel on Impotence definition outlined earlier.

Although ED is clearly a common condition worldwide, published evidence is extremely heterogeneous. Prevalence rates for any degree of ED from the selected population-based studies vary markedly from 12.1% in Spanish men aged 25-70 years [121] to 74% in Finnish men aged 50-70 years [120]. Rates from community-based studies range from 39% in general medical practice patients aged 18-91 years in Australia [28], to 75% in men attending prostate cancer health screening aged 30-90 years in the USA [114]. A large difference in population-based prevalence rates between countries can be expected due to differences in the sociocultural characteristics of populations and exposure to relevant medical and lifestyle risk factors. For example, in a population-based study Martin-Morales et al [121] found a 12.1% prevalence in the general non-institutionalised population of Spanish men aged 25-70 while Selvin et al [119] found a 35% prevalence in the general non-institutionalised population of American men over 20 years of age. These two studies are comparable as they are both nationally representative, investigate a similar age strata and used a similar self-administered single-item self-reporting question. However, sociocultural differences in men's attitude towards ED may affect self-reporting and it is not certain to what extent this impacts on prevalence rates [40]. As there is a paucity of NZ specific data available, this review will focus mainly on studies conducted in Australia and the USA in addition to relevant multinational studies. These countries are deemed the most culturally and geographically appropriate for comparison.

Table 2.4. A summary of selected population and community-based studies investigating the prevalence of erectile dysfunction (ED).

Author, year (Study)	Study population	Selection method and sampling frame	Method of administration	Tool used to evaluate ED, period covered	Respondents n, total Sample (N), response rate %	Age (years)	Prevalence of ED (%)		
							Mild	Moderate /Severe	Overall
Australia									
Pinnock 1999 [166] (Omnibus Survey)	General population of South Australia	Subset of 1997 Omnibus multiple-user household interview survey	Postal questionnaire	UCLA prostate cancer index, 3 months	427(745) 57%	>40	ND	21	ND
Chew 2000 [28]	General medical practice population in Perth, Australia	Consecutive patients across 62 general medical practices	Self-administered questionnaire	3-item question (Dichotomous question followed by duration and frequency)	1240(3100) 40%	18-91	9.6	27.5	39.4
Chew 2008 [5] (WAMHS)	General population of Western Australia	Age-stratified random selection, WA electoral roll	Postal questionnaire	IIEF-5	1770(4228) 42%	>20	16.6	23.8	40.3 (23.4)
Holden 2010 [162, 167] (MATeS)	General population of Australia	Age and geographically stratified random selection from telephone directory	CATI	Single-item question (ability to "get and keep", 4 response categories)	5990(7636) 78%	>40	ND	21.3	ND
Martin 2012 [168] (FAMAS)	General population of Adelaide, Australia	Random selection, residents in north-western suburbs	Self-administered questionnaire	IIEF	1195(ND)	35-80	35.2	17.7	52.9
Weber 2013 [169] (45 and Up Study)	General population of New South Wales, Australia	Random selection, Medicare enrolment database	Self-administered questionnaire	Single-item question (ability to "get and keep", 4 response categories)	108477(ND) 18%	≥45	ND	38.0	ND

Author, year (Study)	Study population	Selection method and sampling frame	Method of administration	Tool used to evaluate ED, period covered	Respondents n, total Sample (N), response rate %	Age (years)	Prevalence of ED (%)		
							Mild	Moderate /Severe	Overall
USA									
Feldman 1994 [1] (MMAS)	General non-institutionalised population of Massachusetts, USA	Probabilistic stratified random sampling from the Massachusetts census list in 11 cities and towns in the Boston area	Face-to-face and self-administered questionnaire	Single-item question (ability to "get and keep", 4 categories)	1290(1709)	40-70	17	35	52
Panser 1995 [170] (Olmsted County)	General population with regular sexual partner and without certain surgical, medical or neurological conditions in Olmsted County, Minnesota, USA	Age and geographic-stratified random selection from population	Face-to-face and self-administered questionnaire	Single-item question ("ability to have" rated on 6-point scale), 1 month	2115(5135) 41%	40-79	ND	12	ND
Laumann 1999 [3] (NHSLS)	General population of USA	National probability sample	Face-to-face with standardised questionnaire	Single-item question ("arousal difficulties, dichotomous response), 12 months	1244(1410)	18-59	ND	5	ND
Ansong 2000 [163]	Rural counties population of Central New York State, USA	Age-stratified random sampling from census data	Postal questionnaire	Dichotomous question, 6 months	1438(5198) 28%	50-76	ND	ND	46.2
Bacon 2003 [164] (HPFS)	Health professionals, USA	Surviving active participants in the HPFS cohort (1986)	Postal questionnaire	Single-item question (ability to "have and maintain", 5 point scale), 5 years	34282(43235) 79%	>50	ND	33	ND

Author, year (Study)	Study population	Selection method and sampling frame	Method of administration	Tool used to evaluate ED, period covered	Respondents n, total Sample (N), response rate %	Age (years)	Prevalence of ED (%)		
							Mild	Moderate /Severe	Overall
Monga 2002 [115] (Rancho Bernado)	General population of Rancho Bernado, California, USA	Surviving members of community-based cohort of a heart disease risk factor study (1972)	Postal questionnaire	IIEF-5, 6 months	976(1525) 64%	>30	24	27	51
Barqawi 2005 [114] (PCAW)	Men attending PCAW screening program USA	Volunteers from men attending PCAW	Self-administered questionnaire	IIEF-5	6641(12679)	30-90	ND	ND	75
Selvin 2007 [119] (NHANES)	General population USA	Complex multi-stage probability sampling from non-institutionalised population	Computer-assisted self-administration	Single-item question (ability to "get and keep", 4 categories)	2126	>20	16.5	18.5	35
Kupelian 2010 [131] (BACH)	General population, Boston, USA	Multi-stage stratified random sampling from Boston city	Face-to-face and self-administered questionnaire	IIEF-5	2301	30-79	37.4	9.2	46.6
Londoño 2012 [118]	Men enrolled local health plans, Kaiser Permanente, Southern California, USA	Volunteers from 2 Kaiser Permanente health plans enrolled in California Men's Health Study	Postal questionnaire	Single-item self-report ("getting and keeping", 4 categories)	37712	45-69	28.1	28.5	56.5
Europe									
Helgason 1996 [34]	General population of Stockholm, Sweden	Random selection, Swedish Population Registry of residents	Postal questionnaire	Radiumhemmet's Scale of Sexual Function (frequency of erection stiffness "usually sufficient for intercourse", 8 ordinal categories)	319(435) 73%	50-80	ND	32	ND

Author, year (Study)	Study population	Selection method and sampling frame	Method of administration	Tool used to evaluate ED, period covered	Respondents n, total Sample (N), response rate %	Age (years)	Prevalence of ED (%)		
							Mild	Moderate /Severe	Overall
Ventegodt 1998 [171]	General population of Denmark	All persons born on a set date every 5 yrs, 1904-1974, Danish population (CPR Register)	Postal questionnaire	Single dichotomous question	1494(2460) 61%[741 men]	18-88	ND	5.4	ND
Braun 2000 [172] (Cologne Male Survey)	General population of Cologne, Germany	Age-stratified sampling in Cologne district	Postal questionnaire	Validated Cologne ED questionnaire (6-item question and composite ED score)	4489(8000) 56%	30-80	ND	19.2	ND
Koskimaki 2000 [120]	General population of Finland	Random selection of men in Tampere and 11 municipalities, national population register	Postal questionnaire	Two-item question ("getting" and "maintaining" each with 4 response categories)	2198(3143) 70%	50-70	48	26	74
Blanker 2001 [173] (Krimpen Study)	General population without urogenital disorders in a Dutch municipality, The Netherlands	Eligible men from total population of men 50-75 yrs in a Dutch municipality near Rotterdam	Self-administered questionnaire	International Continence Society (ICS) sex questionnaire	1688(3924) 43%	50-78	ND	6.4	ND
Martin-Morales 2001 [121]	General population of non-institutionalised men in Spain	Stratified probabilistic multi-stage random sampling of non-institutionalised residents of Iberian peninsula	Face-to-face and self-administered questionnaire	Single-item self-assessment ("incapacity", 4 response categories) and IIEF	1857(2476) 75%	25-70	5.2 16.2	6.9 2.7	12.1 18.9

Author, year (Study)	Study population	Selection method and sampling frame	Method of administration	Tool used to evaluate ED, period covered	Respondents n, total Sample (N), response rate %	Age (years)	Prevalence of ED (%)		
							Mild	Moderate /Severe	Overall
Mak 2002 [122]	General population of urban Belgium	Age-stratified random selection, official population registers of Ghent and Charleroi	Face-to-face structured interview	Single-item self-report ("get and maintain", 4 response categories) and IIEF	799(1615) 50%	40-70	26.6	34.8	61.4
Lyngdorf 2004 [7]	General population enrolled in medical practice in Zealand, Denmark	All patients enrolled at 12 GP practices, Naestved Zealand, Denmark	Postal questionnaire	Single-item self-report ("achieve and maintain", 4 response categories) and IIEF	2246(4310) 52%	40-80	ND	ND	52
Ponholzer 2005 [132]	General population of men volunteering for health examinations in Vienna, Austria	Consecutive series of volunteers participating in free health examinations, Vienna	Self-administered questionnaire	IIEF-5	2869	20-80	23.7	8.5	32.2
Korfage 2008 [174]	Elderly men without prostate cancer in Rotterdam, The Netherlands	Selection of consecutive participants in the European Randomised study on Screening for Prostate Cancer (ERSPC)	Postal questionnaire	Dutch module SAc	3892(4822) 81%	58-78	ND	ND	19.1
Multinational									
Morillo 2002 [128] (DENSA)	Urban population of Colombia, Ecuador and Venezuela	Multi-stage random sampling from densely populated cities based on local census data	Face-to-face with self-administered questionnaire	Single-item question ("achieve and maintain", 4 response categories - Spanish)	1963(2384) 82% Colombia 622 Ecuador 670 Venezuela 654	≥40	33.6 *32.3* *31.8* *36.7*	19.8 *20* *18.6* *19.9*	53.4 *52.8* *52.1* *55.2*

Author, year (Study)	Study population	Selection method and sampling frame	Method of administration	Tool used to evaluate ED, period covered	Respondents n, total Sample (N), response rate %	Age (years)	Prevalence of ED (%)		
							Mild	Moderate /Severe	Overall
Nicolosi 2003 [129] (Pfizer Cross-National Study)	General population of Brazil, Italy, Japan and Malaysia	Sampling of households in each country	Face-to-face (Brazil), Telephone interview (Italy), Telephone or in person (Malaysia), postal questionnaire (Japan)	Single-item question ("get and keep", 4 response categories)	2400(ND) Brazil 600 92% Italy 600 72% Japan 600 51% Malaysia 600 16%	40-70	 25 49 44 42	 15.5 17.2 34.5 22.4	 40.5 66.2 78.5 64.4
Rosen 2003 [27] (MSAM-7)	General population of USA, UK, France, Germany, the Netherlands, Italy, Spain	Complex multi-stratified sampling from household database	Postal survey	DANPSS and IIEF	14254(34800) 41%	50-80	ND	ND	48.9
Rosen 2004 [161] (MALES)	General population in USA, UK, Germany France, Italy, Spain, Mexico, Brazil	Subsample of the MALES participants (N=27839) recruited via random-digit dialling (80%), random selection of email addresses of participants in a consumer survey participants (20%)	CATI with standardised questionnaire (80%), Internet-based interview (20%)	Single-item question ("erection difficulties", dichotomous response)	27839(ND) *USA 45%* *UK 48%* *Germany 45%* *France 48%* *Italy 53%* *Spain 50%* *Mexico 55%* *Brazil 51%*	20-75	ND	16 *22* *13* *13* *11* *13* *10* *14* *14*	ND

Author, year (Study)	Study population	Selection method and sampling frame	Method of administration	Tool used to evaluate ED, period covered	Respondents n, total Sample (N), response rate %	Age (years)	Prevalence of ED (%)		
							Mild	Moderate /Severe	Overall
Corona 2010 [130] (EMAS)	Representative sample of urban community dwelling men in Italy, Belgium, Sweden, UK, Spain, Poland, Hungary, Estonia	Age-stratified random sampling from population registers in one major centre in each country	Interviewer-assisted questionnaire	EMAS-SFQ incl. single-item question ("get and keep", 4 response categories), 1 month	3369(8416) 41% *Italy* *Belgium* *Sweden* *UK* *Spain* *Poland* *Hungary* *Estonia*	40-79	ND	30 *25* *32* *24* *31* *23* *36* *30* *43*	ND
Laumann 2005 [26] (GSSAB)	General ageing urban population of 29 countries	Random digit dialing or door-to-door sampling in major cities or intercept sampling in major cities depending on country	CATI/ face-to-face/ self-completed standardised questionnaire	Two-item question ("erectile difficulties", 3 response categories), 12 months	27516(191310) 14% [13618 men]	40-80	ND	10	ND
Moreira 2005 [175] (GSSAB)	General ageing urban population of Spain	Random digit dialing	CATI	*As above*	1500(6584) 23% [750 men]	40-80	4.4	7.7	12.7
Moreira 2005 [176] (GSSAB)	General ageing urban population of Germany	Random digit dialing	CATI	*As above*	1500(8608) 17% [750 men]	40-80	2.0	5.9	7.9
Moreira 2005 [177] (GSSAB)	General ageing urban population of Brazil	Random digit dialing	CATI	*As above*	1199(6510) 18% [471 men]	40-80	4.1	9.0	13.1

Author, year (Study)	Study population	Selection method and sampling frame	Method of administration	Tool used to evaluate ED, period covered	Respondents n, total Sample (N), response rate %	Age (years)	Prevalence of ED (%)		
							Mild	Moderate /Severe	Overall
Moreira 2006 [178] (GSSAB)	General ageing urban population of Korea	Arbitrary intercept method in public areas	Face-to-face and self-completed questionnaire	*As above*	1200(3691) 33% [600 men]	40-80	15.7	16.1	31.9
Moreira 2008 [179] (GSSAB)	General ageing urban population of Australia	Random-digit dialing	CATI	*As above*	1500(8875) 17% [750 men]	40-80	6.0	15.1	21.1
Moreira 2008 [180] (GSSAB)	General ageing urban population of UK	Random-digit dialing	CATI	*As above*	1500(8820) 17% [750 men]	40-80	5.3	12.6	17.8
Laumann 2009 [6] (GSSAB)	General ageing urban population of USA	Random-digit dialing	CATI	*As above*	1491(16560) 9% [742 men]	40-80	9.6	12.4	22.5
Nicolosi 2006 [181] (GSSAB)	General ageing urban population of Europe [Sweden, UK, Belgium, Germany, Austria, France, Spain, Italy]	Random-digit dialing	CATI	*As above*	10000(~54300) 18% [4977 men] *Sweden 750* *UK 750* *Belgium 250* *Germany 750* *Austria 227* *France 750* *Spain 750* *Italy 750*	40-80	ND	8 *7* *13* *13* *6* *6* *9* *8* *8*	ND

Author, year (Study)	Study population	Selection method and sampling frame	Method of administration	Tool used to evaluate ED, period covered	Respondents n, total Sample (N), response rate %	Age (years)	Prevalence of ED (%)		
							Mild	Moderate /Severe	Overall
Nicolosi 2006 [8] (GSSAB)	General ageing urban population of Anglophone countries [USA, Canada, UK, Australia, NZ]	Random-digit dialing	CATI	*As above*	6012(47543) 13% [2992 men] *USA 742* *Canada 500* *UK 750* *Australia 750* *NZ 250*	40-80	ND	11 *10* *7* *13* *16* *25*	ND
Shaeer 2011 [24] (GOSS)	Internet users, Middle East [Egypt, Libya, Tunisia, Algeria, Morocco, Sudan, Saudi Arabia, Yemen, Palestine, Lebanon, Jordan, Syria, Iraq, Kuwait, Qatar, UAE, Bahrain]	Volunteer participation through random advertising on Facebook and other online media	Online survey	IIEF-5 (Arabic) and single-item question ("do you suffer from ED/impotence?" dichotomous response)	3110(6030)	>18	42.5	2.6	45.1 (47)
Shaeer 2012 [136] (GOSS)	English-speaking male internet users, USA	*As above*	Online survey	IIEF-5 (English) and single-item question as above	2022(10814) 19%	>18	26.7	11	37.7 (33.7)

Response rates are provided where possible and calculated by dividing initial response number by total sample size of those eligible to support comparison. Where a study included both genders, the number of male respondents has also been included. Different tools are used to classify ED: where ED prevalence has been reported as occasionally, sometimes or frequently these have been reported as mild (occasionally) or moderate to severe (sometimes or frequently) and where ED prevalence has been reported as mild, mild-moderate, moderate and severe categories, these have been reported as mild (mild or mild-moderate) or moderate/severe (moderate or severe) respectively. The prevalence of ED is provided as a percentage but is calculated differently in each study: most often either crude prevalence in total respondents, crude prevalence in sexually active respondents, age-adjusted prevalence to reflect the population age distribution, or the World Standard Population (WSP-adjusted) prevalence rate adjusted to reflect the World Health Organizations WSP to support international comparison (shown in parentheses where available). BACH, Boston Area Community Health Survey; CATI, Computer Assisted Telephone Interview; Cologne Male Survey, Kölner Erfassungsbogen der Erektilen Dysfunktion (KNEED); EMAS, European Male Ageing Study; FAMAS, Florey Adelaide Male Ageing Study; GOSS, Global Online Sexuality Survey; GSSAB, Global Study of Sexual Attitudes and Behaviours; HPFS, Health Professionals Follow-Up Study; IIEF-5, 5-item International Index of Erectile Function; MALES, Multinational Men's Attitudes to Life Events and Sexuality; MATeS, Men in Australia Telephone Survey; MMAS, Massachusetts Male Aging Study; MSAM-7, Multinational Survey of the Aging Male; ND, not determined/divulged; NHANES, National Health and Nutrition Examination Survey; NHSLS, National Health and Social Life Survey; Olmsted County Study, Olmsted County Study of Urinary Symptoms and Health Status Among Men; PCAW, Prostate Cancer Awareness Week; Pfizer Cross-National Study, Pfizer Cross-National Study of the Prevalence and Correlates of Sexual Dysfunction; WAMHS, Western Australia Men's Health Study.

3.2.1 New Zealand

Reliable NZ specific data on the prevalence of ED are currently lacking. Extension of the prevalence rates reported in the MMAS [1] or in the Australian population [5, 28, 162, 166, 168, 169] is not ideal due to differences in the socio-cultural environment, ethnic profile and occurrence of associated risk factors between NZ and the populations examined. To date, there is only one study investigating the prevalence of ED in NZ. The 2000-2001 multinational GSSAB included NZ as one of the 29 countries examined. Nicolosi et al [8] found that of the five Anglophone countries examined, ED was most frequently reported by men aged 40-80 years in NZ (n=250, 25%), compared to Australia (n=750, 16%), the UK (n=750, 13%), USA (n=742, 10%) and Canada (n=500, 7%). However, this research is not only out-dated, but is also not cited in NZ reports which rely on data from other countries, most commonly the MMAS [182]. There are several limitations to the study design: 1) data collection in NZ was via a structured telephone interview with a standardised questionnaire which lacks anonymity and may alter responses to questions on such a sensitive health issue; 2) only two questions were used to assess ED which is incongruent with the multi-dimensional definition of ED, and these were not validated and did not collect information on the severity of ED; 3) this study focussed on sexual attitudes and behaviours, not ED per se; and 4) it does not provide information on the medical and lifestyle correlates in the NZ population. Further research is needed to examine whether the higher prevalence reported in NZ is reliable and whether this is due to differential exposure to associated risk factors such as medical and lifestyle correlates, socioeconomic factors, genetic predisposition or cultural perception of ED.

3.2.2 Australia

Prevalence rates for moderate-severe ED in Australia range from 17.7% in men aged 35-80 years [168] to 38% in men aged ≥45 years [169]. Among population-based studies, in 1997 Pinnock et al [166] found 21% moderate-severe ED using the UCLA-Prostate Cancer Index in a postal survey of South Australian men (n=427, >40 years). Similarly, in 2002 Chew et al [5] found 23.8% moderate-severe ED using the IIEF-5 in a postal survey in the WAMHS of men (n=1770, >20 years). Also in 2002, Martin et al [168] found 17.7% prevalence of moderate-severe ED using the IIEF in a self-administered survey in the FAMAS (n=1195, 35-80 years). Similarly, the results from the 2000-2001 multinational GSSAB [8] show 16% prevalence of moderate-severe ED in Australian men using a two-item question in a CATI of urban males (n=750, 40-80 years). In 2003, Holden et al [162] found 21.3% prevalence of moderate-severe ED using the MMAS question in a CATI in the nationally representative MATeS (n=5990, >40 years). Excluding the study by Chew et al [5] as it included younger men (>20 years), these

earlier findings are very similar and suggest that the prevalence in men >40 years ranges from 16% to 21.3%. However, more recently in 2013, Weber et al [169] reported 38% moderate-severe ED using the single-item question in a self-administered questionnaire in the 2006-2010 45 and Up Study (n=108,477, ≥45 years) in New South Wales. This suggests that the prevalence of moderate-severe ED in the general ageing male population in Australia may be increasing.

3.2.3 United States

In the USA, the prevalence rate for moderate-severe ED ranges from 5% in men aged 18-60 years [3], to 35% in men aged 40-70 years [1]. Among nationally representative population-based studies, in 1992 Laumann et al [3] found 5% moderate-severe ED using a single-item question with dichotomous responses conducted in a face-to-face interview in the National Health and Social Life Survey (NHSLS, n=1244, 18-59 years). In contrast, in 2001-2002 Selvin et al [119] found 18.5% moderate-severe ED using a single-item question with 4 response categories conducted via computer-assisted self-administration in the NHANES (n=2126, >20 years). These studies both used nationwide probability sampling and a similar age range; however, they differed in the method of administration and the instrument used to evaluate ED. Additionally, data collection for the NHANES occurred after the introduction of PDE-5 inhibitors to the marketplace. Public advertising of an effective treatment option is likely to have increased public awareness of ED and the willingness of men to self-report ED, leading to higher reporting rates in the later NHANES. The more recent NAHNES figure was much lower than the 35% found in Massachusetts men aged 40-70 years in the 1987-1989 MMAS [1] and 27% in Californian men over 30 years of age in the 1998-1999 Rancho Bernado study [115]. The older age range in these two studies can be expected to result in a higher prevalence as age is strongly and consistently associated with ED. However, the NHANES results were higher than those found in some other studies in the USA including 9.2% in men aged 30-79 years in Massachusetts in the 2002-2005 BACH study [131]. In contrast to the NHANES, the BACH study used face-to-face interview, which may have altered participant responses due to a lack of privacy and confidentiality. Furthermore, while the NHANES used the single-item question (16.5% mild, 18.5% moderate-severe ED) the BACH study used the IIEF-5 (37.4% mild, 9.2% moderate-severe ED). The tools differ in their sensitivity to classify mild cases. The NHANES results are higher than that found at a similar time in the 2000-2001 multinational GSSAB [8] which shows 12.4% prevalence of moderate-severe ED in American men using a two-item question in a CATI of urban males (n=1491, 40-80 years). This is contrary to expectations given the older age range in the GSSAB, suggesting that the use of an unvalidated tool and the low response rate (9%) may limit the reliability of these results. The most recent study by Londoño

et al [118] found 28.5% prevalence of moderate-severe ED amongst 37,712 men aged 45-70 enrolled in local health plans in Southern California in 2002-2003. These results are similar to an earlier study in 2000 by Bacon et al [164] which found 33% prevalence amongst 34,282 health professionals aged >50 years in the nationwide HPFS. Again, these studies investigated prevalence in an older age group and therefore higher prevalence rates are not unexpected. The complex random selection method and nationally representative sampling frame used in the NHANES [119] suggest that this study provides the most reliable estimation of the prevalence of moderate-severe ED in the general adult male population in the USA. However, the age of the data (2001-2002) limits its current reliability and new data are needed. The differences reported highlight the importance of the standard adoption of a validated instrument and consistent method of administration to evaluate ED.

Prevalence rates need to be established and monitored in individual countries using well-designed nationally representative population-based cross-sectional studies. Furthermore, the heterogeneity of study designs internationally has lead to a call for multinational studies that employ a consistent methodological approach to support international monitoring and cross-country comparison. Although large multinational studies [24, 26, 27, 128-130, 161] have been conducted they remain fraught with design issues and the data continues to vary widely both within and between studies.

3.2.4 Multinational studies

The 1997-1998 Pfizer Cross-National Study [129] (n=2400, 40-70 years) used a single-item question with 4 response categories and found a prevalence rate for moderate-severe ED of 15.5% in Brazil, 17.2% in Italy, 34.5% in Malaysia, and 22.4% in Japan. However the method of administration and response rates differed significantly between countries (face-to-face interviews in Brazil (92% response), telephone interviews in Italy (72% response), postal questionnaires in Japan (51% response) and either telephone or face-to-face interviews in Malaysia (16%)). This may have affected the reliability of the information for cross-comparison. The 2001 MALES study [161] (n=27839, 20-75 years) used a single-item question with dichotomous responses and found variable prevalence rates for moderate-severe ED in 8 diverse countries (Spain 10%, France 11%, UK, Italy and Germany 13%, Mexico and Brazil 14%, USA 22%). They used two methods of administration (80% computer-assisted telephone interview and 20% internet-interview) but the response rates were similar across the countries (45-55%). The MALES study found a lower prevalence of ED in the countries sampled (10-22%) compared to those examined in the Pfizer Study (15.5-34.5%) but similar rates for the two common countries: Italy (13% MALES vs 17.2% Pfizer) and Brazil (14% MALES vs 15.5% Pfizer).

In comparison to the Pfizer and MALES studies, the 2003-2005 EMAS [130] (n=3369, 40-80 years) used both a single-item question with 4 response categories and a consistent method of administration (face-to-face interviewer-assisted questionnaire) and found evidence of variable prevalence between 8 countries in Europe (Spain 23%, Sweden 24%, Italy 25%, Hungary 30%, UK 31%, Belgium 32%, Poland 36%, Estonia 43%). Comparing common countries to the MALES and Pfizer studies shows high variability between studies (Italy 13% MALES, 17.2% Pfizer, 25% EMAS; Spain 10% MALES, 23% EMAS; UK 13% MALES, 31% EMAS). Likely reasons for discrepancies include the wider age range sampled in the MALES (20-75 years MALES, 40-80 years EMAS), the tools used to measure ED (dichotomous response vs 4 response categories) and the classification of ED (ED present if "yes" response vs self-report of moderate-complete ED) [161]. A younger age range would lead to lower prevalence rates in the MALES study and the dichotomous response used could lead to greater misclassification of ED as absent when it is in fact present and persistent but not causing concern. The single-item self-reported question with 4 responses in the Pfizer and EMAS studies has greater sensitivity to correctly classify ED, especially distinguished as moderate-severe ED. However, the EMAS results are representative only of the urban population as sampling was limited to the main centres of each country [130].

The largest population-based multinational study to date has been the 2001-2002 GSSAB, which investigated sexual activity and relationships in 27,516 men (13618) and women (13898) aged 40-80 years in 29 countries [26, 32]. It used a standardised questionnaire, either self-completed or in a structured interview and assessed sexual function using a two-item question of self-assessment. Respondents were asked whether they had ever experienced one or more specified sexual problems for ≥2 months during the previous year, and if so, if they experienced it occasionally, sometimes or frequently. Prevalence was calculated using those who were sexually active in the past year. The results are published in a series of papers by country surveyed or grouped to allow comparison between countries and continents [8, 26, 32, 175-181]. Overall results show that although sex and the maintenance of relationships into middle and older age remains important to individuals, the prevalence of sexual problems is high and increases with age in many countries throughout the world. Moderate-severe prevalence rates published for individual populations (as opposed to pooled multinational results) are as follows: Germany 5.9% [176], Spain 7.7% [175], Brazil 9.0% [177], USA 12.4% [6], UK 12.6% [180], Australia 15.1% [179], Korea 16.1% [178]). This study had many methodological problems including sample bias, inconsistent sampling protocols, and methods of standardised questionnaire administration between countries; however, this was found to

influence neither estimates of sexual behaviours, nor the likelihood of reporting sexual problems. The unvalidated tool used to measure ED and the definition of sexual dysfunction may have led to under-reporting and results cannot be compared with other studies as few are based on true population samples and many sample a different age strata and use different definitions of "sexual problems". However, overall it is evident that the prevalence of moderate-severe ED reported was generally lower than other multinational studies shown in Table 2.4, and varied greatly between countries from 6% in Germany [176] to 16% in Korea [178].

The most recent multinational study is the 2010-2012 GOSS [24, 136] (n=3310 Middle East, n=2022 USA, >18 years), which used the multi-item well-validated IIEF-5 tool in an online survey offered to web surfers and advertised on Facebook and other web sites. It found a 2.6% prevalence of moderate-severe ED in Middle East and 11% in USA Internet users. The data from other countries is yet to be reported. The prevalence rate for the USA is lower than that reported in the MALES study (11% vs 22%) but comparable to that found in the GASSAB (12.4%). Strengths of this study include the consistent use of the Internet to administer the survey (non-confrontational, supporting privacy, anonymity and self-paced completion, allowing a broad geographic reach and cross-country comparison) and the use of the IIEF-5 (a quantifiable and validated tool in multiple languages). However, although interesting, the reliability of data gathered on the Internet with the obvious selection bias is questionable. The participation rates varied across countries, and as expected were highest amongst younger, urban and highly educated men. The data are unlikely to reflect the general population and as suggested by the authors, men without Internet access are likely to be more disenfranchised and suffer a disproportionate burden of poor health. Furthermore, the use of the Internet may heighten cultural differences in reporting ED and attract more sexually aware individuals. Despite these limitations, the GOSS will be conducted every 5 years and this should provide valuable comparable longitudinal information to support the monitoring of prevalence internationally.

3.3 Incidence

Although a plethora of data is available on the prevalence of ED, any temporal pattern is currently concealed by the high degree of variation in study design. Six population-based longitudinal studies report incidence rates (Table 2.5). Johannes et al [183] used the results from the MMAS (baseline 1987-1989, follow-up 1995-1997) to analyse the incidence of ED in men in Massachusetts and found a crude rate of 26 cases/1000 man-years. The incidence rates reported in the USA (26/1000 [183] and 28/1000 [184]) are comparable to those found in the Netherlands (28/1000 [185]), but much lower than those reported in Finland (39/1000 [186, 187]) and Brazil (66/1000 [188]). It is interesting that in the Dutch study, after follow-up of 2.1 years the incidence rate was 28/1000, but after a second follow-up at 4.2 years a lower incidence rate of 14/1000 was reported [185]. The reasons for this are unclear. The most recent data were obtained in the Australian FAMAS [189], which investigated the incidence in 1195 randomly selected men living in Adelaide (baseline data 2002-2005, follow up 2007-2010). ED was measured during a clinical interview using the IIEF and defined as a score ≤16 ("significant ED") in men with normal erectile function at baseline. The crude incidence rate reported was 36/1000. This is similar to the incidence rate found in Finland [186, 187], higher than those found in the USA [183, 184] and the Netherlands [185] and lower than that found in Brazil [188].

Incidence rates were consistently found to increase with age [183-188]. Johannes et al [183] found that incidence rates in the MMAS increased from 12/1000 to 30/1000 and 46/1000 for men in their 40s, 50s and 60s respectively. Gades et al [184] found that incidence rates in the Olmstead Study increased from 6/1000 for men in their 40s, to 118/1000 for men in their 70s. Based on the MMAS data, the overall incidence rate for 40-69 year old Caucasian men in the USA in 2000 was estimated at approximately 618,000 new cases annually [183]. Aytaç et al [190] applied the United Nations projected male population distribution by quinquennial age groups data for 2025 to calculate a conservative projected age-adjusted global incidence [190]. The incidence of ED was projected to increase by 170 million new cases from 152 million cases in 1995, to 322 million cases in 2025 [190]. ED is not fatal and as treatment options support temporary symptom reduction but do not offer a cure the only limitation on growth is mortality. It is evident that there is a need for more longitudinal studies to support comparison across all countries and regions of the world [22].

Table 2.5. Longitudinal cohort studies reporting incidence rates for erectile dysfunction (ED).

First author, year (Study)	Country	Data collection period	Study population and selection	Age-range (years)	Respondents (n), sample size (N)	Method of administration, tool, definition of ED, period covered	Mean follow-up period (years)	Crude incidence rates (cases/1000 man-years)
Johannes 2000 [183] (MMAS)	USA	1987-1989 1995-1997	Probabilistic stratified random sampling from the Boston Massachusetts census list in 11 cities and towns	40-70	1290 (1709)	Self-administered questionnaire, validated single-item question "moderate-complete ED", ND	8.8	26
Gades 2009 [184] (Olmsted County)	USA	1996 biennial until 2004	Age and geographic-stratified random selection from population of Olmsted County, Minnesota	40-79	2215 (3874)	Self-administered questionnaire, validated BMSFI tool score ≤3, 1 month	2.0	28
Martin 2014 [189] (FAMAS)	Australia	2002-2005, 2007-2010	Random selection residents in Northwestern region of Adelaide	35-80	899 (1195) 810 (899)	Self-administered questionnaire, validated IIEF "significant ED" (score ≤16), ND	5.0	32
Moreira 2003 [188] (Brazilian)	Brazil	1998 2000	Stratified cluster sampling of households from census data in city of Salvador	40-70	602 (654) 501(602)	Face-to-face interview, validated single-item question "moderate-complete ED", ND	2.0	66
Shouten 2005 [185] (Krimpen)	The Netherlands	1995-1997 1997-2000 2000-2002	Eligible men (without urogenital disorders) from total population of men 50-75 years in a Dutch city near Rotterdam	50-78	1204 (1661) 882 (1204)	Self-administered questionnaire, ICS sex questionnaire "clinically relevant ED", ND	2.1 4.2	28 14
Shiri 2003 [186] Shiri 2004 [187] (Tampere)	Finland	1994 1999	All men born 1924/1934/1944 residing in Tampere and 11 surrounding cities in 1994, National Population Register	50, 60, 70	1683 (3143) 1442 (1683)	Postal questionnaire, unvalidated 2-item question "moderate-complete ED", ND	5.0	39

Brazilian, Brazilian Men's Health Study; FAMAS, Florey Adelaide Male Ageing Study; HPFS, Health Professionals Follow-Up Study; Krimpen, Krimpen Study; MMAS, Massachusetts Male Aging Study; Olmsted County Study, Olmsted County Study of Urinary Symptoms and Health Status Among Men; Tampere, Tampere Aging Male Urological Study; ND, not defined.

3.4 Comparability issues

The paucity of nationally representative population-based studies means it is not possible to adopt any single figure as representative of prevalence or incidence in a given country. There is a high level of heterogeneity in published international epidemiological data for ED. It is not known whether these data are reliable, or if the variation is an artefact due to inherent differences in sociocultural aspects affecting the reporting of ED or differences in study design. These include sampling bias (random selection from population databases [119, 131, 162, 168, 169] or urban Internet users [24, 136] to convenience sampling from consecutive patients in general medical practices [28] or volunteers at public health screening programmes [114]); the age strata investigated (men over 20 [3, 5, 119, 161], 30 [131, 168], 40 [1, 26, 118, 128, 129, 162, 166, 169] or 50 years of age [27, 163, 164]); the method of collecting data (postal questionnaire [5, 27, 115, 118, 163, 164, 166], face-to-face-interview [3, 129], self-administered questionnaire [1, 28, 114, 116, 131, 168, 170], computer-assisted self-interview [119], CATI [26, 161, 162]) or Internet [136]); the tool used to assess ED (unvalidated questions [3, 26, 28, 161, 163, 164, 170], validated single-item questions [1, 118, 119, 128-130, 136, 162], validated multi-item questions [BMSFI [116], IIEF [27, 168] or IIEF-5 [5, 114, 115, 131, 136]]); the definition of ED and the time period used for assessment (1 month [116, 130, 170], 12 months [3, 26] or 5 years [164]); and reporting of prevalence rates (crude [1, 3, 28, 114, 115, 163, 164, 170], weighted [5, 119, 131, 162, 166, 168] and/or World Standard Population-adjusted rates [5, 136]).

3.4.1 Sample size, sampling frame and method, study population

Population-based sample surveys are used as a proxy for complete population surveillance, which is often logistically and financially impractical. The study population should be selected in a manner that generates an accurate representation of the actual population and the sample size large enough to provide the power to detect true associations. As shown in Table 2.4, very few studies had a sample size <500 participants, while many had over 5,000 participants offering a high level of statistical power. However, the sampling frame and methods vary widely.

A robust sampling frame should ensure that the true population is represented with equal probability of selection. For example, while population registers or census [128, 130, 163] and household databases [26, 27, 166, 168] are compulsory and therefore supposedly contain 100% of the population, registering on the national electoral roll [5] and medical databases [169] are voluntary and likely to exclude some sectors of the community. The choice of selection method is also important, as any selection bias will affect the representativeness of

the data. For example, the use of convenience sampling from patients in a medical practice [28, 118] cannot be considered as a proxy for the wider population, as there may be inequalities in healthcare access, especially for minority groups. Similarly, convenience sampling from consecutive volunteers attending a health-screening programme [114] is unlikely to represent the prevalence of ED in the general population as there is likely to be a bias towards health-conscious men. There are many valid selection methods available ranging from simple random selection [116] from a population database to more complex multi-stage probabilistic [119] or proportional stratified sampling [5, 131, 163, 170] strategies to ensure fair representation of major relevant groups within the wider population. The best option is the one which gives the most representative sample of the various demographic and health parameters in a population.

Although many studies have selected randomly from the general population [27, 115, 119, 129] and can therefore be considered representative of the true population examined, others have focussed on specific populations such as urban [3, 128, 130, 131], rural [163], sexually active [116], or those without specific medication conditions such as urogenital disorders or prostate cancer [173, 174, 184]. This limits the comparability of resultant data and can have a large impact on apparent prevalence. For example, ED is highly prevalent in men with prostate cancer and excluding these men would result in a lower prevalence of ED. All studies have limited the age strata investigated to adult men, however some include younger men [3, 5, 119, 136, 161] while many have restricted it to ageing men (>40 or >50 years) [1, 26, 27, 116, 118, 128-130, 162-164, 166, 170]. This has a clear impact on prevalence rates of ED as age is strongly and consistently associated with ED: focusing on older men will usually result in a higher prevalence rate, especially in the moderate-severe category.

3.4.2 Method of administration, assessment tool and definition

The method of administration is likely to significantly impact study results. Due to the private and sensitive nature of sexual function, anonymity and privacy should be ensured. Methods that involve direct contact such as face-to-face interview [1, 26, 28, 119, 129-131, 168, 170], especially in a public setting [114, 178], could result in stress, embarrassment and concerns about social stigmatisation. This could generate underreporting bias, incomplete responses or reluctance to participate altogether [3]. Shaeer et al [136] suggest that non-confrontational methods offering greater privacy and anonymity such as telephone [26, 129, 161] or online surveys [136, 161] may provide a margin of excellence and yield higher participation rates and lower reporting bias. Postal surveys reach a wide geographic span and can support privacy and anonymity [5, 27, 115, 118, 129, 163, 164, 166], however the collection of personal data such

as name or contact details in postal surveys may also contribute to a response bias and/or low response rate. Response rates for postal surveys in Table 2.4 are highly variable: 28% in rural countries of Central New York State, USA [163], 37% in Western Australia [5], 57% in South Australia [166], 64% in Rancho Bernado, California, USA [115] and 79% in health professionals in the USA [164]. Online and postal surveys have the added benefit of allowing the respondent to complete in their own time, providing time for more thoughtful answers and allowing for more extensive questioning. The best method of administration should ensure privacy and anonymity and be selected to maximise response rates and representation within the selected population, within the logistical and financial constraints of the study.

The importance of the assessment tool is highlighted by evidence of significant variation within studies using multiple tools [121, 136]. Martin-Morales et al [121] conducted a population-based study in Spain and found 12.1% prevalence with a single-item question and 18.9% prevalence with the IIEF-5. In the GOSS, Shaeer et al [24, 136] reported highly variable results in the Middle East and USA using two different tools: 7% and 4.9% using the single-item question compared to 45.1% and 37.7% using the IIEF-5 respectively. These results indicate that the validated IIEF-5 is more likely to report a higher ED prevalence than the single-item question and that prevalence rates are likely to differ significantly depending on which assessment tool is used. Despite the availability of well-validated sensitive and specific tools such as the IIEF-5 [110], studies continue to use unvalidated poorly designed tools that do not support robust and comparable data. The IIEF-5 is sensitive, specific, well validated, short and convenient with low subject burden. However, it would be advisable to include the single-item self-assessment question to support both comparison with past research and internal consistency.

The issue of comparability is compounded by differences in the definition of ED used in data reporting. ED is often defined by a self-report of moderate-severe ED, as the mild category may be a mix of both mild persistent and intermittent situational ED. This conservative approach is common in reported studies [26, 129, 130] and although it reduces the likelihood of misclassification, it may also underestimate the prevalence of ED. Table 2.4 provides prevalence data on mild, moderate-severe and overall ED categories where possible; however, while some studies report prevalence data on all categories of ED [1, 115, 118, 119, 131], others do not [3, 114, 116, 163, 164, 170]. In some cases this data is not available as the tool used limits data collection. For example, the use of a dichotomous question to assess ED in the nationally representative NHSLS study [3] resulted in a 5% prevalence rate in the USA. This may represent either moderate-severe ED or overall ED (including mild cases), depending on

assumptions made regarding participant interpretation of the question. In some cases it appears to be the author's decision to omit certain results. For example, Barqawi et al [114] investigated the prevalence of ED amongst men attending the multi-centre Prostate Cancer Awareness Week (PCAW) screening program using the IIEF-5 in a self-administered questionnaire. Although prevalence data on all categories were gathered, only the overall prevalence of 75% was reported, limiting the ability to compare this to other published studies. As shown in Table 2.4, the time stipulated to define ED also ranges markedly between 1 month [130], 1 year [6, 8, 26, 175, 177-181] and 5 years [164] but is often not reported [24, 27, 128, 129, 136, 161]. It is not known what effect this will have on self-reporting; however, future studies should clarify this both during data collection and in reporting data. A clinical diagnosis of ED generally requires 3-month duration of symptoms [22] therefore any tool should ideally refer to at least 3 months.

3.4.3 Data reporting

Prevalence data are calculated as a percentage by dividing the number of cases by the corresponding population; however, the denominator varies between studies. The majority of available studies present crude prevalence rates in the total [114, 118, 163, 170, 191] or sexually active population [3, 115, 116]. However, some population-based studies present adjusted prevalence data [5, 131, 166, 168], weighted to reflect the age distribution in the target population. The age distributions of different countries and continents vary widely and some are more inclined towards younger or older populations. This can have a clear impact on sexual function and ED prevalence. Standardisation to an external reference population, such as the World Health Organizations (WHO) World Standard Population (WSP), has been suggested to support meaningful international comparison [5, 24, 136]. Very few epidemiological studies have reported WSP-adjusted prevalence data: Chew et al [5] reported 23.4% in Australia, Shaeer et al [136] 33.7% in the USA and 47% in the Middle East [24].

The variation in available data highlights the need for ED prevalence rates to be established and monitored within each country. Future studies should focus on gathering reliable population-based data on prevalence and risk factors in ageing men using randomised selection from population databases, well-validated tools and a consistent method of administration, ideally repeated every 5 to 10 years. Furthermore, reported prevalence rates should be weighted to accurately represent the age-distribution of the population sampled, and standardised to a stable reference population such as the WSP [192] to enable meaningful international comparisons. However, it is evident that ED is highly prevalent worldwide and is

expected to increase [183-188]. This is due to ageing populations and an increase in the risk factors associated with ED including diabetes, CVD, obesity and smoking [190, 193].

4.1 RISK FACTORS FOR ORGANIC ED

Epidemiological research, including cross-sectional (Table 2.4) and prospective cohort (Table 2.5) studies, supports the gathering of information on risk factors for disease. ED and its risk factors do not manifest uniformly in all populations and there are marked variations in the prevalence rates of ED, its risk factors and the relative significance of those risk factors in published literature. This review will focus mainly on risk factors for organic ED.

Sociodemographic factors such as race or ethnicity [114, 118, 131, 167, 194], marital status [1, 3, 5, 168], education [119, 163], occupation [5] and socioeconomic status (SES) [129, 131, 178] can affect the risk of developing ED. However, the most established risk factors are as follows: ageing [1, 3, 5-7, 26-28, 114, 118-120, 122, 124-129, 131-133, 160, 161, 163-165]; presence of comorbidities (e.g., diabetes mellitus [1, 24, 27, 128-130, 136, 161, 175, 179, 195], metabolic syndrome (MetS) [24, 45, 130] and CVD [1, 24, 27, 129, 164, 194, 196, 197]); LUTS [27, 114, 129, 130, 194]; prostate disease [128, 178]; medications [1, 24, 118, 128, 129, 198]; recreational drugs [199, 200]; trauma or surgery affecting vascular or nerve function in the spine or pelvis (e.g., prostate surgery [129, 201-203]); endocrine disorders [1, 114, 204-206]); anatomical disorders [15, 207]; depression [1, 6, 24, 129, 130, 136, 161, 175, 177]; and lifestyle factors (e.g., smoking [1, 27, 129, 161, 178, 200] and physical inactivity [175, 178, 179]). Ageing, comorbidities and their associated medical treatments appear to be the most significant risk factors with multiple comorbidities [161] and polypharmacy [118] further compounding the risk. It is likely that the relative importance of these risk factors differs between populations due to differences in sociodemographic profile and differential exposure. Laumann et al [194] reported that established risk factors are of varying levels of importance in different racial/ethnic groups. This supports the need to establish risk factors in both individual countries and specific racial/ethnic groups. In the following section, risk factors will be grouped into sociodemographic, medical and lifestyle risk factors. Where possible, age-adjusted or multi-adjusted odds ratios (OR), hazard ratios (HR) or relative risks (RR) and their 95% confidence intervals [95%CI] will be used for comparative purposes.

4.2 ED and sociodemographic factors

4.2.1 Ageing

The prevalence of ED increases with age, and age has been well established as a strong independent predictor of ED in both cross-sectional [1, 3, 5-7, 26-28, 114, 118-120, 122, 124-

129, 131-133, 160, 161, 163-165] and longitudinal [183-189] studies. Feldman et al [1] reported a progressive increase in ED with each decade in men in the MMAS: approximately 40% in men in their 40s, 50% in their 50s, 60% in their 60s and 70% in their 70s. It has been supported to varying degrees in studies worldwide (USA [119], Australia [5, 166, 168], Germany [172], Sweden [34], Finland [120], the Netherlands [174, 208], Spain [121], Belgium [122], Austria [132], Egypt [209], Morocco [123], Iran [120, 124], Brazil [125, 126], Turkey [127], China [133], Singapore [160], Malaysia [134, 135]). Multinational studies add further support to the association between age and ED [27, 130, 161]. The MSAM-7 [27], conducted across the USA and 6 European countries (UK, France, Germany, the Netherlands, Italy and Spain) confirmed age as a strong independent predictor of ED with men in their 70s at near 7-fold increased risk of ED compared to men in their 50s (OR=6.86 [6.02-7.82]). Most recently, the longitudinal FAMAS in Australia [189] has shown that older age is a strong and significant independent predictor of incident ED with every 10.6-year increase in age conferring a 2.6-fold increased likelihood of incident ED and a 1.9-fold reduced likelihood of remission. Age remained one of the strongest determinants of incident ED even after controlling for other sociodemographic, medical and lifestyle factors.

Elderly men often suffer from a greater number of comorbidities and certain common medications have been shown to impair sexual function [141]. However, independent of concurrent pathologies, ageing is characterised by alterations in the vascular system, resulting in tissue remodelling, reduced elasticity, increased fibrosis and arterial stiffness [210]. Even amongst “healthy” men, the ultra-structural anatomy of CC tissue in older men (60-70 years) shows increased connective tissue and a decrease in the proportion and cytoskeletal organisation of SMC compared to younger men (18 – 28 years) [211]. Ageing is also associated with a reduction in NO levels with endothelial damage leading to down-regulation of eNOS, the excessive release of adrenergic compounds and the activation of endothelin and ROK, and/or the impairment of SMC relaxation by endogenous factors [212]. When combined with earlier animal experimental results indicating an increased apoptotic index in trabecular and SMC of ageing rat penile tissue [213] this may help explain the loss of CC compliance and altered erection kinetics in ageing men. Further molecular studies are required to elucidate age-related mechanisms of ED; however, although ageing is reported as the single greatest risk factor for ED, ED is not an inevitable result of ageing. It is more likely that as age increases the frequency of neurovascular insults increases, resulting in an increased prevalence of ED. Although ED appears to be an age-related issue, many of the contributing factors may be treated or even reversed given the correct medication, nutrition and/or lifestyle intervention.

4.2.2 Other sociodemographic factors

4.2.2.1 Race/ethnicity

Epidemiological evidence suggests a relationship between ED and race/ethnicity [3, 114, 118, 131, 167, 194]. In the Australian MATeS, Holden et al [167] found an association between ED and ethnic origin. Middle-Eastern and Asian born men living in Australia reported lower rates of ED than those from European countries; however, the low sample size of ethnic subpopulations limited the power to detect a significant relationship. In the USA, the prevalence of ED appears to be higher in African-American men compared to Caucasian men [114, 118, 131, 194]. In the American PCAW study, Barqawi et al [114] conducted a large survey of age-matched racially diverse men (75% Caucasian, 17% African-American, 8% other). After adjusting for age, comorbidities and smoking, Caucasian men had a significantly higher IIEF-5 score (+6.57 [4.39-8.76]), indicating better erectile function, compared to African-American men. Laumann et al [194] conducted the Male Attitudes Regarding Sexual Health (MARSH) study, a cross-sectional population-based nationally representative survey of ED in 2001-2002 (n=2173, >40 years, White 41.5%, Black 27.4%, Hispanic 31.1%) which oversampled minority groups to ensure adequate sample size to estimate prevalence specific to a particular race or ethnic group. Sampling was performed using random-digit dialing from national telephone lists stratified by race/ethnic group and age in decade. In the CATI, both the IIEF-5 and the single-item question were included, with ED defined by self-report of moderate-complete ED in the single-item question. They found 22% overall weighted prevalence with 19.9% in Hispanics, 21.9% in Whites and 24.4% in Blacks. Although the prevalence rates were not significantly different, the medical and lifestyle risk factors were shown to play significantly different roles amongst the three groups. For example, the risk of ED was significantly higher in white men >70 years and those with diabetes; black men with severe LUTS; and Hispanic men >60 years and those suffering from moderate LUTS, hypertension or depression. Furthermore, the risk was significantly lower among white men who had sexual intercourse in the last 3 months; black men who were sexually active in the last 3 months, drank ≥5 alcoholic drinks per day on ≥1 day during the past year, exercised, or had a good relationship with their partner; and Hispanic men who had a high school or higher education. In the 2001-2005 BACH study, Kupelian et al [131], multi-stage random sampling stratified by age, gender, race and ethnic group ensured adequate representation of minority groups (White 36.2%, Hispanic 33.3%, Black 30.4%). After age-adjustment, ED was significantly correlated with race ($p<0.01$), and although it was not significant in a full model including all significant covariates ($p=0.09$), or after excluding men with comorbid conditions ($p=0.07$), Black and Hispanic men were

observed to have poorer erectile function scores than white men. More recently, Londoño et al [118] obtained a diverse racial sample (61.6% white, 8.9% African-American, 5.5% Asian, 18.8% Hispanic, 3.4% other) in a population-based postal survey of men enrolled in a health plan in Kaiser Permanente, Southern California. Using the single-item self-report, they found a significant difference in ED prevalence between racial groups ($p<0.001$), particularly evident in the moderate-severe categories (Caucasian 27.9%, Asian 30.9%, Hispanic 31.3%, African American 33.1%).

Many available epidemiological studies have either not collected or reported racial and ethnic data [6, 179], not examined its association with ED [5, 28, 166, 168] or failed to achieve adequate representation of minority groups thus limiting the statistical power to detect differences between racial/ethnic groups [3, 167]. Furthermore, there is uncertainty surrounding the reliable collection of racial and ethnic data. Race and ethnicity are two distinct concepts. Race is biologically determined and an ascribed attribute whereas ethnicity is a self-perceived measure of cultural affiliation and therefore a fluid concept [214]. Individuals may not understand the concept, confuse ethnicity with other aspects of cultural identity (e.g., race, nationality or ancestry), identify with or belong to more than one ethnic group, object to being asked, refuse to answer or answer flippantly [214]. This presents difficulties in collecting reliable data on ethnicity. The results of available literature indicate that racial/ethnic group may be an important confounder in studies investigating the prevalence of ED and its associated risk factors. Further research is needed to elucidate the mechanisms accounting for racial/ethnic differences. It is clear that extending the findings of one population group to another is not recommended and studies investigating specific racial/ethnic groups are required.

4.2.2.2 Marital status/sexual activity

While some cross-sectional studies report a higher prevalence of ED in men without a regular partner and those who are widowed or separated/divorced [5, 28], this is not supported by longitudinal studies [184, 189]. Chew et al [5] reported a higher prevalence of any degree of ED (IIEF-5 score <22) amongst Australian widowers (70%) and men who were separated or divorced (53%) compared to men who were married or in a *de facto* relationship (38%) or who had never been married (28%). However, the age-adjusted odds for ED were higher amongst men who had never been married (OR=6.5 [3.4-12.7]), those who had been separated or divorced (OR=2.6 [1.6-4.1]), and widowers (OR=2.5 [0.8-8.0]) compared to those married or with a partner. Similarly, Laumann et al [3] found significant increased odds for ED in American men who had never been married (OR=1.73 [1.00-2.97]) compared to currently married men,

but the odds were not significantly different in widowed/divorced/separated men. In a longitudinal cohort study in the USA, Gades et al [184] found that men with a regular sexual partner at baseline had better sexual function but experienced a more rapid annual decline in sexual function than those without a regular sexual partner [184]. In contrast, in the longitudinal FAMAS, Martin et al [168] found that although moderate-severe ED was significantly more prevalent in men without a partner than those with a partner at baseline (44.5% vs 17.3%, p<0.001), and having no regular partner was significantly independently associated with an increased risk of both mild and moderate-severe ED even after adjusting for multiple confounders (OR's not provided), it was not a predictor of incident ED in Australian men [189].

Many studies either do not gather and/or report data on marital status [131], do not examine its relationship with ED [1, 6, 170, 179] or have insufficient sampling size of subpopulations to provide sufficient power to detect a relationship. The inconsistency in published research may be partially explained by confounding with sexual activity. The relationship between ED and lack of regular sexual partner has been well established. Some studies, including the large multinational GSSAB study [26], restrict reporting to sexually active participants or men with a regular partner. However this is likely to underestimate prevalence as men with ED may cease to be sexually active as a result of sexual difficulties [179]. Married men and men in *de facto* relationships are more likely to be sexually active and have regular sexual intercourse than men not in a stable relationship. Chew et al [5] found that 73% of Australian men in a stable relationship were sexually active and 61% of these had regular sexual intercourse, while 54% of men not in a stable relationship were sexually active and 39% of these had regular sexual intercourse. The relationship between marital status and ED is likely to be both complex and bidirectional: marital status may affect sexual function but ED may also affect marital status. The results of these studies indicate that marital status and sexual activity may be important confounders in studies investigating the prevalence of ED and its associated risk factors.

4.2.2.3 Socioeconomic factors

Available research provides moderate but inconsistent evidence to support a relationship between ED and socioeconomic factors such as education [119, 163, 188], income [5, 188] employment status [5], occupation [5], or socioeconomic indices [129, 131, 178]. Cross-sectional [119, 163] and longitudinal [188, 189] studies suggest that ED is less prevalent in men with a higher education. Moreira et al [188] in the longitudinal Brazilian Men's Health Study found that men with <5 years education had a 2.7-fold increased risk of incident ED compared to men with >12 years education. Martin et al [189] in the longitudinal FAMAS found that

Australian men with higher educational backgrounds had a lower age-adjusted risk of ED and this was independent of income, providing support for a protective effect of education. Conversely, in a cross-sectional study in the USA, Laumann et al [3] found no significant association between ED and education; however, there was a higher age-adjusted likelihood of ED in men who had experienced a significant change in household income (20%) between 1988-1991 (OR=2.11 [1.01-4.38]). Similarly, in the USA results from the GSSAB, Laumann et al [6] found no significant difference in the likelihood of ED in men with secondary school or above compared to primary school or below (OR=0.60 [0.15-2.35]) or in men with a medium or high household income compared to a low income (OR=0.90 [0.44-1.85]). However, longitudinal studies [188, 189] suggest that ED is less prevalent in men with a higher household income. Results from the longitudinal Brazilian Men's Health Study [188] and the Australian FAMAS [189] support an independent association between ED and household income. There appears to be a 2.7-fold increased risk of incident ED in men from low-income households [188, 189]. In cross-sectional studies, Chew et al [5] found ED prevalence to be highest in areas of socioeconomic disadvantage in Australian men measured using the Socio Economic Index for Area (SEIFA) index (45% vs 39.5% in the highest to lowest areas respectively); however, there was no significant difference in the age-adjusted odds (OR=1.1 [0.7-1.6]). Few studies have investigated employment status and occupation. Chew et al [5] found that Australian men who had retired for health reasons had a higher risk of developing ED compared to currently employed men (OR=3.4 [2.0-5.9]). Using the Australian Standard Classification of Occupations (ASCO), "clerical, sales, and service workers" had a higher crude prevalence of ED (47% compared to 37-42% in other occupational categories) but the age-adjusted odds were significantly higher only in the lowest skilled category of "intermediate production and transport workers and laborers" (OR=1.4 [1.0-2.8]). However, there was no significant difference in the likelihood of ED in blue-collar workers compared to white-collar workers (OR=1.2 [0.9-1.6]) [5].

As sociodemographic profiles differ between countries and are associated with differential exposure to medical and lifestyle risk factors, the relationship between these variables and ED is likely to differ between countries. It is evident that such data should be routinely collected and reported in future epidemiological studies to support the gathering of country-specific data and allow pooling of data for meta-analysis to clarify the relationship between ED and sociodemographic variables.

4.3 ED and medical factors

Any medical condition that affects the neurovascular or endocrine system, disrupting nerve function, impairing arterial flow in the penile arteries or disturbing hormonal transmission of sexual stimulation, may affect the quality and duration of an erection.

4.3.1 Metabolic disorders

4.3.1.1 Diabetes mellitus and prediabetes

Defined as self-reported diagnosis, use of diabetes medication, 8-h fasting plasma glucose (FPG) ≥126 mg/dL, non-fasting glucose ≥200 mg/dL or glycated haemoglobin A1c (HbA_{1c}) ≥6.5%) [215, 216], DM is a significant risk factor for CVD [217, 218] and has a well established association with ED [1, 219]. Epidemiological studies support diabetes (both TIDM and T2DM) as one of the key risk factors for ED [1, 28]. The 2008-2009 NZ Adult Nutrition Survey (NZANS) [220] reported that 7% of NZ adults have diabetes (8% of men) and a further 26% have prediabetes (26% of men). In the absence of intervention, prediabetes (defined in Table 2.6) usually leads to T2DM [215]. T2DM accounts for 90-95% of all diagnosed cases [221] and will be the focus of this discussion.

Table 2.6. Classification of diabetes and glycaemic control using fasting plasma glucose (FPG) and glycated haemoglobin A1c (HbA_{1c}) according to cut-offs recommended by the American Diabetes Association [216].

Classification of laboratory tests	Desirable	Prediabetes	T2DM
FPG mg/dL (mmol/L)	<100 (5.6)	100-125 (5.6-6.9)	≥126 (7.0)
HbA_{1c} % (mmol/mol)	<5.7 (38.8)	5.7-6.4 (38.8-46.4)	≥6.5 (47.5)

Compared to the general population, ED is highly prevalent in diabetics and the prevalence increases with age, duration, and severity of the disease [1, 172, 222]. Indeed, published prevalence rates range from 28% to 90% [219, 223]. Early studies suggested that 75% of diabetic males aged 40-70 years were at risk of ED, compared to 52% in the general population [1, 224, 225] and the prevalence remains high even after adjusting for age [1, 119, 189]. In the USA, the MMAS [1] found 28% age-adjusted prevalence of complete ED in treated diabetics aged 40-70 years and the NHANES [119] observed 39% age-adjusted moderate-severe ED in diabetic men >20 years. Indeed, diabetes had the highest age-adjusted OR compared to all other risk factors measured (OR=3.90 [2.16-7.04]) and multivariate analysis adjusting for sociodemographic and medical factors showed that diabetes was an independent risk factor for ED (OR=2.91 [1.47-5.73]). The 45 and Up Study [169] in Australia also reported a higher prevalence of ED in diabetics compared to non-diabetics (62% vs 32% respectively). After adjusting for both age and sociodemographics there was a 2.6-fold increased risk (OR=2.6 [2.54-2.79]) and this remained significant after adjustment for comorbidities (OR=2.39 [2.27-

2.51]). Recent longitudinal data from the FAMAS [189] in Australia supported this: diabetics had both a significantly higher likelihood of incident ED and a significantly lower likelihood of remission. It is clear diabetes is one of the most important risk factors for ED.

Poor glycaemic control is associated with a higher risk of microvascular conditions, macrovascular disease and all-cause mortality [226, 227]. A review by Lewis et al [22] indicates that: insulin-dependent DM, >10 years DM, high levels of HbA_{1c} (a measure of average plasma glucose concentration over time and thus an indicator of glycemic control), medicated diabetes, and a history of diabetes-associated disease (arterial, renal or retinal) and neuropathy all indicate higher odds for ED. Indeed, several longitudinal studies have demonstrated an inverse relationship between HbA_{1c} and ED [228, 229], that glycaemic control is an independent predictor of IIEF scores ($p<0.001$) in men with T2DM even after adjusting for diabetic peripheral neuropathy ($p=0.023$) [229] and that HbA_{1c} increases with the severity of ED [228, 230]. In 2013, Weinberg et al [231] analysed data from the 2001-2004 NHANES (n=3306 men, ≥20 years) in the USA and reported that men with FPG 100-126 mg/dL (5.6-7 mmol/L) and ≥126 mg/dL (>7 mmol/L) had increasingly higher odds of ED (OR=1.22 [0.83-1.80] and OR=2.68 [1.48-4.86] respectively). Men with HbA_{1c} 5.7-6.4% (38.8-46.4 mmol/mol) and ≥6.5% (47.5 mmol/mol) also had increasingly higher odds of ED (OR=1.73 [1.08-2.76] and OR=3.70 [2.19-6.27] respectively). Multivariate analysis showed that HbA_{1c} remained a strong independent predictor of ED (OR=3.19 [1.13-9.01]). This suggests that prediabetes also increases the risk of ED, that there may be a temporal relationship between early adverse metabolic changes and the development of ED, and that the relationship may be dose-dependent: the severity of ED symptoms increases with the severity of metabolic change.

However, studies on the benefits of glycaemic control for ED symptoms show conflicting results: some show that reduction in HbA_{1c} is associated with improvement in ED symptoms, while others show no significant change even in the presence of well-controlled glucose levels [223, 232]. The high prevalence of ED in diabetics is suggested to result from an increased susceptibility to vascular disease, autonomic neuropathy and gonadal dysfunction [1], indicating a complex pathology. The efficacy of improved glycaemic control in ameliorating ED symptoms would naturally depend on the aetiology and degree of pathophysiological change. Adverse changes have been shown in the penile vasculature of diabetic men with ED [233] including ultra-structural changes to the SMC in the CC [234] and impaired neurogenic and endothelium-dependent SMC relaxation [235]. Furthermore, hypogonadism is also highly prevalent in diabetic men with ED. Corona et al [236] found 24.5% hypogonadism in diabetic males with ED, compared with 12.6% in other men with ED. Unsurprisingly, PDE_5 inhibitors are

less effective in diabetics [237]: they address vasculogenic aetiology but cannot alter ED in diabetic men with possible neurogenic or endocrinological ED.

Diabetes is well established as an important risk factor both for ED and CVD. In a meta-analysis of 3 longitudinal and 9 cross-sectional studies (n=22,586), Yamada et al [238] found evidence from cohort studies to support significantly higher odds for CVD events (OR=1.74 [1.34-2.27]) and coronary heart disease (CHD, OR=1.72 [1.5-1.98]) and cross-sectional studies to support significantly higher odds for CVD events (OR=3.39 [2.58-4.44]), CHD (OR=3.43 [2.46-4.77]), and peripheral vascular disease (PVD, OR=2.63 [1.41-4.91]) in diabetic men with ED compared to those without ED. Diagnosed T2DM and prediabetes both confer a higher risk of CVD. Indeed, there appears to 2-3 times the risk of CVD in diabetics [238] and CVD is the major risk factor for diabetic-associated morbidity and mortality [239]. ED may be an excellent predictor of silent CVD in diabetics, independent of glycemic control and ED severity [22]. This emphasises the importance of early diagnosis of ED and subsequent cardiometabolic examination.

4.2.1.2 Metabolic Syndrome (MetS)

MetS is a widely used tool to describe a collection of clinical signs that indicate increased cardiometabolic risk: central obesity, dyslipidemia (elevated triglycerides (TG) and lowered high density lipoprotein cholesterol (HDL-c), impaired glucose metabolism, and elevated blood pressure (BP) [240-242] (as shown in Table 2.7). The two major risk factors for MetS are obesity and insulin resistance (IR), which are exacerbated by physical inactivity, ageing, endocrine changes and genetics. A progressive disorder, it often culminates in T2DM and increased risk of CVD [243]: indeed it has been reported that MetS increases the risk of diabetes 6-fold [244] and MI or stroke 2-fold [245]. There is considerable debate over the terminology and guidelines used to define MetS. However, in 2009 a consensus was reached to combine the two most commonly used criteria: the International Diabetes Federation (IDF) [240] and the American Heart Association/National Heart, Lung, and Blood Institute (AHA/NHLBI) Adult Treatment Panel III (ATP III) [241]: the presence of any 3 or more of the 5 criteria shown in Table 2.7 constitutes MetS. However, more data is needed to establish a universal cutoff for central adiposity and until then the IDF criteria should be used for non-European males and either the IDF or the AHA/NHLB1 criteria for European males [246].

Table 2.7. Classification of the Metabolic Syndrome (MetS) in adult men using the American Heart Association/National Heart, Lung, and Blood Institute (AHA/NHLBI) Adult Treatment Panel III (ATP III) [241], the International Diabetes Federation (IDF) [240] or most recently the joint IDF and AHA/NHLBI [246] criteria.

Criteria	AHA/NHLB1 ATP III	IDF	IDF and AHA/NHLBI
	Presence of ≥3 following:	**Presence of central obesity plus ≥3 of the following:**	**Presence of ≥3 following:**
Central obesity	*WC ≥102 cm*	*WC ≥94 cm*	*Population- and country-specific definitions*
Lowered HDL-c*	HDL-c <1.03 mmol/L (40 mg/dL)	HDL-c <1.03 mmol/L (40 mg/dL)	HDL-c <1.0 mmol/L (40 mg/dL)
Elevated serum triglycerides*	TG ≥1.7 mmol/L (150 mg/dL)	TG ≥1.7 mmol/L (150 mg/dL)	TG ≥1.7 mmol/L (150 mg/dL)
Elevated blood pressure**	≥130/85 mmHg	≥130 SBP or ≥85 DBP mmHg	≥130 SBP and/or ≥85 DBP mmHg
Impaired glucose metabolism***	FPG >5.6 mmol/L (100 mg/dL)	FPG ≥5.6 mmol/L (100 mg/dL)	FPG ≥5.6 mmol/L (100 mg/dL)

All three criteria include as alternate indicators *any specific treatment for this lipid abnormality, **treatment of previously diagnosed hypertension, ***previously diagnosed T2DM (replaced with drug treatment for elevated glucose in the joint IDF and AHA/NHLB1 definition).

Approximately 27% of American adults meet these criteria for MetS and the prevalence is increasing [247]. Several cross-sectional and longitudinal studies have reported that MetS is an independent risk factor for ED [248-252]. Recently, Weinberg et al [231] reported that MetS carried a 2.5-fold increased risk of ED (OR=2.55 [1.85-3.52]) in American men. This was further supported in a 2014 meta-analysis of 10 observational studies (n=4092) [253], which found that MetS was associated with a significant increased risk of ED (RR=1.60 [1.27–2.02]). Moreover, a 2014 observational study [254] of 107 urology outpatients with LUTS or ED found that 50 had MetS and that IIEF-5 scores were significantly lower in the men with MetS (14.52 vs 17.42, p=0.03). The association of MetS with ED has also been reported to be greater than the effect of any of the MetS criteria alone [255]. However, it is unclear whether it is MetS per se, or the associated increase in susceptibility to T2DM and CVD that confers increased risk of ED. The paucity of intervention trials on the impact of improving MetS on sexual dysfunction leaves this area open for further investigation into causation.

4.2.1.3 Insulin resistance and hyperinsulinaemia

A reduction in the normal ability of insulin to increase glucose uptake and utilisation [256], IR is well-established as a precursor of T2DM [257] and may also lead to CVD independent of T2DM. Prior to the development of overt diabetes, excess insulin production by pancreatic beta cells creates a hyperinsulinaemic state to ensure adequate glucose uptake and maintain normal blood glucose levels despite declining insulin sensitivity [256]. More pronounced IR is associated with a higher risk of micro and macrovascular conditions and mortality [226, 227]. It is a feature of other disorders (e.g., obesity, hypertension and cancer [258]) and is considered a metabolic defect linking the cardiometabolic components that define MetS. Although the mechanism underlying the relationship between IR and CVD is unclear, it has been suggested that environmental insults converge with IR, which subsequently promotes inflammation and alters gene expression with detrimental metabolic and haemodynamic consequences: including endothelial dysfunction and disturbed NO production [256]. Early studies [259] reported a positive relationship between insulin sensitivity and eNO production in adult men. Insulin acts to promote eNOS expression and activation along the PI3K-dependent signalling pathway to increase production of NO [260]: IR therefore would decrease eNOS expression and NO production.

Direct measurement of insulin-mediated glucose uptake (IMGU) using the hyperinsulinaemic euglycaemic clamp technique [261] or the frequently sampled intravenous glucose tolerance test (FSIVGTT) [262] is not convenient or cost-effective in large-scale epidemiological and clinical studies. The most commonly used surrogate measurement is the Homeostatic Model Assessment of IR (HOMA-IR) [263] which directly approximates IR and is calculated from a single measurement of fasting serum insulin (FSI) and FPG using the following formula: FSI (μU/ml) × FPG (mmol/L)/22.5. However, several accepted limitations affect the reliability of HOMA-IR scores. These include: high coefficients of variation, skewed distribution, limited use in certain populations (e.g., non-obese diabetics), and large variability in the cut-off points used to define IR (≥1.6 [264] to ≥3.8 [265]). Cut-off points are sometimes defined arbitrarily (IR is often considered present with an index ≥3) or determined based on a specific percentile (ranging from 75^{th}– 90^{th} [266]) of the HOMA-IR distribution in a given population. A 2013 study by Gayoso-Diz [266] suggested that cut-offs should also take into account gender, age and MetS criteria; however, further work is needed in this area to define clinically relevant cut-off values. To overcome these limitations, fasting insulin is often included in studies and tertiles of both this and HOMA-IR are usually reported [231].

Results from the NHANES [267] suggest that IR is a common metabolic condition affecting 25% of adults in the USA and recent analysis [231] revealed a graded relationship between ED and both FPI and HOMA-IR in the top tertiles. Furthermore, Rey-Valzacchi et al [268] conducted a prospective, randomised, placebo-controlled, double-blind intervention study (n=30) to investigate the effect of Metformin on the response to sildenafil in men with ED (IIEF-5 <22) and IR (HOMA-IR ≥3). The treatment group showed significant improvement in IIEF-5 score and a decrease in HOMA-IR compared to the placebo group at both 2 months (IIEF-5: 17.0±6.0 vs 14.3±3.9, p=0.01; HOMA: 3.9±1.6 vs 5.5±2.4, p=0.01) and 4 months (IIEF-5: 19.8±3.8 vs 14.3±3.9, p=0.005; HOMA: 4.5±1.9 vs 5.5±2.4, p=0.04). This supports the relationship between IR and ED and suggests that IR may be a causative factor in the development of ED. IR is suggested to be the early sign of endothelial dysfunction and may help predict vasculogenic ED [269].

4.2.1.4 Obesity

Obesity is highly prevalent amongst adult men and increasing worldwide [270, 271]. The 2008/2009 NZANS [271] showed that 27.7% of men >15 years of age were obese (BMI ≥30 kg/m^2) and 69.0% were either overweight or obese (BMI ≥25 kg/m^2). Obesity is a component of MetS [246] and well established as a risk factor for both T2DM and CVD [272-274]. Both the amount of excess adiposity and its distribution contribute to the increased risk of chronic disease: excess central adiposity poses a greater health risk than peripheral adiposity. Visceral fat distribution is associated with modified lipoprotein metabolism [275] and is an independent risk factor for chronic disease and all-cause mortality. Obesity is a complex multifactorial disease resulting from environmental (social, cultural and behavioral), physiological (metabolic) and genetic factors; however, for the purposes of this review it is considered a metabolic disorder due to its association T2DM and inclusion as a criteria for MetS.

Accurate quantification and localisation of body fat requires magnetic resonance imaging (MRI), computerised tomography (CT), or dual-energy x-ray absorptiometry (DEXA)[276]; however, these methods are often impractical as they are complex, expensive imaging techniques requiring skilled technicians and specialist equipment. The BodPod (Life Measurement Incorporation©), a method of analysing body composition using air displacement plethysmography and whole body densitometry, is simple, quick and convenient and has been shown to be accurate in measuring percentage body fat (BF%) in comparison to hydrostatic weighing and DEXA [277]. However, while it provides information on BF% and fat-free mass, it does not provide information on fat distribution. Obesity is generally defined

using simple anthropometric indices that act as a proxy for total body fat or abdominal fat to evaluate disease risk. Measurements such as Body Mass Index (BMI), waist-to-hip ratio (WHR), waist circumference (WC) and waist-to-height ratio (WHtR) are convenient and have been shown to correlate with abdominal fatness measurements from advanced imaging methods (BMI r=0.69, WHR r=0.54, WC r=0.75, WHtR r=0.83) [278]. There is a convincing level of evidence to support associations between all four indices and T2DM, hypertension, CVD risk factors, CVD risk and overall mortality [279]. The commonly used cut-offs to define obesity in men are shown in Table 2.8.

Table 2.8. Anthropometric indices and commonly used cut-offs to define obesity and increased risk of cardiometabolic disease in men: Body Mass Index (BMI) [280], waist-to-hip ratio (WHR) [281], waist circumference (WC) [280], and waist-to-height ratio (WHtR) [282].

Anthropometric index	Cut-off
BMI	≥30 kg/m^2
WHR	>0.9
WC	>102 cm
WHtR	>0.5

While a BMI >30 kg/m^2 is widely used to classify obesity [272, 280] (see Table 2.9), it is a proxy measurement of total body fatness that does not distinguish between fat and muscle or provide information on distribution. A WHR >0.9 indicates central obesity in men and confers a substantially increased risk of metabolic complications [281]; however, a person can lose weight and decrease their overall fatness without altering their WHR. While BMI, WHR and WC are all significantly associated with abdominal adiposity measured using MRI, WC has been found to most reliably predict abdominal fat distribution [278, 283]. The WC is therefore a preferable proxy for body fatness and obesity-associated disease and mortality risk. A WC >102 cm [272, 280] is a clinically accepted measurement to identify central obesity in men. Although the IDF suggests cut-off points for different ethnic groups (Europids >94 cm, South Asians, Chinese and Japanese >90 cm)[284], further research is needed to determine whether sex, age and population-specific cut-off points are needed [279]. WHtR is the most recent addition to the proxy measurements for body fatness. A 2008 meta-analysis [285] of 9 cross-sectional and one longitudinal study reported that WHtR is superior to BMI, WC and WHR in detecting CVD risk factors (T2DM, hypertension and dyslipidemia). In 2010 Schneider et al [286] conducted a longitudinal study (n=11,000, duration 8 years) and also reported that WHtR was a better measure for incident CVD events and mortality than the BMI. Conversely, in 2011 Mørkedal et al [287] reported the results of a longitudinal study (n=60,000, duration 13 years) and found that after adjustment for BMI, WHR was superior to WHtR in predicting ischemic heart disease (IHD) mortality. It is evident that more research is needed in this area to clarify

which of the indices is most accurate in determining disease risk and mortality. However, in contrast to WC alone, the correction of WC for height means that a single cut-off value may be valuable in predicting disease risk associated with abdominal fatness in different sex, age and ethnic groups. In 2010, a large systematic review [282] of 22 longitudinal studies and 57 cross-sectional studies suggested that a WHtR >0.5 signifies the critical boundary for increased disease risk and may be an appropriate global value.

Table 2.9. Classification of overweight and obesity based on Body Mass Index (BMI) and waist circumference (WC) in men [280]

			Disease risk* compared to normal BMI and WC	
	Obesity Class	**BMI kg/m^2**	**≤102 cm**	**>102 cm**
Underweight		<18.5	-	-
Normal		18.5-24.9	-	-
Overweight		25.0-29.9	Increased	High
Obesity	Grade I	30.0-34.9	High	Very High
	Grade II	35.0-39.9	Very High	Very High
Extreme obesity	Grade III	≥40	Extremely High	Extremely High

*Disease risk for Type 2 Diabetes Mellitus (T2DM), hypertension and cardiovascular disease (CVD)

Some researchers suggest that obesity should be considered an independent risk factor for ED [288, 289]; however, its role is controversial [22] and further research is needed to clarify the association and determine causation. Both cross-sectional [119, 164, 168, 290-295] and prospective cohort [12, 183, 189] studies report an association between obesity and ED with higher prevalence rates of ED in obese men and an increased risk with increasing weight. Overweight and obese men appear to have 2-3 times the risk of ED compared to non-obese men [290, 296]. Clinic-based studies have shown that more than a third of men presenting with ED are overweight or obese [297], and that obesity is associated with more severe symptoms [298] and greater reductions in sexual quality of life [299]. While there are many studies focusing on BMI, WC and WHR, there is a paucity of data on the association between ED and WHtR, and also BF% using air displacement plethysmography and fat distribution using imaging techniques. Research using these techniques may help clarify the association between obesity and ED.

In cross-sectional studies, the American NHANES [119] reported an age-adjusted prevalence rate of 20% moderate-severe ED in obese men (BMI >30 kg/m^2) compared with 15% in overweight men (BMI 25-29.9 kg/m^2) and 14% in healthy men (BMI <25 kg/m^2). After adjusting for age, obesity was associated with almost double the risk of ED (OR=1.8 [1.03-3.14]) however it was not a significant predictor after adjusting for other CVD risk factors (OR=1.48 [0.89-2.45]). In the BACH survey [131], WHR but not BMI was significantly associated with IIEF-5 scores: the greater the central adiposity the worse the ED symptoms. This is not unexpected as

fat distribution not body mass predicts metabolic and cardiovascular complications. However, again this was no longer significant after adjusting for sociodemographic, medical and lifestyle factors. In multivariate analysis, the importance of obesity and/or central adiposity as a predictor is likely to be lost due to confounding with T2DM and CVD. Interestingly, the Australian MATeS [162] showed that after age-adjustment, a small WC was significantly protective against moderate-severe ED (<94 cm: OR=0.7 [0.6-0.9]) and a large WC was associated with a slight increased risk (≥102 cm: OR=1.3 [1.0-1.6]), whereas being underweight (BMI <20 kg/m^2: OR=2.4 [1.3-4.3]) or obese (BMI ≥30 kg/m^2: OR=1.8 [1.4-2.2]) were both significantly associated with an increased likelihood, while being overweight was not (BMI 25-29.9 kg/m^2: OR=1.1 [0.9-1.3]). After adjusting for all medical and lifestyle factors, having a small WC or being underweight remained significant independent predictors of decreased and increased risk respectively (ORs not provided). A U-shaped association with BMI is likely as having both low and high BMI can be indicators of poor health. Indeed, the 2013 45 and Up Study [169] showed that the likelihood of moderate-complete ED increased 45% in underweight (BMI<18.5 kg/m^2: OR=1.45, p<0.05), 61% in class I obese (BMI 30-35 kg/m^2: OR=1.61, p<0.05), 200% in class II obese (BMI 35-40 kg/m^2: OR=2.25, p<0.05) and 300% in class III obese men (BMI >40 kg/m^2: OR=3.24, p<0.05) without prostate cancer (PCa) compared to men with a BMI 18.5-25 kg/m^2. However, in 2015, Maseroli et al [300] and Park et al [301] reported significantly higher prevalence rates of central obesity in men with ED in cross-sectional studies in Italy (WC>102 cm: 31.7% vs 22.8%, p<0.05) and Korea (WHR ≥0.93: 69.3% vs 58.7%, p<0.001); however, there were no significant differences in the prevalence rates of overweight or obesity by BMI in either study.

In longitudinal studies, although the baseline MMAS [1] results showed no association between BMI or WHR and ED, prospective results [302] demonstrated that BMI was a significant independent predictor: 22% of overweight men (BMI ≥28 kg/m^2) had incident ED compared to 13% of normal weight men (BMI <28 kg/m^2) and being overweight doubled the likelihood of incident ED (multi-adjusted OR=1.96 [1.17-3.28]). Interestingly, baseline obesity also predicted a higher risk of incident ED, regardless of subsequent weight loss. In contrast, the baseline HPFS [164] results showed that obesity (BMI >28.7 kg/m^2) was associated with higher risk for ED (RR=1.3 [1.2-1.4]) compared to normal weight men (BMI <23.2 kg/m^2) and prospective results [12] demonstrated that compared to a BMI <25 kg/m^2 at baseline, the risk of incident ED increased 19% with a BMI 25-26.9 kg/m^2, 33% with a BMI 27-29.9 kg/m^2, and 90% with a BMI >30 kg/m^2 (RR=1.9 [1.6-2.2]). In Australia, the baseline FAMAS [168] results were the first to show that ED was significantly associated with abdominal fat mass measured

using the DEXA. As abdominal fat mass increased, the severity of ED increased (no ED: 10.86 [7.88-11.39] kg/m^2; mild ED: 11.38 [7.14-14.78] kg/m^2; moderate-severe ED: 11.94 [7.48-13.37] kg/m^2; p=0.038). This relationship remained after multivariate adjustment: every 1 standard deviation (SD) increase in kg/m^2 abdominal fat mass independently predicted an increased likelihood of ED. The prospective results [189] also showed that visceral adiposity was associated with ED. The mean abdominal fat mass was significantly higher in men with incident ED compared to those without (36.0±7.1% vs 32.4±7.7%, p≤0.05) and visceral adiposity was a significant independent predictor of both incident ED and a lower likelihood of remission of symptoms after controlling for sociodemographic, lifestyle factors and comorbidities.

Strong evidence comes from several intervention studies that have found that lifestyle changes resulting in weight reduction improves erectile function [143-148]. Esposito et al [143] conducted an RCT in 110 men with ED. The intervention group (n=55, mean age=43.5 years, mean BMI=36.9 kg/m^2) received monthly group sessions with instruction on reducing caloric intake and increasing PA to achieve 10% reduction in body weight. The control group (n=55, mean age=43 years, mean BMI=36.4 kg/m^2) received general information on healthy eating and PA. After 2 years, the intervention group showed significantly improved BMI (-5.7 vs -0.7 kg/m^2, p<0.001) and IIEF scores (+3.1 vs +0.1, p<0.001) compared to the control group. The same research group [144] conducted another RCT in 209 men with or at risk of ED. The treatment group (n=104, mean age=45.3 years, mean BMI=31.9 kg/m^2) received specific advice on weight reduction, diet quality and PA. The control group (n=105, mean age=45.7 years, mean BMI=31.5 kg/m^2) received general information regarding healthy food choices and PA. After 2 years, the intervention group had lost more weight (-8.9 vs -2.1 kg, p<0.001) and lowered their BMI (-2.7 vs -0.5 kg/m^2, p<0.001) and WC (-4.9 vs -0.9 cm, p<0.001) compared to the control group. IIEF-5 scores had improved in the intervention group and a significantly greater proportion of men had normal erectile function compared to the control group (+22 vs +2%, p=0.015). In another small RCT in 20 morbidly obese men (intervention group: mean age=36.7 years, mean BMI=55.7 kg/m^2; control group: mean age=42.2 years, mean BMI=54.0 kg/m^2), Reis et al [146] reported significant reduction in both BMI (p<0.0001) and erectile dysfunction (p=0.022) 24 months after an exercise and diet modification intervention with subsequent gastric bypass surgery. Khoo et al [145] conducted an RCT in centrally obese men (n=70, mean age=49.7 years, BMI ≥30 kg/m^2, WC ≥102 cm) with and without T2DM (n=19 and n=25 respectively) and a control group (n=26). After 8 weeks on a low-calorie meal replacement diet, a 10% weight loss was significantly associated with improved IIEF-5 scores in

both diabetic and nondiabetic men and the degree of improvement was significantly associated with the degree of weight loss. The same group conducted another small uncontrolled trial [148] with 31 obese men with T2DM placed on either a low caloric diet (n=19, mean age=58 years) or a high-protein low-fat diet (n=12, mean age=62 years) for 8 weeks followed by 44 weeks on the high-protein low-fat diet. They reported a 5-10% weight loss and WC reduction with other cardiometabolic health improvements. Both IIEF-5 scores and LUTS were significantly improved and again, this was associated with the degree of weight loss and WC change. Further large-scale well-designed RCTs are needed to clarify the effect of both surgical and non-surgical weight loss on erectile function in overweight and obese men with ED. However, current evidence supports that it is possible to improve erectile function through weight loss.

4.2.2 Cardiovascular disorders

4.2.2.1 Cardiovascular diseases

The term CVD refers to a range of diseases involving the cardiovascular system: coronary artery disease (CAD) or IHD, angina, myocardial infarction (MI), cerebrovascular disease, atrial fibrillation (AF), heart failure (HF), heart valve disease, congenital heart disease, cardiomyopathy, pericardial disease, aortic disease, and PVD [303]. These diseases, particularly CAD and cerebrovascular disease, are the main causes of death worldwide [304] and have similar key pathological processes including vascular injury, inflammation and calcification. Based on well-established risk factors for CVD, the AHA [305] encourages people to meet 7 ideal health goals for cardiovascular health: do not smoke; eat a healthy diet; be physically active; maintain a healthy body weight; and normal BP, FPG and TC levels. Meeting a greater number of these health metrics has been shown to lower the risk of CVD and all-cause mortality [306].

Epidemiological research has clearly established that ED is strongly associated with self-reported CVD and CVD outcomes [1, 17, 24, 27, 129, 196, 197, 291, 292]. The prevalence of ED is higher in men with self-reported diagnosed CVD [168], even after adjusting for age [1, 119]. Prevalence rates as high as 75% have been reported [307]. In the USA, the NHANES [119] results showed an age-adjusted prevalence rate of 25% moderate-severe ED in men >20 years with a history of CVD compared to 18.5% in the general population. The BACH survey [131] found a significant age-adjusted association between the IIEF-5 score and self-reported heart disease (β=-2.95 [-4.22, -1.68], $p<0.01$); however, in contrast the GSSAB in the USA showed no significant increase in odds for ED in men with self-reported heart disease [6]. In Australia, similar to the NHANES results, the baseline FAMAS [168] results showed a higher prevalence of

moderate-severe ED in men aged 35-80 years with self-reported CVD compared to men without CVD (29.3% vs 17.2%, p<0.001); however, multivariate analysis showed no significant independent effect of CVD. At follow-up [189], angina was not a significant predictor of incident ED after 5 years but it was a significant negative predictor of remission. In contrast, AF has recently been shown to be an independent risk factor for ED (HR=1.53 [1.05-2.24]) in a large prospective cohort study [308] (n=19258, 3853 cases, 15406 controls, duration 5 years). The incidence of ED in the AF cohort was 1.65 times that of the control cohort (20.6 vs 12.5 cases/10,000 man-years, p<0.001).

Clinical-based studies show that IHD is prevalent in men with ED [309-311] and ED is highly prevalent in men with IHD [312-314]. In 2013, Pauker-Shanon et al [312] conducted a clinical-based study (n=171, mean age=64.2 years) in men with IHD (≥50% stenosis in ≥1 coronary artery diagnosed by coronary angiography, a history of MI or a combination of the two) investigating the association between CVD risk factors (age, DM, hypertension, smoking, hyperlipidaemia, left ventricular ejection fraction (LVEF), and 3-vessel disease) and ED. Of these men, 83% had ED (IIEF-5 <22): 36% had mild (IIEF-5 17-21) and 47% had significant ED (IIEF-5 ≤16). Age (p<0.0001), occluded coronaries (p=0.049), 3-vessel disease (p=0.037) and hypertension (p=0.024) were significantly higher or more prevalent in men with ED. Not surprisingly, 59% had ≥3 concomitant CVD risk factors and the mean number of risk factors increased as the severity of ED increased. Furthermore, up to 89% of HF patients are reported to suffer from ED [315-317].

Although there is some inconsistency in results from epidemiological studies, the variability in study design including the way CVD data were collected (e.g., self-report of disease outcomes, self-report of CVD medication, or direct measurement of heart function or vessel disease) makes them difficult to compare. Furthermore, while they may appear inconsistent for self-reported CVD and CVD outcomes, there is strong evidence supporting the association between ED and risk factors for CVD (both self-reported and measured directly): ageing [1, 3, 5-7, 26-28, 114, 118-120, 122, 124-129, 131-133, 160, 161, 163-165, 292], T2DM [1, 24, 27, 128-130, 136, 161, 175, 179, 195], obesity and MetS [24, 45, 130, 292], hypertension [1, 24, 27, 128, 129, 136, 161, 175, 176, 318], hyperlipidaemia [1, 27, 161, 292], atherosclerosis [319], smoking [1, 27, 129, 161, 178, 200, 292] and physical inactivity [175, 178, 179, 291, 292]. Studies consistently report that ED is more prevalent among men with one or more of these CVD risk factors, and that having these risk factors significantly increases the odds of having ED. Further modifiable CVD risk factors including SES, diet, alcohol consumption and stress [320] have also been suggested to be risk factors for ED. Some risk factors for ED may be specific to CVD

patients: decreased exercise capacity (inadequate cardiac output for sexual performance), atherosclerosis (atherosclerotic plaques, endothelial dysfunction and arterial stiffness) and medications (thiazide diuretics, digoxin, aldosterone, traditional β-blockers) [321-324]. Furthermore, CVD, ED and depression, a triad of conditions often found in the same patient, commonly exacerbate each other [325]. As such, studies now support that ED is an early marker of CVD [326, 327]. In a 2011 meta-analysis of 12 prospective cohort studies, Dong et al [328] provided strong evidence to support the significant and independent association between ED and CVD risk, CHD, stroke and all-cause mortality. Atherosclerosis may also occur in the arteries supplying blood to the CC, beginning as endothelial dysfunction and leading to the development of atherosclerotic plaques which block blood flow. The small size of the penile blood vessels makes them extremely sensitive to vascular change [329]. The penile arteries are narrower than coronary arteries and are thus more likely to become occluded as a result of atheroma, resulting in reduced blood flow and poor quality erections [17]. ED is now widely accepted as synonymous with endothelial dysfunction and is thus considered a precursor for systemic vascular disease in many men [10, 330, 331]. Evidence supporting the use of ED as an early marker of CVD will be discussed further in Section 5.0.

4.2.2.2 Hypertension

Hypertension (defined as self-reported hypertension, use of antihypertensive medication, and/or >140 mmHg SBP and >90 mmHg DBP [332]) is a highly prevalent condition worldwide [333, 334] and a major risk factor in CVD [335]. The 2008/2009 NZANS [334] showed that 34% of NZ men over 15 years of age had hypertension and 14% used hypertensive medication. Although a clinical cutoff value of >140/>90 mmHg is often used, the 2003 WHO and International Society of Hypertension guidelines for the diagnosis of hypertension [332] further classify hypertension into Grade 1, 2 and 3 as shown in Table 2.10. Elevated BP is well established as a risk factor for CVD; however, this effect is modified by age with raised DBP being a better predictor in young people and elevated SBP in the middle-aged and elderly [335]. The Joint National Committee (JNC) on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure [336, 337] guidelines state that: SBP>140 mmHg is more important than DBP in persons >50 years; CVD risk doubles in 20/10 mmHg increments from 115/75 mmHg and there is a 90% lifetime risk of hypertension in people who are normotensive at 55 years; and those with a SBP 120-139 mmHg or a DBP 80-89 mmHg are prehypertensive and require lifestyle modification. Additionally, pulse pressure (PP) and mean arterial pressure (MAP) are important markers of peripheral and arterial vascular resistance respectively and may be superior predictors of CVD risk in middle-aged and elderly people [335].

Table 2.10. Classification of hypertension according to the 2003 World Health Organization/International Society of Hypertension (WHO/ISH) guidelines [332].

Blood pressure (BP)	Grade 1	Grade 2	Grade 3
Systolic BP (mmHg)	140-159	160-179	≥180
Diastolic BP (mmHg)	90-99	100-109	≥110

ED is highly prevalent in the hypertensive population [119, 318, 338] and hypertension is extremely common in men with ED [161]. Although an early RCT [321] suggested this was not the case - the Treatment of Mild Hypertension Study (TOMHS) reported that ED was relatively rare (12%) in mild hypertensives (n=902, 557 men, 45–69 years) - the inclusion criteria of this study would have excluded most ED cases. However, the results did support an association between ED and both SBP and use of antihypertensive medication. A later clinical-based study [318] (n=476) of patients with hypertension reported 68% prevalence of ED and 45% had severe ED. ED is also more prevalent in hypertensive men compared to the general population [119, 338]. In the USA, the MMAS [1] showed a 15% age-adjusted prevalence of complete ED in treated hypertensives versus 9.6% in the entire sample and ED was associated with both the duration and severity of the hypertension. The NHANES [119] found an age-adjusted prevalence rate of 15% moderate-severe ED in men with untreated hypertension and 28% in men with treated hypertension. Hypertension was present in 36% of men with ED in the multinational MALES study [161] compared with 19% in men without ED ($p<0.0001$). Furthermore, in a survey of general medical patients (n=7689, mean age=58.9 years), Giuliano et al [338] reported ED (IIEF-5 score <22) in 67% of hypertensive patients, 71% of diabetic patients, and 77% of patients with both conditions. Also, the prevalence was affected by the history and characteristics of the hypertension and the number and type of antihypertensives used. It is uncertain whether it is hypertension *per se* or antihypertensive medications or a combination of the two causing the higher prevalence of ED in epidemiological studies.

It is well established that antihypertensive therapy can cause ED [339]. However, results from the FAMAS [168] cohort showed that significantly more Australian men with hypertension had that mild ED and hypertension was a significant predictor of only mild ED (OR=1.79 [1.18-2.71]). When hypertension was further split into diagnosed hypertension, use of antihypertensives and measured hypertension, the largest contributor to the increased risk was measured BP (OR=1.67 [1.05-2.61]), suggesting that it is predominately the hypertension and not the medication behind the higher prevalence of ED in hypertensives. They also ranked the antihypertensive medications by effect size and found the following therapies contributed significantly to the effect of hypertensive medication on mild ED (OR=1.44 [1.02-2.66]): angiotensin II inhibitors, Ca^{2+}-channel blockers, thiazides, angiotensin-converting enzyme (ACE)

inhibitors, high ceiling diuretics and beta-blockers. This contrasts with an earlier review by Baumhakel et al [340] which found evidence to support the adverse effect of thiazide diuretics and beta-blockers (except Nebivolol) but either no effect or a favourable effect of ACE inhibitors, angiotensin-receptor blockers and Ca^{2+}-channel blockers. Further research is needed to clarify the effect of specific medications on erectile function.

Blood flow is essential for tumescence and the primary pathogenesis of ED in hypertension is suggested to be atherosclerosis with resultant altered haemodynamics reducing penile blood flow [341]. Jensen et al [342] reported 27% of men with hypertension had ED and their SBP was significantly higher than men without ED (p=0.046). Further examination of penile function (including flaccid and dynamic PDS) revealed that the vast majority of cases (89%) were vasculogenic in aetiology and due to arterial dysfunction: IHD was a significant determinant of hypertensive ED (p=0.005). Furthermore, experimental studies have shown that Nebivolol, a beta-receptor blocker, reduces oxidative stress and improves endothelial function in the aorta and CC of apolipoprotein-e knockout mice [343]. It also appears to increase sinusoidal eNOS expression, improve relaxation and protect against structural changes to CC tissue in spontaneously hypertensive rats [344]. Sustained hypertension may result in increased oxidative stress and damage to both endothelial cells and SMC resulting in a reduction in dilation and penile blood flow [345]. PDE_5 inhibitors have been shown to improve hypertensive ED and also cause a mild reduction in overall BP; however, they are contraindicated in some cases as they may have a synergistic effect with certain hypertensive medications (e.g., organic nitrates) leading to hypotension [345]. Hypertension is well established as a risk factor for ED and the development of ED in hypertensive men indicates further deterioration in vascular health.

4.2.2.3 Dyslipidaemia

Dyslipidaemia (defined as self-reported dyslipidaemia, use of dyslipidaemia medication, elevated serum low density lipoprotein cholesterol (LDL-c) ≥160 mg/dL (>4 mmol/L), total cholesterol (TC) ≥240 mg/dL (>6 mmol/L), TG ≥ 200 mg/dL (>2.3 mmol/L) and/or HDL-c <40 mg/dL (<1.0 mmol/L)) is a highly prevalent condition [346, 347] and a major cause of CVD [348, 349]. The NHANES [347] showed that amongst American adults >20 years of age in 2008, 44% had borderline or high TC (≥200 mg/dL), 15% had high TC (≥240 mg/dL) indicating hypercholesterolemia and 19% had low HDL-c (<40 mg/dL) [347]. Moreover, in 2010, 38% had high LDL-c and 70% of those were being treated [346]. The National Cholesterol Education Program-ATP III (NCEP-ATP III) classification of lipid levels is shown in Table 2.11. The major atherogenic lipoprotein and target of cholesterol lowering therapy is LDL-c and this is the most

widely used and accepted lipid biomarker for CVD risk [349]. However, both TG and HDL-c are modifiable risk factors [348] and strong predictors of adverse cardiovascular outcomes [350, 351]. Mixed dyslipidaemia can be considered present with ≥2 lipid abnormalities (high LDL-c, low HDL-c and/or high TG) [352]. Simple indices involving HDL-c including the ratio of TC:HDL-c (≥5.0) and TG:HDL-c (≥3.5) have been suggested to be more predictive than LDL-c alone [350].

Table 2.11. Classification of lipid and triglyceride levels according to the National Cholesterol Education Program-Adult Treatment Panel III (NCEP-ATPIII) [349].

Classification of lipid biomarkers	**Desirable/ Optimal**	**Near optimal/ above optimal**	**Borderline High**	**High**	**Very High**
Total cholesterol (mg/dL)	<200	-	200-239	≥240	-
LDL cholesterol (mg/dL)*	<100	100-129	130-159	160-189	≥190
Triglycerides (mg/dL)	<150		150-199	200-499	≥500
HDL cholesterol (mg/dL)	**Low**			**High**	
	<40			≥60	

* LDL cholesterol is the primary target of lipid lowering therapy

Dyslipidaemia appears to be a risk factor for ED [353]. Epidemiological evidence [1, 119, 161, 354] shows that dyslipidaemia is highly prevalent amongst men with ED [355, 356] [161], that ED is more prevalent in men with dyslipidaemia than in the general population [119, 161], and that raised TC and LDL-c and lowered HDL-c are all associated with ED [1, 354, 357]. Bodie et al [355] examined the laboratory data of 3,547 patients with ED and found that 48% had TC levels >200 mg/dL. Similarly, Roumeguere et al [356] conducted a prospective case-control study (n=315, 215 cases, 100 controls) and found that hypercholesterolaemia was more prevalent in ED cases than in controls (71% vs 52% respectively, p=0.06). Furthermore, LDL-c (p=0.043), HDL-c (p=0.029) and TC:HDL-c (p<0.001) were significantly correlated with ED with a strong trend observed for TC (p=0.067) and TG (p=0.079). Age, HDL-c and TC:HDL-c remained significant independent predictors of ED in a multiple logistic regression model. In a longitudinal cohort study (n=3250, 26-83 years, mean follow-up 22 months), Wei et al [354] found that with every 1 mmol/L increase in TC or decrease in HDL-c, the risk of ED increased by 34% and decreased by 62% respectively. In contrast, the MMAS [1] found no correlation between TC and the probability of ED; however, results supported the inverse relationship between HDL-c and ED. A reduction in HDL-c from 90 to 30 mg/dL increased the age-adjusted probability of moderate ED by 18% in men aged 40-55 years and complete ED by 15% in men aged 56-70. The multinational MALES study [161] found that high cholesterol (self-reported or receiving treatment) was significantly more prevalent in men with ED than without (29% vs 4% respectively, p<0.0001). Nikoobakht et al [357] conducted a case-control study (n=200, 100 organic ED cases, 100 healthy controls) and found significantly higher TC (p=0.04) and LDL-c

(p=0.02) levels in men with ED (IIEF-5) and significant associations between these lipid levels and risk of ED (OR=1.74 and OR=1.97 respectively). For every 1 mg/dL increase in TC or LDL-c there was a 0.036 and 0.035 decrease in IIEF-5 scores respectively (indicating worsening symptoms) and 4% of ED was accounted for by differences in TC or LDL-c (r^2=0.04). However, there was no significant association with TG or HDL-c levels. The NHANES [119] found 23% prevalence of ED in men with hypercholesterolemia (TC≥240 mg/dL), self-reported diagnosis or advice to take lipid-lowering medication); however, hypercholesterolemia did not significantly increase the age-adjusted odds of ED (OR 1.09 [0.79-1.50]). In the BACH study, Hall et al [358] found no significant positive association between hyperlipidaemia and ED (IIEF-5); however, men with treated hyperlipidaemia were twice as likely to report ED compared to men without or with untreated hyperlipidaemia, and there was a higher risk amongst younger men (<55 years) on hyperlipidaemia treatment who also had diabetes and/or CVD. More recently, results of the Australian FAMAS [168] found no association between self-reported high cholesterol and the prevalence of ED at baseline (p=0.134) or the incidence of ED at follow-up (p>0.05); however, it was found to significantly predict a lower odds of remission (p<0.05). [189].

There is inconsistent evidence on the effects of lipid-lowering treatment on ED. Saltzman et al [359], Bank et al [360] and Hong et al [361] observed improvement in ED (IIEF scores) following treatment with atorvastatin, quinapril or combined treatment with a PDE_5 inhibitor. However, Bruckert et al [362] and Solomon [363] found that lipid-lowering therapies worsened ED symptoms. In 2014, Kostis et al [364] conducted meta-analysis of 11 randomised trials investigating the effects of statin therapy on IIEF scores. They found that overall statin therapy significantly improved IIEF scores (+3.4 points [1.7-5.0], p=0.0001) compared to controls and this was suggested to be larger than the effects reported for lifestyle modification and approximately half the effect reported for PDE_5 inhibitors. The common pathophysiology is considered to be endothelial dysfunction as excess oxidised LDL-c may impair NO activity and thus endothelium-dependent relaxation in the CC, resulting in ED [365].

4.2.2.4 Atherosclerosis, arterial stiffness, endothelial dysfunction

Atherosclerosis (the accumulation of lipids and fibrous elements in the arterial wall resulting in the stiffening and/or narrowing of the blood vessel and reduced blood flow) is a progressive and multifactorial disease and the primary cause of CVD. It is a chronic inflammatory condition that develops in response to vascular injury from atherogenic insults such as hypertension, hyperlipidaemia and smoking [366]. Haemodynamic forces combined with oxidised LDL-c damage the endothelium, increasing its permeability and altering the expression of endothelial cell genes (e.g., eNOS) resulting in impaired NO activity and reduced arterial elasticity.

Endothelial dysfunction is a systemic disorder that precedes atherosclerosis, and arterial stiffness is associated with subclinical atherosclerosis and increased CVD risk [367-369]. Defective NO activity plays a key role in the pathogenesis of vasculogenic ED [370] and endothelial damage appears to be the link between the metabolic and cardiovascular ED risk factors mentioned previously. The initial event in clinical atherosclerosis is the retention of lipoproteins in the intima, followed by the recruitment of monocytes and the formation of 'foam cells' (cholesterol-engorged macrophages) in the subendothelium. These develop into 'fatty streaks' and subsequently 'fibrous lesions'. These plaques expand to block blood flow and advance to become calcified, ulcerated or haemorrhagic; however, they can also erode or rupture forming a blood clot and leading to an acute cardiac event [366]. The majority of ED cases are vasculogenic; atherosclerosis in the penile arteries and/or its precursors, endothelial dysfunction and arterial stiffness, are likely to be the primary cause.

There are many methods currently used to clinically diagnose atherosclerosis (e.g., electrocardiography (ECG), echocardiography, chest x-ray, angiography, stress testing, CT, MRI, and positron emission tomography (PET) scanning). These are generally lengthy, invasive procedures that can be expensive and require highly skilled technicians and complex medical equipment. However, the assessment of risk factors has long been used to identify those at risk of atherosclerosis. There are many risk factors used: age, gender, family history, diabetes, MetS, obesity, inflammation and raised inflammatory markers, elevated BP, dyslipidemia, raised haemostatic markers, depression, and environmental factors (e.g., smoking, a high fat diet and low PA). The risk factors for atherosclerosis are multiplicative and prediction algorithms have been developed using multiple risk factors to calculate the future risk of CVD events.

4.2.2.5 CVD risk prediction algorithms

The most commonly used is the Framingham risk model, proposed in 1976 [371] to calculate CHD risk based on a USA cohort, adapted in 1991 [372] to include HDL-c, and again in 2008 [373] to create a single prediction tool that can be used to predict the risk of general or specific atherosclerotic CVD events (coronary, cerebrovascular, PVD or HF). The latest Framingham risk model [373] uses assessment of age, gender, TC, HDL-c, SBP, use of hypertension medication, smoking, and diabetes status to generate an individual's risk of atherosclerotic CVD events in the next 10 years. Scores are arbitrarily classified into low (<10%), intermediate (10-20%) or high (>20%) risk. Other CVD risk assessment models have been formulated, such as the QRISK [374] and ASSIGN [375] scores based on UK cohorts. These incorporate Framingham risk factors with the addition of family history and social deprivation; however, there is doubt

regarding claims that these offer improved predictive value over the Framingham risk score [376].

Several cross-sectional [377, 378] and longitudinal cohort studies [379, 380] have reported an association between ED and Framingham risk scores. In 2005, results of a cross-sectional observational study [377] of men without history of CHD or stroke participating in a health-screening project in Vienna (n=2,561 men, age range=30-74 years) reported that those with moderate-severe ED (IIEF-5 ≤16) had a significantly higher 10-year risk of developing CHD (13% vs 8% respectively, p<0.001) and stroke (13% vs 9% respectively, p=0.041). In 2006, results of a study [378] of primary care patients in Canada (n=3921 men, age range=40-88 years) showed that 49.4% had ED (IIEF <26), and in the absence of CVD or diabetes a 1% increase in the 10-year risk of CHD independently predicted a 1% increase in the risk of ED (OR=1.03 [1.02-1.05]). Furthermore, the longitudinal population-based Krimpen Study [379] (n=1248 men without CVD, age range=50-75, mean follow-up=6.3 years) showed that Framingham CVD risk scores increased with the severity of ED (12% vs 14% vs 18% for normal, reduced and severe ED respectively, p<0.05) and the incidence of CV events also increased with the severity of ED (5 vs 10 vs 19 cases/1000 man-years for normal, reduced and severe ED respectively, p< 0.05). The risk of CV events increased with increasing ED severity (HR=2.0 [1.4-2.7] vs HR=3.8 [2.0-7.3] for reduced and severe ED respectively, p<0.05) and ED remained a significant independent predictor of CV events even after adjustment for age and Framingham score (HR=1.6 [1.2-2.3] vs HR=2.6 [1.3-5.2] for reduced and severe ED respectively). These results support that ED, although associated with traditional CVD risk factors, has an independent relationship with CVD events and may be a valuable addition to risk prediction models. Most recently, the results from the longitudinal BACH Survey [380] (n=965 men free of CVD, age range=30-79 years, BACH I 2002-2005, BACH II 2006-2010, BACH III 2010-2012) supported the relationship between changes in ED and Framingham risk scores over time. They showed that "transient" and "persistent" ED were both significantly associated with 10-year CVD risk and a greater increase in this risk over time. Amongst men with "persistent" ED, 10-year CVD risk was 1.58% higher in younger men and 2.54% higher in older men. This supports the importance of assessment of CVD risk in men presenting with ED.

A systematic review in 2012 [381] reported that although ED was an independent predictor of CVD, it provided no benefit over the traditional risk factors in risk prediction. However, a 2013 systematic review and meta-analysis [382] of 16 longitudinal studies (n=92,757, mean follow-up=6.1 years) investigating the ability of ED to predict the risk of CVD events reported pooled results for the risk of each of the reported CVD endpoints for men with ED: total CV events

(RR=1.44 [1.27-1.63]), CV mortality (RR=1.19 [0.97-1.46]), MI (RR=1.62 [1.34-1.96]), cerebrovascular events (RR=1.39 [1.23-1.57]), and all-cause mortality (RR=1.25 [1.12-1.39]). They found that ED is associated with an increased risk of CV events and all-cause mortality and that the risk is greatest in younger men and those in intermediate Framingham CVD risk scores. This is important as these two groups may benefit the most from the addition of a novel independent marker like ED to currently accepted risk prediction algorithms to support reclassification and ensure appropriate treatment.

4.2.2.6 Imaging biomarkers

Accurate, non-invasive procedures to assess atherosclerosis, endothelial function and arterial stiffness are also available. Although they are not widely used clinically, they show great promise in research. Ultrasonic measurement of the intima-media thickness (IMT) of the common carotid artery is non-invasive, reproducible and the most widely accepted imaging biomarker of subclinical atherosclerosis [383]. Increased carotid IMT (above the 75th percentile within the population) is well established as a marker of atherosclerosis [384]. Carotid IMT is now recommended to improve CVD risk stratification in asymptomatic adults [384]. Furthermore, it has recently [385] been shown that measurement of cavernosal artery IMT is possible and correlates positively with carotid IMT ($r=0.61$, $p<0.001$). Cavernous IMT may be a valuable addition to the available tools used to predict vasculogenic ED and may help identify systemic atherosclerosis in its early stages.

High-resolution ultrasonic measurement of flow-mediated vasodilation (FMD) of the brachial artery can be used to measure endothelial function – the ability of endothelial cells to stimulate vasodilation [370]. Reactive hyperemia (an endothelium-dependent transient increase in blood flow following a period of restricted blood flow) induces FMD, which can be measured in the peripheral arteries using the percentage change in brachial FMD after occlusion [386]. Pharmacologically induced endothelium-independent FMD can also be measured in response to sublingual administration of nitroglycerin (NTG). Reduced FMD has been shown to correlate with CVD risk in low risk populations and may be independently related to cardiovascular events in asymptomatic subjects; however, it is not yet clear whether it provides any benefit beyond traditional risk factor assessment in a clinical setting [384]. The use of a combination of FMD and carotid IMT has been suggested as an alternative method to determine vasculogenic ED (100% sensitivity, 59.2% specificity) [387]. This may be valuable in a research setting as it is less intrusive than the current use of PDS.

Mechano-transducers, applanation tonometers, echo-tracking and Doppler flow meters can be used to assess central and peripheral arterial stiffness via the measurement of pulse wave

velocity (PWV) and pulse wave analysis (PWA) [388]. PWV is the velocity of the waveform as it passes between two arterial points (the distance between the two recording sites divided by the time between the feet of two pulse waves). It is increasingly used in research and is considered the gold standard measurement of arterial stiffness [389]. Although PWV can be measured using the carotid-radial (crPWV), femoral-posterior tibial (legPWV), brachial-radial (armPWV) or brachial-ankle (baPWV) arteries, it is ideally measured between the common carotid and femoral arteries (cfPWV). A higher cfPWV is indicative of increased central aortic stiffness and has been shown to be an important predictor of major adverse cardiac events (MACE) and all-cause mortality [368]: a cfPWV >10 m/s is suggested to predict CV events [390] and >12 m/s is suggested to be indicative of organ damage [391]. It is associated with increased mortality in both high-risk [392, 393] and apparently healthy individuals [394, 395]. In a prospective cohort study [394] of community-living older adults in the USA (n=2075, 1491 males, mean age=74 years, average follow-up=4.6 years), being in the highest quartile for cfPWV was shown to significantly increase the risk of all-cause mortality (RR=1.7 [1.2-2.5]), cardiovascular mortality (RR=2.3 [1.2-4.3]), CHD (RR=1.5 [1.1-2.1]) and stroke (RR=3.6 [1.8-7.2]) compared to the lowest quartile. In another prospective cohort study [396] of 1678 Danish subjects (n=1678, 878 males, age range=40-70 years, median follow-up=9.4 years) after adjustment for sex, age, BMI, MAP, current smoking, and alcohol intake, every 1 SD increase in cfPWV (3.4 m/s) increased the risk of CHD by 16% (HR=1.16 [1.00-1.35], $p<0.05$), a cardiovascular event by 17% (HR=1.17 [1.04-1.32], $p<0.05$) and cardiovascular mortality by 20% (HR=1.20 [1.01-1.41], $p<0.05$). However, the use of PWV in clinical settings is limited by technical concerns, issues with the standardisation of measurement protocols, quality control issues, and the lack of well-established thresholds defining risk [388]. PWA can also be used to assess the arterial pulse waveform to determine other haemodynamic parameters: the reflection of the pulse wave (aortic augmentation index (AIx)), the brachial pulse pressure (BPP), carotid pulse pressure (CPP) and carotid distension (CD). These parameters have been shown to have a modest ability to predict CVD outcomes [388]. PWV is the preferred measurement for arterial stiffness and commercial companies have developed specialised equipment such as the SphygmoCor® (AtCor Medical Pty Ltd, Sydney, Australia) using applanation tonometry, simultaneous ECG measurements and proprietary algorithms to support PWA and measure and calculate PWV.

Some researchers suggest that atherosclerosis should be considered an independent risk factor for ED; however, further research is needed to clarify the association and determine causation. There is currently a paucity of data from prospective longitudinal cohort [397] and

population-based cross-sectional studies [398] that have included measurement of imaging biomarkers of atherosclerosis, arterial stiffness or endothelial dysfunction to determine their contribution to the prediction of ED. However, many non-population based [399], clinical-based [387, 400-402] and case-control studies [329, 403-407] have investigated the association between one or more of these markers and ED. Results are generally consistent, depending upon the technique used.

Increased carotid IMT has been established as a risk factor for ED in men without clinical atherosclerosis but with CVD risk factors (T2DM, obesity, hyperlipidaemia, hypertension) [400]: a high carotid IMT significantly increases the risk of ED (OR=2.6 [1.1-5.9]) and is correlated with ED severity [400, 401]. A 2014 cross-sectional observational study [399] investigated the relationship between carotid IMT and ED in South Korean men (n=799, median age=57 years) and found 62% had atherosclerosis, the median IIEF-5 scores were significantly lower in men with atherosclerosis (15 vs 16 respectively, p=0.006), there was a significant negative correlation between IIEF-5 score and maximum IMT (p<0.001) and ED severity increased with increasing plaque size (p<0.001). In 2016, Lahoz et al [408] conducted a population-based study of Spanish men randomly selected from the general population (n=614, mean age=61 years): 59.7% had ED (IIEF-5 <22), mean carotid IMT was significantly higher in men with ED (0.762 ± 0.151 mm vs 0.718 ± 0.114 mm, p<0.001) and increased significantly with ED severity (p-trend=0.004). Even after adjusting for age, CVD risk factors and treatment, carotid plaques were more common (63.8% vs 44.8%, p<0.001) and this also increased significantly with ED severity (p-trend<0.001).

Impaired brachial FMD is common in men with ED in the absence of clinical cardiometabolic disease [329, 387, 403-405]. Several case-control studies [405, 406] in men without clinical CVD have reported significantly lower endothelium-dependent brachial FMD in response to occlusion, and also endothelium-independent brachial FMD in response to sublingual administration of NTG, in men with ED compared to men without ED. For example, Kaya et al [329] assessed the endothelial function of ED patients with no clinical cardiometabolic disease compared to healthy controls (n=57, 32 cases, 25 controls) and found no significant baseline differences in BMI, HR, BP, FPG or lipid levels; however, the percentage change in brachial FMD was significantly lower after occlusion and in response to 0.4 mg NTG in ED patients (6.0±2.9% vs 12.3±3.5% and 12.8±4.2% vs 17.8±5.2% respectively). Also, in a clinic-based study, Ucar et al [387] investigated the relationship between PDS, carotid ITM and brachial FMD in ED patients (n=56). The PDS showed 29 had vasculogenic ED (17 cases of CVOD and 12 of arterial insufficiency). Carotid IMT was significantly higher in men with vasculogenic ED and brachial

FMD was significantly more impaired in men with ED due to arterial insufficiency ($p<0.05$). Furthermore, a 2013 study [402] in CAD patients showed that impaired brachial FMD was also a significant predictor of ED in men with clinical disease (OR=2.33 [0.59-9.23], p=0.03): patients with ED had lower FMD (6.40±4.60% vs 9.10±4.87%, $p<0.001$) and FMD was negatively correlated with ED severity (r=-0.22, p=0.004). Most recently, in 2015 Gerber et al [398] conducted a cross-sectional population-based study and found that brachial FMD was significantly lower in men with ED (97.1±2.5 vs 106.0±1.6 cm/s, p=0.003) indicating endothelial dysfunction; however, response to NTG administration was similar to that of men without ED (6.6±0.33% vs 7.2 ± 0.24%, p=0.147) suggesting no difference in non-endothelium dependent vasodilation. These studies consistently support a relationship between ED and endothelial dysfunction.

There is limited data available on the association between PWV and ED. In 2013, a case-control study [407] (n=45, 21 cases, 24 controls) investigating the association between ED and asymptomatic vascular dysfunction in middle-aged men found that carotid IMT was significantly higher in men with ED (598.57 vs 535.54 $mm \cdot 10^{-3}$, p=0.03) but found no significant differences in PWV. However, a 2014 longitudinal patient cohort study [397] investigated the ability of PWV, beyond traditional risk factors, to predict MACE in men with ED but without known CVD (n=344, mean age=56 years, mean follow-up=4.7 years). A PWV of 7.81 m/s correctly predicted no MACE in 98% of cases. Men who had MACE had a significantly higher baseline PWV compared to those who did not (9.2±1.5 vs 8.2±10.2, $p<0.001$) and being in the highest PWV tertile (>8.8 m/s) conferred a 4-fold higher risk of MACEs (HR=3.97, p=0.035) compared to the lowest tertile (<7.6 m/s). PWV improved the ability of a conventional risk factor model to correctly classify CVD risk in patients with ED by 27.6% (p=0.033). Measurement of PWV may help determine which ED patients are at higher risk of CVD.

4.2.2.7 Blood biomarkers

Blood biomarkers, such as the lipid profile, are widely used in both clinical and research settings to assess atherosclerotic risk. In addition to dyslipidaemia, elevated markers and mediators of inflammation (e.g., C-reactive protein (CRP), interleukin-6 (IL-6), interleukin-1β (IL-1β), and tumour necrosis factor-α (TNF-α)) and endothelial-prothrombotic compounds (e.g., endothelin-1 (ET-1); intracellular adhesion molecule-1 (iCAM-1); vascular adhesion molecule-1 (vCAM-1)); monocyte chemo-attractant protein-1 (MCP-1); fibrinogen and homocysteine (Hcys)) are indicative of a pro-atherogenic state. Although inflammation is an innate protective immunovascular response to insult, chronic inflammation is implicated in the initiation and progression of atherosclerosis [409]. It is both a response to a vascular insult and

an insult itself: inflammation leads to vascular injury, endothelial dysfunction and an altered prothrombotic state which further promotes inflammation and prepares the way for atherosclerotic lesion formation. In particular, CRP has well-established pro-inflammatory and pro-atherogenic properties [410]. *In vitro* studies have shown CRP inhibits eNO production, reduces its bioavailability and activity, stimulates release of ET-1 and IL-6, upregulates the expression of adhesion molecules, stimulates MCP-1 and monocyte migration, facilitates endothelial cell apoptosis and inhibits endothelial growth factor-stimulated angiogenesis [411, 412]. An elevated CRP level indicates systemic inflammation of the vascular wall and is a significant independent predictor of CV events [413, 414]. It has been shown to be a stronger predictor of CV events than LDL-c [415]. Elevated levels of these markers and mediators may precede both atherosclerosis and vasculogenic ED.

Studies have consistently reported significantly higher inflammatory markers/mediators [406, 416-420] and/or raised endothelial-prothrombotic compounds [416, 417, 421] in men with ED, even in the absence of other CVD risk factors, suggesting they play an important role in the pathogenesis of ED. In 2006, Vlachapoulos et al [417] conducted an observational study of 141 consecutive patients with or without ED and CAD (38 CAD/ED, 25 CAD/no ED, 46 no CAD/no ED, 32 with no CAD/no ED, mean age=58.8 years). Vasculogenic ED was measured using PDS and defined as a peak systolic velocity (PSV) <35 cm/s and/or an end-diastolic velocity (EDV) >5 cm/s. CAD was either documented or determined using coronary angiography and excluded using exercise stress test and stress echocardiography. They investigated the association between ED and inflammation, endothelial dysfunction and an altered prothrombotic state. Overall, ED patients had significantly increased levels of all inflammatory (CRP, IL-6, IL-1β, TNF-α) and endothelial-prothrombotic (von Willebrand factor (vWF), plasminogen activator inhibitor-1 (PAI-1), tissue type plasminogen activator (tPA), fibrinogen) markers/mediators. These substances all showed significant correlations with IIEF-5 scores (r_s=-0.22 to -0.48, all p≤0.01). Interestingly, the effect of ED on these markers (with the exception of IL-6) did not depend on the presence of CAD, supporting earlier studies that inflammation is a key element to ED and may be indicative of subclinical CAD. All of these markers/mediators were also independent predictors of ED in multivariate models adjusting for age, SBP, TC, FPG and BMI (OR 1.1–2.6, all p<0.05); however, the accuracy of inflammatory substances in predicting ED was generally poor (AUC 0.66-0.69, all p<0.01) with slightly better performance from endothelial-prothrombotic substances (AUC 0.63–0.79, all p<0.01). Inflammation may be a marker but not a risk factor for ED; however, further work is needed in this area.

In a 2007 retrospective cross-sectional study of a cohort of health professionals (n=988, age range=46-81 years, 1995-2000), Eaton et al [421] found poor to very poor erectile function was present in 27.5% of the men in 1995 and 39.6% in 2000. At the univariate level, ED was significantly correlated with many atherosclerotic biomarkers (e.g., TG, HDL, TG:HDL-c, TC:HDL-c, CRP, IL-6, TNF receptor 1, iCAM-1, vCAM-1, Factor VII, fibrinogen, all $p<0.05$) but not non-HDL-c, Lp(a) or Hcys (all $p>0.05$). After multivariate adjustment, ED was associated in a graded fashion with elevated levels of some atherosclerotic biomarkers of dyslipidemia (TG: OR=1.8, p=0.007; and TC:HDL-c: OR=2.1, p=0.02), endothelial function (iCAM: OR=2.0, p=0.06) and thrombosis (Factor VII: OR=2.9, p=0.03) but not inflammation. This study supported the need for further prospective cohort studies into the association between ED and endothelial function. Ideally such studies would include longitudinal measurement of risk factors, biochemical markers and imaging biomarkers such as carotid IMT, brachial FMD or PWV measurement.

Over the past 5 years, inflammation and endothelial dysfunction have also been linked to ED presence and severity in T2DM patients without symptomatic CHD [418]. Arana Rosainz Mde et al [418] conducted a cross-sectional study of T2DM patients (n=190, 150 ED, 40 no ED). They reported higher inflammatory cytokines (raised TNF-α, lowered IL-10 and elevated TNF-α:IL-10) and endothelial dysfunction (raised E-selectin) markers in T2DM patients with ED (IIEF-5 score) compared to those without ED. In contrast to earlier studies [416, 421], there was no significant difference in the endothelial activation marker iCAM-1. The severity of ED increased with increasing TNF-α and E-selectin levels and multivariate analysis (adjusted for age, diabetes duration, insulin medication, hypertension, IR, glycaemic control and MetS) showed that TNF-α:IL-10 and E-selectin levels were independent predictors of ED. In a 2013 case-control study (192 ED cases, 33 controls, age range=<40 years), Yao et al [419] found that younger men with ED had significantly higher SBP, HOMA-IR, CRP and IMT and significantly lower FMD values compared with controls. HOMA-IR (AUC 0.759, $p<0.001$) and FMD (AUC 0.933, $p<0.001$) were significant predictors of ED in men <40 years of age with ED of unknown etiology. Overall, the evidence suggests that inflammation, endothelial dysfunction and thrombosis are involved the pathogenesis of ED.

Chronic inflammation is implicated in the pathogenesis of cardiometabolic disease and may be the link between these conditions and ED [422]. It is clear that atherosclerosis, arterial stiffness and endothelial dysfunction are more common and severe in men with vasculogenic ED and that these defects occur even in men with no signs of clinical CVD. ED is now accepted as an early marker of endothelial dysfunction, atherosclerosis and CVD.

4.2.3 Endocrine disorders

The hypothalamic-pituitary-gonadal (HPG) axis is central to male androgen production. In brief, CNS stimulation triggers the secretion of gonadotropin-releasing hormone (GnRH) from the hypothalamus, leading to the release of follicle stimulating hormone (FSH) and luteinising hormone (LH) from the anterior pituitary. LH stimulates testosterone production in the Leydig cells of the testis. Circulating total testosterone (TT) is predominately inactive and tightly bound to sex hormone-binding globulin (SHBG, 60-80%): the remaining fraction is considered bioavailable and either weakly albumin-bound (20-40%) or free testosterone (FT, 2-3%) [423]. Testosterone and its secondary metabolites (dihydrotestosterone (DHT) and estradiol (E_2)) play an important role in many physiological functions including the development of the male reproductive organs and secondary male sexual characteristics. Hypogonadism is a clinical condition defined by low TT in the presence of clinical signs and symptoms of androgen deficiency (e.g., reduced libido, ED, infertility, lethargy, depression, diminished intellectual capacity, osteoporosis, reduced muscle mass and strength) [424, 425]. It can be either inherited or acquired and is classified as primary (due to testicular dysfunction and classified by low TT and raised LH and FSH) and/or secondary (due to hypothalamic or pituitary dysfunction and classified by low TT and low-normal LH and FSH) [425]. Although hypogonadism can occur at any age, testosterone levels decline with advancing age and late-onset hypogonadism is common. This has important effects not only on sexual function, but also on the cardiovascular and metabolic systems, the brain, and musculoskeletal system [426]. In younger men it is necessary to distinguish between primary and secondary hypogonadism [425]; however, with advancing age SHBG levels increase causing a decrease in bioavailable testosterone. An ageing man can present with low-normal TT levels yet show the signs and symptoms of late-onset hypogonadism. Therefore, the measurement of TT, SHBG and albumin becomes important as it allows the calculation of bioavailable testosterone and FT (according to the Vermeulen formula). Repeated measurement is highly recommended in the case of low TT as fluctuations, whether real or false, may lead to misdiagnosis [427]. Testosterone levels should be taken in the early morning (8:00-11:00 AM) [427] as they can decrease by 15-20% over the course of a day [425].

There is no clear consensus on diagnostic cutoff levels. The latest guidelines [424] recommend that TT <8 nmol/L (230 ng/dL) indicates treatment would be beneficial; TT 8-12 nmol/L (230–350 ng/dL) indicates further assessment is needed; and TT >12 nmol/L (350 ng/dL) indicates that treatment is not required. However, studies report using various TT levels to define hypogonadism: earlier studies generally used <10.4 nmol/L (300 ng/dL) [428] while more

recent studies have used <12.1 nmol/L (350 ng/dL) [429, 430]. The most recent study investigating endocrine and metabolic disorders in ED [300] used the following cut-offs: primary hypogonadism TT <10.5 nmol/L (300 ng/dL) and LH >9.4 UL, secondary hypogonadism TT <10.5 nmol/L (300 ng/dL) and LH ≤9.4 mIU/L. There appears to be no accepted threshold for LH, FSH, or bioavailable T (combined albumin-bound and FT); however, an FT level <225 pmol/L (65 pg/ml) is suggested to also indicate testosterone treatment would be beneficial [424]. It is recommended that FT be calculated using the Vermeulen formula and not measured directly as current methods have proven unreliable [427]. For comparative purposes, normal reference values for TT, FT, LH and FSH [431] and recent guidelines [424] are shown in Table 2.12.

Table 2.12. Normal reference values for fasting total testosterone (TT), free testosterone (FT), luteinising hormone (LH) and follicle stimulating hormone (FSH) levels in men (taken from [431]) and guidelines for the definition of hypogonadism (adapted from [424]).

Laboratory test	Normal reference values	Classification of hypogonadism
TT	270-1070 ng/dL	Low <8 nmol/L (230 ng/dL) Borderline 8-12 nmol/L (230–350 ng/dL) Normal >12 nmol/L (350 ng/dL)
FT	50-210 pg/ml	Low <225 pmol/L (65 pg/ml)
LH	1.42-15.4 mIU/L	-
FSH	1.24-7.8 mIUU/L	-

Current evidence suggests a relationship between hypogonadism and ED. Cross-sectional [114, 432] and longitudinal [433-435] studies have shown that TT and/or FT decline with age. Hypogonadism is common in ageing men: the Baltimore Longitudinal Study on Aging [433] (n=890, mean age=53.8 years) revealed 20% prevalence of hypogonadism (TT <11.3 nmol/L (325 ng/dL) in healthy men in their 60s, 30% in their 70s and 50% in their 80s. This mirrors the increase in ED with advancing age: indeed, the majority of patients with hypogonadism are reported to present with decreased libido and/or ED [436]. However, Barqawi et al [114] found that although hypogonadism (TT <10.4 nmol/L (300 ng/dL)) was highly prevalent in the PCAW population (30%) and TT levels were significantly correlated with IIEF-5 scores at the univariate level ($p<0.05$), it did not remain so in multivariate analysis ($p=0.104$). Wu et al [437] conducted a multi-centre European population-based observational study (n=3219, mean age=59.7 years) and found that sexual symptoms (poor quality morning erections, low sexual desire and ED) were significantly related to androgen levels ($p<0.001$). A TT level of 8.0-13.0 nmol/L (230-370 ng/md) and a FT level of 160-280 pmol/L (46-81 pg/ml) significantly increased the probability of these sexual symptoms. Also, a cumulative effect was seen with more sexual symptoms related to a lower androgen level. They suggested that late-onset hypogonadism could be diagnosed by the presence of ≥3 sexual symptoms in addition to TT <11 nmol/L (320 ng/dL)

and a FT level <220 pmol/L (64 pg/ml). Recently, Maseroli et al [300] conducted a comparative observational study, investigating endocrine and metabolic disorders in a large ED patient cohort (n=3847) and a population-based cohort (n=202) from the Florence arm of the EMAS. They found that secondary hypogonadism (TT <10.5 nmol/L (300 ng/dL) and LH ≤9.4 mIU/L) was significantly more common in ED patients than the general population (18.9 vs 8%, p<0.001) even after adjusting for age. This evidence from epidemiological studies supports a simple association but does not imply causation.

In addition to testosterone, there is ongoing debate about the role of hyperprolactinaemia in erectile function. Prolactin, a non-androgenic hormone that acts to inhibit GnRH release and decrease circulating androgen levels, is produced by the pituitary [438]. It has been recommended that prolactin be routinely measured in the presence of low TT [439]. Severe hyperprolactinaemia (>735 mu/L (35 ng/ml)) is associated with reduced sexual desire [440]; however, the role in ED is yet to be elucidated. Interestingly, Maseroli et al [300] found that hypoprolactinaemia (PRL <113 mU/L (5ng/ml)) was significantly more prevalent in ED patients than the general population (28.2% vs 17.8%, p=0.001), even after adjusting for age, BMI, and TT (OR=1.95 [1.32-2.87]). However, there was no difference in the prevalence of either mild (>420 mU/L (20 ng/ml) or severe (>735 mU/L (35 ng/ml) hyperprolactinaemia. Further research is needed to clarify what appears to be a complex relationship between prolactin and ED and to elucidate the mechanism involved.

Although androgens play an established role in sexual desire and behavior, there is debate regarding a direct role in adult erectile function [22, 427]. This appears to be predominately due to conflicting opinions regarding the benefits of universal screening and testosterone replacement therapy (TRT) in men with ED. TRT comes with risks and requires strict monitoring as it is associated with liver dysfunction and may result in prostate hypertrophy. However, results of some RCTs have shown that TRT improves sexual function and other cardiometabolic risk factors [441]. Despite this debate, there is some evidence from animal studies that testosterone may play a role in all stages of the erectile process: from modulation and timing of sexual desire in the CNS, and regulation of peripheral sexual stimulation in penile neurons, to mediation of vasodilation and eNO production and smooth muscle relaxation in trabecular arteries [427, 442, 443]. It appears that testosterone may have a direct role in sexual function; however, further research is needed to elucidate the mechanism in humans. It is likely that the mechanism is similar to cardiometabolic disease: vasculogenic and neurogenic disruption of normal erectile function. Indeed, recent meta-analyses have reported significant associations between androgen deficiency and CV morbidity and mortality (p<0.0001) [444], and T2DM

($p<0.0001$) [445]. It is clear that it is important to assess endocrine health as a potential cause of ED and that ideally this would include measurement of TT, LH, FSH and also SHBG and albumin to calculate FT in ageing men.

4.2.4 Depression, anxiety and stress

Depression is a common mental health disorder characterised by despondency, loss of interest and/or pleasure, reduced perception of self-worth, feelings of guilt or worthlessness, altered appetite and weight change, change in sleep patterns, lethargy, and loss of concentration [446]. It can be short-term or long-lasting and is classified as mild, moderate or severe. It causes an impaired ability to cope and function in daily life and can, in some severe cases, lead to suicide. However, it can be reliably diagnosed and, depending on the severity, effectively treated with either self-help and psychosocial support or pharmacological intervention and psychotherapy [446]. Depression is a serious concern amongst the elderly: European studies [447] show 15-25% prevalence in community dwelling adults >85 years compared to 5% in the general population. It often comes with symptoms of anxiety, which is a psychophysiological signal of stress. Symptoms of anxiety include the following: persistent anxious thoughts; overwhelming fear, panic or unease; persistent self-doubt; sleep problems; sustained muscle tension; chronic digestive problems; shortness of breath; heart palpitations; dry mouth; cold and sweaty, tingling or numb hands and feet; and the inability to be still and calm [448]. Anxiety disorders are extremely common. The World Mental Health Survey [448] reported lifetime prevalence estimates of 31% in the USA and 24.6% in NZ.

Major depressive disorder and depressive episodes are generally clinically diagnosed according to the American Psychiatric Associations Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR or DSM-5) or the WHO International Statistical Classification of Diseases and Related Health Problems (ICD) criteria. However, their use requires extensive clinical training, therefore researchers use alternate methods including direct self-report of diagnosed depression or symptoms of depression. The most common methods are as follows: the Centre for Epidemiologic Studies Depression Scale (CES-D, 20 items), the Beck Depression Inventory (BDI, 21 items)[449, 450], the Hamilton Rating Scale for Depression (HAM-D, 17 items) [451] and the Hospital Anxiety and Depression Scale (HADS, 14 items)[452]. However, the Patient Health Questionnaire depression module (PHQ-9, 9 items) [453] is a brief depression severity measure that has been shown to have high sensitivity (88%) and specificity (88%) for major depression with a score of 5, 10, 15, and 20 representing mild, moderate, moderately severe, and severe depression respectively. A PHQ-9 score ≥10 indicates the presence of depressive symptoms [454]. The ability to make criteria-based diagnoses of depressive disorders, the

reliability and validity, low respondent burden and general availability make the PHQ-9 a useful tool. Studies should consider investigating not only self-reported diagnosed and medicated depression, but also depression symptoms measured using a validated instrument as a potential covariate.

Epidemiological evidence from cross-sectional and prospective cohort studies [6, 24, 129, 130, 136, 161, 162, 175, 177, 455] strongly supports an association between depression and ED. Depressive and submissive personalities are more likely to suffer from ED [43, 456, 457]. Self-reported depression and/or taking medication for depression appear to significantly increase the risk of ED [458], although some studies have found no association [128, 176, 178]. Baseline MMAS results [456] revealed that depressive symptoms (CES-D score ≥16) were present in approximately 12% of participants and this did not vary with age; that quintile of CES-D score was linearly positively associated with ED severity; that the presence of depressive symptoms was predictive of moderate-complete ED (OR=2.03 [1.39-2.96]); and that it remained an independent predictor (OR=1.82 [1.21-2.73]) after adjustment for age and other confounding factors (e.g., demographics, anthropometrics, lifestyle, health status, medication use, and hormones). In contrast, the longitudinal results [457] showed that baseline depressive symptoms were not associated with incident ED (OR=0.56 [0.27-1.16]). Studies are increasingly using the PHQ-9 to assess depressive symptomatology [430, 454, 459]. A 2013 retrospective cross-sectional study [454] (n=186 men, mean age=52.6 years) using the PHQ-9 and the IIEF found that total PHQ-9 score and a score ≥10 were significantly correlated with both psychological and physical domains of sexual function ($p<0.01$). However, epidemiological studies establish neither causation, nor the direction of the association.

The relationship is complex and appears to be bi-directional [460, 461]: it is unclear whether depression causes or worsens ED, ED causes or worsens depression, or the two conditions are mutually reinforcing. This is highlighted by a prospective cohort study [461] in Finnish men (n=1683, age range=60-70 years, baseline 1994, follow-up 1999), which found that after adjusting for confounders, incident ED was significantly associated with untreated (OR=2.6 [1.8-3.8]) and treated (OR=3.3 [1.6-7.1]) depression at baseline. The incidence of ED was higher in men with depressive mood at baseline (59 vs 37 cases/1000 man-years), and the incidence of depressive mood was higher in men with ED at baseline (20 vs 11 cases/1000 man-years). Men with treated depression had over 4-times the incidence of ED compared to men free of depression and not medicated, while men with ED had almost twice the incidence of depressive mood. Furthermore, a 2012 meta-analysis [460] found significant heterogeneity between relevant prospective cohort studies (6 studies on depression and risk of sexual

dysfunction (n=3285, follow-up=2-9 years) and 6 studies on sexual dysfunction and risk of depression (n=11171, follow-up=1-10 years); however, overall, the pooled adjusted data showed a definite bidirectional relationship with depression increasing the risk of sexual dysfunction (RR=1.71 [1.05-2.78]) and sexual dysfunction increasing the risk of depression (RR=3.12 [1.66-5.85]). It appears that ED may be a stronger predictor of depression than depression is of ED, although treatment for depression may further increase the likelihood of ED.

Further research is needed to clarify the relationship between ED and depression, and to elucidate the mechanism involved. However, ED is considered a risk factor for depression and this is supported by multiple studies showing that effective treatment of ED results in improved depressive symptoms [459, 462-466]. The evidence suggests that it is important to routinely question men regarding their mental health to establish the potential for psychogenic ED.

4.2.5 Other medical risk factors

There are several other important medical risk factors for organic ED: LUTS and prostate problems [27, 114, 134, 135, 194, 467-469]; urogenital anatomical disorders [470, 471]; vascular, pelvic or spinal trauma or surgery [472, 473]; and medication and drug use [11, 118, 200, 474]. This highlights the need for self-reporting and/or physical examination for these conditions in both clinical and research settings, and the importance of routinely questioning patients or participants regarding their past and present use of prescription and non-prescription drugs. For further information on these risk factors see Appendix 2.

4.3 ED and lifestyle factors

Epidemiological evidence from both cross-sectional and prospective cohort studies supports a role for several lifestyle factors in the aetiology of ED: smoking, alcohol consumption, PA and obesity [475]. The cross-sectional population-based BACH survey [131] in the USA reported that along with age, comorbidities and SES, modifiable lifestyle factors significantly contributed to the prevalence of ED. Amongst men without comorbidities, after age, lifestyle and SES were the most important contributors to ED. The recent 45 and Up Study [169] in Australia (n=123779, age=≥45 years) found that the crude odds of moderate-complete ED were highest amongst men with PCa (OR=9.24 [8.5-10.05]), diabetes (OR=4.08 [3.83-4.34]) or other diseases (OR=1.96 [1.87-2.06]) but remained 26% higher in healthy men with lifestyle risk factors (currently smoking, BMI >25 kg/m^2, >30 alcoholic drinks/wk, being sedentary) compared to healthy men without risk factors (OR=1.26 [1.20-1.33]). It is evident that modifiable lifestyle

factors are important contributors to the risk of ED, particularly in men without comorbid conditions; however, much of the variation in IIEF-5 scores remains unexplained. The limited available clinical evidence into the efficacy of lifestyle intervention in ameliorating the symptoms of ED supports that smoking cessation, increasing PA and weight reduction can reverse ED and restore normal erectile function [12].

4.3.1 Smoking

Tobacco smoking is a major modifiable risk factor for CVD. Manufactured tobacco contains many toxins, in particular nicotine and its metabolites, which induce pathophysiological changes in the myocardium and endothelial cells leading to atherosclerosis and cardiovascular damage. Smoking is generally assessed by self-report with categorisation into nonsmoker versus smoker and further calculation of intensity, type and duration of smoking including pack-years (packs per day multiplied by number of years smoked). ED is more prevalent among smokers than non-smokers [12, 119, 291, 476]. A dose-response relationship has been shown between ED severity and both increasing number of cigarettes smoked [169, 477] and pack-years of smoking [116, 478], and smoking is widely considered an independent risk factor for ED [22]. However, the results of population-based cross-sectional [1, 119, 131, 162, 166, 169] and prospective cohort studies [12, 116, 189, 291] are inconsistent.

The landmark MMAS [1] found that there was no general effect of cigarette smoking on complete ED at baseline: the prevalence was not significantly different (smokers 11% vs non-smokers 9.3%, $p>0.20$), nor was there an effect of dosage, duration or exposure to passive smoking. However, the effect of other established risk factors were amplified in smokers (e.g., in men with treated CHD the rate of complete ED was higher in smokers than non-smokers (56% vs 21% respectively)). In contrast, the follow-up results [291] showed the crude incident rate of moderate-severe ED was higher in cigarette smokers (current 23% vs never 17%), cigar smokers (yes 29% vs no 17%) and passive smokers (at home and work 30% vs none 17%), although these exposures were overlapping. After adjusting for age and multiple confounders (i.e. medical and lifestyle factors including type of exposure), exposure at baseline doubled the odds of moderate-complete incident ED (cigarette smoking (OR=1.97 [1.07-3.63]), passive exposure at home and work (OR=2.07 [1.04-4.13]) and cigar smoking (OR=2.45 [1.09-5.50])). Contrary to these findings, the Australian FAMAS [168, 189] assessed current smoking as a dichotomous variable and found that there was no significant difference in the baseline prevalence of ED between current smokers and non-smokers [168], and that baseline smoking status was an independent predictor of neither incidence nor remission of ED after 5 years [189]. It is likely that the simple dichotomous nature of the question used to assess smoking

limited the sensitivity to detect a difference in this study as it did not account for former smoking or type of exposure.

Similar to the MMAS, the NHANES [119] reported a higher prevalence of moderate-severe ED amongst current smokers and the age-adjusted odds were 60% higher (OR=1.63 [1.01-2.62]) compared to never smokers; however, this was not significant after multivariate adjustment for sociodemographic, lifestyle and medical factors (OR=1.60 [0.83-3.07]). The BACH survey [478] found a significant age-adjusted association between ED and smoking (p=0.01) with a trend in increased risk with cumulative pack-years (OR=1.68 [1.03-2.30] for ≥20 pack-years). The Australian MATeS [162] also found that after age-adjustment, current (any type in the last week) and former smoking (not currently but ≥100 cigarettes or equivalent tobacco over a lifetime) were both associated with an increased risk of ED (OR=1.3 [1.0-1.6] and OR=1.2 [1.0-1.4] respectively) compared to never smoking. More recently, the 45 and Up Study [169] in Australia found that current smokers were more likely to report moderate-complete ED than past smokers or never smokers (18% vs 11% vs 9% respectively) and after adjustment for age, sociodemographic and lifestyle risk factors, former smoking significantly increased the odds of moderate-complete ED by 26% (OR=1.26 [1.22-1.31]) and current smoking by 55% (OR=1.55 [1.46-1.65]) compared to those who had never smoked. Furthermore, current smokers who smoked ≥20 cigarettes a day had greater odds of moderate-complete ED than men who smoked <20 cigarettes a day (OR=1.86 [1.69-2.06] vs OR=1.48 [1.37-1.59] respectively, p<0.001). Overall, these studies support that smoking, particularly heavy smoking, is an important risk factor for ED.

Multinational studies have shown that smoking is significantly associated with both age and geographical location [130]. The majority of multinational studies have reported significantly higher odds of ED in current smokers (OR=1.22 [1.09-1.36] [27], particularly heavy smokers (OR=1.74 [1.11-2.74]) [129], compared to non-smokers after adjusting for age and country. Interestingly, the GSSAB found significantly higher crude odds for moderate-severe ED with ever smoking compared to never smoking in Korea (OR=3.00 [1.33-6.80])[178], but not in America (OR=1.05 [0.61-1.80])[6], Spain (OR=0.79 [0.37-1.68])[175], Germany (OR=1.02 [0.49-2.12])[176] or Brazil (OR=1.37 [0.65-2.88])[177]. Unfortunately, smoking data was not reported in the other GSSAB countries [180, 181] including NZ [8] and Australia [179]. Similarly, in the recent GOSS [24, 136], there was no significant difference in the crude odds of ED in smokers compared to nonsmokers in American (OR=0.74 [0.50-1.10]) or Middle Eastern (OR=1.05 [0.69-1.59]) Internet users. It is evident that some studies present crude odds, others age-adjusted or adjusted for a range of different confounders and covariates. In studies that have adjusted

for comorbidities, smoking was not a significant independent predictor of ED [130]. This is unsurprising due to the strong association between smoking and cardiometabolic disease. In fact, it appears that lifestyle factors, including smoking, may be more important risk factors in younger men and may have little effect on the risk of ED in men over 75 years of age [169], for whom comorbidities and medications may be of greater importance.

Strong support comes from recent meta-analyses and intervention studies. In 2013, Cao et al [476] found that in pooled results from 4 prospective cohort studies and 4 case-control studies (n=28,586), the risk of ED was higher amongst current smokers (OR=1.51 [1.34-1.72]) and former smokers (OR=1.29 [1.07-1.47]) compared to non-smokers. In 2014, Cao et al [477] also reported that in pooled results of one prospective cohort and 9 cross-sectional studies (n=50,360) reporting on the quantity and duration of smoking, there was no evidence of a linear association but the odds of ED increased 14% with every 10 cigarettes smoked per day (OR=1.14 [1.09-1.18]) and 15% with every 10 year increment of smoking duration (OR=1.15 [1.10-1.19]). These studies further support that smoking, in particular current smoking, significantly increases the risk of ED and that a positive dose-response relationship exists between quantity and duration of smoking and the risk of ED. One prospective study [150] investigated the benefit of smoking cessation on ED severity (n=281 smokers with ED requesting nicotine replacement therapy (NRT) without concomitant risk factors, age range=30-60 years, follow-up=1 year) and found IIEF-5 scores at baseline were significantly correlated with smoking intensity (pack-years: r_s=0.533, p>0.05). After 1 year, IIEF-5 scores significantly improved (≥1 grade) in ex-smokers compared to current smokers (25% vs 0%, p=0.009) and the degree of improvement was related to age and severity of ED. Furthermore, one RCT [151] investigated the effect of smoking cessation on ED (n=719 smokers (≥1 cigarette/d) with ED who intended to quit, mean age=49 years). They found that receiving smoking cessation counselling and free NRT for 2 weeks resulted in higher abstinence compared to receiving basic advice on quitting. Quitting smoking was a significant predictor of improved ED symptoms (RR=2.07 [1.61, 2.67]).

Clearly smoking is a risk factor for ED and there is some evidence of causation [151]. However, the way it is measured appears to be important and future studies should include not only smoking status, but also the dosage and duration of smoking and consider assessing passive smoking. Also, its significance as a risk factor is affected by confounding factors such as age, country, SES, diseases and other lifestyle factors. This highlights the importance of establishing risk factors in different countries and adequately measuring all possible confounders and covariates. The current evidence is sufficiently robust to support advising men with ED to quit

smoking.

4.3.2 Alcohol consumption

While chronic alcohol consumption and recurrent binge drinking may have negative health consequences, regular light-moderate consumption has been shown to have cardio-protective effects [479, 480]. Alcohol consumption is generally assessed by self-report including categorisation into drinker versus non-drinker and calculation of alcohol intake (quantity or number of standard drinks per week). ED appears to be more prevalent among heavy drinkers than non-drinkers [1, 169] and a dose-response relationship has been shown between ED severity and increasing number of drinks consumed [1, 131, 168]. Alcohol is assumed to have a negative effect on erectile function and sexual performance; however, moderate consumption may be protective [27, 129]. While some studies have suggested a protective effect, the results of population-based cross-sectional [1, 3, 131, 162, 481] and prospective cohort studies [168, 291] are inconsistent. Additionally, some key studies investigating the prevalence of ED and its associated risk factors omitted to assess alcohol intake [6, 8, 119, 161, 175-181, 184] or did not adequately report their findings [27]. None have investigated the type of alcohol consumed.

The baseline MMAS [1] first reported that excessive alcohol consumption (>600ml/wk) increased the prevalence of minimal impotence from 17% to 29%. Both the quantity consumed and the blood alcohol level (daily consumption normalised for body mass) were associated with mild ED. However, the longitudinal results [291] showed that after adjusting for sociodemographic, lifestyle and medical factors, neither moderate nor heavy alcohol consumption were significant predictors of moderate-severe incident ED compared to <1 drink per day (1-3 drinks/d OR=0.95 [0.54-1.67]; ≥4 drinks/d OR=0.87 [0.41-1.86]). The NHSLS [3] also found that daily alcohol consumption was not a significant independent predictor of ED. In contrast, the BACH survey [131] found that alcohol consumption (categorised as 0, <1, 1-2.9, or ≥3 alcoholic drinks/d) was significantly associated with IIEF-5 scores ($p<0.01$): low-moderate alcohol consumers had better erectile function than abstainers but heavy drinkers had poorer erectile function. However, alcohol consumption was significantly associated with age, comorbidities and SES ($p<0.001$). After adjusting for all covariates, alcohol consumption accounted for 0.3% of the variation in IIEF-5 scores, equal to smoking and PA. Similarly, the Australian WAMHS [481] found that alcohol consumption was associated with age, smoking and CVD. After adjusting for these factors, there remained a modest but statistically insignificant negative association between current alcohol consumption and ED. In contrast, the MATeS [162] assessed alcohol consumption over the past week and categorised men as abstainers, low-risk drinkers (≤6 standard drinks on any one day on ≤3 days a week), risky

drinkers (7-10 standard drinks on any one day), or high-risk drinkers (≥11 standards drinks on any one day). Compared to abstaining, low risk drinking was significantly protective against ED (OR=0.7 [0.6-0.9]) but high risk drinking was not a significant predictor of increased risk (OR=1.1 [0.8-1.6]). Similar to the earlier MMAS results [291], the prospective FAMAS [168] showed that baseline IIEF score was significantly associated with alcohol consumption (p=0.025): moderate alcohol consumption (≤2 standard drinks/d) was independently associated with an increased risk of mild, but not moderate-severe ED (ORs not provided). However, in contrast to the MMAS results [291], the follow-up study [189] found that after adjusting for age, sociodemographic, lifestyle and medical factors, moderate consumption was independently associated with a significantly lower risk of incident ED but not ED remission (ORs not provided). This protective effect of low-moderate alcohol consumption was further supported in the 45 and Up Study [169] which found that in men without PCa, after adjusting for age, sociodemographic and lifestyle risk factors, abstaining significantly increased the risk of ED (OR=1.17 [1.11-1.22]) compared to 1-5 alcoholic drinks/wk, while 6-10 drinks/wk significantly lowered the risk of ED (OR=0.94 [0.90-0.98]), 11-30 drinks/wk had no significant association with ED while >30 drinks/wk was associated with significant increased likelihood of moderate-severe ED (OR=1.28 [1.19-1.385]). The protective effect of moderate consumption was apparent amongst men aged 45-54 years and the detrimental effect of heavy consumption amongst men over 55 years of age.

Alcohol consumption is associated with age and country [130]. The results of multinational studies do not support a significant association between alcohol and ED. In the Cross-National study [129], after age- and country-adjustment there was no significant difference in the likelihood of moderate-severe ED associated with the number of drinks consumed (1-7/wk OR=0.7 [0.59-1.03]; ≥8/wk OR=0.81 [0.60-1.05]) compared to abstaining. In the EMAS [130], after adjusting for age, centre and self-reported health, frequency of alcohol intake (≥1 d/wk) was not a significant risk factor for ED (ORs not provided). Similarly, the recent the GOSS [136] found no significant association between alcohol consumption and ED in American Internet users.

In contrast, meta-analyses of 11 population-based cross-sectional and 2 cohort studies (1990-2006) [152] showed a significant pooled protective effect from cross-sectional studies of regular alcohol consumption (OR=0.79 [0.67-0.92]) – particularly moderate alcohol consumption of ≥8 drinks/wk (OR=0.85 [0.73-0.99]) as opposed to low alcohol consumption (OR=0.73 [0.44-1.20]). However, the two cohort studies (the MMAS [291] and the HPFS [12]) analysed did not show any significant findings. When only age-adjusted ORs were used, the

sensitivity to detect any significant effect was lost. This highlights the importance of future studies measuring and adjusting for all possible confounders when assessing risk factors for ED. There have been no RCTs investigating the effect of altering alcohol consumption on erectile function. It is evident that further research is needed in this area to clarify the relationship between ED and alcohol consumption and that the evidence is not yet sufficient to warrant a potentially controversial message that men with ED may benefit from the consumption of moderate amounts of alcohol.

4.3.3 Physical activity

Physical inactivity is well established as an independent and modifiable risk factor in morbidity and mortality [482]. Being physically active is associated with a 33-35% risk reduction for cardiovascular and all-cause mortality, even after adjusting for other known risk factors [482]. It is included in position statements for the prevention and management of both diabetes [483] and CVD [484]. Physical activity is any physical movement that results in energy expenditure (EE) beyond resting levels, while fitness is an objective term used to describe an individual's ability to be physically active encompassing cardiorespiratory fitness, muscle strength, body composition and flexibility. Both the dose (the total amount of EE in kJ) and the intensity (the absolute rate of EE in metabolic equivalents (METs - where 1 MET equals a resting metabolic rate of approximately 3.5 ml $O_2.kg^{-1}.min^{-1}$) or the percent aerobic power used relative to the maximal heart rate (HR) or maximal oxygen consumption (V O_2max)) are important. The intensity of PA is generally classified as low (4 METS or 40% of V O_2max), moderate (4-6 METS or 40-60% of V O_2max) or vigorous (6 METs or 60% of V O_2max). It can be estimated using either questionnaires to subjectively assess self-reported dose and intensity, or fitness testing to objectively assess exercise capacity. Irrespective of the method used, the strong association between PA and both diabetes and CVD would suggest that physical inactivity would also be a risk factor for ED. Indeed, several cross-sectional [119, 129, 131, 162, 164, 166, 168, 169, 175, 178, 179] and prospective cohort [12, 164, 189, 302] studies have suggested a protective effect of PA [129, 166] and this is supported by two intervention studies that show that increased PA improves erectile function [143, 149].

In prospective studies, the MMAS [291, 302] first assessed PA using a recall of the type, frequency and duration in the past 7 days and calculated total weekly EE by multiplying hours of moderate, vigorous and heavy PA by 17, 25 and 47 kcal/kg/h respectively. Amongst men free of cardiometabolic disease and ED at baseline, after adjusting for sociodemographic, medical and lifestyle factors, PA (measured as (≥200 kcal/d vs <200 kcal/d of moderate-intense PA) was not an independent predictor of incident ED (OR=0.71 [0.42-1.22]) [291]. However,

when men with a history of PCa were excluded [302], a graded inverse association was found between the lowest quartiles of PA with a linear trend observed (p=0.07): men in the 2nd, 3rd and 4th quartile had a 20-50% lower probability of ED (OR=0.8 [0.4-1.4], OR=0.5 [0.3-0.9] and OR=0.6 [0.3-1.2] respectively) compared to men in the lowest quartile. The probability of ED was highest in men who remained sedentary or who became sedentary, and lowest in men who remained active or became active over the 8-year follow-up period, highlighting the importance of maintaining PA levels. The HPFS [12, 164] supported these findings amongst health professionals. After adjusting for medical and lifestyle factors, baseline PA (measured as >32.6 vs <2.7 MET h/wk) was associated with a 30% lower risk of ED (RR=0.7 [0.6-0.7]) and after adjusting for sociodemographic and lifestyle factors, higher intensities of PA (2.7-7.6, 7.7-16.5, 16.6-32.6, >32.6 MET h/wk) were associated with increasingly lower probabilities of incident ED (RR=0.9 [0.8-1.0], RR=0.9 [0.8-1.0], RR=0.8 [0.7-0.9] and RR=0.7 [0.7-0.8] respectively) [12]. In contrast to these earlier studies, the FAMAS [168] found a significant association between leisure time PA and ED at baseline: insufficient PA (<150 min over a typical fortnight) was significantly less common in men with normal function and more common in men with ED (normal EF 34.8% vs 36.4%; mild ED 47.1% vs 38.6%; moderate-severe ED 21.8% vs 18% respectively, p=0.007) compared to sufficient PA (>150 min over a typical fortnight). However, after adjusting for sociodemographic, lifestyle and medical factors, insufficient PA significantly increased the likelihood of mild but not moderate-severe ED (ORs not provided). In the follow-up study, baseline low PA was significantly more common both among men with incident ED [189] (57.5% vs 50%, p≤0.05) but those with ED remission (48.2% vs 35.8%, p≤0.05). After adjusting for age, sociodemographic, lifestyle and medical factors, insufficient PA was a significant independent predictor of neither incidence nor remission. The relative simplicity of the method used to assess PA would limit its ability to detect a significant difference compared to more sensitive and complex methods used in the MMAS [291, 302] and HPFS [12, 164].

Cross-sectional studies have also reported a protective effect of vigorous exercise [119, 131, 166, 169] and a detrimental effect of a sedentary lifestyle [119, 162] on erectile function. The NHANES [119] defined PA as participation in moderate or vigorous activity in the past 30 days and included assessment of sedentary behaviours (TV watching, computer and video use in h/d), muscle strengthening activities, walking and cycling, and self-comparison to others of the same age. After age-adjustment, ED was more prevalent in men who had not been physical active in the past 30 days compared to those moderate or vigorous PA (23 vs 17 vs 13% respectively). The age-adjusted odds for ED were significantly higher amongst inactive men

compared to those who were vigorously active (OR=1.94 [1.32-2.83]), although this became insignificant after further adjustment for sociodemographic, lifestyle and medical factors (OR=1.51 [0.95-2.38]). Increasing hours of sedentary behaviours were also associated with an increased prevalence of ED (1-2 h OR=2.61 [1.56-4.37]; 3-4 h OR=2.62 [1.22-5.59]; ≥5 h OR=3.52 [1.92-6.44]) and these associations were all slightly smaller but remained significant after multi-adjustment - in particular, ≥5 hours of TV, video or computer use per day tripled the risk of ED compared to <1 hour (OR=2.94 [1.56-5.44]). The absence of muscle-strengthening activities in the past 30 days and negative self-comparison to the PA level of others of the same age were both significantly associated with ED in age-adjusted and multi-adjusted models respectively. This study highlights the importance of the method used to assess PA. Sedentary behaviours may be more important than PA in predicting the risk of ED. The BACH survey [131] measured PA using the Physical Activity Scale for the Elderly (PASE) and categorised it as low, medium and high. After adjustment for age, PASE was significantly associated with IIEF-5 scores ($p<0.01$): the more PA the less severe the ED symptoms. In the multi-adjusted model, a trend was observed ($p=0.07$) but when men with comorbid conditions were excluded, there was no significant association between PA and IIEF-5 scores ($p=0.27$). This is not surprising given that men with comorbidities are both less likely to be physically active and more likely to have ED. In the cross-sectional MATeS [162], PA was measured as self-reported intensity, frequency and type in past week and subsequently categorised into sedentary (no participation in PA), insufficient (some PA but not enough to meet sufficient criteria) and sufficient activity (≥5 separate sessions of vigorous PA). A sedentary lifestyle was significantly associated with an age-adjusted increased likelihood of ED (OR=1.5 [1.2-1.8]) but insufficient PA was not (OR=1.1 [0.9-1.3]) compared to sufficient PA. After adjusting for all lifestyle, medical and medication factors, being sedentary remained a significant independent predictor of ED. Further supporting the relationship between PA and ED, in the 45 and Up Study Weber et al [169] found that an increasing number of PA sessions in the past week was associated with a decreasing odds of moderate-severe ED (1-5 session OR=0.77 [0.72-0.83]; 5-7 session OR=0.61 [0.57-0.66]; 11-15 session OR=0.52 [0.48-0.55]; 16-20 sessions OR=0.47 [0.43-0.50]; >20 sessions OR=0.47 [0.43-0.50]) and this was consistent across all age strata.

The results of multinational studies are unclear regarding any potential protective effect of PA and many did not assess PA as a potential risk factor [24, 27, 130, 136, 161]. The Pfizer Cross-National study [129] described PA as “less than average”, “average” or “more than average” and found that after adjusting for age and country, men who were more physically active than average had a 45% lower likelihood of moderate-severe ED (OR=0.55 [0.40-0.75]). This

remained significant after adjusting for sociodemographic, medical and lifestyle factors (OR=0.64 [0.45-0.92]). Contrary to this finding, the GSSAB used the same classification for PA and found no significant association between below average PA and erectile difficulties amongst sexually active men in the USA [6] (OR=1.21 [0.64-2.30]), Germany [176] (OR=1.44 [0.73-2.86]) or Brazil [177] (OR=0.9 [0.37-2.23]); however, it was associated with double the likelihood of ED in Australia [179] (OR=2.5 [1.26-4.97]), Spain [175] (OR=2.46 [1.14-5.31]) and Korea [178] (OR=1.98 [1.02-3.84]) compared to average and above PA. The results for many countries [180, 181], including NZ [8], were not reported. As only crude odds have been presented it would be interesting to see the results of more complex analysis. However, the results of these studies illustrate that country specific assessment is necessary to determine whether PA is protective against ED. Furthermore, it is important that future studies not only present crude and age-adjusted odds, but also adjust for other potential confounders such as sociodemographical, medical and lifestyle factors.

Evidence from meta-analyses and intervention studies strengthens the support for PA as protective against ED. A 2007 meta-analysis [485] of 7 cross-sectional studies reporting adjusted ORs for PA indicated significant heterogeneity ($p<0.000$); however, pooled analysis provided support for the protective effect of PA. In a 2-category response model, there was a 47% lower likelihood of ED in men with 'above average' PA compared to 'average' PA (OR=0.53 [0.31-0.91]). In a 3-category response model a dose response relationship was observed with 'moderate PA' and 'high PA' associated with increasingly lower risks of ED (OR=0.63 [0.43-0.93]; and adjusted OR=0.42 [0.22-0.82] respectively) compared to 'low PA'. Furthermore, there are two intervention studies that provide strong support for the protective effects of PA. Esposito et al [143] conducted a single-blind RCT in 110 obese men (BMI ≥30 kg/m^2) with ED (IIEF score ≤21) but not actively seeking treatment, without diabetes, hypertension or hyperlipidaemia (mean BMI=36 kg/m^2, mean IIEF score=13.7, age range=35-55 years). The intervention group (n=55) received comprehensive advice on caloric restriction and increased PA to achieve a ≥10% loss of total body weight. The control group (n=55) received basic educational material about healthy eating and exercise. After 2 years, the intervention group lost more weight (-15 vs -2 kg), had a greater increase in PA (+195 vs +84 min/wk) and a greater decrease in BMI (-5.7 vs -0.7 kg/m^2) compared to the control group. Most importantly, they had a significant improvement in mean IIEF score (13.9 to 17.0, $p<0.001$ vs 13.5 to 13.6, $p=0.89$) and more men reported normal erectile function (IIEF score ≥22) (17 vs 3 respectively) than in the control group. Lamina et al [149] also conducted an RCT (n=50, 50-70 years) in men with diagnosed ED and chronic stable hypertension (>1 year duration of SBP 140-180 and DBP

90-109 mmHg). Men who were underweight or obese, smokers, alcoholics, with a range of diseases, or involved in vigorous PA or had above average fitness for their age (VO_2max >33 ml/kg/min for men over 50 years and >27 ml/kg/min for men over 60 years of age) were excluded. After a 1-week wash-out period of placebo antihypertensive medications, blood samples were taken and a submaximal cycle ergometer stress test performed to assess aerobic power. The intervention group (n=22, mean age=62.1 years) then received an 8-wk training program of 45-60 min/d at 60–79% of their HR maximum reserve (HR_{max}) three times per week, while the control group remained sedentary (n=21, mean age=64 years). All subjects were placed on methyldopa, an antihypertensive treatment that does not affect normal haemodynamic response to exercise. After 8 weeks there was another 1-wk washout period followed by blood sampling and a submaximal fitness test. The intervention group had a significant reduction in both SBP and DBP, and a significant increase in VO_2max (all $p \leq 0.001$) compared to the control group. Most importantly, IIEF values were significantly improved in the intervention group compared to the control group (+3.64 vs +0.85 respectively, p=0.000). Although further large-scale prospective studies and RCT are needed to establish causality, current evidence supports a positive effect of increased PA on erectile function. Combined with the well-established health benefits of PA, the evidence is sufficient to warrant advising men with ED to increase PA levels to help improve their symptoms.

4.3.4 Obesity

Obesity can be considered to be either a medical factor or a lifestyle factor. In this thesis, obesity was discussed as a medical and metabolic risk factor for ED in Section 4.2.1.4.

4.3.5 Diet

There are various dietary assessment methods used to assess food consumption, allowing researchers to describe the intake within a population and individual intakes for group-level analysis. The most commonly used self-reporting methods in epidemiological research are as follows: the multiple-pass 24-hour Dietary Recall (24-h recall), the weighed or estimated Food Record (FR) and the Food Frequency Questionnaire (FFQ). These methods can be used to calculate average intakes of specific foods or nutrients, or to analyse dietary patterns (for further information see Appendix 2). Nutrient biomarkers can be used both to provide an accurate measurement of specific nutrient levels and to validate these dietary assessment methods.

Epidemiological evidence suggests a role for diet in the pathogenesis of ED [291]; however, very few cross-sectional studies [119] and prospective cohort studies [12, 291] have included dietary analysis in their assessment of risk factors. Others have assessed diet but are yet to report the results [12, 119, 164]. Furthermore, there is very limited data available from RCTs to support the effect of dietary change on the symptoms of ED.

4.3.5.1 Macronutrient intakes and food groups

The MMAS [291] first assessed nutritional intakes using the Willett semi-quantitative 1-year FFQ, calculating saturated and unsaturated fat intake as a percentage of total energy intake, and dietary cholesterol (mg/1000kcal/d) and dietary fibre (g/1000kcal/d) intake. In multivariate analysis, there was no relationship between saturated fat intake per 1% of energy (OR=0.99 [0.88-1.11]), dietary fibre per SD increase (OR=1.11 [0.85-1.45]) or dietary cholesterol per SD increase (OR=1.27 [0.99-1.63]) and incident ED; however, unsaturated fat intake per 1% of energy was associated with a significantly lower likelihood of moderate-severe incident ED (OR=0.92 [0.85-1.00]). This suggests that unsaturated fat may be protective against ED. The prospective FAMAS [168, 189] is the only other prospective study to assess dietary intake of macronutrients using a semi-quantitative FFQ but these results have not yet been reported.

Two small observational studies have suggested a protective role of fruits and vegetables. A study in diabetic Iranian men [486] (n=312 men with T1DM or T2DM, age range=20-83 years) assessed fruit intake (daily vs weekly/seldom) and found a significant association between fruit intake and ED: after adjusting for age, low fruit intake was a significant predictor of an increased risk of ED (OR=2.6 [1.2-5.3]) and this remained significant after adjusting for other factors (OR=3.2 [1.4-7.9]). More recently, results of a 2013 study in diabetic Canadian men [487] (n=1466 men with T1DM or T2DM, mean age=65.1 years) supported an independent association between consumption of fruit and vegetables and ED: after adjusting for sociodemographic, lifestyle and medical factors every additional serving of fruit and vegetables per day was found to decrease the risk of ED by 10% (OR=0.9 [0.82-0.98]). Fruit and vegetable consumption may be protective against ED. None of the key multinational studies have assessed diet as a predictor of ED [6, 8, 24, 27, 129, 130, 136, 161, 175-181]. Large-scale cross-sectional multinational studies are needed to investigate both specific food and nutrient intakes and dietary patterns as predictors of ED. This would provide important data upon which to base future research in this field.

4.3.5.2 Dietary patterns

Dietary pattern analysis [488] is increasingly used in nutritional epidemiological research to examine the relationship between overall diet and disease risk. Either an "a posteriori" (factor or cluster analysis) or an "a priori" (dietary indices) approach can be used with a diet score generated as a measure of dietary quality, by summing up foods considered to be important for a specific disease [488]. For example, cross-sectional studies [489, 490] have shown that a "Western" dietary pattern (high in red and processed meats, high-fat dairy products and refined grains) is positively correlated with biomarkers of cardiometabolic risk (i.e. insulin, c-peptide, leptin, Hcys) [490], CRP, IL-6, iCAM, vCAM, E-selectin, P-selectin [490]) while the "prudent" dietary pattern (higher in fruit, vegetables, legumes, whole grains, fish and poultry) is negatively correlated with insulin, Hcys [489], CRP and E-selectin [490]. As inflammation plays a role in the pathogenesis of ED, a dietary pattern that reduces inflammation may be protective against ED. Indeed, there is accumulating evidence from a group of authors in Italy who suggest that the Mediterranean dietary pattern (high in vegetables, legumes, fruit and nuts, cereals, fish and olive oil and low in meat and dairy products [491]) may be beneficial to erectile function.

Esposito et al [154] conducted a case-control study in 200 men (100 ED cases, mean age=54.1 years, mean IIEF-5=15.4; 100 age-matched controls, mean age=53.1 years, mean IIEF=23.6). They used an FFQ with 140 food items listed in 14 all-inclusive food groups or nutrients. Portion sizes were assessed using household units and portion aid photographs and used to calculate intake in grams per day. Men with ED (IIEF-5 <21) were significantly less likely to adhere to a Mediterranean diet, had lower intakes of vegetables and fruits and nuts and a lower ratio of MUFA to SFA (all $p<0.05$), and were observed to have lower legume and fish intakes (both $p=0.08$). After adjusting for hypertension, hypercholesterolemia, BMI, WC, PA and total energy intake, the intake of fruits and nuts ($p=0.02$) and the ratio of MUFA to SFA ($p=0.04$) remained associated with ED. This study highlighted the importance of dietary factors in the development of ED, supported the earlier suggestion of a protective effect of unsaturated fat [291] and fruit intake [486, 487] and reinforced the need for intervention studies. The same group had earlier shown that a Mediterranean-style diet was effective in reducing weight and inflammatory markers and improving endothelial function in patients with MetS [492]. They subsequently conducted a dietary intervention study [155] in 65 men with both MetS (defined by ATP III criteria) and ED (IIEF-5 ≤21) who did not have signs of CVD, psychiatric issues, alcohol abuse, smoking or any medication. After 2 years, men on the Mediterranean diet (n=35, mean age=44.3 years, mean IIEF score=14.4) had significantly

decreased FPG, insulin, LDL-c, TG, SBP and CRP (all $p<0.05$) and increased HDL-c ($p<0.05$) and improved endothelial function ($p<0.01$) compared to the control group maintaining their usual diet (n=30, mean age=43.5 years, mean IIEF score=14.9). Most importantly, their IIEF-5 scores significantly improved compared to control group (14.4 to 18.1 vs 14.9 to 15.2 respectively, p=0.01) and 13 subjects achieved remission (IIEF-5 >22) compared to two in the control group (p=0.015). A dietary pattern that is high in vegetables, fruits, nuts, whole grains and fish and low in refined grains, red meat and processed meat may be protective against ED [493].

4.3.5.3 Caffeine intake

Caffeine consumption may be protective against ED; however, there is a paucity of research in this area. Very few epidemiological studies have investigated caffeine intake as a risk factor for ED and no intervention studies have been conducted. An early population-based survey [494] of elderly men (≥60 years) in Michigan, USA reported that consuming at least one cup of coffee per day was associated with significantly better erectile function. However, a 2003 cross-sectional survey [495] (n=1814, age range=35-70 years) of primary care patients in Pakistan, Egypt and Nigeria found that overall, consuming caffeinated beverages was independently associated with an almost 2-fold increased age-adjusted risk of ED (OR=1.91 [1.35–2.72]). Moreover, the longitudinal Tampere study [187] in Finland (n=1442, aged 50, 60, or 70 years, follow-up=5 years, 1994-1999) found that among the 1130 men free of ED at baseline, there was no association between the amount of coffee consumed and the age-adjusted incidence of ED (2-4 cups/d OR=0.9 [0.6-1.4]; ≥5 cups/d OR=1.1 [0.7-1.6]).

Most recently, analysis of the 2001/2002 and 2003/2004 NHANES [153] results from men (n=3724, mean age=49 years) with complete data for ED (assessed using the global single-item question) and diet (assessed using the first of two multiple-pass 24-h recalls) assessed caffeine using both quintiles of mg/d (1^{st} quintile (0–7), 2^{nd} quintile (8–84), 3^{rd} quintile (85–170), 4^{th} quintile (171–303), and 5^{th} quintile (304–700)) and daily intake (yes/no) of specific caffeinated beverages (coffee, tea, total soda (regular and low-calorie), and energy and sports drinks). The mean caffeine intake was 188.3 mg/d and daily intake of coffee, tea, total soda, and energy and sports drinks was prevalent in 55.4%, 20.6%, 58.5% and 3.1% of men respectively. Results suggested that after adjusting for age, education, race/ethnicity, obesity, PA, smoking, total water intake, total energy and alcohol intake, moderate caffeine intake in the 3^{rd} and 4^{th} quintiles (85-303 mg/d) was associated with a lower risk of moderate-severe ED (OR=0.58 [0.37-0.89] and OR=0.61 [0.38-0.97] respectively) compared to intakes in the 1^{st} quintile (0-7 mg/d). This study was consistent with the early cross-sectional study by Diokno et al [494] but contrasts with both the null finding of the prospective study by Shiri et al [187] and the finding

of a positive association in the cross-sectional study by Shaeer et al [495]. This latest study suggests that consumption of 2-3 cups of coffee per day (170-375 mg/d) may be protective against ED. Further research is needed in this area including large-scale cross-sectional population-based studies and prospective cohort studies to determine whether caffeine is an independent predictor of ED, and intervention trials to determine whether altering caffeine consumption affects ED symptoms.

5.1 ED AS A MARKER OF CVD

CVD is the major cause of mortality worldwide. In 2008, 13.5 million deaths (23.6%) occurred as a result of IHD, stroke or other cerebrovascular disease [496]. The global burden of non-communicable diseases like CVD threatens not only human health but also development and economic growth. It is suggested that the cost of CVD is likely to increase 22% by 2030 [497, 498]. Many CVD-related deaths are suspected to be both premature and preventable as early detection and effective intervention are both possible with modern medicine. The marked increase in CVD-associated social and economic costs over coming decades may be more effectively met if emphasis is placed on prevention and identifying the early stages of the disease whilst the progression can be slowed, halted or preferably reversed**.** Improvement of risk prediction for CVD is vital to identify high-risk individuals still in the early stages of disease, who could thus most benefit from targeted intervention [499].

Penile erections are largely vascular events [500], and it is not coincidental that CVD shows similar age-related patterns and shares the same risk factors as ED: ED acts as an early warning or sentinel marker of CVD in many men [501, 502]. Therefore, individuals with ED could be targeted for early intervention aimed at reducing CVD risk. The use of ED as a novel, sensitive and specific marker for CVD may add to the current suite of established clinical risk factors and improve risk prediction and monitoring of disease progression or response to intervention. It therefore meets the requirements of a biomarker: to be accurate, reproducible, acceptable and widely accessible [503].

Extensive evidence exists supporting ED as an early marker of CVD. The relationship appears to be (1) temporal (ED precedes the onset of CVD); (2) robust (independent of and comparable to conventional CVD risk factors) and consistent (across different studies and population groups) and; (3) to exhibit a clear dose-response (the risk of CVD increases with increasing severity of ED symptoms) [328]. Furthermore, there are several plausible biological mechanisms to explain the link between ED and CVD. Vasculogenic ED is now established as a risk factor for the presence of silent CVD [504] and 80% of asymptomatic men with ED (presumed vascular) have been found to have multiple cardiovascular risk factors [505].

5.2 Temporal

The relationship between ED and CVD has been found to be temporal, with ED symptoms often manifesting 2-3 years before CAD symptoms [506-508] and 3-5 years before the incidence of a coronary event [509]. This highlights the importance of clinical investigation into CVD risk in patients presenting with ED. Strong support exists for the establishment of ED as a standard component of clinical CVD risk assessment. The Second Princeton Consensus on sexual dysfunction and cardiac risk recognised this stating that asymptomatic men presenting with ED should be treated as cardiac patients until proven otherwise, and that "men with ED and other cardiovascular risk factors (e.g., smoking, obesity, sedentary lifestyle) should be counselled in lifestyle modification" [10]. Identifying ED as an early sign of endothelial dysfunction and atheroma offers early identification of men at risk of adverse cardiovascular outcomes, supporting timely and effective aggressive intervention to reduce vascular risk [506]. This is especially important given the seven-fold increase in risk of a cardiovascular event in men with ED under 40 years of age with no cardiac history [510]. Approximately 50% of sudden cardiac deaths occur in patients with asymptomatic IHD or silent CVD [511].

5.3 Robust and consistent

The relationship between ED and CVD is robust and consistent. Strong epidemiological research, observational and clinical studies, support ED as an important marker of generalised silent vascular dysfunction and a strong predictor of later CV morbidity [197, 313, 379, 406, 508, 510, 512-520]. The MMAS first confirmed in a large population-based random sample that ED is both highly prevalent and highly correlated with CVD [1]. Hypertensive patients had a 15% probability of complete ED, 20% if they also smoked cigarettes, whilst cardiac disease patients had a 39% probability of complete ED, 56% if they also smoked cigarettes. Atherosclerosis is associated with almost 40% of cases of ED in men over 50 years of age [521]. In a study by Billups and Friedrich [522], 60% of healthy men with ED exhibited abnormal lipid profiles and of these 90% showed evidence of penile arterial disease [522]. The prevalence of ED in men with diabetes is approximately 50% (depending on age and severity) while in men with CVD it ranges from 39-64% (depending on the type and severity) [523]. A 2011 meta-analysis of the prospective cohort studies (12 prospective studies, mean follow-up 4–16.2 years, n=36,744) examining the association between ED and CVD identified a significant and independent association between ED and increased risk of CVD (48% increased risk), CHD (46% increased risk), stroke (35% increased risk) and all-cause mortality (19% increased risk) [328]. ED is a strong predictor of both all-cause mortality and cardiovascular outcomes including MI, stroke and HF in men with CVD [524].

5.4 Dose-response

Early research into the relationship between ED and CVD suggested a dose-response relationship. Greenstein et al [525] showed a significant positive correlation between the severity of ED and the severity of CVD. Patients with IHD involving only one vessel had significantly better erectile function than those with disease in multiple vessels. However, this study involved established clinical CVD patients and used an un-validated self-reporting tool to assess erectile function. More recent studies conducted using validated measures, such as the IIEF questionnaire [19, 110] which supports capturing more reliable information on the severity of ED, indicate that the risk of CVD increases as the severity of the ED symptoms increases even in those without diagnosed CVD [297, 379, 526]. This supports a dose-response relationship between ED and CVD.

5.5 Possible mechanisms

There are several biologically plausible mechanisms proposed to explain the link. As ED and CVD share the same risk factors and exist concomitantly, the underlying primary pathophysiology is suggested to be endothelial dysfunction. Atherosclerosis (characterised by the development of atheromata, atherosclerotic plaque, vascular calcification and arterial stiffness as a result of vascular injury, endothelial dysfunction and inflammation) affects all vascular beds to a similar extent. The small diameter of blood vessels and high content of endothelium in the penis [527] mean that the clinical consequences of vascular disease will manifest as ED in the penile arteries long before any other signs of CVD in the coronary arteries are evident. ED has been suggested to be synonymous with both endothelial dysfunction and early death [528]. The integrity of endothelial cells is fundamental to cardiovascular health and endothelial dysfunction is associated with a range of adverse cardiovascular outcomes. Endothelial dysfunction leads to reduced SMC relaxation in the walls of the arterioles causing impaired vasodilation. Vasodilation is essential to normal erectile function: penile tumescence occurs as a result of NO-mediated smooth muscle relaxation and vasodilation [529]. The same insults that result in endothelial damage and ED are the precursors to atherosclerosis and CAD. Endothelial response (commonly measured by changes to BP and platelet aggregation in response to L-arginine) has been shown to be significantly reduced in diabetic men with ED compared to diabetic men without ED [530]. Additionally, endothelial dysfunction (with or without concurrent atherosclerosis) is likely to narrow the small penile arteries causing ED before narrowing of the larger coronary arteries causes angina [531]. Furthermore, SMC degeneration in the penile CC tissue has been shown in rabbits as a result of a high cholesterol diet and impaired lipid metabolism [532] – two major risk factors

for CVD. Smooth muscle dysfunction, such as a peripheral vascular defect in the endothelium-dependent and independent vasodilation (NO-cGMP) systems, coexisting with endothelial dysfunction may occur in men with ED before systemic vascular disease becomes manifest [405].

Thus the clinical consequences of ED often manifest 2-3 years before the consequences of coronary atherosclerosis [509], and ED acts as a silent marker of CVD [501, 502]. This has led to a strongly supported view that ED is a valuable marker of generalised vascular disease in asymptomatic men and can therefore be used in the early identification of potentially reversible vascular deterioration and significant risk for CVD. In practical terms, this means we have 3-5 years from the presentation of ED symptoms to reduce the risk of a CV event: aggressive intervention is required.

6.0 CONCLUSION

Our knowledge of ED has improved dramatically over the past 20 years: the development of an accepted definition of ED, a means for its assessment and diagnosis, a change of focus from subjective to objective measures, and recognition of the condition's complex nature have allowed significant advancement. Progress has been made in understanding the pathophysiology, identifying the prevalence, incidence and risk factors, and in the development of effective pharmaceutical treatments. ED is highly prevalent in many countries throughout the world and although high levels of variability in study design hinder reliable comparative evaluation of epidemiological studies, the prevalence, incidence and risk factors appear to vary greatly between countries. There are currently no reliable data available on the prevalence of ED and its associated risk factors in NZ. The changing dietary patterns, increasingly indoor lifestyles, sedentary behaviour, and burgeoning obesity and diabetes rates indicate a high level of risk for ED in NZ and predict an increase in the prevalence of ED in the coming decades. Furthermore, Maori and Pacific Island populations suffer a disproportionate burden of the associated risk factors and comorbidities and are likely to have a higher prevalence of ED. The combination of assumed increased prevalence and established inequalities in access to healthcare highlights the need for research into ED in NZ and the development of cost effective treatment interventions. A population-based cross-sectional survey is needed to support future research in this area and to inform health promotion, healthcare and treatment subsidisation policies. Reliable and comparable data on current ED prevalence rates worldwide are necessary to allow us to follow these patterns in an international context.

ED has serious implications for the individual, their partner, society and the health care system. Effective treatment results in the re-establishment of normal sexual function in many men and a range of interventions are currently available. However, the first line treatment is an oral erectogenic and these are not effective, appropriate or acceptable in all men. Evidence suggests that there is often a significant delay between the onset of symptoms and seeking treatment, which can lead to a situation of reduced sexual activity resulting in the breakdown of normal relationship dynamics or social isolation. It is clear that the stigma surrounding ED needs to be addressed to encourage more men to come forward in order to support effective diagnosis and treatment of both the ED and any underlying comorbidities.

ED is clearly well established, if not widely known, as an early maker of silent CVD. Its diagnosis can therefore be used to identify men at risk of CVD at an early stage allowing timely intervention. As the common denominator is endothelial dysfunction resulting from a combination of inflammation and oxidative stress, reducing inflammation and oxidative stress may be a novel approach in the treatment of ED. A focus on affordable and accessible alternative treatments for ED, including dietary and lifestyle interventions, may offer significant potential for advancement in the prevention and effective treatment of this disorder. The projected increasing prevalence signals a significant new public health concern that affects the quality of life of older men in an ageing population, is associated with concomitant medical risks, and is costly to the healthcare system. Further research into the aetiology of ED and measures to reduce or prevent it will improve the quality of life of older men and reduce the burden on society by cutting associated healthcare costs.

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CHAPTER 3

ERECTILE DYSFUNCTION – A POPULATION-BASED CROSS-SECTIONAL SURVEY OF ITS PREVALENCE AND ASSOCIATED SOCIODEMOGRAPHIC, LIFESTYLE AND MEDICAL FACTORS IN NEW ZEALAND

1.0 INTRODUCTION

Erectile dysfunction (ED) is a common condition amongst ageing men worldwide. Its prevalence has been investigated in many countries and populations including Australia [1-4], the United States of America (USA) [5-10], South America [11, 12], Europe [13-15], the Middle East [16, 17] and Asia [18-20]. Published rates are highly varied which may reflect differences in study design and reporting rather than inherent differences in prevalence. The most commonly reported figure is from the landmark Massachusetts Male Aging Study (MMAS) which found that 52% of men over 40 years of age suffer from some degree of ED [5]. Epidemiological research includes several large multinational studies [21-26]. The Global Study of Sexual Attitudes and Behaviours (GSSAB) [27] investigated a range of sexual problems in the general ageing population aged 40-80 years in 29 countries, including New Zealand (NZ). They reported a 25% prevalence of moderate to severe erectile difficulties in 250 NZ men. This was the only NZ data available and due to the sample size (small), sampling frame (telephone directory) and sampling method (random-digit dialling with substitutions allowed), it is unlikely to be nationally representative. Furthermore, compared to the widely used and validated 5-item International Index of Erectile Function (IIEF-5) [28], the unvalidated question used to assess ED lacked the sensitivity to assess its severity. Risk factors were not assessed in this study and are yet to be established in the NZ population.

The most well established risk factor for ED is ageing [29-34]. However, ED is not an inevitable result of ageing, but the result of an accumulation of negative health insults over time and is therefore amenable to change. Sociodemographic (e.g., ethnicity [10, 35, 36], marital status [1, 37, 38], education [9, 31, 32, 39], household income [31, 32], employment status and occupation [1]), lifestyle (e.g., smoking [4, 9, 40-43], alcohol consumption [4, 5, 10, 22, 44, 45], physical activity (PA) [3, 4, 9, 10, 22, 45-48] and caffeine intake [49-51]) and medical (e.g., cardiovascular disease (CVD) [22, 40, 44, 52-55], hypertension [3-5, 23, 54, 56], hyperlipidaemia [5, 23, 53], diabetes [5, 21-23, 25, 54], obesity [25, 53, 54], metabolic syndrome (MetS) [57], lower urinary tract symptoms (LUTS) and prostate issues [19, 35, 44, 58, 59], hypogonadism [60, 61] and depression [22, 23, 25, 39, 45, 54, 62-64]) factors have been associated with an increased risk of ED in some studies and populations, although the evidence remains highly heterogeneous. Establishment of risk factors can aid in both the identification of men at risk and the direction of appropriate interventions to address the aetiology. Many of these risk factors are shared with CVD; indeed, ED is now considered an important early sign of underlying organic disorder, most commonly vascular dysfunction and subclinical CVD. However, it has been reported that the majority of men with ED do not seek treatment [1, 27,

39]. Others may receive treatment without appropriate medical assessment. The lost opportunity to diagnose and treat underlying causes and to increase awareness of the complexity of the disorder has serious implications for chronic disease rates.

This population-based cross-sectional observational study aimed to assess the prevalence of ED in NZ men 40-70 years of age and to examine its associated factors.

2.1 METHODS

Postal questionnaires were sent to a sample of 2000 men aged 40-70 years, age-stratified by decade and randomly selected from the NZ Electoral Roll. Once a participant was identified, no replacement or substitution was allowed. A modified Dillman method [65] was employed with three approaches (Appendix 3) made: 1) all 2000 men were sent a survey pack consisting of an invitation/information letter, a booklet-style survey, a return card and a reply-stamped envelope; 2) non-responders were sent a reminder postcard; and 3) non-responders were sent a final survey pack including an additional form outlining frequently asked questions. A prize draw was used as a motivator. The survey contained no personal identifiers. Participation was voluntary and a returned survey was taken as informed consent. Ethical approval was granted by the Massey University Human Ethics Committee (HEC Southern A, Application 10/75).

2.2 Sample size

The resident male population of NZ aged 40-70 years in 2013 was estimated at 768,801 [66]. Based on a 5% margin of error, a 95% confidence level and an estimated response rate of 20%, a sample size of 1925 was required to obtain 385 complete responses. Two-thousand were selected to allow for population morbidity, mortality and mobility due to the use of the NZ Electoral Roll as a sampling frame, with inherent non-sampling error issues including under-coverage, differential coverage and declining coverage between elections. The study sample (2000) represented 0.3% of the target population.

2.3 Postal survey

The survey (Appendix 3) was designed in four sections (sociodemographics, sexual activity and function, lifestyle, medical history) with a mix of 42 open and closed questions, six of which were multi-item tools including various validated tools. The initial survey was piloted twice before use, on six men from varied demographic backgrounds. It took approximately 10 minutes to complete. All data were collected by self-report only.

2.3.1 Sociodemographic factors

Ethnicity was by self-identification into single or multiple ethnic groups according to the 2013 NZ Census [66] with multi-ethnicity cases categorised into the minority group for analysis, and as European or non-European (NZ Maori, Pacific Peoples or Asian). Occupation category was classified according to the Australian and NZ Standard Classification of Occupations (ANZSCO) [67+ with categories reduced from eight to five to reduce complexity: "white collar" occupations (managers, professionals) and "blue collar" occupations (technicians and trades workers; community, personal service, clerical, administrative and sales workers; machinery operators, drivers and labourers).

2.3.2 Sexual activity and function

The validated IIEF-5 [28] was used to assess ED. The five questions applied to the previous six months and covered four domains: (i) erection confidence, (ii) erection firmness, (iii) erection maintenance, and (v) sexual satisfaction. Each question had 5 response options allowing the calculation of a score from 5-25 for erectile function. Scores were categorised according to established levels: ≤21 ED (17-21 mild, 12-16 mild to moderate, 8-11 moderate and 5-7 severe) and 22-25 no ED. The validated single-question self-assessment tool [68] was included for comparative purposes and required self-reporting into one of four categories: not impotent, minimally impotent, moderately impotent or completely impotent.

2.3.3 Lifestyle factors

The 9-item shortened European Prospective Investigation into Cancer and Nutrition Physical Activity Questionnaire (EPIC-PAQ) was used to assess PA [69]. This comprised of four main questions concerning occupational activity, time spent doing recreational and household activities (walking, cycling, gardening, household chores, do-it-yourself and sports), participation in vigorous non-occupational activities, and the number of flights of stairs climbed daily, over the previous year. From these responses, participation in vigorous activities was categorised as yes versus no. The well-established Cambridge index [70] (based on a cross-tabulation of occupational activity and the sum duration of hours per week spent in cycling and sports activities) was used to assign participants to one of four categories: inactive, moderately inactive, moderately active or active. This method has been found to correlate well with PA energy expenditure and time spent in moderate and vigorous PA compared to other similar indices [71]. Sun exposure was measured using an unvalidated 15-item tool adapted from von Hurst et al [72], designed to identify sun exposure and sun protection behaviours. Responses were scaled from one to five. Responses to questions 1-6 and 14 allowed the calculation of a

score for sun protection (ranging from 5-35). Responses to questions 7-8 and 11-13 allowed the calculation of a score for sun exposure (ranging from 5-30). Scores were classified into quartiles.

2.3.4 Medical factors

Medical factors included self-reported diagnosed or undiagnosed medical conditions and the use and type of medications and dietary supplements (both prescribed and non-prescribed). In addition to self-reported depression, current depression symptoms were assessed using the brief 9-item Patient Health Questionnaire (PHQ-9) [73] to categorise individuals by the presence (PHQ-9 score ≥10) and severity of depressive symptoms (minimal (1-4), mild (5-9), moderate (10-14), moderately severe (15-19), severe (20-27)). This is a simple and brief self-reporting tool that incorporates DSM-IV depression diagnostic criteria alongside other important symptoms of major depressive disorder, rates frequency of symptoms supporting a score for severity index, and includes a question asking the degree to which problems identified affect an individual's function. This method has been well validated with scores ≥10 indicating high sensitivity (88%) and specificity (88%) for major depression[73].

2.4 Data analyses

Surveys were de-identified and data checked by a third party. Sample proportions were age-weighted to reflect the 2013 NZ Census population age distribution [66]. The sociodemographic characteristics were compared to expected proportions based on available data from the 2013 NZ Census using χ^2-test. ED was defined as an IIEF-5 score ≤21 over the past 6 months [74]. The prevalence of ED and the 5 categories of severity were assessed overall and for each age group within the study and presented as crude, age-weighted, and adjusted for the average age distribution worldwide from 2000-2025 using the World Health Organization World Standard Population (WSP) for adults aged 40-69 years [75]. The prevalence of ED was also assessed using the single-question self-assessment tool [68] and categorised by category of ED severity and any degree of ED for comparison with the IIEF-5. Cohen's Kappa coefficient (κ) was calculated to determine consistency between the two tools. Age-weighted associations between sexual function and age, and subsequently ED (IIEF-5 ≤21) and sociodemographic, lifestyle and medical variables were assessed for statistical significance using χ^2 and Fisher's exact tests. Using un-weighted data, crude and age-adjusted odds ratios (OR) and 95% confidence intervals [95% CI] were calculated using binomial logistic regression. Separate models were created for each block of variables (sociodemographic, lifestyle and medical). All predictors within a block with an age-adjusted p-value <0.1 were entered and non-significant variables eliminated in a stepwise backward (Likelihood Ratio) elimination

algorithm to determine the final minimal models. Finally the OR [95% CI] of having ED were estimated for each covariate in a combined model adjusting for sociodemographic, lifestyle and medical factors. Analyses were performed using Microsoft Excel® 2010 (Microsoft Corporation, Redmond, WA, USA) and SPSS statistical software package version 20.0 (SPSS Inc., Chicago, IL, USA). The significance level was two-tailed and set at $P<0.05$.

3.1 RESULTS

3.2 Response rate and respondent profile

The response rate was 30% (599) with 28% (562) of surveys deemed complete for analysis based on completion of the IIEF-5. The age specific response fractions are shown in Table 3.1. There was an over representation of older men suggesting a participation bias. Sample proportions were subsequently age-weighted [66].

Table 3.1. Age-specific survey response rates comparative to the New Zealand (NZ) male population and the World Health Organization World Standard Population (WSP).

	Well-LaD Study[ŧ]			NZ male population[ǂǂ]			WSP[ǂǂǂ]		
Age (years)	n	%	Response fraction	n	%	Weighting factor Nk/nk	%	%	Weighting factor Nk/nk
40-49	157	27.94	23.54	288411	37.51	1.34	12.6	43.15	1.54
50-59	190	33.81	28.49	270837	35.23	1.04	9.9	33.90	1.00
60-69	214	38.08	32.13	209553	27.26	0.71	6.7	22.95	0.60
Other*	1	0.18	-	-	-	-	-	-	-
Total	562	100	28.1	768801	100	-	29.2	100	-

*The 'Other' category includes a respondent who did not provide age data. [ŧ]The Well-LaD Study included 2000 surveys sent to an age-stratified sample with approximately equal numbers of men in each decade. [ǂǂ]Descriptive data were subsequently age-weighted according to the 2013 NZ Census population age distribution. This work is based on/includes Statistics New Zealand's data which are licensed by Statistics New Zealand for re-use under the Creative Commons Attribution 3.0 New Zealand licence [66]. [ǂǂǂ]The WSP data for adults aged 40-69 years[75].

A description of the respondents is presented in Table 3.2. Comparison between age-weighted sociodemographic data and available data from the 2013 NZ Census [66] showed that a diverse cross-sectional sample was obtained that was generally representative of the population. However, there was evidence of participation bias, particularly towards European men, with a partner, with post-secondary school education and living in rural areas. The statistical significance of differences between observed and expected proportions was tested using χ^2-test and results showed a significant difference ($p<0.05$) across all sociodemographic characteristics. The study sample cannot be considered nationally representative.

Table 3.2. Age-weighted prevalence of sociodemographic characteristics amongst survey respondents (n=562) comparative to the 2013 NZ Census data for men aged 40-69 years (n=768,801).

		Age-weighted prevalence		NZ 2013 Census Prevalence	X^2-test	
Characteristic*		**n**	**%**	**%**	**statistic**	**p-value****
Age range (years)	40-49	210	37.5	37.5	0.002	0.999
	50-59	198	35.2	35.2		
	60-69	153	27.3	27.3		
Ethnicity	European	492	87.9	77.6	43.037	**<0.001**
	NZ Maori	35	6.3	10.4		
	Asian	17	3.1	8.8		
	Pacific Peoples	15	2.7	4.6		
Relationship status	Married/de facto/civil union	475	85	65.9	93.322	**<0.001**
	Single/dating	52	9.3	16.4		
	Separated/divorced/widowed	32	5.7	17.7		
Education	None	108	19.2	19.9	27.215	**<0.001**
	Secondary school	149	26.6	36		
	Post-secondary school	304	54.3	44		
Current employment status	Employed	487	87	79.8	20.994	**<0.001**
	Not employed and seeking work	17	3.1	3.1		
	Not employed and not seeking work	55	9.9	17.1		
Household income	Low (0-59,999)	225	41.1	39.8	7.344	**0.025**
	Middle (60,000-99,999)	151	27.5	32.6		
	High (100,000+)	172	31.4	27.6		
Occupational category	Managers	128	26.3	26.1	28.590	**<0.001**
	Professionals	113	23.3	19.8		
	Technicians and Trades Workers	119	24.5	18		
	Community, Personal Service, Clerical, Administrative and Sales Workers	57	11.7	15.2		
	Machinery Operators and Drivers and Labourers	69	14.2	20.9		

Characteristic*		Age-weighted prevalence		NZ 2013 Census Prevalence	X^2-test	
		n	%	%	statistic	p-value**
Location	North Island	400	71.4	76.5	8.434	**0.004**
	South Island	161	28.6	23.5		
Residence	Urban	359	64.7	85.8	203.101	**<0.001**
	Rural	196	35.3	14.2		

*Missing values for each characteristic not shown. **Age-weighted characteristics that are significantly different (p<0.05) from the expected proportions are highlighted in bold.

3.3 Non-respondent and incomplete respondent profile

The contact rate was 97%: 3% (64) of the 2000 surveys were returned as the recipient had moved or was deceased. The refusal rate was 67% (1337): 53% (1057) did not respond and 14% (280) actively declined. As the surveys contained no personal identifiers, the characteristics of non-responders could not be assessed; however, 37 (2%) surveys were returned with an incomplete IIEF-5, the characteristics of whom are known. Their sociodemographic profile was similar to respondents, although they were mostly employed in lower skilled "blue collar" occupations. Importantly, 24 were not sexually active in the past month and 25 usually had sexual intercourse less than once a month. Thirty-two completed the single-question self-assessment and 22 had some degree of ED (10 minimal, six moderate, six complete). The same 32 also completed at least one question in the IIEF-5: question one was the most and question three the least frequently completed. Of the 24 men who were not sexually active in the past month, 13 reported their usual frequency of sexual intercourse as "never", and they generally omitted question three of the IIEF-5 regarding maintenance of erection post penetration and question five regarding satisfaction of sexual intercourse. They were also more likely to have ED according to the single-question self-assessment: 14 had some degree of ED (three minimal, five moderate, six complete). The high number of incomplete responders who were not sexually active and had ED, suggests that the true prevalence of ED will be underestimated.

3.4 Sexual function

The erectile function of respondents was stratified into categories of severity based on established IIEF-5 score definitions. The prevalence and severity of ED in the various age groups is shown in Table 3.3. The crude prevalence of ED was 42% (22% mild, 10% mild-moderate, 6% moderate, 4% severe). In comparison to the IIEF-5, the crude prevalence of ED using the single-question self-assessment (n=555) was 45% (15% minimal, 25% moderate, 6% severe). Based on the commonly used cut-off for ED as moderate-severe, 30% had ED. Using the Kappa statistic, substantial agreement was found between the two tools in the discrimination of dichotomous categories (κ=0.75 *0.70-0.81], $p<0.001$), but only moderate agreement in the discrimination of multiple categories (κ=0.55 *0.50-0.61], $p<0.001$). The IIEF-5 (≤21) was used to define ED as a dichotomous variable for subsequent analysis. The prevalence of any degree of ED was 38% when adjusted for the age distribution of NZ males, and 37% when adjusted to the WSP. There was a highly significant relationship between ED and age (Figure 3.1): older men were more likely to have ED than younger men with approximately 20% in their 40s, 40% in their 50s and 60% in their 60s ($p<0.001$).

Table 3.3. Crude, age-weighted and World Standard Population (WSP) adjusted prevalence of erectile dysfunction (ED) in various age groups in survey respondents (n=562) using the 5-item International Index of Erectile Function (IIEF-5).

	Prevalence (%) of ED by IIEF-5 scores					
		Mild to severe ED				
Age group (years)	No ED (22-25)	Mild 17-21	Mild-Moderate 12-16	Moderate 8-11	Severe <8	ED (≤21)
40-49 (n=157)	76.4	15.9	4.5	1.9	1.3	23.6
50-59 (n=190)	62.1	24.7	6.8	4.7	1.6	37.9
60-69 (n=214)	40.7	23.9	17.3	9.8	8.4	59.4
Crude prevalence	57.8	21.9	10.3	5.9	4.1	42.2
Age-weighted prevalence*	61.6	21.2	8.8	5.1	3.3	38.4
WSP-adjusted prevalence†	63.4	20.7	8.2	4.7	3.0	36.6

*Adjusted for the age distribution of the New Zealand male population aged 40-69 years in 2013 (n=768,801). †Adjusted to the World Health Organization World Standard Population (WSP) of people aged 40-69 years

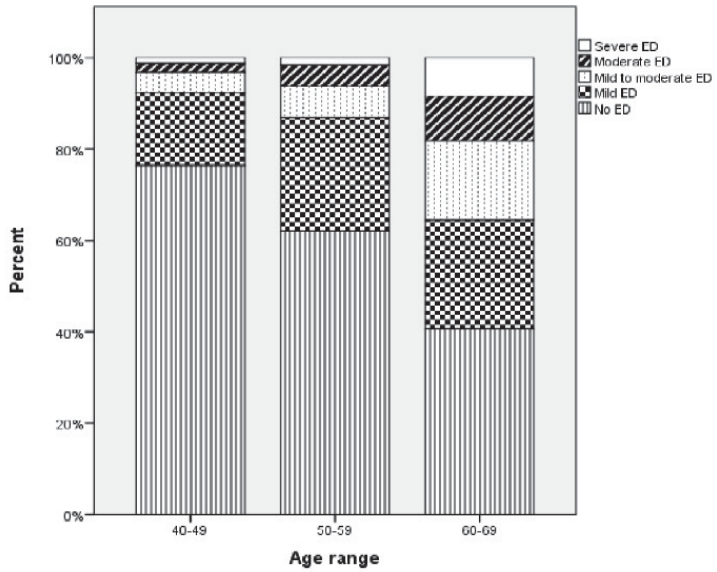

Figure 3.1. Age-weighted prevalence and severity of erectile dysfunction (ED) by age in survey respondents (n=562).

A summary of the age-weighted characteristics of the respondents regarding sexual activity and function, and their relationship with age, is shown in Table 3.4. Despite a 38% prevalence of ED, only 8% had been medically diagnosed and 10% were taking ED medication: most commonly prescription oral medication, followed by natural remedies (data not shown). Of men with ED, only 17% had been diagnosed and 22% were taking some form of treatment. Premature ejaculation (PE) was present in 25% of men and delayed ejaculation (DE) in 13% but neither condition was related to age ($p>0.05$). The majority of men were satisfied with their current sexual function (57%); however, satisfaction was less likely as men aged ($p<0.001$). There was a relationship between ED and sexual satisfaction: 70% of men with ED were less than satisfied and only 30% were satisfied with their current sexual function ($\chi^2=99.81$, N=556, $p<0.001$). Few men (11.3%) reported suffering from anxiety or depression as a result of lack of sexual activity or the inability to perform; however, the prevalence significantly increased with age ($p=0.023$). The majority of men were sexually active in the past month (73%) although this was significantly less likely in older men ($p<0.001$). The reported usual frequency of sexual intercourse and sexual thoughts were more than once a week in 42% and 85% of men respectively, indicating discordance between activity and desire. Increasing age was associated with a lower frequency of both sexual activity and sexual desire (both $p<0.001$). The majority of men felt confident about the future of their current relationship (81%) and this was not related to age ($p=0.587$); however, men with ED were less likely to feel confident than men without ED (71% vs 88% respectively; $\chi^2=23.35$, N=517, $p<0.001$). Furthermore, the more severe the ED, the less likely men reported feeling confident in their relationship (74% in mild, 70% in mild-moderate, 69% in moderate, 59% in severe; $\chi^2=24.95$, N=515, $p<0.001$).

Table 3.4. Age-weighted prevalence of sexual activity and function characteristics amongst survey respondents (n=562) by age group in New Zealand men aged 40-70 years.

		Age-weighted prevalence									
		Overall		40-49 years		50-59 years		60-69 years		X^2-test	
Characteristic/condition*		**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**statistic**	**p-value****
ED (IIEF-5)	No	346	61.6	161	76.3	123	62.1	62	40.5	48.025	**<0.001**
	Yes	216	38.4	50	23.7	75	37.9	91	59.5		
Diagnosed ED	No	513	91.6	202	96.2	181	91.4	130	85.5	13.058	**0.001**
	Yes	47	8.4	8	3.8	17	8.6	22	14.5	-	-
Treated ED	No	503	90.0	198	94.3	178	89.9	127	84.1	10.100	**0.006**
	Yes	56	10.0	12	5.7	20	10.1	24	15.9	-	-
Premature ejaculation	No	423	75.4	170	81.0	144	72.7	109	71.2	5.679	0.058
	Yes	138	24.6	40	19.0	54	27.3	44	28.8	-	-
Delayed ejaculation	No	484	86.6	188	89.1	167	84.8	129	85.4	1.879	0.391
	Yes	75	13.4	23	10.9	30	15.2	22	14.6	-	-
Feelings regarding current sexual function	Satisfied	314	56.5	138	66.0	110	56.4	66	43.4	18.298	**<0.001**
	Less than satisfied	242	43.5	71	34.0	85	43.6	86	56.6	-	-
Anxiety or depression resulting from sexual activity or function	No	488	88.7	188	90.8	176	91.2	124	82.7	0.759	**0.023**
	Yes	62	11.3	19	9.2	17	8.8	26	17.3	-	-
Sexual intercourse in past month	No	149	26.7	40	19.0	48	24.4	61	40.1	20.865	**<0.001**
	Yes	410	73.3	170	81.0	149	75.6	91	59.9	-	
Usual frequency of intercourse	<once a month	132	23.6	35	16.7	43	21.8	54	35.3	23.322	**<0.001**
	at least monthly	193	34.5	70	33.3	68	34.5	55	35.9	-	-
	>once a week	235	42.0	105	50.0	86	43.7	44	28.8	-	-
Usual frequency of sexual thoughts	<once a month	14	2.5	0	0.0	5	2.6	9	6.0	24.431	**<0.001**
	at least monthly	70	12.6	16	7.7	26	13.3	28	18.5	-	-
	>once a week	472	84.9	193	92.3	165	84.2	114	75.5	-	-
Feelings about current relationship	Confident	420	81.4	161	83.4	146	81.1	113	79.0	1.067	0.587
	Less than confident	96	18.6	32	16.6	34	18.9	30	21.0	-	-

*Missing values for each risk factor not shown. **Age-weighted characteristics/condition that show a significant relationship ($p<0.05$) with age are highlighted in bold. ED, erectile dysfunction; IIEF-5, 5-item International Index of Erectile Function

3.5 Sociodemographic factors

The prevalence of ED and its association with sociodemographic, lifestyle and medical factors and their crude and age-adjusted odds ratios are shown in Table 3.5. The χ^2 analysis showed a significant relationship between ED and all of the sociodemographic variables assessed ($p \leq 0.001$), with the exception of residence in an urban or rural/semi-rural zone ($p=0.119$). Further analysis using binomial logistic regression supported these relationships. Compared to men in their 40s, the odds of ED increased 2-fold for men in their 50s and 5-fold for men in their 60s. After adjusting for age, all sociodemographic variables, except residence, remained significant predictors of ED prevalence ($p<0.05$). Most importantly, the age-adjusted odds of ED increased 6-fold in non-European men compared to European men, over 3-fold in men who were unemployed and seeking work compared to employed men and 2-fold in men with no regular partner compared to men in married/de facto/civil union relationships. Men in lower-skilled occupations also had over 2-fold higher odds of ED, evident in the lowest skill category of machinery operators, drivers and labourers. On the contrary, the odds of ED reduced by 75% in men with a high income compared to a low income, by 45% in men with post-secondary school qualifications compared to no formal qualifications and by 40% in men living in the South Island compared to the North Island. Residential zone was not associated with ED in this study.

3.6 Lifestyle factors

As shown in Table 3.5, there was a significant relationship between ED and smoking ($p=0.012$): current smokers had a higher prevalence of ED compared to former and never smokers. After adjusting for age, the odds of ED remained 3-fold higher amongst current smokers compared to never smokers. The relationship between ED and alcohol consumption was highly significant ($p=0.001$): current drinkers had a lower prevalence of ED than never and former drinkers, particularly beer and red wine drinkers ($p=0.002$ and $p=0.026$ respectively). After adjusting for age, the likelihood of ED remained 50% lower amongst current drinkers compared to never drinkers and although alcohol consumption was not a statistically significant age-adjusted predictor of ED ($p=0.064$), both beer and red wine consumption predicted ED and were associated with a 30-40% lower risk. Less than a quarter of all respondents drank >15 standard drinks per week and there was no relationship observed between this exposure and ED ($p>0.05$). There was no relationship observed between consumption of caffeinated beverages, or type of beverage consumed and ED (all $p>0.05$); however, after adjusting for age, drinking black tea was a significant predictor ($p=0.019$) of a 46% lower likelihood of ED. Furthermore, drinking herbal tea decreased the odds by 40% and energy drinks increased the odds by 93%,

although neither reached statistical significance as predictors (p=0.072 and p=0.054 respectively). Although most smokers, drinkers and caffeine consumers indicated the type consumed, many did not provide the number consumed per week, therefore more detailed analysis was not possible. The majority of respondents were physically active. Both vigorous PA and the Cambridge Index were significantly related to ED (p<0.001 and p=0.028 respectively): there was a lower prevalence of ED amongst physically active men and after adjusting for age, PA remained a significant predictor of ED. Men who regularly participated in vigorous PA or were classified as moderately active or active had an approximately 50% lower likelihood of ED. There was no significant relationship observed between ED and quartile of sun protection or exposure behaviours (both p>0.05), but a lower prevalence of ED was observed amongst men with mild exposure. In contrast, although while there was no difference in the odds of ED by sun exposure quartile (p>0.05), high sun protection behaviour was a significant predictor of ED (p=0.047) and associated with a 40% lower risk; however, it did not remain after adjusting for age(p=0.059).

3.7 Medical factors

Both CVD (defined as atherosclerosis, heart disease, angina, heart attack, heart failure, or stroke) and its major risk factors hypertension, hypercholesterolaemia and type two diabetes mellitus (T2DM) were associated with a higher prevalence of ED (all p<0.01): 65.5% of men with CVD had ED compared with 35.3% of men without CVD. After adjusting for age, these were all significant predictors of ED (p=0.002, p<0.001, p=0.018 and p=0.025 respectively). Indeed, CVD, hypertension and T2DM each more than doubled the likelihood of ED. Only 5.2% of men had prostate cancer, benign prostatic hyperplasia (BPH), prostatitis or Peyronie's disease; however, these conditions were associated with a higher prevalence of ED (p=0.022). They increased the crude odds of ED 2-fold although this was not a significant predictor after age-adjustment (p>0.05). Less than 1% of men had hypogonadism and this limited the power to detect a significant relationship (p=0.073); however, 4/5 of these men had ED and after adjusting for age, although not a significant predictor (p=0.090), hypogonadism was observed to increase the risk of ED 7-fold. Self-reported depression, post-traumatic stress disorder (PTSD) or a psychiatric condition were present in 13.8% and this was not related to ED (p>0.05); however, depression symptoms assessed using the PHQ-9 showed that 8.1% suffered from major depression and there was a higher prevalence of ED amongst these men, although this did not reach statistical significance (p=0.057). Further analysis by category of depression showed 30.2% of men suffered from some degree of depression and there was a significant relationship between the severity and ED (p<0.001). After age-adjustment, mild and moderate

symptoms and major depression were all significant predictors of ED ($p<0.001$, $p=0.017$ and $p=0.016$ respectively): mild and moderate symptoms increased the risk 3-fold while major depression doubled the risk of ED. There was no relationship observed between ED and any of the other medical conditions assessed ($p>0.05$). There was a significant relationship between taking medication and ED ($p=0.002$): this exposure was a significant predictor of ED ($p=0.04$) and increased the age-adjusted odds by 50%. There was no relationship between supplementation use and ED ($p>0.05$).

Table 3.5. Age-weighted prevalence of sociodemographic, lifestyle and medical characteristics amongst survey respondents (n=562) and their relationship with the prevalence of erectile dysfunction (ED). The crude and age-adjusted odds ratios and 95% confidence intervals (OR [95% CI]) for these exposures and ED are provided.

		Age-weighted prevalence							
		Overall		With ED		X^2 or Fisher's Exact		Crude ORs	Age-adjusted ORs
Characteristic/condition*		**n**	**%**	**n**	**%**	**Statistic**	**p-value**	**OR [95% CI]**	**OR [95% CI]**
SOCIODEMOGRAPHIC									
Age group	40-49	210	37.5	50	23.7	48.025	**0.000**	Referent	Referent
	50-59	198	35.2	75	37.9	-	-	**1.98 [1.24-3.17]†**	**1.98 [1.24-3.17]†**
	60-69	153	27.3	91	59.5	-	-	**4.73 [2.99-7.49]†**	**4.73 [2.99-7.49]†**
Ethnicity	European	492	87.9	168	34.1	29.725	**0.000**	Referent	Referent
	Non-European	68	12.1	46	68.7	-	-	**4.02 [2.26-7.15]†**	**6.29 [3.35-11.81]†**
Relationship status	Partner	475	85.0	168	35.4	12.017	**0.001**	Referent	Referent
	No regular partner	84	15.0	46	55.4	-	-	**2.15 [1.35-3.43]†**	**2.15 [1.32-3.50]†**
Education	None	108	19.2	58	53.7	13.619	**0.001**	Referent	Referent
	Secondary school	149	26.6	54	36.2	-	-	**0.52 [0.32-0.86]†**	0.63 [0.38-1.06+ǂ
	Post-secondary school	304	54.3	103	33.9	-	-	**0.48 [0.31-0.74]†**	**0.55 [0.35-0.87]†**
Current employment status	Employed	487	87.0	171	35.0	19.067	**0.000**	Referent	Referent
	Not employed and seeking work	17	3.1	11	64.7	-	-	2.39 [0.84-6.83]	**3.55 [1.18-10.73]†**
	Not employed and not seeking work	55	9.9	34	60.7	-	-	**2.62 [1.58-4.34]†**	1.38 [0.79-2.40]
Household income	Low (0-59,999)	225	41.1	116	51.6	43.593	**0.000**	Referent	Referent
	Middle (60,000-99,999)	151	27.5	61	40.4	-	-	**0.64 [0.43-0.97]†**	0.72 [0.47-1.10]
	High (100,000+)	172	31.4	33	19.2	-	-	**0.22 [0.14-0.34]†**	**0.26 [0.16-0.41]†**
Occupational category	Managers	128	26.3	33	25.6	20.775	**0.000**	Referent	Referent
	Professionals	113	23.3	31	27.2	-	-	1.06 [0.60-1.86]	0.98 [0.54-1.78]
	Technicians and Trades Workers	119	24.5	51	42.9	-	-	**2.12 [1.24-3.62]†**	**2.06 [1.17-3.60]†**
	Community, Personal Service, Clerical, Administrative and Sales Workers	57	11.7	28	49.1	-	-	**2.43 [1.26-4.68]†**	**2.30 [1.16-4.57]†**
	Machinery Operators and Drivers and Labourers	69	14.2	33	47.8	-	-	**2.58 [1.39-4.78]†**	**2.44 [1.28-4.66]†**
Location	North Island	400	71.4	169	42.2	8.806	**0.003**	Referent	Referent
	South Island	160	28.6	46	28.8	-	-	**0.60 [0.41-0.87]†**	**0.60 [0.40-0.89]†**
Residence	Urban	359	64.7	145	40.4	2.427	0.119	Referent	Referent
	Rural	196	35.3	66	33.7	-	-	0.77 [0.54-1.10]	0.79 [0.54-1.15]

Characteristic/condition*		Age-weighted prevalence: Overall n	Overall %	With ED n	With ED %	X^2 or Fisher's Exact Statistic	p-value	Crude ORs OR [95% CI]	Age-adjusted ORs OR [95% CI]
LIFESTYLE									
Smoking status	Never	344	61.4	118	34.3	8.906	**0.012**	Referent	Referent
	Former	152	27.1	63	41.4	-	-	**1.48 [1.01-2.16]†**	1.29 [0.87-1.93]
	Current	64	11.4	34	53.1	-	-	**2.47 [1.41-4.34]†**	**3.52 [1.91-6.48]†**
Cigarette smoker	No	506	90.4	186	36.8	7.276	**0.007**	Referent	Referent
	Yes	54	9.6	30	55.6	-	-	**2.42 [1.33-4.39]†**	**3.56 [1.86-6.84]†**
Pipe, cigar, cheroot or cigarillo smoker	No	552	98.6	211	38.2	-	0.437	Referent	Referent
	Yes	8	1.4	4	57.2	-	-	1.84 [0.41-8.29]	2.48 [0.54-11.42]
Alcoholic beverage consumer	Never	40	7.1	22	55.0	13.279	**0.001**	Referent	Referent
	Former	20	3.6	14	66.7	-	-	1.68 [0.57-4.98]	1.52 [0.49-4.77]
	Current	501	89.3	179	35.7	-	-	**0.52 [0.27-0.99]†**	0.53 [0.27-1.04+‡‡
Beer drinker	No	176	31.5	84	47.7	9.138	**0.002**	Referent	Referent
	Yes	382	68.5	131	34.2	-	-	**0.64 [0.45-0.91]†**	**0.67 [0.46-0.98]†**
White wine drinker	No	377	67.5	154	40.8	2.637	0.104	Referent	Referent
	Yes	181	32.5	61	33.7	-	-	0.78 [0.55-1.12]	0.75 [0.51-1.10]
Red wine drinker	No	328	58.8	139	42.4	4.974	**0.026**	Referent	Referent
	Yes	230	41.2	76	33.0	-	-	**0.65 [0.46-0.92]†**	**0.61 [0.42-0.87]†**
Spirits drinker	No	434	77.9	166	38.2	0.030	0.862	Referent	Referent
	Yes	124	22.1	48	39.0	-	-	0.96 [0.64-1.43]	0.85 [0.56-1.30]
RTD or pre-mixed drinker	No	519	93.0	200	38.5	0.107	0.744	Referent	Referent
	Yes	39	7.0	14	35.9	-	-	0.84 [0.41-1.71]	1.26 [0.59-2.69]
Standard drinks per week	<=15	398	76.6	144	36.2	0.001	0.981	Referent	Referent
	>15	122	23.4	44	36.1	-	-	1.00 [0.66-1.52]	1.00 [0.65-1.55]
Caffeinated beverage consumer	Never	14	2.5	5	35.7	0.381	0.827	Referent	Referent
	Former	13	2.4	6	46.2	-	-	1.33 [0.28-6.28]	1.92 [0.38-9.72]
	Current	534	95.2	204	38.2	-	-	0.97 [0.33-2.82]	0.99 [0.32-3.07]
Coffee drinker	No	127	22.9	54	42.5	1.275	0.259	Referent	Referent
	Yes	429	77.1	159	37.0	-	-	0.86 [0.58-1.27]	0.88 [0.58-1.33]
Tea drinker	No	215	38.7	89	41.4	1.476	0.224	Referent	Referent
	Yes	341	61.3	124	36.3	-	-	0.83 [0.59-1.18]	**0.64 [0.44-0.93]†**

Characteristic/condition*		Age-weighted prevalence				X^2 or Fisher's Exact		Crude ORs	Age-adjusted ORs
		Overall		With ED					
		n	%	n	%	Statistic	p-value	OR [95% CI]	OR [95% CI]
Herbal tea drinker	No	492	88.4	193	39.2	1.739	0.187	Referent	Referent
	Yes	65	11.6	20	30.8	-	-	0.63 [0.36-1.08+ǂǂ	0.59 [0.33-1.05+ǂǂ
Green tea drinker	No	460	82.7	177	38.5	0.137	0.711	Referent	Referent
	Yes	96	17.3	35	36.5	-	-	0.89 [0.57-1.39]	0.89 [0.55-1.42]
Soft drink drinker	No	389	69.8	148	38.0	0.210	0.886	Referent	Referent
	Yes	168	30.2	65	38.7	-	-	1.00 [0.68-1.45]	1.37 [0.91-2.05]
Energy drink drinker	No	507	91.1	192	37.8	0.485	0.486	Referent	Referent
	Yes	49	8.9	21	42.9	-	-	1.22 [0.65-2.28]	1.93 [0.99-3.77+ǂǂ
Cups of coffee per day	<=2 cups	359	68.5	141	39.3	1.076	0.300	Referent	Referent
	>2 cups	165	31.5	57	34.5	-	-	0.89 [0.61-1.30]	0.89 [0.60-1.32]
Vigorous PA	No	123	22.1	69	56.1	21.314	**0.000**	Referent	Referent
	Yes	434	77.9	144	33.2			**0.40 [0.27-0.59]ǂ**	**0.48 [0.32-0.73]ǂ**
Cambridge Index	Inactive	67	12.1	36	53.7	9.105	**0.028**	Referent	Referent
	Moderately inactive	114	20.7	43	37.7	-	-	**0.49 [0.27-0.89]ǂ**	0.55 [0.30-1.04+ǂǂ
	Moderately active	153	27.7	60	39.2	-	-	**0.50 [0.28-0.88]ǂ**	**0.55 [0.31-1.00]ǂ**
	Active	219	39.6	73	33.3	-	-	**0.41 [0.24-0.70]ǂ**	**0.52 [0.29-0.93]ǂ**
Sun protection quartile	Q1	130	24.4	60	45.8	4.750	0.191	Referent	Referent
	Q2	150	28.1	58	38.4	-	-	0.72 [0.45-1.16]	0.78 [0.47-1.28]
	Q3	134	25.0	51	38.1	-	-	0.70 [0.43-1.14]	0.74 [0.45-1.24]
	Q4	121	22.5	39	32.5	-	-	**0.60 [0.36-0.99]ǂ**	0.60 [0.36-1.02+ǂǂ
Sun exposure quartile	Q1	167	31.1	65	38.9	5.231	0.156	Referent	Referent
	Q2	165	30.6	53	32.1	-	-	0.71 [0.45-1.11]	0.77 [0.48-1.22]
	Q3	97	18.2	44	45.4	-	-	1.21 [0.73-2.00]	1.33 [0.78-2.25]
	Q4	108	20.1	45	41.7	-	-	1.11 [0.69-1.81]	1.15 [0.69-1.90]

Characteristic/condition*		Age-weighted prevalence: Overall n	Overall %	With ED n	With ED %	X^2 or Fisher's Exact Statistic	p-value	Crude ORs OR [95% CI]	Age-adjusted ORs OR [95% CI]
MEDICAL									
CVD	No	502	90.1	177	35.3	19.137	**0.000**	Referent	Referent
	Yes	55	9.9	36	65.5			**3.31 [1.91-5.75]†**	**2.42 [1.37-4.30]†**
Hypertension	No	402	72.2	127	31.6	27.038	**0.000**	Referent	Referent
	Yes	155	27.8	86	55.5			**2.78 [1.92-4.02]†**	**2.23 [1.52-3.29]†**
Hypercholesterolaemia	No	354	63.6	116	32.8	12.316	**0.000**	Referent	Referent
	Yes	203	36.4	97	47.8			**1.92 [1.36-2.72]†**	**1.55 [1.08-2.24]†**
T2DM	No	521	93.5	191	36.7	8.524	**0.004**	Referent	Referent
	Yes	36	6.5	22	61.1			**2.75 [1.40-5.38]†**	**2.25 [1.11-4.56]†**
Cancer	No	535	95.9	203	37.9	0.911	0.340	Referent	Referent
	Yes	23	4.1	11	47.8			1.28 [0.58-2.87]	0.98 [0.43-2.25]
Restless Leg Syndrome	No	525	94.4	200	38.1	0.183	0.669	Referent	Referent
	Yes	31	5.6	13	41.9			1.49 [0.75-2.96]	1.17 [0.56-2.46]
Auto-immune disorders	No	531	95.5	200	37.7	2.076	0.150	Referent	Referent
	Yes	25	4.5	13	52.0			1.74 [0.82-3.70]	1.32 [0.60-2.90]
Prostate problem or Peyronie's disease	No	528	94.8	197	37.3	5.277	**0.022**	Referent	Referent
	Yes	29	5.2	17	58.6			**2.01 [1.01-3.98]†**	1.22 [0.59-2.52]
Nerve damage	No	530	95.2	202	38.1	0.435	0.509	Referent	Referent
	Yes	27	4.8	12	44.4			1.20 [0.56-2.58]	1.21 [0.51-2.49]
Hypogonadism	No	552	99.1	209	37.9	-	0.073	Referent	Referent
	Yes	5	0.9	4	80.0			5.58 [0.62-50.21]	6.97 [0.74-65.48+‡‡
Depression, PTSD or a psychiatric condition	No	481	86.2	178	37.0	2.667	0.102	Referent	Referent
	Yes	77	13.8	36	46.8			1.59 [0.98-2.56+‡‡	1.54 [0.93-2.55+‡‡
PHQ-9 depression	No	485	91.9	177	36.5	3.619	0.057	Referent	Referent
	Yes	43	8.1	22	51.2			**2.00 [1.06-3.78]†**	**2.28 [1.16-4.47]†**
PHQ-9 category of depression	None	369	69.8	115	31.2	25.023	**0.000**	Referent	Referent
	Mild	116	22.1	62	53.4			**2.32 [1.51-3.58]†**	**3.39 [2.09-5.50]†**
	Moderate	25	4.7	14	56.0			**2.74 [1.20-6.27]†**	**3.24 [1.34-7.85]†**
	Moderately severe	16	3.0	6	37.5			1.37 [0.47-4.03]	2.34 [0.74-7.38]
	Severe	2	0.4	2	100.0			2951581898 [0.00-.]	1503509825 [0.00-

Characteristic/condition*		Age-weighted prevalence							
		Overall		With ED		X^2 or Fisher's Exact		Crude ORs	Age-adjusted ORs
		n	%	n	%	Statistic	p-value	OR [95% CI]	OR [95% CI]
Substance abuse	No	521	93.5	198	38.0	0.590	0.442	Referent	Referent
	Yes	36	6.5	16	44.4			1.23 [0.60-2.51]	1.78 [0.83-3.81]
Medication	No	318	57.2	104	32.7	9.875	**0.002**	Referent	Referent
	Yes	238	42.8	109	45.8			**1.75 [1.25-2.46]ǂ**	**1.46 [1.02-2.08]ǂ**
Supplementation	No	349	63.1	138	39.5	0.770	0.380	Referent	Referent
	Yes	204	36.9	73	35.8			0.82 [0.58-1.16]	0.75 [0.52-1.08]

*Missing values for each risk factor not shown. **Age-weighted characteristics/conditions that show a significant relationship ($p<0.05$) with erectile dysfunction (ED) are highlighted in bold. ǂVariables that provide a statistically significant contribution to the prediction of ED ($p<0.05$), also highlighted in bold. ǂǂVariables that do not provide a statistically significant ($0.05>p<0.1$) contribution to the prediction of ED but may be of interest in a combined logistic regression model to predict ED. CVD, cardiovascular disease; PA, physical activity; PHQ-9, 9-item Patient Health Questionnaire; PTSD, post-traumatic stress disorder; T2DM, type 2 diabetes mellitus.

3.8 Multivariate analysis

Multivariate regression analyses are presented in Figure 3.2. The full model included: sociodemographic (age, ethnicity, household income and location), lifestyle (smoking, alcohol consumption and vigorous PA) and medical (CVD, hypertension, T2DM and prostate problems or Peyronie's disease) predictor variables. The independent predictors of increased risk of ED were: increasing age, non-European ethnicity and current smoking. Men in their 50s had 2.3-fold (OR=2.32 [1.31-3.95], p=0.004) and men in their 60s had 4.9-fold (OR=4.91 [2.75-8.74], p<0.001) the risk of ED compared to men in their 40s. Non-European men had 3.5-fold (OR=3.50 [1.72-7.13], p=0.001) the risk of European men. Current smoking increased the risk of ED 2.8-fold (OR=2.80 [1.41-5.57], p=0.003) compared to never smokers; however, former smoking was not an independent risk factor. Not having a partner (OR=1.66 [0.94-2.92], p=0.080), having a "technician and trades worker" occupation (OR=1.76 *0.92-3.35], p=0.087), having hypertension (OR=1.47 [0.94-2.32], p=0.093) and depression (OR=1.98 [0.93-4.21], p=0.077) were observed to increase the risk of ED; however, their contribution to the prediction of ED did not reach statistical significance. A high household income (OR=0.39 [0.24- 0.65], p<0.001) and regular participation in vigorous PA (OR=0.58 [0.36-0.92], p=0.02) were both independent predictors of a lower risk of ED. Drinking black tea (OR=0.67 [0.43-1.02], p=0.063) was observed to decrease the risk of ED; however, this did not reach statistical significance.

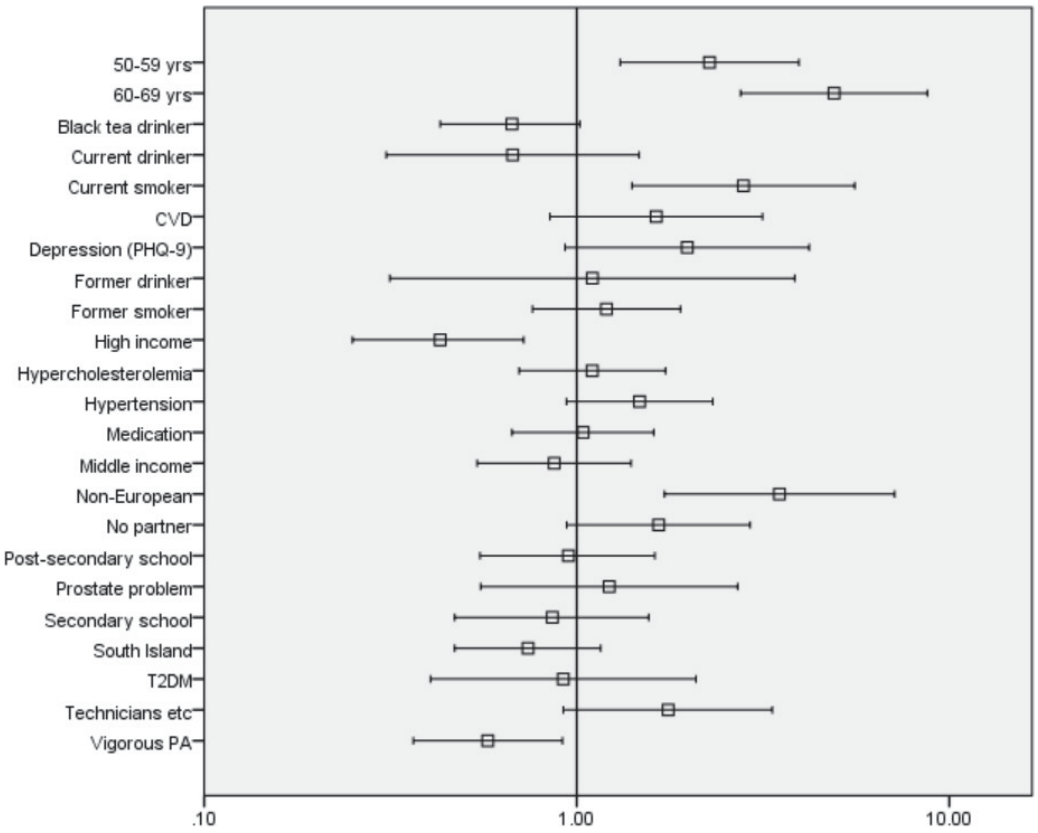

Figure 3.2. Forest plot of the multivariate estimates for ED in survey respondents (n=562). Data are presented as ORs and 95% CI from binomial logistic regression of ED prevalence (IIEF-5; referent category: score >21). The final model included age, ethnicity, income, location, smoking status, alcohol consumption, vigorous PA, CVD, hypertension, T2DM, and prostate problems or Peyronie's disease. Model fit was assessed through pseudo-R^2 (Nagelkerke): it explained 30.5% of the variance in ED prevalence and correctly classified 71.7% of cases. Reference categories for categorical predictors (not shown) were as follows: 40-49 years; European ethnicity; partner; no formal education; low household income; occupational category "professionals"; North Island; never smoker; never drinker (alcohol); not a black tea drinker; no vigorous PA; no diagnosis of hypertension, hypercholesterolaemia, CVD, T2DM; no depression (PHQ-9 score <10); no prostate problems or Peyronie's disease. CI, confidence interval; CVD, cardiovascular disease; ED, erectile dysfunction; IIEF-5, 5-item International Index of Erectile Function; ORs, Odds Ratios; PA, physical activity; PHQ-9, 9-item Patient Health Questionnaire; T2DM, type 2 diabetes mellitus.

4.0 DISCUSSION

The 42% crude and 38% age-weighted prevalence of ED in this cross-sectional cohort of NZ men is comparable to other findings internationally [1, 3, 5, 9]. It is lower than the crude 52% found in 1290 American men aged 40-70 years in the landmark MMAS [5] using the single-item self-assessment tool, and the age-weighted 53% found in 1195 Australian men aged 35-80 years in the Florey Adelaide Male Ageing Study (FAMAS) [3] using the 15-item IIEF. However, it is higher than the crude 35% reported in 2126 American men aged >20 years in the nationally representative National Health and Nutrition Examination Survey (NHANES) [9] using the single-item self-report question, and the age-weighted 25% reported in 1580 Australian men aged >20 years in the Western Australian Men's Health Study (WAMHS) [1] using the IIEF-5 – although both of these studies included younger men. Published prevalence rates are highly heterogeneous between countries and studies, highlighting the need for establishment and monitoring of country-specific rates. Differences in study design limit the comparability of results. Few studies have presented prevalence rates weighted to represent the age-distribution of the population sampled [1], or standardised to a stable reference population to enable meaningful international comparisons [1, 26, 54]. The WSP-adjusted prevalence of 37% in this study is comparable to the WAMHS [1] which reported 23% in Western Australia, and the Global Online Sexuality Survey (GOSS) [26, 54] which reported 34% in American and 47% in Middle Eastern internet users. Both studies used the IIEF-5 to assess ED. Previous data in NZ were limited to the GSSAB [27], which reported a 25% prevalence of moderate to severe "erectile difficulties" in NZ men. The results of our study show the prevalence of moderate to severe is 17.2% (when including mild-moderate ED cases) suggesting that the GSSAB may have overestimated the prevalence in NZ men. Applying the age-weighted prevalence to the 2013 NZ Census population estimates suggests that approximately 295,000 NZ men have some degree of ED: 163,000 mild, 68,000 mild-moderate, 39,000 moderate, and 25,000 severe cases.

As expected, there was a strong age-association: both the prevalence and severity of ED increased with age; however, we found positive associations between ED and several other factors, which remained after adjusting for age. Ethnicity was an important risk factor. Self-identification as non-European was associated with a 6-fold increased risk of ED: this remained 3.5-fold after adjusting for multiple confounders. Several studies in the USA reported poorer erectile function scores in African Americans and Hispanics compared to Caucasians [10, 35, 36]; however, many studies did not examine ethnicity as a risk factor or had a limited ability to detect a difference due to inadequate representation [2]. One study showed that the

importance of specific risk factors might differ by ethnicity [58], highlighting the need for future research into ED in specific NZ minority groups. Having no regular partner, being unemployed and seeking work and having a lower skilled "blue collar" occupation were all associated with twice the age-adjusted odds of ED, although none remained significant independent predictors. Few studies have investigated employment status and occupational category [1]. The Australian WAMHS [1] reported higher age-adjusted odds of ED in men who were not in a current relationship and unemployed men; however, in contrast to our results they found no difference between ANZSCO categories. Our results showed a protective effect of income, education and living in the South Island, although only income remained a significant independent predictor. This is consistent with limited available evidence supporting a relationship between ED and socioeconomic factors such as education [9, 31, 32, 76], income [1, 77] or socioeconomic indices [10, 22, 78], including longitudinal studies [31, 32] which suggest a lower risk of ED in highly educated men and an independent association between ED and household income with a 2.7-fold increased risk of incident ED in men from low-income households.

The cross-sectional population-based BACH study [10] reported that along with age, comorbidities and socioeconomic status, modifiable lifestyle factors (smoking, alcohol consumption, and PA) significantly contributed to the prevalence of ED. Another recent large cross-sectional population-based study in 123,779 men over 45 years in New South Wales, Australia [4] found that the crude odds of moderate-complete ED were highest amongst men with comorbidities but also 26% higher in healthy men with lifestyle risk factors (currently smoking, BMI >25 kg/m^2, >30 alcoholic drinks per week, and being sedentary) compared to healthy men without risk factors (OR=1.26 [1.20-1.33]). The limited available clinical evidence into the efficacy of lifestyle intervention supports that both smoking cessation, increasing PA and weight reduction can reverse ED and restore normal erectile function [47]. Our study supported the significance of current smoking as an independent risk factor and PA as an independent protective factor for ED. ED is reported to be more prevalent among heavy drinkers [4, 5] with a dose-response relationship between ED severity and the number of drinks consumed [3, 5, 10]; however, moderate consumption is suggested to be protective [22, 44]. The results of population-based cross-sectional [5, 10, 38, 45, 79]) and longitudinal studies [3, 40] are inconsistent. Our results showed a significant association between ED and alcohol consumption, with current drinkers having a lower prevalence of ED; however, the association was lost after adjusting for age and multiple confounders. We found that consumption of beer and red wine in particular was associated with a lower prevalence of ED and this protective effect remained after adjusting for age. Further research into this is warranted.

Caffeine consumption has been suggested to be protective against ED [34, 49,-51]; however, there is a paucity of research in this area. Very few epidemiological studies have investigated caffeine intake and results of limited cross-sectional [49, 50] and longitudinal [34] studies are conflicting. There have been no intervention studies investigating the effect of altering caffeine consumption on erectile function. Recent analysis of the NHANES [51] reported an independent protective effect of moderate caffeine intakes in quintile 3 and 4 (85-303 mg/day) compared to intakes in the 1st quintile (0-7 mg/day) after adjusting for multiple confounders (OR=0.58 [0.37-0.89] and OR=0.61 [0.38-0.97] respectively) – suggesting that consumption of 2-3 cups of coffee per day (170-375 mg/day) may be protective against ED. We found no association between caffeine consumption or coffee intake and ED. Interestingly, drinking black tea was associated with lower age-adjusted odds of ED and although this was also observed after adjusting for multiple confounders (OR=0.67 [0.43-1.02]) it did not reach statistical significance.

This was the first study to investigate the relationship between sun behaviour and ED. The 4th quartile of protection (high) was associated with 40% lower age-adjusted odds of ED. Although this did not reach significance, it suggests that regular use of sunscreen, clothing, hats, glasses and limiting time in the sun may be protective against ED – possibly related to a higher level of overall health awareness. Additionally, the 2nd quartile of sun exposure (mild) was associated with a 35% lower age-adjusted odds of ED compared to the 1st quartile (low). This did not reach statistical significance but warrants further investigation. The simple unvalidated tool was used as a proxy for personal UV exposure; however, the assessment of UV exposure is complex. Other influences such as geophysical factors affecting ambient UV levels (e.g., latitude, altitude, weather, season, time of day) and personal measures affecting skin exposure (e.g., clothing cover, sunscreen use, activity and skin colour) should also be assessed to provide a more reliable proxy for personal UV exposure.

Associations between CVD [22, 40, 44, 52-55], hypertension [3-5, 23, 54, 56], hypercholesterolaemia [5, 23, 53] and diabetes [5, 21-23, 25, 54] and ED have been well documented and our results supported this showing approximately 2-fold increased age-adjusted odds of ED. Prostate and anatomical conditions have been associated with ED in some studies [21, 78, 80, 81] and our results supported this with the odds of ED 2-fold higher amongst these men; however, this was no longer significant after adjusting for age or multiple confounders. Several studies have shown a significant association between ED and endocrine disorders [5, 35, 82-84]. We observed a higher level of ED amongst men with self-reported hypogonadism and the age-adjusted odds were 7-fold higher; however, the number of men

with this condition was small leading to low statistical power to detect a significant difference. Cross-sectional and prospective cohort studies generally support an association between depression and ED [22, 23, 25, 39, 45, 54, 62-64]; however, some studies have found no significant increased risk of ED in depressed men [21, 78, 85]. The MMAS [86] revealed that the presence of depressive symptoms doubled the odds of moderate-complete ED and it remained an independent predictor after controlling for multiple confounders. However, in follow-up results [87], depressive symptoms were not predictive of incident ED. Later studies supported an association [88], adding a high prevalence of ED in men with a history of diagnosed depression and depressive and submissive personalities [22, 89]. Our results showed that although self-reported depression (combined with PTSD and psychiatric disorders) was not associated with ED, when depressive symptomatology was assessed using the PHQ-9, there was a higher prevalence of ED observed amongst men with major depression and increasing severity of depression. The age-adjusted odds remained over 2-fold higher for men with depression, although after adjusting for multiple confounders it was no longer an independent predictor. The relationship is suggested to be complex [90, 91]: it is uncertain whether depression causes or worsens ED, ED causes or worsens depression, or the two conditions are mutually reinforcing. Further research is needed to clarify the relationship and to elucidate the mechanism involved. However, ED is considered a risk factor for depression and this is supported by studies showing that effective treatment of ED results in improved depressive symptoms [92, 93]. Prescription and non-prescription medications have been associated with an increased risk of ED in several studies [5, 21, 22, 26, 36]. We found a significantly higher prevalence of ED in men taking any form of medication and 1.5-fold increased age-adjusted odds of ED; however, it was not a significant independent predictor of ED after adjusting for multiple confounders. It is possible that this is due to the suppressive effect of ED medication and further research is required to clarify this. Supplementation was found to have no significant association on the prevalence of ED in this study, although future research should consider analysis of the effect of the type of supplementation. After adjusting for multiple confounders, hypertension (OR=1.47 [0.94-2.32], p=0.093) and depression (OR=1.98 [0.93-4.21], p=0.077) were the only medical factors observed to increase the odds of ED; however, their predictive contribution did not reach statistical significance.

Our study is a population-based cross-sectional observational study on ED prevalence and risk factors in a large national age-stratified random sample of NZ men aged 40-70 years. The strengths of our study include (i) the large sample size, random selection and use of a population-based national sampling frame to minimise sampling error and selection bias and

maximise generalisability; (ii) the use of established effective survey methods [94] (i.e. efficient survey design, peer-review and piloting; an interesting topic; follow-up contact with reminders and a second copy of the survey; a prize incentive; inferring an obligatory response; university sponsorship; assurance of confidentiality; and the use of stamped return envelopes) to maximise the response rate and minimise participation, survey and response bias; and (iii) the use of the well-validated, sensitive and specific IIEF-5 [28], a short and convenient tool with a low subject burden, to ensure robust data and maximise comparability with other studies. This study provides the most reliable, comprehensive and current information on ED and its risk factors in NZ men. It is in keeping with international studies on self-reported ED and risk factors and expands upon the NZ results of the multinational GSSAB; however, our results are more robust and generalisable than both smaller clinical cohort studies and larger population-based studies which are often limited by sample size, geographical area, population demographics or the method of assessingED.

The limitations of our study include (i) the inability of cross-sectional data to determine cause and effect; (ii) non-sampling errors associated with the use of the Electoral Roll as a population-based sampling frame (i.e., issues of under-coverage, differential coverage, and declining coverage between elections); (iii) the low response rate reducing the effective sample size and increasing the potential for bias; (iv) the inability to assess nonrespondents due to the anonymity and sensitivity of the survey resulting in the questionable assumption that nonresponders would have responded the same as responders; (v) the possibility of men with ED who were sexually inactive either not responding or not completing the IIEF-5, leading to a conservative estimation of the prevalence of ED and its associations; and (vi) the inherent inability to rule out recall bias due to data collection by self-administration and self-report. Electoral roll extracts are arguably the best available population-based sample frame for research in NZ; however, ethnic minorities and lower socioeconomic individuals may be less likely to enrol and may be underrepresented. As electoral enrolment demographics are not collected, this remains speculative. Although comparison with the 2013 NZ Census suggests that our sample is not nationally representative, neither the Census nor the Electoral Roll is likely to be a perfect match for the true population. The respondent profile shows a diverse demographic generally representative of NZ population. The response rate (30%) was comparable to similar published postal surveys on ED including: 41.9% in the WAMHS [1] and 41% in the Multinational Survey of the Aging Male (MSAM-7) [44]. Furthermore, the response rate was higher than the GSSAB [24] which predominately used a CATI to assess ED and obtained a 14% response rate from 191,310 men and women aged 40-80 years in 29 countries

with 13% in Anglophone countries including NZ [27] – the NZ specific response rate was not specified. As response was voluntary, some degree of non-sampling error is inevitable and response rates are often lower with sensitive topics [94]. While self-administered surveys provide a good alternative to disclose sensitive information, offering higher levels of privacy and confidentiality, it has been reported that individual factors (e.g., interest, motivation and available time) and characteristics (e.g., age, ethnicity, intelligence, education and socioeconomic status) affect response rates [95, 96]: older persons, of European ethnicity, intelligent, educated and with lesser social deprivation are most likely to participate in postal surveys on sexual function and this has been supported in our study.

5.0 CONCLUSION

This is the first comprehensive study into the prevalence of ED and its risk factors in NZ and provides the most reliable data currently available. It is evident that ED is highly prevalent and is associated not only with age but also with many potentially modifiable sociodemographic, lifestyle and medical risk factors. It has been suggested that the relative importance of these risk factors varies with age [4] and between ethnic groups [58], highlighting the importance of further research in the NZ population. Despite an arguable increase in the level of public awareness and acceptance of ED since the development of effective oral pharmacological interventions, public knowledge regarding the risk factors and underlying complexity of the disorder remains poor [97]. ED is now recognised as an early marker of vascular dysfunction and CVD in many men [98], highlighting the importance of increasing awareness and encouraging men to seek full medical assessment and appropriate treatment as soon as symptoms persist.

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CHAPTER 4

LITERATURE REVIEW - VITAMIN D AND ITS LINK TO CARDIOVASCULAR DISEASE AND ERECTILE DYSFUNCTION

1.0 INTRODUCTION

Vitamin D is a unique nutrient in that the primary source is usually exposure to ultraviolet radiation (UVR) resulting in the synthesis of vitamin D_3 (cholecalciferol) in the skin [1]. It is a fat-soluble vitamin naturally present in a narrow range of food sources, used in fortified food products and is available as a nutritional supplement. Vitamin D_3 is a steroid hormone and is considered an essential dietary nutrient only in the absence of adequate sunlight exposure and/or skin synthesis. Under normal circumstances, dietary sources (mainly vitamin D_3 and to a lesser extent vitamin D_2 (ergocalciferol) and other vitamin D metabolites) contribute little to vitamin D status. Structural differences between vitamin D_3 and vitamin D_2 lead to altered metabolism and binding to carrier proteins in the body; however, their metabolites appear to have comparable biological activity [2, 3] and together they are generally referred to as vitamin D. Both endogenous and exogenous vitamin D are hydroxylated to 25-hydroxyvitamin D (25(OH)D – including both 25(OH)D_3 and 25(OH)D_2), the recognised functional indicator of vitamin D status.

The most well-known role of vitamin D is in calcium homeostasis, to ensure normal bone mineralisation: it maintains bone growth and bone remodelling by osteoblasts and osteoclasts. Clinical vitamin D deficiency (currently defined as a serum 25(OH)D level <25 nmol/L (10 ng/ml) in New Zealand (NZ) [4] and <30 nmol/L (12 ng/ml) in the United States of America (USA) [5]) leads to thin, brittle, or misshapen bones which can result in rickets in children and osteomalacia in adults [6]. Adequate vitamin D (currently defined as a serum 25(OH)D level ≥50 nmol/L (20 ng/ml) in both NZ [4] and the USA [5]), in combination with calcium, protects against poor skeletal health outcomes. However, studies suggest that levels previously thought adequate for bone health may be insufficient to support extraskeletal health requirements, leading to a proposed adequate serum 25(OH)D level of ≥75 nmol/L (30 ng/ml) [7].

Vitamin D has a plethora of emerging roles beyond skeletal health with insufficiency associated with an increased risk of all-cause mortality [8], mental health issues [9-11], infectious diseases and viral infections [12-14], respiratory disorders [15, 16], autoimmune disorders [17-20], certain cancers [21-24], metabolic disorders [25-30] and cardiovascular disease (CVD) [31-36]. However, although epidemiological evidence supports an association between vitamin D and these conditions, the results of intervention studies investigating the positive health effects of supplementation are largely inconclusive [37, 38], with the exception of bone health [5] and all-cause mortality [39]. The current level of evidence remains insufficient to confirm the causal relationship between vitamin D and non-skeletal diseases.

There is evidence of a worldwide vitamin D deficiency pandemic [40-45], highlighting the importance of research in this field. Improving vitamin D status (≥75 nmol/L (30 ng/ml)) may have the potential to reduce the risk of many chronic illnesses, including CVD [46]. This review will 1) provide an introduction to vitamin D, 2) examine the vitamin D deficiency pandemic and vitamin D status in NZ, 3) examine its established link with CVD, and 4) assess current evidence supporting a proposed link with erectile dysfunction (ED) as an early marker of CVD.

2.1 BACKGROUND

2.2 Sunlight exposure and photo production

The primary source of vitamin D for most humans is skin exposure to UVR [1, 47]. Vitamin D synthesis occurs mainly within the UVB spectrum (280-320 nm) with maximal synthesis at approximately 297 nm [48]. The mechanism of photo production is well understood. UVB photons penetrate the exposed skin surface, converting provitamin D_3 (7-dehydrocholesterol) in the epidermis to previtamin D_3 [49, 50] (Figure 4.1). Located in the plasma membrane between the fatty acid side chains and the polar head groups, pre-vitamin D_3 goes through a heat-dependent isomerisation of its three double bonds to form vitamin D_3. Sterically incompatible, vitamin D_3 is ejected from the plasma membrane into the dermal capillary bed where it is bound to plasma vitamin D binding protein (DBP) for transportation to the liver [51, 52]. In the liver, it is hydroxylated into 25(OH)D, primarily via vitamin D 25-hydroxylase (25-hydroxylase, also known as cytochrome P450 2R1, a cytochrome P450 enzyme encoded by the CYP2R1 gene) [53]. Any pre-vitamin D_3 remains in the skin for further thermo-isomerisation into vitamin D_3, reaching a plateau at approximately 10-15% of the original pro-vitamin D_3 content of the skin [54]. With prolonged solar exposure, pre-vitamin D_3 and vitamin D_3 are degraded to inactive photoproducts [55]. Therefore vitamin D toxicity through sunlight exposure is not a concern [1, 54] and no cases of toxicity through exposure to sunlight or artificial light have been reported.

Vitamin D status is highly variable worldwide. Many environmental factors impact UV levels between geographical locations, personal behaviour affects exposure, and individual skin characteristics affect the ability to synthesise vitamin D.

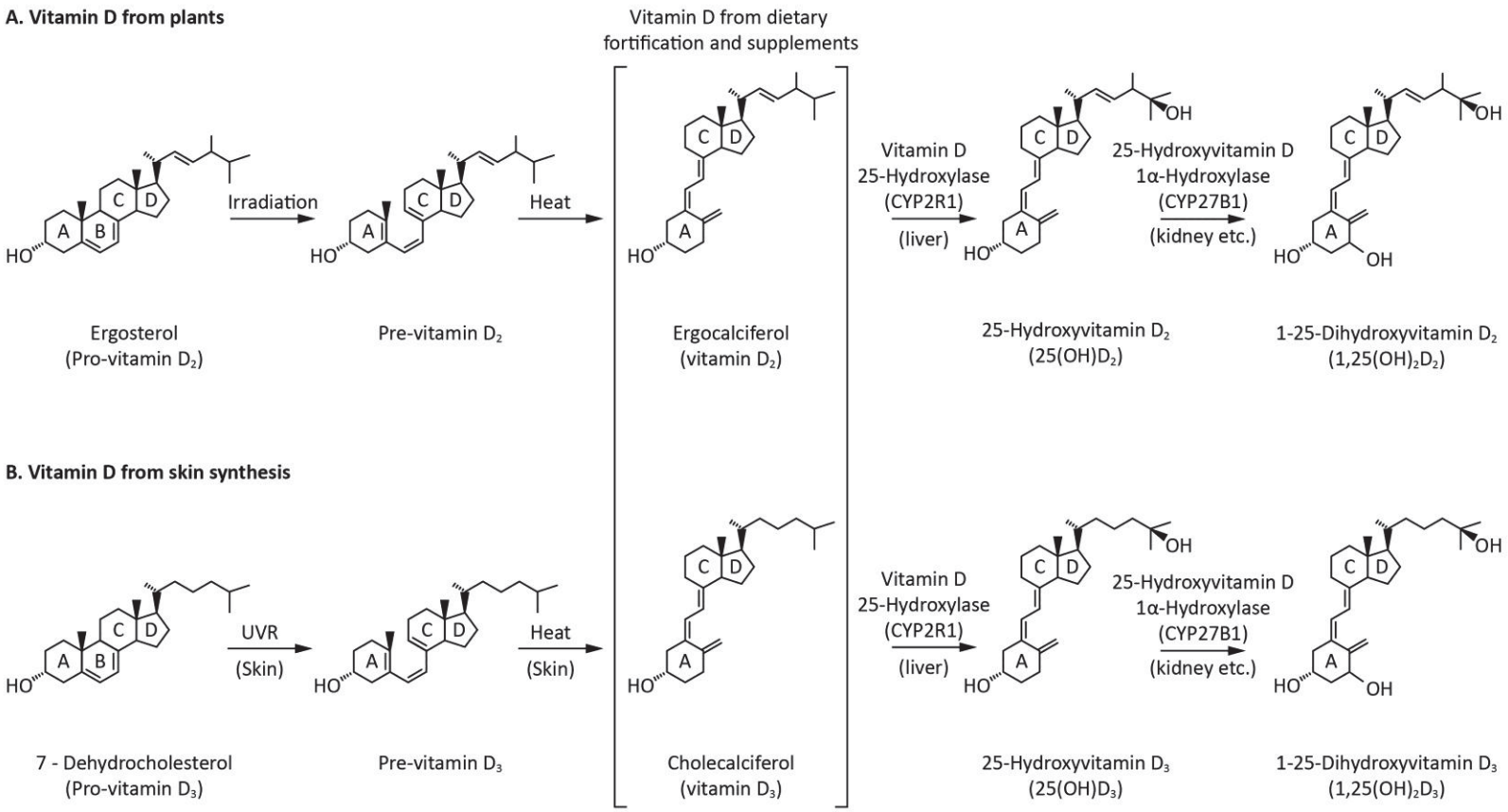

Figure 4.1. The chemical structure of vitamin D_2 and vitamin D_3 including their precursors and main metabolites. Irradiation results in the synthesis of vitamin D_2 (ergocalciferol) in plants from the precursor ergosterol. Ultraviolet radiation (UVR) from sunlight exposure results in the synthesis of vitamin D_3 (cholecalciferol) in the skin from the precursor 7-dehydrocholesterol. Both forms of vitamin D are enzymatically hydroxylated by vitamin D 25-hydroxylase (CYP2R1) to 25-hydroxyvitamin D (25(OH)D), the functional indicator of vitamin D status (which includes both 25-hydroxyvitamin D_2 ($25(OH)D_2$) and 25-hydroxyvitamin D_3 ($25(OH)D_3$). This occurs predominately in the liver. They are further hydroxylated to form 1,25 dihydroxyvitamin D ($1,25(OH)_2D$), the biologically active form of vitamin D (which includes both 1,25 dihydroxyvitamin D_2 ($1,25(OH)_2D_2$) and 1,25 dihydroxyvitamin D_3 ($1,25(OH)_2D_3$). This occurs mainly in the kidneys but also in other tissues containing 25-hydroxyvitamin D 1α-hydroxylase (CYP27B1) (adapted from the Institute of Medicine (US) Committee to Review Dietary Reference Intakes for Vitamin D and Calcium [5]).

2.2.1 Environmental factors affecting photo production

Photo production requires UVB photons to reach the earth's surface and subsequently the skin. The solar zenith angle (SZA) – the angle between vertical and the sun's position at a given time – alters with the earth's orbit and rotation. As it becomes more oblique, UV photons travel a longer path before reaching the surface, increasing interactions with the atmosphere and atmospheric particles (i.e. oxygen, nitrogen, water vapour, aerosols and air pollutants) resulting in greater scattering and absorption of energy [56]. Therefore, vitamin D effective UVR and subsequent photo production of vitamin D_3 vary with latitude, altitude, time of day, season, weather, atmospheric particles and surface reflectivity [56, 57].

Depending on latitude and altitude, it is believed that there is generally sufficient UVB radiation during spring, summer and autumn for vitamin D synthesis to occur [58]. During winter months many people rely on mobilisation of the stores generated throughout the spring to autumn months to maintain vitamin D concentrations [56]. At 0° latitude there appears to be sufficient vitamin D effective UVR all year round, whereas at 40° latitude there is no vitamin D effective UVR for two months of the year (January and December), and at 90° latitude there is no vitamin D effective UVR for 8 months of the year (October – May) [56].

Wellington, the capital city of NZ, lies at a latitude of 41°S and could be assumed to have insufficient UVR for two months of the year. However, the UVR in NZ is approximately 40% greater than equivalent latitudes in the Northern hemisphere due to reduced ozone, lower sun-earth separation and lower pollution levels [59]. Despite its importance, the environmental availability of vitamin D effective UVR is not a consistent predictor of vitamin D status in a population. Even individuals in locations such as NZ, with high levels of vitamin D effective UVR throughout the year, are at risk of vitamin D deficiency due to personal behaviours and individual factors affecting photo production.

2.2.2 Personal behaviours and factors affecting photo production

Mass urbanisation, a reduction in outdoor activity, the use of sunscreen and widespread public health messages to avoid the sun have all affected sunlight exposure. UVB radiation does not penetrate glass [56] making modern indoor lifestyles unconducive to vitamin D synthesis. Furthermore, UVB radiation does not penetrate clothing (depending on weave and colour) [56]. Public health messages promoting the use of protective clothing, religious practices discouraging skin exposure, and the use of sunscreens with a sun protection factor (SPF) over 8 (designed to block UVB rays) lower vitamin D synthesis. An individual may live in a location

with high vitamin D effective UVR exposure year-round but avoidance of outdoor activities and aggressive skin protection may result in low personal UV exposure.

Individual factors such as skin colour and age can also affect photo production of vitamin D_3. Skin pigmentation (melanin) blocks UV radiation and therefore acts as a form of sunscreen. Darker skinned people, such as Maori and Pacific peoples, have higher levels of melanin providing a natural SPF of approximately 8-30. This means they are at a much higher risk of vitamin D deficiency, especially if they live at higher latitudes [60]: they require 5-10 times the sun exposure to synthesise the same amount of vitamin D as a Caucasian in the same location [61], depending on skin colour and environmental factors. Vitamin D deficiency is also common amongst the elderly. In addition to reduced outdoor activity and sun exposure, ageing is associated with a decrease in cutaneous 7-dehydrocholesterol [62]: the same degree of sun exposure in an older person will result in lower vitamin D synthesis than in a younger person. Thus, even in the presence of sufficient unprotected UVB exposure, individual factors impact upon the ability to synthesise vitamin D.

2.2.3 Assessment of sun exposure

The objective measurement and assessment of personal day-to-day UV exposure is complex and must take into account both geophysical factors affecting the availability of vitamin D effective UVR and personal factors affecting skin exposure and photo production. Current measurements (e.g., dosiometers) are best used in conjunction with UV exposure diaries that include geophysical, behavioural and personal measures (i.e. location, weather, activity, clothing cover, sunscreen use and skin colour). The measurement of real-life UV exposure remains a controversial and complex area in vitamin D research.

2.2.4 Recommendations for sun exposure

The importance of sunlight exposure is highlighted by a strong seasonal variation in vitamin D status [1, 47, 63] and evidence that brief sun exposure to one minimal erythemal dose (MED, causing light pinkness without burn 24 hours post exposure) causes a rise in vitamin D status comparable to ingesting a single dose of 10,000-20,000 international units (IU) of supplemental vitamin D [64]. Sunlight exposure may confer additional benefits independent of vitamin D synthesis including maintaining normal circadian rhythms [65], improving mood [66] and reducing blood pressure (BP) [67]. However, assessment of vitamin D requirements cannot currently safely address the level of sun exposure required due to the associated public health concerns.

It is prudent to limit exposure to UV radiation from both natural and artificial sources. However, over the past three decades, there has been widespread avoidance of sunlight together with aggressive skin protection behaviours, promoted by dermatology experts choosing to portray sunlight exposure as threatening [68]. These photo protective measures, such as the use of sunscreen and protective clothing, remain highly recommended by dermatology experts, who encourage people to meet their vitamin D requirements through diet and supplementation [69]. Recently, more moderate messages have begun to appear. In the 2012 Consensus Statement on Vitamin D and Sun Exposure in NZ [4], the Cancer Society, Ministry of Health (MOH) and Accident Compensation Commission (ACC) concluded that a balanced approach towards sun exposure is needed. They recommend that full sun protection is used from September to April between 10 a.m. and 4 p.m., but encourage daily outdoor physical activity (PA) in the early morning or late afternoon. From May to August they recommend daily outdoor PA around midday with face, arms and hands exposed [4].

In contrast, prominent international vitamin D researchers [70] suggest that 5–30 minutes of unprotected sun exposure to the face, arms, legs, or back at least twice a week between 10 a.m. and 3 p.m. is needed to maintain adequate vitamin D status. Furthermore, they suggest that moderate use of commercial sun beds (2%–6% UVB radiation) may also be effective at maintaining adequate levels [70]. However, the use of tanning beds and solaria is not recommended by the Cancer Society of NZ; they emit predominately damaging UVA radiation and are associated with increased risk of early-onset melanoma [4].

2.2. Dietary vitamin D

Dietary intake has a low biological importance in terms of its contribution to maintaining vitamin D concentrations. As cutaneous synthesis provides approximately 80-100% of vitamin D [71], in the presence of adequate sunlight exposure dietary vitamin D may be unnecessary [72]. However, where there is reduced exposure and/or a reduced ability to synthesise vitamin D, such as in the elderly, dietary vitamin D becomes more important.

2.2.1 Food sources

Very few foods naturally contain significant levels of vitamin D, with the exception of oily fish (e.g., salmon, tuna, sardines and mackerel), fish liver oils, beef liver, dairy products, and egg yolks [73]. Vitamin D is generally present as both vitamin D_3 and metabolites in food of animal origin [74]. Low levels of vitamin D_2 are present in plants. Particularly high levels have been reported in both wild and commercially cultivated irradiated mushrooms (8.4-1192.8 IU (0.2-29.8 µg) per 100 g fresh weight) [75].

The vitamin D content of foods can vary widely both between and within countries [73, 76] depending on differences in the methods used to measure vitamin D and in environmental factors such as UV exposure, dietary intake (e.g., farmed salmon has been found to contain only 10-25% of the vitamin D content of wild salmon [77]) and fortification practices. The NZ Food Composition Database (NZFCD) provides information on nutrient values, including the vitamin D content, of approximately 2600 commonly consumed foods [76]. It is widely used to quantify dietary intake in NZ despite well-known issues with the accuracy of the data [78] (see Appendix 4). Table 4.1 provides a comparison of the estimated vitamin D content of rich food sources in NZ [76] and the USA.

Table 4.1. Vitamin D content of selected food sources (adapted from Plant and Food Research [76] and USDA [73]).

	NZ amount per serve IU* (µg)	**USA amount per serve IU* (µg)**
Cod liver oil, 1 tbsp	1160 (29)	1,360 (34)
Salmon, cooked, 100 g	800 (20)	360 (9.0)
Sardines (canned in oil, drained) 50 g	96 (2.4)	250 (6.3)
Tuna fish, canned in oil, drained, 85 g	48 (1.2)	200 (5.0)
Milk, reduced fat (fortified 40-100 IU/100 ml) 1 c	56 (1.4)	100-250 (2.5-6.3)
Egg, 1 large, whole	40 (1.0)	20 (0.5)
Liver, beef, cooked, 100 g	4 (0.1)	15 (0.4)

*IU = International Units (40 IU = 1 µg)

Fortified foods provide the majority of vitamin D in some countries (such as Canada and the USA) due to mandatory fortification of staple foods [79]. Mandatory fortification was widely introduced in the 1930s to combat rickets [80] but was later banned in many countries due to fears of over-fortification following an outbreak of hypercalcaemia [80]. Fortification is voluntary in NZ and is permitted in a limited range of products [81]. In general, NZ margarine (1.0 µg/10 g) and some dairy products such as yoghurt (1.0 µg/150 g) and reduced-fat milk (1.0 µg/200 ml) are often fortified. Imported products (e.g., ready-to-eat breakfast cereals and fruit juice) also often contain added vitamin D. In comparison to Canada where all milk is mandatorily fortified with 35–40 IU/100 ml and the USA where all milk is voluntarily fortified with 40 IU/100 ml, in NZ only reduced–fat milk can be voluntarily fortified with 40 IU/200 ml. Fortification can be a valuable tool to increase dietary intake and the presence of vitamin D fortified foods on the NZ market has risen dramatically over the past decade [82]. However, additional supplementation may be required in some populations.

2.2.2 Supplementation

Those most at risk of vitamin D deficiency: newborns, infants, pregnant women and lactating women, dark-skinned peoples, veiled women and the elderly living in residential care [83], should consider taking a nutritional supplement to maintain adequate levels of vitamin D. A wide variety of supplements is available worldwide and vitamin D is sold commercially either alone or in combination with other vitamins and minerals. Supplements are available in two forms: D_3 (manufactured by irradiation of 7-dehydrocholesterol in lanolin) and D_2 (manufactured by irradiation of ergosterol in yeast). Both effectively raise serum 25(OH)D levels [2, 3]; however, at high doses (50,000 IU) vitamin D_2 may be less effective at raising and maintaining 25(OH)D in humans [84]. It appears to have a markedly lower potency and duration of activity. Most vitamin D supplements in NZ contain vitamin D_3. Studies have confirmed the safety of supplementation of vitamin D_3 at doses up to 10,000 IU/d (250 µg/d) [85-88]. Commercial supplements are generally ≤1,000 IU/d and the clinical standard supplement is 50,000 IU/m, however larger doses up to 500,000 IU [89] are available for research purposes and daily, weekly, monthly, quarterly and annual dose regimes are possible (see Appendix 4). Promoting supplementation in otherwise healthy people is contentious and there is serious doubt concerning the long term safety and efficacy of achieving and maintaining optimal vitamin D status via supplementation [90].

2.2.3 Assessment of dietary intake

Given the lack of accurate and reliable methods currently available to assess and quantify the dietary intake of vitamin D (see Appendix 4), the low contribution of natural dietary sources to vitamin D status, the limited fortification of foods and the availability of year-round vitamin D effective UV exposure in NZ, the assessment of dietary intake may be both unreliable and unproductive. Focusing on the assessment of supplementation, sun exposure and sun protection behaviours may be more physiologically relevant.

2.2.4 Recommendations for dietary intake

Dietary recommendations vary by age and gender and between countries [5, 91](Table 4.2). These recommendations are based on the assumption of minimal solar exposure and are set to avoid negative impacts on bone health and calcium metabolism. However, studies assessing dietary intake of vitamin D have shown that very few adults reach the recommended 5-10 µg/day [83, 92-97]. Indeed intakes in NZ range from 2.0-2.4 µg/d [98]. Moreover, experts are concerned that these recommendations are actually insufficient to maintain adequate serum 25(OH)D concentrations with minimal sunlight exposure [71]. It has been suggested that 100

IU/d of dietary vitamin D results in only a 2.5 nmol/L (1 ng/ml) increase in serum 25(OH)D [2, 88]. Therefore, to raise a baseline serum 25(OH)D level from 25 nmol/L to >50 nmol/L and sustain it would require 1000 IU/d. In NZ this would mean daily consumption of approximately: 1 Tbsp of cod liver oil, 125 g of cooked salmon, 520 g of canned sardines, 1770 g of canned tuna, or 1.8 L of fortified reduced fat milk (based on Table 4.1 [76]). As this is neither practical nor economically feasible for the majority of people, it would be near impossible to obtain adequate vitamin D from natural and fortified food sources without additional supplementation in the presence of restricted sunlight exposure.

Table 4.2. Dietary recommendations for vitamin D in Australia and New Zealand, the USA and Canada.

	Australia and NZ [91]**		**USA and Canada [99]**	
Age	**AI**	**UL**	**RDA**	**UL**
0–12 months	200 IU (5 µg)	1000 IU (25 µg/d)	400 IU (10 µg)*	*0-6 m* 1000 IU (25 µg/d) *7-12 m* 1500 IU (37.5 µg/d)
1–13 years	200 IU (5 µg)	3200 IU (80 µg/d)	600 IU (15 µg)	*1-3 y* 2500 IU (62.5 µg/d) *4-8 y* 3000 IU (75 µg/d) *9-13 y* 4000 IU (100 µg/d)
14–18 years	200 IU (5 µg)	3200 IU (80 µg/d)	600 IU (15 µg)	4000 IU (100 µg/d)
19–50 years	200 IU (5 µg))	3200 IU (80 µg/d)	600 IU (15 µg)	4000 IU (100 µg/d)
51–70 years	400 IU (10 µg)	3200 IU (80 µg/d)	600 IU (15 µg)	4000 IU (100 µg/d)
>70 years	600 IU (15 µg)	3200 IU (80 µg/d)	800 IU (20 µg)	4000 IU (100 µg/d)
Pregnancy	600 IU (15 µg)	3200 IU (80 µg/d)	600 IU (15 µg)	4000 IU (100 µg/d)
Lactation	600 IU (15 µg)	3200 IU (80 µg/d)	600 IU (15 µg)	4000 IU (100 µg/d)

*Figure is for AI only. Intakes provided in both International Units (IUs) and micrograms (µg): 40 IU is equal to 1 µg. RDA, recommended dietary allowance; AI, adequate intake; UL, tolerable upper intake level.

2.3 Vitamin D metabolism and mechanism of action

Endogenous vitamin D_3 (from sun exposure) enters the blood stream via the skin while exogenous vitamin D_3 and D_2 (from food and supplements) is absorbed via the intestinal enterocytes, packaged into chylomicrons and transported via the lymphatic system into the venous circulation. All vitamin D is inert and must undergo bio-activation via two successive stages of hydroxylation (Figure 4.2).

Firstly, vitamin D is transported to the liver where it is hydroxylated into 25-hydroxyvitamin D (25(OH)D), primarily via 25-hydroxylase [53]. This stage is relatively unregulated and 25(OH)D has a long half-life (50-90 days) [100, 101]. Vitamin D metabolites are predominately (90%) bound to DBP for circulation in the blood. This protein is a low affinity, high capacity binder with a higher affinity for 25(OH)D relative to other forms of vitamin D. The remainder is either loosely bound to albumin (10%) or circulating as unbound 25(OH)D (0.1%) [102]. According to the "free hormone hypothesis", these free forms of 25(OH)D are biologically active [103].

Secondly, 25(OH)D is further hydroxylated to form 1,25-dihydroxyvitamin D ($1,25(OH)_2D$ or calcitriol) via 25-hydroxyvitamin D_3 1-alpha-hydroxylase (1α-hydroxylase, also known as

cytochrome p450 27B1, a cytochrome P450 enzyme encoded by the CYP27B1 gene) [104, 105]. Endocrine synthesis of $1,25(OH)_2D$ takes place in the kidneys, however autocrine and paracrine synthesis also take place in a multitude of extra-renal cells containing 1α-hydroxylase. As the active metabolite, $1,25(OH)_2D$ has a short half-life (a few hours) and its concentration in blood is 1000 times lower than 25(OH)D. Its activation is tightly regulated with 1α-hydroxylase activity stimulated by parathyroid hormone (PTH), hypocalcaemia, hypophosphatemia and during high growth stages by sex hormones, prolactin, growth hormones and insulin-like-growth factor 1 (IGF-1) [64] to ensure increased calcium requirements are met. It is inhibited by fibroblast growth factor 23 (FGF23) and $1,25(OH)_2D$ itself which increase the expression of 1,25-dihydroxyvitamin D_3 24-hydroxylase (24-hydroxylase, also known as cytochrome P450 24A1, a cytochrome P450 enzyme encoded by the CYP24A1 gene) resulting in inactive 24-25(OH) metabolites [106].

While the liver and kidneys are the main location for bio-activation, other tissues contain the enzymes required for hydroxylation and can therefore directly activate vitamin D. The action of $1,25(OH)_2D_3$, whether endocrine or autocrine/paracrine, occurs via binding to a vitamin D receptor (VDR) forming a heterodimer complex with the retinoid receptor and acting as a transcription factor to modulate gene expression (see Figure 4.2). This binds to a vitamin D responsive element (VDRE) on a range of responsive genes triggering gene transcription and translation with resultant formation of proteins. The widespread enzymatic capability of activating vitamin D, combined with the presence of the VDR in most organs, tissues and cells throughout the human body [107, 108] supports the wide variety of biological roles for vitamin D.

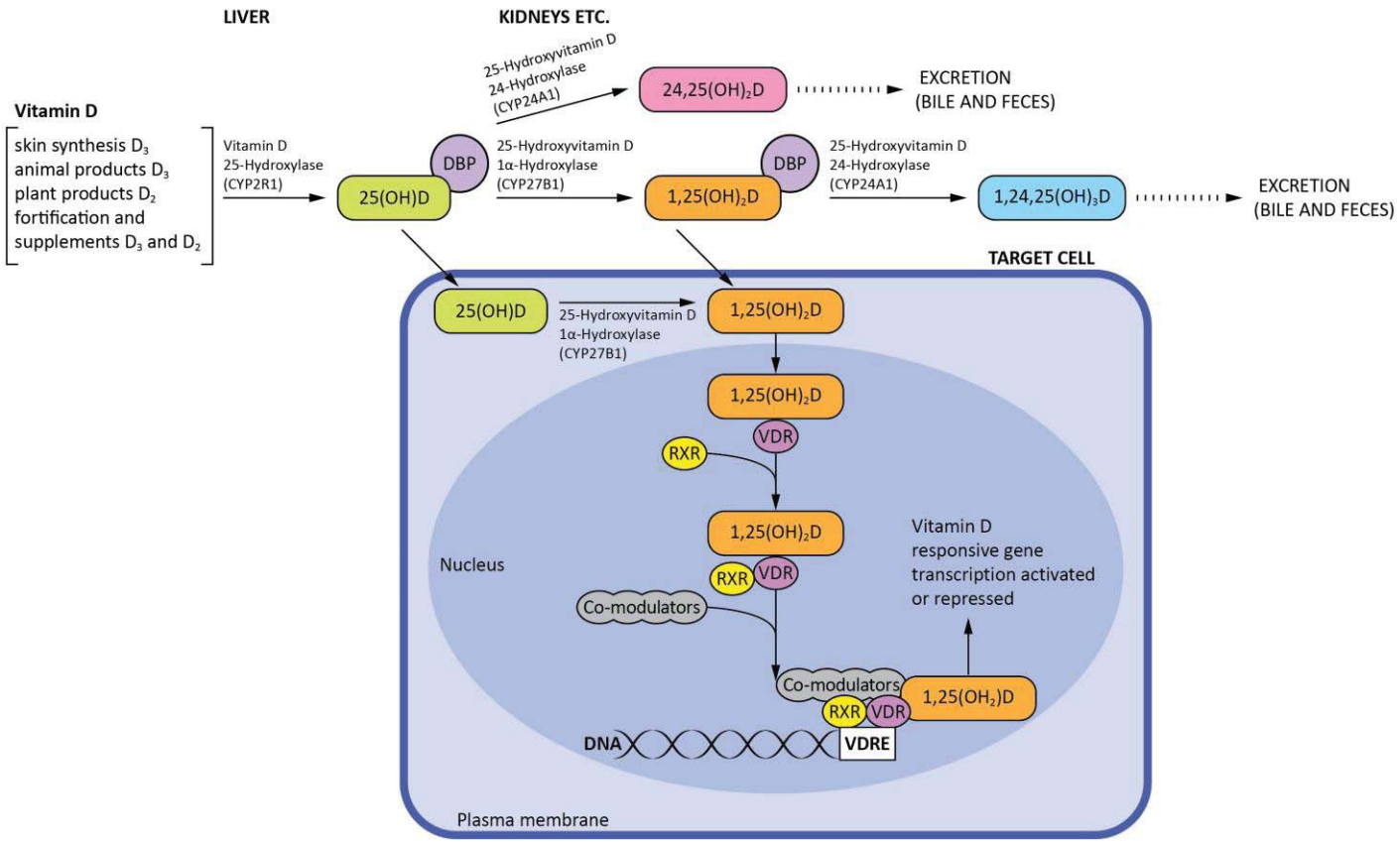

Figure 4.2. A schematic diagram of the metabolism and mechanism of action of vitamin D. The majority of 25-hydroxvitamin D (25(OH)D) and bioactive 1,25-dihydroxvitamin D (1,25,$(OH)_2$D) circulates bound to vitamin D binding protein (DBP). Both bound and free molecules enter the target cell. Local conversion of 25(OH)D to 1,25$(OH)_2$D occurs in cells expressing 25-hydroxyvitamin D 1α-hydroxylase (CYP27B1). Irrespective of renal or local synthesis, 1,25$(OH)_2$D acts via the vitamin D receptor (VDR), binding to the VDR resulting in heterodimerisation with the retinoid X receptor (RXR) after translocating to the nucleus. The VDR–RXR complex binds to the vitamin D response element (VDRE) in various regions of the target gene causing the recruitment of co-modulators. This can either lead to activation or repression of transcriptional regulation in vitamin D responsive genes, thereby altering gene expression. Degradation of vitamin D is via 25-hydroxyvitamin D 24-hydroxylase (CYP24A1) and results in inactive metabolites for excretion in the bile and feces (adapted from Feldman [109] and Institute of Medicine (US) Committee to Review Dietary Reference Intakes for Vitamin D and Calcium [5]).

The long biological half-life of 25(OH)D in the human body suggests a storage capability. Rosenstreich et al [110] supplemented completely deficient rats with radio-labelled vitamin D_3 over a 2 week repletion period before a 10 week deprivation period to investigate the vitamin D content in various tissues and organs. Although all tissues examined (fat, skin, serum, liver, bone, muscle, small intestine, and kidney) contained some radioactivity, adipose tissue contained the greatest quantity of vitamin D and its metabolites. Immediately after supplementation, 50% of radioactivity corresponded to vitamin D_3 and 50% to its metabolites. The total quantity of each form decreased over one month but remained proportional. Levels in adipose tissue were the slowest to decrease and after 6 weeks 80% of all vitamin D was present in adipose tissue [110]. This suggests that adipose tissue serves as a depot to accumulate vitamin D, and that it does so proportionally to its concentration in plasma and releases it at a slower rate than is proportional to its concentration in adipose. The same features may characterise storage and release in humans, suggesting: 1) protection against toxicity in short term overdoses, 2) maintenance of stable plasma concentrations under naturally variable absorption and production conditions, and 3) longer term maintenance of stable plasma concentrations supporting hepatic conversion over periods of reduced intake. However, it has been suggested that vitamin D is sequestered, rather than stored, in adipose tissue [111-113]. Obese individuals have lower plasma 25(OH)D concentrations and are less responsive to increases in vitamin D intake [114]. The contribution of vitamin D stored in adipose and its physiological regulation are unclear. The modern Western obesogenic environment lacks natural seasonal cycles of food availability leading to increased adipose tissue and reduced lipolysis. This may result in sequestration of vitamin D in adipocytes; however there is currently no conclusive evidence supporting this theory. It has also been suggested that the low 25(OH)D levels and reduced response to increased intake in obese individuals is purely a result of dilution due to the larger volume of blood and tissue [111]. This would suggest that supplement doses in intervention studies should be adjusted by body mass.

2.4 Assessment of serum 25(OH)D concentration

The most accurate and integrative indicator of vitamin D status is serum 25(OH)D concentration which reflects both endogenous and exogenous vitamin D and has a long circulating half-life of 50-90 days [100, 101, 115]. It is the most reliable biomarker of vitamin D exposure as it is the predominant form present. Its reliability as a biomarker of health outcomes is unclear [5]. Serum 25(OH)D levels do not provide any indication of the amount of vitamin D stored in body tissues; however, non-hydroxylated vitamin D and

dihydroxymetabolites are present only in relatively low concentrations and cannot be considered reliable biomarkers of vitamin D status. For example, circulating $1,25(OH)_2D$ is generally not considered a reliable indicator of vitamin D status due to its short half-life of 15 hours [115]. Furthermore, serum concentrations are closely regulated by PTH, calcium, and phosphate [115], therefore levels do not decrease until vitamin D deficiency becomes severe [116]. Recent evidence suggests that free 25(OH)D may be more physiologically relevant and a more sensitive biomarker of health status [117], although as it is also produced in cells it is difficult to reliably measure.

Despite the wide acceptance of total 25(OH)D as the functional biomarker of vitamin D status, controversy exists regarding the methods used to measure serum 25(OH)D concentrations. The standard reference method is generally reported as direct UV detection following high performance liquid chromatography (HPLC). It allows individual quantification of $25(OH)D_2$ and $25(OH)D_3$ and is highly accurate and reliable; however, it is expensive, slow and requires large sample volumes. There are several automated chemiluminescence assays frequently used for diagnostic testing [118, 119]. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis has become the preferred method for the accurate quantification of 25(OH)D [120].

The use of a standard reference material [121] enables laboratories to optimise their chosen method and reduce bias to avoid false readings [122]. However, there is wide variability among the results between assays and between laboratories [5, 123, 124]. According to the 2012 Position Statement on Vitamin D and Health in Australia and NZ [125], all NZ laboratories are required to be enrolled with an external scheme such as the international Vitamin D External Quality Assessment Scheme (DEQAS) [126] to monitor 25(OH)D test performance. This should enable laboratories to address performance discrepancies, although the effect of this on method-related variability and imprecision remains unknown [5]. Bias and variability in measurements between both methods and laboratories may be significant enough to misclassify 20-30% of people as deficient [127], affecting clinical decisions. Therefore, serum 25(OH)D levels should be approached with a degree of caution.

2.4.1 Recommendations for serum 25(OH)D concentration

Recommendations for serum 25(OH)D currently focus on the level required to support its primary role in promoting calcium absorption in the intestine to prevent negative bone health outcomes. The current clinical recommendations for serum 25(OH)D levels in the USA and NZ are provided in Table 4.3, alongside those proposed by the Endocrine Society [7]. Both the USA Institute of Medicine in 2011 [5] and the Consensus Statement on Vitamin D and Sun Exposure in NZ in 2012 [4] recommended that 50 nmol/L (20 ng/ml) is adequate to cover the needs of

the majority of the population and support skeletal health, and that >125 nmol/L (50 ng/ml) is associated with potential adverse effects.

The high prevalence of vitamin D insufficiency worldwide and the emerging roles of vitamin D beyond skeletal health have led to debate regarding the current recommendations. Discussion continues over the level of serum 25(OH)D associated with deficiency symptoms, adequacy and optimal health and no consensus has been reached on the recommended cut-off points [128-134]. Studies have begun to show health benefits of a serum 25(OH)D level ≥75nmol/L and the Endocrine Society practice guidelines define adequate as ≥75 nmol/L [7].

Table 4.3. Serum 25-hydroxyvitamin D (25(OH)D) recommendations and the associated health status

USA 25(OH)D recommendations [5]	NZ 25(OH)D recommendations [4]	Endocrine Society 25(OH)D recommendations [7]	Health Status
<30 nmol/L (<12 ng/ml)	<25 nmol/L (<10 ng/ml)	<50 nmol/L (<20 ng/ml)	Associated with deficiency, rickets (infants/children) and osteomalacia (adults)
30–50 nmol/L (12–20 ng/ml)	25–50 nmol/L (10–20 ng/ml)	50-75 nmol/L (20-30 ng/ml)	Associated with inadequacy, poor bone and overall health in healthy people
≥50 nmol/L (≥20 ng/ml)	≥50 nmol/L (≥20 ng/ml)	>75 nmol/L (≥30 ng/ml)	Considered adequate for bone and overall health in healthy people
>125 nmol/L (>50 ng/ml)	>125nmol/L (>50 ng/ml)	>150 nmol/L (>60 ng/ml)	Evidence suggestive of potential adverse effects, particularly >150 nmol/L (>60 ng/ml)

* Serum concentrations of 25(OH)D are reported in both nanomoles per litre (nmol/L) and nanograms per millilitre (ng/ml) in line with international recommendations: 1 nmol/L = 0.4 ng/ml

Toxicity has been shown in adults only after long-term supplementation with extremely high doses (>50,000 IU/d) and is characterised by hypercalcaemia and hypercalcuria with calcification of soft tissues [135]. Vieth [136] proposed that this was primarily due to the high levels of circulating bioactive 1,25(OH)D, not 25(OH)D per se. Indeed, no adverse effects have been reported in populations with high levels of 25(OH)D due to occupational or recreational sun exposure including outdoor workers such as lifeguards (163 nmol/L) [137] and nomadic African tribes (115 nmol/L) [138]. Nor has toxicity been reported in people using tanning beds (95 nmol/L) [139]. Current recommendations for the upper limit of 25(OH)D appear to be set based on perceived risk of toxicity, without any evidence of adverse effects.

3.1 THE VITAMIN D DEFICIENCY PANDEMIC

In many countries there has been an increase in the prevalence of suboptimal vitamin D status in the past decade; including a resurgence of childhood rickets thought of as an eradicated disease [40, 140, 141]. Risk factors for suboptimal vitamin D status are limited exposure to sunlight, chronic sun protective measures, dark skin, older age, obesity, restrictive diets, maldigestion or malabsorption, cholestasis, poor kidney function or kidney disease and certain drugs [142]. The apparent pandemic may be partially due to higher reporting rates resulting from improved accuracy in the measurement of 25(OH)D and changes to the cut-off points used to define deficiency and insufficiency; however, it appears that no one is immune to suboptimal vitamin D status with high rates reported across all age groups worldwide [128, 143] including the USA [70, 144-146], Europe [147], the Middle East [148, 149], India [150], Asia [151, 152], Australia [153, 154] and NZ [40, 42]

3.2 Vitamin D status in New Zealand

In the most recent 2008/2009 NZ Adult Nutrition Survey (NSANS) [155] (n=3099, age ≥15 years, 44% men), the mean level of 25(OH)D in adults was 63.0 nmol/L: 62.4 nmol/L in women and 63.6 nmol/L in men. There was a 32% crude prevalence of vitamin D insufficiency or deficiency (<50 nmol/L) (Table 4.4). Data using the proposed level of <75 nmol/L to define insufficiency were not provided, however the mean 25(OH)D level remains well below this. In comparison, the earlier 1997 NZ National Nutrition Survey (NZNNS) [44] (n=2946, age ≥15 years, 46% men) found a mean serum 25(OH)D level of 50 nmol/L: 47 nmol/L in women and 52 nmol/L in men. There was a 48% crude prevalence of vitamin D insufficiency or deficiency (<50 nmol/L): 52% in women and 45% in men. There was an 84% crude prevalence insufficiency or deficiency based on a proposed level of <80 nmol/L [7]: 86% in women and 82% in men. Data on the prevalence based on a cut-off of <75 nmol/L were not provided. It is unclear whether the apparent improvement in vitamin D status in NZ adults from 1997 to 2008/2009 is real or due to changes in the method used to measure 25(OH)D: radioimmunoassay was used in 1997 and HPLC tandem mass spectrometry in 2008/2009.

Table 4.4. Vitamin D status of New Zealand adults (≥15 years) in 2008/2009 shown as crude prevalence rates (%) with 95% confidence intervals (adapted from MOH [155])

Category	Serum 25-OHD level	Prevalence (%)
Deficient	<25 nmol/L	4.9 [4.0–5.9]
Severely deficient	<12.5 nmol/L	0.2 [0.1–0.5]
Mild to moderately deficient	12.5 - 25 nmol/L	4.6 [3.8–5.7]
Insufficient	25-50 nmol/L	27.1 [24.7–29.5]
Adequate	≥50 nmol/L	68.1 [65.6–70.5]
High	≥125 nmol/L	1.7 [1.0–2.8]

The 2008/2009 NZANS [155] found no significant differences in vitamin D status by age or gender. This contrasts with earlier results from the 1997 NZNNS [44] which found a significant association between increasing age and lower serum 25(OH)D level in women.

Maori had a mean annual serum 25(OH)D concentration of 59.4 nmol/L with a 39.7% prevalence of deficiency or insufficiency (<50 nmol/L) [155]. After age-adjustment, Maori had a significantly higher likelihood of vitamin D insufficiency (RR=1.26 [1.06-1.51]) compared to non-Maori. Pacific peoples had a mean annual serum 25(OH)D concentration of 47.9 nmol/L and a 57.1% prevalence of deficiency or insufficiency (<50 nmol/L). After age-adjustment, Pacific peoples had a 2-fold increase in the likelihood of deficiency (RR=2.32 [1.56-3.46]) and a 77% higher likelihood of insufficiency (RR=1.77 [1.55-2.01]) compared to non-Pacific peoples. This is consistent with the 1997 NZNNS [44] which showed that after adjusting for multiple confounders (ethnicity, age, season, region and BMI category), mean 25(OH)D levels were significantly lower in both Maori and Pacific peoples compared to NZ Europeans (both $p<0.01$).

The highest level of socioeconomic deprivation (NZDep2006 quintile 5) was associated with a lower mean annual serum 25(OH)D concentration compared to the lowest level (56.6 nmol/L vs 69.9 nmol/L) [155]. After adjusting for age, gender and ethnicity, NZDep2006 quintile 5 was associated a 3-fold increase in the likelihood of deficiency (RR=3.15 [1.30-7.64]) and a 66% higher likelihood of insufficiency (RR=1.66 [1.16-2.39]) compared to NZDep2006 quintile 1. Socioeconomic status was not included in the reports from the 1997 NZNNS [44].

The mean annual serum 25(OH)D concentration was lower in obese individuals (BMI ≥30.0 kg/m^2) compared to normal weight individuals (57.0 nmol/L vs 66.3 nmol/L) [155]. After adjusting for age, gender and ethnicity there were no significant differences in the likelihood of deficiency (RR=1.27 [0.68-2.38]) or insufficiency (RR=1.18 [0.95-1.45]). In the 1997 NZNNS [44], individuals categorised as obese (BMI ≥30.0 kg/m^2 in NZ Europeans and ≥32.0 kg/m^2 in Maori and Pacific peoples) compared to overweight or healthy weight by BMI was associated with a lower 25(OH)D level in women ($p<0.01$) but not in men.

The regions of NZ were divided into southern, central and northern based on latitude [155]. There was a lower mean annual serum 25(OH)D concentration in people living in the southern and central regions compared to the northern region (60.5 (56.1-65.0) vs 62.6 (60.8-64.4) vs 65.1 (62.2-68.0) nmol/L respectively). After adjusting for age, sex and ethnicity, people in the southern region were observed to have an 86% higher likelihood of vitamin D deficiency, (RR=1.86 [0.97-3.56)) although this did not reach statistical significance. This is consistent with

the 1997 NZNNS [44] which showed that after adjusting for multiple confounders (ethnicity, age, season, region and BMI category), living in the South Island compared to the North Island was associated with a lower serum 25(OH)D level in women ($p<0.01$).

The 2008/2009 NZANS showed that levels of vitamin D deficiency (<25 nmol/L) were lowest in January-March and peaked in August-October, which reflects reduced sunlight exposure and depletion of serum 25(OH)D stores [155]. The prevalence of deficiency over the August-October months was highest in the southern region and after adjusting for age, sex and ethnicity, people in the southern region had a 3-fold increase in the likelihood of vitamin D deficiency in spring (August-October) compared to those living in the northern regions (RR=3.11 [1.33-7.28]) but no significant increase in the likelihood of insufficiency. This suggests a compounding effect of season and latitude on sunlight exposure. Indeed, in the 1997 NZNNS [44] season was an important determinant in both genders with 25(OH)D levels reportedly lowest in spring (September-November) and highest in summer (December-February), varying by 31 nmol/L in women and 28 nmol/l in men (both p for trend <0.001). In men, the lowest 25(OH)D level was 39 nmol/L in spring (September) and the highest level was 87 nmol/L in summer (January), indicating a strong seasonal variation (48 nmol/L). This is consistent with other populations [64] and suggests that UV exposure is the primary determinant of 25(OH)D levels in NZ.

At a latitude of 34-47°S, NZ has a peak UVI approximately 40% greater than equivalent latitudes in the Northern hemisphere [59], leading to an assumption that the population must obtain sufficient sunlight exposure to maintain adequate vitamin D status [156]. However, while there is sufficient noon UVR throughout the year in Wellington to support vitamin D synthesis, the daily UV index (UVI) can be higher than the level recommended to avoid an increased risk of skin damage ($1\leq$ UVI ≥ 3) [157, 158]. This limits the opportunities to safely synthesise vitamin D. Optimal safe exposure is restricted to mornings (6.30-8.30 a.m.) and evenings (4.30-6.30 p.m.) in summer but includes midday exposure (9 a.m.-4 p.m.) in winter [157]. Data from observational studies indicate that deficiency and insufficiency are widespread within NZ [44, 155], from newborns to the elderly [40-45]. This has been linked to chronic sun protection [159], increasingly sedentary indoor lifestyles and obesity levels [160]. Furthermore, a large portion of the population has darker skin (i.e., Maori and Pacific peoples) increasing the risk of low vitamin D status. Although a 2014 report for the NZ Ministry of Primary Industries [78] identified that dairy products (34.3%), margarine (28.2%), fish (20.8%) and eggs (6.6%) account for 90% of dietary vitamin D intake in NZ men, rich natural sources of dietary vitamin D (e.g., fatty fish and organ meats) are not regularly consumed by New

Zealanders [161] and fortification is voluntary and allowed only in a narrow range of foods [81]. Use of vitamin D supplements appears to be around 30% in adult men over 40 years of age, with a mean intake of 226 IU/day [162]. The 5% prevalence of deficiency alone is concerning, given the serious implications for bone health; however, the additional 27% prevalence of insufficiency is equally concerning due to the emerging research indicating an increased risk of chronic diseases.

4.1 VITAMIN D AND DETERMINANTS OF HEALTH

4.2 Skeletal health

It is well established that clinical vitamin D deficiency causes impaired bone mineralisation resulting in rickets in children and osteomalacia in adults. Characteristics include: impeded growth, soft and deformed bones, bowed legs, bending of the spine, proximal muscle weakness, bone fragility and increased risk of falls and fractures [163, 164]. Less severe deficiency is associated with poor calcium absorption, secondary hyperparathyroidism, bone loss, low bone mineral density (BMD), osteoporotic fracture and increased risk of falls [64, 165]. Bone strength responds to increased muscle load, a relationship modulated by the growth hormone and insulin-like growth factor 1 axis (GH-IGF-1), sex steroids and vitamin D [166]. A functional loop exists; low vitamin D status may lead to functional impairments (sarcopenia, gait and balance issues, and muscle weakness), reduced bone strength (lower bone mass, bone mineral density and skeletal frailty) and therefore increased risk of fractures from falls [167]. Supplementation has been shown to be effective in most clinical trials with bone health as the primary outcome [168-170] and the mechanism behind the effects of vitamin D in bone health is well understood [64, 171, 172].

4.3 Non-skeletal health

Over the past decade, researchers have begun to focus on the role of vitamin D beyond calcium homeostasis: neuromuscular and immunomodulation [173, 174]; the modulation of cell growth, proliferation, differentiation and apoptosis [5]; fetal programming and gene regulation [46]; and renal production, insulin secretion and inflammation [5]. Once the need for vitamin D in the maintenance of calcium homeostasis (essential to short-term survival) is met, excess vitamin D is used by other cells and tissues in the body to support optimal health (essential to long-term survival). Observational studies support an association between low vitamin D status and increased risk of a range of negative non-skeletal health outcomes including CVD [31-36] and its risk factors [25-30].

4.3.1 Vitamin D and cardiovascular health

4.3.1.1 Epidemiological evidence

Observational evidence consistently supports a link between low vitamin D status and increased cardiovascular risk and adverse cardiovascular health outcomes. Early ecological studies reported higher rates of CVD and CVD risk factors with increasing latitude [175-177], a factor associated with lower vitamin D effective UVR exposure. More recently, large cohort studies have confirmed that CVD is more prevalent among subjects with low levels of 25(OH)D (<37.5 nmol/l (<15 ng/ml) [32] and <75 nmol/L (<30 ng/mL) [178]. The rate of cardiovascular events has been shown to be 60% higher in individuals with a 25(OH)D level <37.5 nmol/l (15 ng/mL) (HR=1.62 [1.11-2.36], p=0.01) compared with those with a level >37.5 nmol/l (15 ng/mL) [32]. Furthermore, a recent longitudinal study (2002-2012) of 946 participants with stable CVD [179] found that the rate of secondary cardiovascular events was 30% higher when 25(OH)D levels were <50 nmol/L (20 ng/ml) (HR=1.30 [1.01-1.67]) compared to >50 nmol/L (20 ng/mL) [179] after adjustment for sociodemographic variables, season, health behaviours and comorbidities. Low vitamin D status is also an independent predictor of increased risk of CVD-related mortality [34]. Ginde et al [34] found that after adjusting for demographics, season and CVD risk factors, the risk of CVD-related mortality remained over 2-fold higher in American adults over 65 years of age in the NHANES III (n=3408, median follow-up 7.3 years, median baseline 25(OH)D level = 66 nmol/L, 767 CVD-related deaths) with a 25(OH)D level of <25 nmol/L (10 ng/ml) (HR=2.36 [1.17-4.75]) compared to a level of ≥100 nmol/L (40 ng/ml).

There is also a strong association between low vitamin D status and CVD risk factors (T2DM, obesity, dyslipidaemia and hypertension) [180-182], and vitamin D deficiency is associated with increased risk of CVD even after adjusting for these risk factors [34, 183]. In a large prospective observational study [180] of general healthcare patients (n=41,504, mean age = 55 years, 25% men), both vitamin D deficiency (<37.5 nmol/L (15 ng/ml) and hypovitaminosis D (37.5-75 nmol/L (15-30 ng/ml) were highly prevalent in 16.7% and 46.9% of participants respectively. Hypovitaminosis D and deficiency were highly significantly inversely associated with an increased prevalence of CVD risk factors (T2DM, hyperlipidaemia, hypertension and peripheral vascular disease (all p<0.0001). Furthermore, deficiency in the absence of CVD risk factors still increased the likelihood of developing T2DM, hyperlipidaemia and hypertension, and vitamin D levels remained significantly associated with an increased risk of incident CVD events (CAD, MI, HF and stroke (all p<0.0001), incident death (p<0.0001) and incident adverse events (p<0.0001)).

Observational evidence supports an association between low vitamin D status and type II diabetes [25-27], obesity [28, 29] and the metabolic syndrome (MetS) [29, 30]. Cross-sectional studies report that people with low 25(OH)D levels have raised blood glucose and glycated haemoglobin (HbA1c) levels [184] and higher levels of insulin resistance and beta cell dysfunction [185, 186]. Furthermore, prospective cohort studies show these individuals have a higher risk of developing T2DM [187, 188]. A 2012 meta-analysis [189] of 11 prospective studies (n=3612 cases, 55713 controls) found a strong inverse association between serum 25(OH)D level and incident T2DM: the risk of developing T2DM was 41% lower (RR=0.59 [0.52-0.67]) in the highest quartile of 25(OH)D (>80 nmol/L) compared to the lowest quartile (<50nmol/L). Similarly, a 2013 prospective cohort study (n=9841, follow-up = 29 years) and meta-analysis [188] found that after adjusting for sex, age, smoking, BMI, income, PA, high-density lipoprotein cholesterol (HDL-c) and month of assessment, lower 25(OH)D levels (based on quartiles) were independently associated with a higher incidence of T2DM in the Copenhagen City Heart Study in Denmark. The first (lowest), second and third quartile were associated with 35% (HR=1.35 [1.09-1.66]), 26% (HR=1.26 [1.02-1.55]) and 10% (HR=1.10 [0.88-1.37]) higher risk of incidence T2DM respectively. In the meta-analyses, 14 studies were included (n=72,204) and people in the lowest quartile of 25(OH)D had a 50% higher risk of developing T2DM (OR=1.50 [1.33-1.66]) compared to those in the top quartile, with no evidence of significant heterogeneity or publication bias. These meta-analyses provide strong evidence to support the association between low vitamin D status and T2DM.

Further support comes from the reported association between low vitamin D status and obesity. Vitamin D deficiency (<50 nmol/L) and vitamin D insufficiency (<75 nmol/L) are highly prevalent in obese subjects [190, 191], particularly among the morbidly obese [192] and 25(OH)D levels are significantly lower in obese people compared to non-obese people across a range of ages [181, 193]. One researcher has hypothesised that low vitamin D is actually the cause of common obesity [194]. Vitamin D deficiency has been associated with visceral adiposity in some studies [195, 196]. In one cross-sectional study [28] in Germany (n=131 men and women, aged 66-96 years) total body fat, measured using bioelectrical impedance analysis (BIA), BMI, and hip circumference (HC) were all significant negative predictors of 25(OH)D levels in women, and total body fat remained an independent predictor after controlling for age, lifestyle factors and PTH. Other studies have supported an association between vitamin D deficiency and abdominal obesity [197]. Amongst 276 healthy premenopausal obese women without T2DM or MetS, 25(OH)D levels were significantly negatively correlated with BMI (r=-0.480, p<0.0001), waist circumference (WC, r=-0.480, p<0.0001) and waist-to-hip ratio (WHR,

r=-0.312, p<0.05) and deficiency was significantly associated with a higher BMI, WC and WHR and also category of obesity using BMI and abdominal obesity using WC and WHR [197]. This supports the proposed sequestration of vitamin D in body fat; however, as obesity is also associated with inflammation, it is also possible that the vitamin D in obese individuals is being utilised to reduce inflammation. A recent meta-analysis [198] of 7 cross-sectional and 8 case-control studies (n=3867 obese, n=9342 non-obese) found that 9 studies reported a significant association between vitamin D deficiency (definitions ranged from 25 nmol/L to 75 nmol/L) and obesity and, in pooled results, the risk of vitamin D deficiency was over 3-times higher in obese people (OR=3.43 [2.33-5.06]) compared to non-obese people, irrespective of geographical location.

Vitamin D deficiency and insufficiency are more prevalent in people with MetS and 25(OH)D levels have been found to be significantly lower in adults with MetS and/or its components (abdominal obesity, elevated BP, high glucose, low HDL-c, and high TG concentrations) [30, 199]. In 2013, Lee at al [195] reported that in Korean children (n=1660, 904 boys, 756 girls, age = 9 years) mean 25(OH)D levels were significantly lower in those who had MetS compared to those who did not (16.7±4.1 ng/ml vs 18.9±5.0 ng/ml, p<0.001). The risk of MetS increased significantly as the quartile of 25(OH)D decreased (Q4 referent, Q3 OR=2.6 [1.08-6.30], Q2 OR=4.00 [1.73-9.26], Q1 OR=4.25 [1.84-9.85], p for trend <0.05). However, while most epidemiological studies show a relationship between serum 25(OH)D and MetS and/or its components, particularly amongst adults [200, 201] and those over 40 years [202-205], the results are inconsistent [206-208]. In 2015, Bea et al [209] reported that higher concentrations of both 25(OH)D and $1,25(OH)_2D$ were significantly associated with a lower risk of MetS and its components, particularly triglyceride (TG) levels. Furthermore, higher concentrations of these were associated with a lower risk of abdominal adiposity and lower HDL-c respectively. This study concurred with the earlier studies with respect to 25(OH)D but provides important new information suggesting that $1,25(OH)_2D$ may play an important role in the transcriptional regulation of genes involved in metabolic dysregulation [210]. To our knowledge, only one prospective cohort study has investigated vitamin D and MetS. Fung et al [211] investigated the effect of dietary and supplementary vitamin D intake (measured using a diet history covering consumption of 100 food and beverages over the past 30 days) on the incidence of MetS (defined by the Adult Treatment Panel III (ATP III) criteria) and the prevalence of its components in 4727 young black and white adults from the Coronary Artery Risk Development in Young Adults (CARDIA) cohort in multiple centers in the USA. The dietary intakes were as follows: Q1 0.22-3.70, Q2 3.71-6.09, Q3 6.10-8.99, Q4 9.0-13.5, Q5 >13.5 ug/d. After adjusting

for sociodemographic factors (age, race, gender, education, centre) and energy intake, vitamin D intake was inversely associated with the cumulative prevalence of abdominal obesity, high glucose and low HDL-c. After additional adjustment for smoking, PA, alcohol, dietary and supplemental calcium intake, there was also significantly lower risk of incident MetS as vitamin D intake quintile increased (Q1, referent, Q2 HR=0.81 [0.66-1.00], Q3 HR=0.83 [0.67-1.02], Q4 HR=0.69 [0.54-0.86], Q5 HR=0.77 [0.60-1.00], p for trend = 0.02). The prevalence of MetS was higher in the lowest quintile compared to the highest quintile over the 20 years (208 vs 168 cases). Serum 25(OH)D concentrations were measured in a subsample of 402 black and white, male and female participants aged 25-36 years. There was a weak but significant correlation between dietary vitamin D intake and serum 25(OH)D concentrations (r=0.13, p=0.016) and the results of validation to multiple 24-HDR were not provided [211]. The epidemiological evidence to date suggests that vitamin D plays an important role in metabolic function.

Vitamin D appears to be associated with measurements of endothelial health and arterial stiffness. In 2012, a cross-sectional observational study [212] in Korean adults with T2DM (n=305, mean age = 54 years, 43% men, mean baseline 25(OH)D level = 28 nmol/L (11 ng/ml)) found a high prevalence of vitamin D deficiency (86% serum 25(OH)D <50 nmol/L (20 ng/ml)) and insufficiency (11% serum 25(OH)D 50-73 nmol/L (20-30 ng/ml)) and that arterial stiffness (assessed by Pulse Wave Velocity (PWV) between the brachial and ankle arteries) was significantly greater in individuals with a serum 25(OH)D level <50 nmol/L (20 ng/ml)) compared to those with a level ≥50 nmol/L (20 ng/ml) (42.21±82.76 vs 26.84±21.79 $cm.s^{-1}$, p<0.05). Furthermore, the duration of hypertension (6.57±8.07 vs 1.37±3.09 years, p<0.05) and levels of total cholesterol (TC, 186.14±42.38 vs 163.78±38.25 mg/dl, p<0.05), TG (1869.71±128.98 vs 143.63±63.99 mg/dl, p<0.05), low-density lipoprotein cholesterol (LDL-c, 107.03±35.42 vs 105.40±35.45 mg/dl, p<0.05) and HbA1c (4.61±1.10 vs 4.58±1.09 %, p<0.05) were all significantly higher in the group with serum 25(OH)D levels <50 nmol/L (20 ng/ml)), indicating higher cardiovascular risk; however, there was no difference in other vascular health measurements (ankle-brachial index (ABI), carotid intima thickness (IMT), or either systolic or diastolic BP), BMI, duration of diabetes, serum creatinine, albumin, calcium, or phosphate, HDL-c or the inflammatory marker C-reactive protein (CRP, all p>0.05). Serum 25(OH)D concentrations were inversely correlated with PWV (r=-0.303, p<0.01) and serum 25(OH)D level remained a significant independent predictor of PWV (p<0.001) after adjusting for all the other variables measured.

Vitamin D plays a role in muscle strength and physical performance [5, 213]. Observational studies report a positive correlation between 25(OH)D status and muscle function and

deficiency is associated with reduced muscle mass and muscle strength resulting in sarcopenia [214-218]. Evidence for the relationship between vitamin D and muscle strength is particularly strong amongst the elderly: low 25(OH)D is associated with lower grip strength and muscle mass amongst adults over 65 years of age [219]. Adequate vitamin D may also help improve muscle strength in healthy adults [220]. Higher 25(OH)D status has recently been associated with higher handgrip strength in middle-aged healthy adults in the Netherlands (n=802, age range = 40-80 years) [221] and the association was most pronounced below a threshold of 60 nmol/L where handgrip strength increased 0.09 [40.8-118.4] kg per nmol/L ($p<0.01$). A recent systematic review by Redzic et al [222] found 10 studies that directly investigated the relationship between vitamin D status and muscle strength in healthy adults. Five studies reported a significant positive linear relationship between serum 25(OH)D levels and muscle strength and overall there was a moderate-strong effect size indicating a positive relationship between higher 25(OH)D and increased muscle strength. Handgrip strength was the most common method used to test muscle strength, although different equipment and techniques may contribute towards variability in outcomes. Vitamin D supplementation at doses ≥800 IU/d with [223, 224] or without [225] co-administration of calcium has been shown to significantly improve muscle strength in older adults. Studies at lower doses have shown no significant effect of supplementation on handgrip strength [226].

Vitamin D may also be related to maximal oxygen consumption (VO_2max) [227], the gold standard assessment of cardiorespiratory fitness; however, few studies have investigated this relationship. Positive associations have been reported between serum 25(OH)D concentrations and VO_2max in younger adults [228, 229]. Ardestani et al [228] reported that VO_2max, measured using a graded treadmill protocol with metabolic gas analysis in healthy adults (n=200, 46% men), was positively associated with serum 25(OH)D concentration ($r=0.29$, $p=0.0001$) independent of age, gender and BMI. However, there was an interaction with self-reported participation in moderate to vigorous PA: the effect was greatest in adults with low (16 h/wk, $p=0.0001$) or moderate levels of PA (35 h/wk, $p=0.0004$) and was no longer significant with high levels of PA (>64 h/wk, $p=0.900$). Furthermore, both serum 25(OH)D and VO_2max decline with age [230] and a 2014 observational study [231] reported that after adjusting for body fat percentage, serum 25(OH)D concentration was also positively associated with VO_2max ($r=0.316$, $p=0.010$) in 67 healthy but sedentary older women aged 60-74 years. However, both serum 25(OH)D and VO_2max were significantly lower amongst African American women and on further analysis, the association was significant only amongst African American ($r=0.727$, $p=0.005$), not European American women ($r=0.064$, $p=0.643$).

Low vitamin D status appears to be an independent risk factor for the development of CVD [31, 64]. Although existing epidemiological evidence is promising, it is difficult to isolate the effects of vitamin D from the confounding effects of other nutrient and non-nutrient factors. Furthermore, observational evidence does not prove causation; indeed the association may be a case of reverse causation, whereby people at risk of CVD are also less likely to go outside and thus have lower sunlight exposure [232].

4.3.1.2 Intervention evidence

Recent meta-analyses examining the risk of all-cause mortality in participants involved in vitamin D RCTs found minimal heterogeneity between studies and that vitamin D appears to decrease the relative risk (RR) of all-cause mortality (RR ranging from 0.93 [233] to 0.97 [39]). However, meta-analyses investigating the effects of vitamin D supplementation specifically on cardiometabolic outcomes show the results of relevant RCT are relatively homogenous but unclear and inconclusive [36, 234, 235]. A systematic review of two relevant RCTs found a moderate but statistically insignificant 10% reduction in CVD risk (pooled RR=0.90 [0.77-1.05]) in intervention groups supplemented with 1000 IU/d [236]. The 2011 report on Dietary Reference Intakes for Calcium and Vitamin D by the Institute of Medicine (IOM) controversially summarised the evidence of nonskeletal benefits including CVD as "inconsistent and/or conflicting or did not demonstrate causality" [5]. Most recently, Bolland et al [237] conducted a meta-analysis of the effect of vitamin D supplementation (with or without calcium) on vascular outcomes and concluded that supplementation does not meaningfully reduce (>15%) the RR of any CVD outcomes (MI or ischemic heart disease (9 trials, 48 647 patients), stroke or cerebrovascular disease (8 trials, 46 431 patients)). However, as CVD is a complex cluster of diseases it is likely that vitamin D would be most effective in the early stages of its development in preventing or delaying vascular deterioration. Therefore, not only the dose and duration of supplementation and baseline 25(OH)D levels, but also the timing of the intervention in terms of the health of participants may be important considerations in future trials. Some intervention studies in participants with specific CVD risk factors (T2DM, obesity, MetS, hypertension, hyperlipidemia, endothelial dysfunction and arterial stiffness) have shown promising results.

Long-term trials show no significant reduction in the incidence of T2DM in healthy adults treated with vitamin D [238-240]. The Women's Health Initiative [238] study in the USA showed no significant reduction in the incidence of T2DM in healthy postmenopausal women (n=33,951, mean age = 62 years, mean baseline 25(OH)D = 42 nmol/L) in a placebo-controlled double-blind trial following daily supplementation with 1000 mg of calcium plus 400 IU of

vitamin D_3 for 7 years (HR=1.01 [0.94-1.10]). Similarly, the RECORD [239] trial in the UK, a placebo-controlled double-blind trial of adults over 70 years of age with a recent record of osteoporotic fracture (n=5292, mean age = 77 years, mean baseline 25(OH)D = 38 nmol/L, 15% men), showed no significant reduction in the incidence of self-reported T2DM following daily supplementation with 100 mg of calcium and/or 800 IU of vitamin D_3 for 24-62 months (adjusted OR=1.11 [0.77-1.62]). These studies were both were limited by the small doses of vitamin D_3 used. However, in 2016, the Tromsø study [240] in Norway (2008-2015) also showed no significant difference in the progression to T2DM in prediabetics (n=511, mean age = 62 years, mean baseline 25(OH)D = 60 nmol/L, 61% men) in a placebo-controlled trial following 20,000 IU/week for 5 years. There was no significant difference in the number of new cases of T2DM between treatment and placebo groups (103 vs 112 respectively, HR=0.90 [0.69–1.18]), nor were there any differences in changes to glucose levels, insulin resistance, serum lipids or BP. The dose regime and duration of this study (approximately 2850 IU/d for 5 years) mean it provides the strongest evidence to date that vitamin D supplementation does not prevent T2DM; however, the mean baseline 25(OH)D level was 60 nmol/L (24 ng/ml) and the benefits of supplementation are likely to be limited in people who are already vitamin D replete. Further large long-term supplementation trials using similar doses designed to achieve the proposed optimal vitamin D status (serum 25(OH)D level ≥75 nmol/L) in people who are insufficient at baseline (serum 25(OH)D <50 nmol/L) are warranted.

A 2012 meta-analysis [241] of 15 randomised trials found no significant benefit of vitamin D supplementation in improving glycemic control or insulin resistance in people with either T2DM, impaired glucose tolerance or normal fasting glucose levels. The studies varied greatly in the sample size and characteristics of the study population: from 16 healthy males (mean age = 26 years) in Germany [242] and 33,951 healthy post-menopausal women (mean age = 62 years) in the USA [238]) to 71 centrally obese men without T2DM (mean age = 44 years) in India [243] and 81 South Asian women with insulin resistance (mean age = 42) in NZ [244] to 281 adults with T2DM and nephropathy (mean age = 64 years) in Europe and the USA [245]. Although vitamin D_3 [238] was the predominant form used, some studies used vitamin D_2 [246], 1-alphacalcidol (1α-hydroxyvitamin D3), calcitriol [242], and one study used the synthetic analogue paricalcitol (19-nor-1,25-$(OH)_2$-vitamin D_2)[245]. The dose regimes ranged from 400 IU/d vitamin D_3 [238] to a one off dose of 200,000 IU/fortnightly vitamin D_3 [247], and in duration of supplementation from 7 days [242] to 7 years [238]. Only one study focused on ageing men using vitamin D_3. Nagpal et al [243] reported that supplementation of 120,000 IU/fortnight over 6 weeks in 35 healthy middle-aged Indian men (mean age = 44 years, mean

baseline 25(OH)D = 33 nmol/L, 35 in intervention group, 36 in control group) without T2DM but centrally obese resulted in a significant improvement in insulin sensitivity compared to a placebo. The intervention group had significantly improved serum 25(OH)D levels compared to the control group (+35.1±27.28 vs +0.60±11.61 nmol/L, p<0.001). Postprandial insulin sensitivity (oral glucose insulin sensitivity (OGIS ($ml.min^{-1}.kg^{-1}$)) was significantly improved (+21.17±67.86 vs -11.43±60.97, p=0.038) and this remained significant after adjusting for multiple confounders (group, age, WHR, baseline 25(OH)D level and betel-nut chewing) with a mean difference in the adjusted change in OGIS between the two groups reported as 41.1±15.4 (r^2=0.204, p=0.01). Both a higher WHR and a lower baseline 25(OH)D level were predictors of a greater improvement in insulin sensitivity. However, there was no significant difference in fasting insulin sensitivity (homeostasis model assessment (HOMA) scores), blood pressure or lipid profile in this study.

Vitamin D may aid in reducing body fat in obese subjects. A 2012 double-blind placebo-controlled RCT [248] (n=77 healthy obese women, mean age = 38 years, mean BMI 29.8 kg/m^2) of 1000 IU (25 µg) daily vitamin D_3 versus a placebo (25 µg lactose) for 12 weeks found a significant decrease in body fat mass following supplementation in healthy obese women (-2.7±2.1 vs -0.47±2.1 kg, p<0.001) with a significant correlation between the change in serum 25(OH)D level and the amount of body fat mass lost (r=-0.319, p=0.005).

Similarly to the Nagpal et al [243] study, in a long-term high-dose double-blind placebo-controlled RCT, Scragg et al [249] reported that after supplementation with 200,000 IU of vitamin D_3 per month for two months followed by 100,000 IU monthly for 18 months (n=322, mean age = 47.6 years, 25% men, 94% European, mean baseline 25(OH)D level = 72 nmol/L) there was no significant benefit on BP: neither systolic, diastolic nor pulse rate were affected. This was despite achieving an optimal mean 25(OH)D level in the intervention group compared to the placebo group (73 to 124 nmol/L vs 71 to 56 nmol/L respectively). However, at 72 nmol/L, the baseline 25(OH)D levels were not particularly low and the benefit of supplementation in replete individuals is unlikely.

Low vitamin D has been associated with hyperlipidemia in observational studies, however an RCT [250] using a high dose (50,000 IU/week) of vitamin D_3 found no significant effect on lipid parameters (TC (p=0.14), LDL-c (p=0.13), HDL-c (p=0.71), TG (p=0.43)) after 8 weeks (n=151 adults with increased risk of CVD, mean age = 48 years, 55% men, mean baseline 25(OH)D level = 34.5 nmol/L) compared to a placebo. The short duration may have limited the strength of this study to detect a significant difference. Further large scale, high-dose and long-duration intervention trials in subjects with low baseline 25(OH)D levels and specific CVD risk factors are

needed to determine the effect of vitamin D supplementation on CVD risk factors.

Recently, a 2015 meta-analysis [251] of the effects of fat soluble vitamins on fasting flow-mediated vasodilation (FMD) of the brachial artery – the gold standard measurement of endothelial function – in adults identified nine parallel-designed RCTs investigating vitamin D (n=658, 345 treatment, 313 controls, mean age = 59.8 years, mean BMI = 28.4 kg/m^2). There was no evidence to support an overall positive effect of supplementation on FMD (weighted mean difference +0.15%, 95% CI -0.21-0.51%, p=0.41). Seven of those studies supplemented with vitamin D_3 with doses ranging from 2500 IU/d [252] to 5000 IU/d [253] and from 60,000 IU/month [254] to 100,000 IU/quarter [255]. Two studies supplemented with vitamin D_2 and used a single dose of 100,000 IU [246, 256]. The duration ranged from eight weeks [257] to 12 months [255]. Only two of these studies found a significant benefit of supplementation on FMD [246, 254]. Sugden et al [246] reported that a single dose of 100,000 IU vitamin D_2 after 8 weeks resulted in a significant improvement in endothelial function (FMD +2.29%, 95% CI 0.10-4.48) in patients with T2DM (n=34, mean age = 64 years, 53% men, mean baseline 25(OH)D = 38.3 nmol/L). Harris et al [254] also reported that 60,000 IU/month of vitamin D_3 over 16 weeks in African American adults (n=45, mean age = 30 years, 47% men, mean baseline 25(OH)D level = 36.3 nmol/L) raised mean serum 25(OH)D levels from 34.3±2.2 to 100.9±6.6 nmol/l and improved endothelial function (FMD +3.10%, 95% CI 0.29-5.91) compared to the placebo group. In contrast, Gepner et al [252] reported no effect of 2500 IU/d vitamin D_3 over 4 months in healthy post-menopausal women (n=114, mean age = 63.9 years, mean baseline 25(OH)D level = 78.3 nmol/L) on FMD (+0.6%, 95% CI -1.08-1.20, p=0.77), carotid-femoral PWV (p=0.65), augmentation index (AIx, p=0.10) or CRP (p=0.97). In comparison with Sugden et al [246] and Harris et al [254] with their mean baseline 25(OH)D level <40 nmol/L, the higher baseline 25(OH)D level in this study is likely to have affected the results as supplementation is unlikely to be effective in replete individuals. Further trials are needed to clarify the effect of supplementation on endothelial function and arterial stiffness in subjects with a low baseline 25(OH)D level.

Although epidemiological evidence supports an association between vitamin D and CVD, this does not prove causation and the results of intervention studies are largely inconclusive. The results of relevant meta-analyses of the limited data currently available [37, 236, 251] have concluded that there is insufficient evidence to support a beneficial effect of vitamin D supplementation on cardiometabolic outcomes (T2DM, hypertension, CVD), CVD risk factors (T2DM, obesity, hypertension and hyperlipidemia) or vascular health measurements (endothelial function and arterial stiffness) at the low doses often used (1000 IU/d); however,

supplements at moderate to high doses may yet prove beneficial, particularly in those with low baseline serum 25(OH)D concentrations (<50 nmol/L). Although there are many accepted limitations in the design of published RCTs, there are at least three large-scale RCTs currently underway investigating the benefits of vitamin D for CVD outcomes: The Vitamin D Assessment Trial (ViDA, ACTRN12611000402943, n=5110, 200,000 IU at baseline with 100,000 IU/m thereafter for 4 years, commenced June 2011 in NZ) [258]; the Vitamin D and Omega-3 Trial (VITAL, NCT01169259, n=20000, 2000 IU/d for 5 years, commenced July 2010, USA) [259]; and the Finnish Vitamin D Trial (FIND, NCT01463813, n=2500, 1600-3200 IU/d for 5 years, commenced September 2012 in Finland) [259]. It is hoped that these large trials will contribute clear and conclusive evidence for the role of vitamin D in CVD.

4.2.1.3 Possible mechanisms

The mechanisms underlying the effect of vitamin D on cardiovascular and metabolic health converge. Cardiovascular (e.g., endothelial cells [260, 261], vascular smooth muscle cells [262, 263] and cardiomyocytes [1, 264, 265]) and metabolic (e.g., pancreatic β-cells [266, 267], skeletal muscle cells [268] and adipocytes [269]) tissues have both been found to express 1-α-hydroxylase and the VDR [270]. This strongly supports a role for vitamin D in cardiovascular and metabolic health as it suggests that these are tissues for the autocrine/paracrine effects of vitamin D. Several biologically plausible mechanisms have been proposed including an effect of 1-25$(OH)_2$D on immunoregulation and inflammation [70, 271-273], oxidation [274], insulin secretion and responsiveness [185, 210, 243, 275-279], adipogenesis and fat distribution [280, 281], the activity of the renin-angiotensin system (RAS) [282], and endothelial function [283, 284].

Cardiovascular disorders are associated with a pro-inflammatory milieu and vitamin D may play an important role in the regulation of chronic low grade inflammation which is a precursor to cardiometabolic disease [285]. The innate immune system is the first line of defence against both internal and external insults, initiating an inflammatory response to destroy pathogens, remove damaged cells and material and help repair and restore tissue integrity. Atherosclerosis begins with elevated plasma lipids which infiltrate the arterial wall and are subsequently modified (i.e. oxidised), promoting inflammation and the expression of adhesion molecules [286]. Macrophages transmigrate and process and present elements of the modified lipoprotein to T lymphocytes, activating and augmenting atherosclerotic lesion progression [287]. As chronic inflammation is harmful to host tissues, it is essential that the inflammatory response is tightly regulated [288]. This requires not only the absence of inflammatory stimuli, but also the actions of gene products to regulate inflammatory reactions and suppress the

response when necessary [288]. The expression of many of these inflammatory-related genes is mediated by 1-25$(OH)_2$D via the VDR [5, 289].

In vitro [271], animal [272] and human studies [273] increasingly support that vitamin D decreases systemic inflammatory markers of vascular disease, provides immune cells with anti-inflammatory properties, and decreases macrophage transmigration into tissue. For example, in response to an acute cardiac injury, innate immune mechanisms that increase the expression and activity of both vascular cell adhesion molecule (VCAM) and inflammatory cytokines are activated to promote the adhesion of leukocytes to endothelial cells and support the migration of inflammatory cells into the injury site. Arnson et al [273] conducted an RCT to examine the effects of 4000 IU/d for 5 days on inflammatory cytokine levels in patients following an acute MI (n=50, mean age = 65.7 years, 78% men, mean baseline 25(OH)D = 45 nmol/L (18 ng/mL). The treatment effectively diminished the inflammatory response to the cardiac injury by reducing the circulating levels of adhesion molecules and some inflammatory cytokines. There was a significant difference between the intervention and control groups for VCAM-1 (p=0.03), CRP (p=0.03), and IL-6 (p=0.05); however there was no significant difference in IL-8 (p=0.1), ICAM-1 (p=0.22), E-selectin (p=0.15), VEGF (p=0.29) or TNF-α (p=0.16) levels. This study supports the acute anti-inflammatory cardio-protective effects of vitamin D in response to cardiac injury.

Studies have demonstrated an association between vitamin D status and inflammatory conditions [246, 290, 291], suggesting it may regulate the expression of pro- and anti-inflammatory cytokines or have anti-inflammatory and/or antioxidant properties, possibly via a direct anti-oxidant role in scavenging free radicals before damage ensues [292]. However, it remains uncertain whether low vitamin D status leads to inflammation, or vice versa [293]. Recently researchers [274] suggested that the low vitamin D status seen in inflammatory conditions is a result of its biodegradation and interference with normal vitamin D metabolism due to oxidative stress caused by chronic, immune-mediated vascular and systemic inflammation. This implies that contrary to current opinion, vitamin D may not play an active role in CVD and this could explain the inconsistent evidence supporting the benefits of supplementation on CVD risk factors and CVD outcomes.

Vitamin D also plays a role in insulin secretion and sensitivity. Both *in vitro* and *in vivo* studies have shown that VDR knockout impairs insulin secretion [275]. Vitamin D deficiency also impairs insulin secretion and this ameliorates after vitamin D status is replenished [276, 277]. The expression of calbindin is promoted by 1,25$(OH)_2$D [172, 294, 295] and *in vivo* studies have shown that calbindin-D_{28k} in pancreatic β-cells modulates insulin release by regulating

intracellular calcium with calcium buffering possibly protecting against cytokine mediated destruction of β-cells [278]. Vitamin D deficiency is also associated with impaired insulin sensitivity [185], which ameliorates after vitamin D supplementation [243, 277]. *In vitro* studies have shown that $1,25(OH)_2D$ stimulates both insulin receptor expression [210] and insulin responsiveness for glucose transport in skeletal muscle cells [279].

In obesity there is evidence of alterations to the vitamin D-endocrine system including: lowered 25(OH)D and increased circulating $1,25(OH)_2D$, serum PTH and urinary cAMP and renal tubular reabsorption of calcium [112]. Following weight loss in obese individuals, 25-hydroxylase and 1α-hydroxylase enzymes have been shown to decrease significantly and serum 25(OH)D levels to increase in subcutaneous adipose tissue indicating dynamic local control of vitamin D metabolism [296] - adipocytes are reducing conversion rates and releasing 25(OH)D into circulation. There appears to be a direct action of $1,25(OH)_2D$ in adipocytes to regulate visceral adiposity: *in vitro* studies in human adipocytes have shown that $1,25(OH)_2D$ upregulates the expression of 11β-hydroxysteroid dehydrogenase type 1 resulting in decreased cortisol production - a glucocorticoid hormone that is involved in visceral fat distribution [280]. Furthermore, *in vitro* studies have shown that $1,25(OH)_2D$ inhibits adipogenesis, halting the differentiation of pre-adipocytes to mature adipocytes [281]. Therefore, while adipose tissue is a storage site for vitamin D, it also appears to be an important target tissue for vitamin D in the regulation of metabolism. The physiological mechanisms behind the role of vitamin D in obesity appear to be complex, including genetic and cell signalling mechanisms, and much remains to be elucidated [297].

Vitamin D deficiency has also been suggested to activate the renin-angiotensin system (RAS) and macrophage endoplasmic reticulum (ER) stress proteins to promote hypertension and atherosclerosis [282]. $1\text{-}25(OH)_2D$ is a potent stimulator of renin production although the mechanism has not been well defined. Animal studies suggest that both absence of VDR [298] and vitamin D deficiency [282] result in increased BP and accelerated atherogenesis via local renal activation of the RAS, but that these are reversed with replenishment of vitamin D status. Vitamin D deficient mice on a high fat diet had over 2-fold the level of atherosclerosis (increased macrophage infiltration, fat accumulation, ER stress activation and macrophage foam cell formation) compared to vitamin D sufficient mice on a high fat diet. However, the evidence from human trials on the benefit of vitamin D supplementation on hypertension [243, 249] or markers of atherosclerosis such as endothelial function [251] is inconclusive.

Recent research suggests that vitamin D is essential to the health of endothelial cells. Animal studies have shown that vitamin D insufficiency results in impaired vasodilation resulting from

deficient production of two essential factors; nitric oxide (NO) and endothelium-derived hyperpolarising factor [299]. A potent inducer of smooth muscle cell relaxation and vasodilation, NO plays a pivotal role in the regulation of the endothelium-dependent processes maintaining vascular wall homeostasis [300]. A vitamin D "micro-endocrine system" appears to exist in endothelial cells [283, 284]. *In vitro* and animal studies have suggested that vitamin D supplementation may improve endothelial function [301]. However, while emerging evidence supports the role for vitamin D in endothelial health, the results of human intervention studies are inconclusive [251, 302]. The integrity of endothelial cells is fundamental to cardiovascular health and endothelial dysfunction is associated with a range of adverse cardiovascular outcomes, including ED.

It is clear that further research is needed to clarify the association between vitamin D and cardiovascular health and illuminate the physiological mechanisms involved. It remains uncertain whether low vitamin D levels cause cardiovascular dysfunction or are an indication of deteriorating cardiovascular heath.

5.0 THE NOVEL LINK BETWEEN VITAMIN D AND ERECTILE DYSFUNCTION

Although the rationale for the hypothesis is strong, there is little evidence available to support the hypothesised link between vitamin D and ED. Two early studies conducted relevant investigations. Massry et al [303] suggested that elevated PTH played a role in the development of impotence in men with uraemia. In this study, supplementation with $1,25(OH)_2D_3$, the active metabolite of vitamin D, led to the inhibition of PTH release, an increase in plasma testosterone levels, a reduction in plasma gonadotropin concentrations, and improved overall sexual function in men. However, these results were not supported in a later trial of $1,25(OH)_2D_3$ therapy. Blumberg et al [304] conducted a cross-over designed single-blind placebo-controlled trial investigating the effect of 0.25-1.5 µg/d $1,25(OH)_2D_3$ over 4 months on sexual function and endocrine health in 10 male and 5 female dialysed patients (11 with sexual dysfunction). Sexual performance (libido, frequency of intercourse or masturbation) was measured using a semi-structured interview and serum biochemical and endocrine parameters (calcium, PTH, phosphorous, alkaline phosphatase, luteinising hormone (LH), follicle stimulating hormone (FSH), testosterone, oestradiol and prolactin) were tested. They found a significant increase in serum calcium and a decrease in PTH in both sexes, with a slight rise in testosterone in men. However there was no difference in sexual function after 2-4 months treatment [304]. They concluded that supplementation with $1,25(OH)_2D_3$ improved secondary hyperparathyroidism in this patient group but provided no benefit to sexual function. However, the small sample size, use of patients on dialysis, few male subjects, the

use of an unvalidated measure to assess sexual function, lack of specific measurement of ED, the low dosage and use of $1,25(OH)_2D_3$ instead of vitamin D_3 and the lack of measurement of serum 25(OH)D level limit the value of these results in providing evidence against the hypothesis. If renal function is normal, vitamin D_3 is the preferred supplement as it is hydroxylated to form 25(OH)D (allowing the monitoring of vitamin D status in response to supplementation) and subsequently to the bioactive $1,25(OH)_2D_3$ thus supporting the normal function and regulation of the vitamin D micro-endocrine system. Although $1,25(OH)_2D_3$ has a multitude of potential therapeutic applications, it does not alter 25(OH)D levels and, as it is already active, toxicity is more likely. The resultant hypercalcaemic effects (increased bone resorption and soft-tissue calcification) limit its use in human studies with subjects with normal renal function; indeed despite impaired renal function, 11 of the 15 dialysis patients became hypercalcaemic (>11 mg/100 ml) during the treatment, requiring a reduction in dosage during the trial [304].

Subsequent to the initiation of this PhD research in 2010, a hypothesis paper about the link between vitamin D, ED and CVD was published by Sorensen and Grant [305] in the USA in 2012. In 2014 Barassi et al [306] showed that in 143 Italian patients with ED, a large proportion (49.5%) had hypovitaminosis D (<50 nmol/l (20 ng/mL) 25(OH)D) and this was more prevalent in men with arteriogenic ED (measured using penile colour Doppler ultrasound at baseline and after intracavernosal injection of prostaglandin E1). Vitamin D levels were significantly lower in men with severe/complete ED compared to mild ED (49 nmol/L (19.8 ng/mL) vs 65 nmol/L (26.1 ng/mL) respectively) indicating a potential dose-response relationship (severity of ED may be positively correlated with severity of vitamin D insufficiency) however this was not found to be statistically significant. Furthermore, vitamin D levels were significantly lower in men with arteriogenic ED compared with borderline and non-arteriogenic ED (45 nmol/L (18.2 ng/mL) vs 56 nmol/L (22.5 ng/mL) and 63 nmol/L (25.3 ng/mL). This was the first study to investigate the link between vitamin D and arteriogenic ED and offered new evidence to support an association. It provided promising preliminary data that support the hypothesis presented in this thesis and show the need for further research to establish the association, and ultimately address the question of causation.

6.0 CONCLUSION

Vitamin D deficiency (a serum 25(OH)D level <30 nmol/L (12 ng/mL)), is highly prevalent worldwide [307], affecting every segment of society across a diverse range of populations [1, 40-42, 44, 144, 145, 147, 150, 151, 308, 309]; indeed it is now considered a worldwide pandemic [310]. Furthermore, approximately 50% of adults appear to have vitamin D insufficiency (a serum 25(OH)D level <50 nmol/l (20 ng/mL)) [1]. The prevalence is even higher if the Endocrine Society recommendation of a serum 25(OH)D concentration of <75 nmol/l (30 ng/mL) is used to define insufficiency. This is thought to be largely as a result of reduced personal UV exposure associated with modern indoor lifestyles and chronic sun protection behaviours. It presents a significant public health concern as there is increasing evidence that in addition to the well-known effects of vitamin D deficiency on skeletal health, vitamin D insufficiency may have a multitude of negative non-skeletal health consequences including the development of cardiometabolic diseases.

It has been suggested that the non-skeletal roles for vitamin D are achieved only when serum 25(OH)D levels are ≥75 nmol/L (30 ng/ml) [310]. Almost every cell and tissue in the body appears to have a VDR and enzymatic capability of converting 25(OH)D to $1,25(OH)_2D$, if supplied with sufficient substrate. However, there may be a hierarchy for its utilisation. Only when the endocrine need for vitamin D in the maintenance of calcium homeostasis for short-term survival is met, will other tissues receive sufficient substrate to support autocrine and paracrine requirements for vitamin D in long-term survival and optimal health. This supports the need to re-evaluate current recommendations to ensure they support not only the avoidance of deficiency symptoms, but also optimal health. In countries such as NZ with high vitamin D effective UVR but also high rates of skin cancer, increasing fortification of staple foods should be considered to raise intake in the general population. More importantly, safe and sensible supplementation and sun exposure recommendations are required, especially for those at high risk of vitamin D insufficiency. Most individuals reach a peak serum 25(OH)D level at the end of summer with a nadir at the start of spring [44]. It has been shown that a summer level of >40 nmol/L is required to support a winter level of approximately 20 nmol/L [93], suggesting that a summer level of 150 nmol/L would be required to ensure a winter level of approximately 75nmol/L. This is a level commonly found in outdoor workers [137, 138], suggesting that even in the general population with increasingly indoor sedentary lifestyles, supplementation may be required to maintain the proposed optimal level of ≥75 nmol/L.

Although the observational evidence supporting an association between low vitamin D status and CVD is strong, evidence supporting low vitamin D status as a causative factor in CVD development is limited and inconclusive. This is largely due to the lack of large-scale well-designed RCTs with a high dosage and long duration in people with a low baseline 25(OH)D level without clinical CVD. Such trials are clearly needed to establish causation, along with *in vitro* and *in vivo* studies to further elucidate the mechanism involved. Whilst debate continues to flourish, vitamin D insufficiency may be a factor in the establishment of CVD and it can be safely and easily restored through supplementation and/or sensible sun exposure. Vitamin D insufficiency is most likely to play a role in the early development of vascular dysfunction. As an early marker of CVD, ED – specifically vasculogenic ED in the absence of clinical CVD – supports the early identification of men at high risk of developing CVD and may be associated with vitamin D status.

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CHAPTER 5

VITAMIN D STATUS, ERECTILE FUNCTION AND CARDIOVASCULAR DISEASE RISK IN 100 APPARENTLY HEALTHY MEN AGED 40-70 YEARS IN THE MANAWATU, NEW ZEALAND

1.0 INTRODUCTION

Epidemiological studies consistently support an association between low vitamin D status and cardiovascular disease (CVD) [1-7]. The evidence from large prospective cohort studies clearly shows a strong association between serum 25-hydroxyvitamin D (25(OH)D) concentrations and CVD risk factors (e.g., type 2 diabetes mellitus (T2DM), hypertension, dyslipidaemia [1-3] and arterial stiffness [8]), the risk of developing clinical CVD [1, 4, 5], secondary cardiovascular events [6] and CVD-related mortality [1, 7]. Furthermore, vitamin D status remains an independent predictor of increased risk of CVD even after adjusting for its risk factors [1, 7, 9]. The results of intervention studies remain inconclusive [10-12], although vitamin D supplementation in participants at risk of CVD but without clinical disease has been shown to significantly improve insulin resistance [13], obesity [14] and endothelial function [15, 16]. This supports a role for vitamin D insufficiency in the development of vascular dysfunction. Despite this evidence, the optimal 25(OH)D concentration required to support sufficiency remains unclear. The current New Zealand (NZ) Ministry of Health (MOH) [17] recommendation of ≥50 nmol/L may be sufficient to support the well-established role for vitamin D in skeletal health; however the Endocrine Society [18] recommendation of ≥75 nmol/L is increasingly used as it is more likely to support the plethora of other emerging roles for vitamin D.

The results of the 2008/2009 NZ Adult Nutrition Survey (NZANS) revealed that 27% of adults over 15 years of age had serum 25(OH)D levels considered insufficient (<50 nmol/L) and a further 5% had levels considered deficient (<25 nmol/L) [19]. This indicates approximately one third of the adult population is at risk of the adverse health implications of low vitamin D status. The impact of vitamin D insufficiency on the cardiovascular health of NZ adults, particularly those who appear healthy, is unknown. As in many developed nations, NZ has an ageing population [20], and CVD – largely a disease of older age – accounts for 32% of all deaths annually and 27% of deaths in men aged 40-70 years [21]. These deaths are considered predominately premature and preventable; however, early identification and timely effective intervention are vital to curbing the rapid rise of CVD.

Erectile dysfunction (ED) is increasingly accepted as an early marker of CVD [22-25] and can be measured via self-reporting using the validated 5-item International Index of Erectile Function questionnaire (IIEF-5) [26]. There is a high prevalence of ED in NZ men aged 40-70 years and after adjusting for a range of sociodemographic, lifestyle and medical factors, ageing, being of non-European ethnicity, and current smoking are independent risk factors while a high household income and regular physical activity (PA) are protective. Other studies have reported that ED shares additional risk factors with CVD, including: T2DM [27-37], obesity and

metabolic syndrome (MetS) [33, 34, 38, 39], hypertension [27, 29-32, 34-36, 40, 41], hyperlipidaemia [27, 31, 32, 39] and atherosclerosis [42]. Indeed, epidemiological research supports a strong independent association between ED and the risk of developing CVD and adverse cardiovascular outcomes [27, 30, 31, 34, 39, 43-46]. While ED is a multifactorial disorder with both organic and psychogenic factors often affecting erectile function [47], it is predominately organic in aetiology [48] and considered synonymous with endothelial dysfunction [22, 49-51]. Therefore it is a valuable marker to identify men at risk of developing CVD at an early stage, supporting timely intervention.

It is therefore hypothesised that men living in NZ are at increased risk of vitamin D insufficiency, ED and CVD as they age, and that these factors are related. This is a novel hypothesis and could provide new information upon which to base future research into the potential for vitamin D as a treatment or adjunct treatment for ED and CVD prevention. This cross-sectional observational study had four aims: 1) to describe the vitamin D status, erectile function and cardiovascular health of a group of apparently healthy men aged 40-70 years living in the Manawatu, NZ; 2) to investigate the relationship between vitamin D status and CVD risk, ED and CVD risk; and 3) to examine the relationship between vitamin D status and erectile function.

2.1 METHODS

2.2 Recruitment

Between October 2012 and March 2013, postal invitations were sent to a randomly selected computer-generated age-stratified sample of 600 men aged 40-70 years from the Manawatu electorate (including Palmerston North city) of the NZ Electoral Roll. Packages included a letter of invitation and an information sheet (Appendix 5). A motivator was provided in the form of a prize draw. The study was also advertised on the radio and in a local newspaper. Eligible self-selected volunteers were enrolled. Responses were received from 125 men who were screened for participation: 22 did not meet criteria for eligibility, 3 were eligible but later pulled out due to time constraints. Of the 100 participants, 62 were directly invited and 38 were self-selected. All subjects provided written informed consent. Ethical approval was granted by the Central Health and Disability Ethics Committee (LRS/10/07/032/AM01).

2.3 Inclusion/exclusion criteria

Initial inclusion criteria were: male, 40-70 years old, living in the Manawatu region, healthy or with lifestyle associated diseases (e.g., cardiovascular disease (CVD) or type two diabetes mellitus (T2DM)). Volunteers were excluded if a subsequent telephone interview (see Appendix 5) revealed they suffered from: advanced or uncontrolled CVD or T2DM with any incidence of myocardial infarction (MI), heart failure or stroke or recent hospitalisation (in the past 2 years) for coronary heart disease (CHD), cardiomyopathy, hypertensive heart disease, cardiac dysrhythmia, inflammatory heart disease, valvular heart disease, cerebrovascular disease or peripheral arterial disease, hypoglycaemic seizure or coma). They were also excluded if they suffered from: clinical depression, post-traumatic stress disorder (PTSD) or a psychiatric condition; autoimmune disorders such as type 1 diabetes, lupus, multiple sclerosis, myalgic encephalomyelitis, rheumatoid arthritis or psoriasis; prostate cancer, benign prostatic hyperplasia, prostatitis or Peyronie's disease; multi-system atrophy, spinal cord injury or tumors, prolapsed intervertebral discs of tumors, disease to the parasympathetic nerves of the pelvic, pelvic surgery or abdominal surgery; chronic renal failure; hyperprolactinaemia; hyper or hypogonadism; smooth muscle dysfunction; malignant disease; recent surgery in the past year; substance abuse; were competitive cyclists or were unable to safely provide a blood sample. Use of vitamin D supplements or ED treatments were not exclusion criteria.

2.4 Assessment

All participants attended the Human Nutrition Research Unit for 2.5-3 hours and underwent a comprehensive health assessment (see Appendix 5). Assessments were staggered and commenced between 8:00am and 9.30am with participants in a fasting state with food and beverage consumption, smoking and physical activity (PA) avoided for 12 hours prior. Assessment included: medical history (including medication and supplement use); anthropometric measurements (height, weight, waist circumference (WC), hip circumference (HC) and assessment of body fat percentage (BF%) and android to gynoid fat ratio (A:G) using dual energy X-ray absorptiometry (DEXA®, Hologic Discovery A, Wisconsin, MA, USA); and vascular health measurements (blood pressure (BP), heart rate (HR) and assessment of arterial stiffness (aortic augmentation pressure (AP@HR75) and augmentation index (AIx@HR75) adjusted to a HR of 75 bpm, pulse wave velocity (PWV)) using arterial tonometry (SphygmoCor CPV®, AtCor Medical, Sydney, Australia). Fasting venous was drawn using standard venepuncture technique by a trained phlebotomist in five (maximum 26 ml) BD Vacutainer® tubes (BD Diagnostics, Auckland, NZ) with appropriate additives. One tube was chilled for later DNA analysis (see Chapter 7). Plasma or serum was separated by centrifugation at 3500 rpm

(2000 g) and 4°C for 10 minutes (see Appendix 5). One tube was chilled and transferred to MedLab (MedLab Central Ltd, Palmerston North, NZ) for same-day lipid profile analysis (triglycerides (TG), total cholesterol (TC), low-density lipoproteins (LDL-c), high-density lipoproteins (HDL-c)). Other samples were stored at -80°C for later measurement of biochemical markers (serum 25(OH)D, plasma glucose (FPG), plasma insulin (FPI), total testosterone (TT), sex hormone binding globulin (SHBG)) outsourced to Canterbury Health Laboratories (CHL, Christchurch, NZ). Participants were provided with a standard breakfast after phlebotomy before completing a private computer-based survey, which included sociodemographic and lifestyle assessment and the IIEF-5 and single-question self-report for assessing erectile function. Finally, subjects underwent fitness testing using the YMCA cycle ergometer submaximal test (YMCAsub) to calculate the estimated maximal oxygen consumption (VO_2peak) and strength testing using a hand-held dynamometer to assess maximal handgrip strength.

2.5 Main outcome measures

2.5.1 Vitamin D status

Serum 25(OH)D was analysed by Canterbury Health Laboratories (CHL, Christchurch, NZ) and determined by HPLC tandem mass spectrometry. Vitamin D status was categorised according to both the current NZ Ministry of Health (MOH) recommendations [17] (deficient <25 nmol/L, insufficient 25-49.9 nmol/L, adequate 50-124.9 nmol/L, high >125 nmol/L), and the Endocrine Society recommendations [18] (deficient <50 nmol/L, insufficient 50-74.9 nmol/L, adequate ≥75 nmol/L, high >250 nmol/L). In subsequent analysis, vitamin D insufficiency was defined according to the Endocrine Society recommendation as <75 nmol/L.

2.5.2 Erectile function

Erectile function was assessed using the validated IIEF-5 [26]. The five questions applied to the previous 6 months and covered 4 domains: (i) erection confidence, (ii) erection firmness, (iii) erection maintenance, and (iv) sexual satisfaction. Each question had five response options allowing the calculation of a score from 5-25. Established criteria were used to describe ED severity: 22-25 no ED, 17-21 mild ED, 12-16 mild to moderate ED, 8-11 moderate ED, and 5-7 severe ED. However, in subsequent analysis ED was defined as a score ≤21 and therefore any degree of ED. The single-question self-report [52] was included for comparative purposes and required self-reporting into one of four categories: not impotent, minimally impotent, moderately impotent or completely impotent.

2.5.3 Cardiovascular disease risk factors

2.5.3.1. Sociodemographic and lifestyle

Sociodemographic (age, ethnicity, relationship status, education, employment status, household income, occupational category and residence) and lifestyle (smoking status, alcohol consumption and Cambridge PA Index) were assessed as in Chapter 3.

Cardiorespiratory fitness was objectively assessed in men deemed physically able (i.e. without physical injury or undiagnosed/uncontrolled hypertension) using the well-established YMCAsub test to estimate VO_2peak from information on HR and work rate [3]. This protocol follows a multistage format with subjects cycling on a Monark Ergomedic Cycle at a low to moderate intensity (starting at 150 kpm (0.5 kp)) at a pedal rate of 50 rpm, through three or more consecutive 3-minute increasing workloads designed to raise the HR to between 110 bpm and 85% of the age-predicted maximum HR. The HR during the final minute of the first workload determines the workload during subsequent stages. HR and oxygen consumption (VO_2) were monitored throughout with the use of a Polar FT1 Heart Rate Monitor with T31 coded™ transmitter and the TrueOne® 2400 Metabolic Measurement System (Parvo Medics Inc., Sandy, Utah, USA). The system was calibrated daily to ensure consistency and a standardised technique was used to ensure reliability. It is fast (approximately 20 minutes per test), simple, safe, non-invasive, suitable for repeat testing and for use with older and potentially sedentary adults. The Borg scale of perceived exertion [53] was used and testing ceased when the score exceeded 17 points indicating “very hard” exertion. Blood pressure was monitored throughout and testing ceased in the event of a hypertensive response (BP >240/110 mmHg) [54]. A PowerLab ECG monitor (PowerLab Ltd, Christchurch, NZ) was also used to monitor potential cardiac complications.

Handgrip strength was assessed using a Jamar® Hydraulic Hand Dynamometer (Patterson Medical, Auckland, NZ). Equipment was calibrated and adjusted to grip size to ensure consistent and reliable results. The seated participant gripped the dynamometer with maximum isometric effort for 5 seconds with their elbow supported at their side. Measurements were taken in triplicate in each hand and hand dominance recorded. Grip strength was recorded to the nearest 1 kg and the highest value used for analysis.

2.4.3.2 Anthropometric

Anthropometric measurements were taken in duplicate or triplicate if the difference between the two initial measurements exceeded a given level (i.e. 1 cm or 5 g). The average of the two closest measurements was used. Height (cm) was measured using a stadiometer. The subject stood with their head in the Frankfurt plane and the measurement was made during a deep breath. Weight (kg) was measured using a calibrated digital floor scale. BMI (kg/m^2) was calculated. WC (cm) was measured at the mid-point between the lower margin of the last palpable rib and the top of the iliac crest. HC (cm) was measured anteriorly at the level of the symphysis pubis and posteriorly at the level of the maximal protrusion of the gluteal muscles. WHR and WHtR were calculated. DEXA® was used by a trained technician according to established protocol to assess fat mass as BF% and fat distribution as A:G fat ratio.

2.4.3.3 Vascular health

Using a brachial cuff and an Omron Automatic Digital Blood Pressure and Pulse Monitor with IntellisensE™ Model T3 (Omron Corp., Tokyo, Japan), BP (mmHg) and HR (bpm) were assessed in the left arm while in a supine position following 15 minutes of rest and 5-6 deep breaths. Two measurements were taken with 5 minutes between readings. The SphygmoCor CPV® system (AtCor Medical, Sydney, Australia) was used in both Pulse Wave Analysis (PWA) and Pulse Wave Velocity (PWV) mode following established protocol to noninvasively assess central blood pressure and arterial stiffness. PWA uses arterial tonometry to derive the central aortic pressure waveform from external measurement of the radial pressure waveform. PWV uses arterial tonometry to measure the peripheral pressure waveforms at two sequential arterial sites with simultaneous electrocardiogram (ECG) signal recording to calculate the velocity of the waveform travelling the measured distance between the two peripheral sites – in this study the carotid and radial arterial sites. Measurements were taken in triplicate and the average of the two closest measurements with an operator index >70 used. Parameters of interest in this study were the AP@HR75, AIx@HR75 and PWV (m/s). Raised AP@HR75 and AIx@HR75 indicate augmented central aortic pressure resulting from a reflected arterial pulse wave, suggestive of systemic arterial stiffness – an early sign of subclinical atherosclerosis. Given a constant ratio of vessel wall thickness to vessel radius, the PWV is proportional to the square root of the incremental elastic modulus of the vessel wall; therefore, increased central pressure and arterial stiffness are reflected in raised PWV.

2.4.3.4 Biomarkers and health conditions

Lipid profiles (TG, TC and HDL-c) were analysed by MedLab (MedLab Central Ltd, Palmerston North, NZ) using an Abbott Architect Ci8200 chemistry module (Abbott Laboratories (NZ) Ltd, Auckland, NZ): TG and TC concentrations were measured using the enzymatic colorimetric method and HDL-c using accelerator selective detergent assay; LDL-c fraction was calculated using the Friedewald equation (LDL-c=TC-[HDL-c+(TG/2.2)]) in samples with a TG <4.5 mmol/L; and ratios of TC:HDL-c and TG:HDL-c were calculated.

Other biomarkers were analysed by Canterbury Health Laboratories (CHL, Christchurch, NZ): FPG was determined by enzymatic glucose hexokinase assay (Abbott c series analyser, Abbot Laboratories (NZ) Ltd, Auckland, NZ); FPI was analysed by immunoassay following polyethylene glycol (PEG) precipitation of immunoglobulins (Roche Cobas e411 analyser, Roche Diagnostics NZ Ltd, Auckland, NZ). Homeostatic Model Assessment Index (HOMA-IR) scores were calculated as HOMA-IR=(FPG (mmol/L)*(FPI (pmol/L)/6))/22.5 [55]. For the hormone profile, TT was analysed using the enzyme-linked immunosorbent assay (ELISA) extracted method, SHBG was determined using the sandwich ELISA method, FT (pmol/L) was derived from TT and SHBG levels using the Vermeulen formula [56], and FAI was calculated using the formula FAI=1000*(TT (nmol/L))/SHBG (nmol/L)).

Health conditions were defined according to established criteria as shown in Table 5.1. The Framingham Heart Study algorithm [57-59], a tool widely adopted in cardiovascular treatment guidelines internationally, was used to estimate the 10 year risk (%) of developing CVD, coronary heart disease (CHD), myocardial infarction (MI), stroke, death from CVD and death from CHD. Framingham scores were calculated using an online calculator [60] according to established formula based on the traditional risk factors: age, gender, smoking, diabetes, SBP, TC and HDL-c levels.

Table 5.1. The criteria used to define the health conditions assessed in the study.

Condition	Definition
Obesity	BMI >30 kg/m2 [61] WC ≥102 cm [61] WHR of >0.9 [62] WHtR of >0.5 [63]
Hypertension	SBP >140 and/or a DBP >90 mmHg [64]
Dyslipidaemia	Presence of ≥2 NCEP-ATPIII lipid abnormalities [65]
Diabetes or prediabetes	FPG ≥5.6 mmol/L [66]
Hypogonadism	TT level <8nmol/L OR a 8-12 nmol/L with a FT level <225 pmol/L [67]
Depression	PHQ-9 score ≥10 [68, 69]
Metabolic syndrome	Presence of ≥3 AHA/NHBL-ATPIII criteria [70]

AHA/NHBL-ATPIII, American Heart Association/National Heart, Lung, and Blood Institute (AHA/NHBL) - Adult Treatment Panel III; BMI, Body Mass Index; DBP, diastolic blood pressure; FPG, fasting plasma glucose; FT, free testosterone; NCEP-ATPIII, National Cholesterol Education Program - Adult Treatment Panel III; PHQ-9, Patient Health Questionnaire 9-Item; SBP, systolic blood pressure; TT, total testosterone; WC, waist circumference; Well-LaD, Wellness, Lifestyle and Diet; WHR, waist-to-hip ratio; WHtR, Waist-to-height ratio.

2.5 Data analyses

The categorical characteristics of the study sample are presented as the absolute frequency (count). Normally distributed data (verified using the Kolmogorov–Smirnov criterion) are reported as mean ± standard deviation (SD). Data not normally distributed are reported as median (interquartile range (IQR)). No transformations were used. Outliers were not removed as they were of *a priori* interest. All tests were two-tailed and p-values ≤0.05 were considered statistically significant. Analyses were performed using Microsoft Excel® 2010 (Microsoft Corp., Redmond, WA, USA) and SPSS Statistics version 20.0 (IBM Corp., Armonk, NY, USA).

The sociodemographic characteristics were compared to expected national proportions based on available data from the 2013 NZ Census [71] using χ^2 or binomial tests.

Cohen's Kappa coefficient (κ) was calculated to determine consistency between the IIEF-5 and single-question self-assessment tools.

Differences between two groups were assessed using: independent *t*-tests for comparing group means in normally distributed data; Mann–Whitney U tests for comparing group distributions and independent samples median tests for comparing group medians in data not normally distributed; and χ^2 or Fisher's exact tests for comparing frequencies between categories in categorical data. Univariate correlations were assessed using the Spearman's rank correlation coefficient (r_s) to investigate relationships between variables and assess collinearity. Differences between multiple groups were assessed using independent samples median tests for comparing group medians and Kruskal-Wallis tests for comparing distributions.

Predictors of ED with a univariate association p-value<0.100 were assessed using binomial logistic regression to calculate crude and age-adjusted odds ratios (OR) and 95% confidence intervals [95% CI] and determine their effect on the likelihood of having ED. The p-value indicates the significance of its predictive capability in the model, not its effect size.

The performance of serum 25(OH)D level as a predictor of ED was evaluated by receiver operator characteristic (ROC) curve analysis to determine sensitivity and specificity level as a diagnostic test.

3.1 RESULTS

3.2 Response rate and respondent profile

Responses were received from 81 (13.5%) of the 600 men directly invited to take part and a further 44 were received from volunteers in response to advertising or word-of-mouth. Of these 125 men, 103 (62 invited, 41 volunteers) were eligible to take part and three volunteers withdrew before their appointment due to time constraints. All appointments took place over the spring/summer months (October 2012 - March 2013). The respondent profile is provided in Table 5.2 and compared to the 2013 NZ Census data for men aged 40-69 years. The median age was 53 years. The sample was diverse and nationally representative in terms of both age and education; however, participants were predominately of European ethnicity, in a relationship, employed and living in a rural or semi-rural environment.

Table 5.2. Sociodemographic characteristics of study participants (n=100) compared to expected proportions based on the 2013 New Zealand (NZ) Census[†] (n=768,801).

		Study[**] prevalence	NZ 2013 Census prevalence	χ^2 or binomial test	
Characteristic[*]		**n**	**%**	**statistic**	**p-value**[‡‡]
Age range (years)	40-49	37	37.5	1.317	0.518
	50-59	31	35.2		
	60-69	32	27.3		
Ethnicity	European	93	77.6	15.790	**0.001**
	NZ Maori	5	10.4		
	Asian	1	8.8		
	Pacific Peoples	1	4.6		
Relationship status	Married/de facto/civil union	94	65.9	39.298	**<0.001**
	Single/dating	2	16.4		
	Separated/divorced/widowed	2	17.7		
Education	None	17	19.9	1.244	0.537
	Secondary school	33	36		
	Post-secondary school	49	44		
Current employment status	Employed	93	79.8	10.811	**0.004**
	Not employed and seeking work	1	3.1		
	Not employed and not seeking work	6	17.1		
Household income	Low (0-59,999)	25	39.8	15.602	**<0.001**
	Middle (60,000-99,999)	30	32.6		
	High (100,000+)	44	27.6		
Occupational category	Managers	35	26.1	10.245	**0.036**
	Professionals	22	19.8		
	Technicians and Trades Workers	17	18		
	Community, Personal Service, Clerical, Administrative and Sales Workers	16	15.2		
	Machinery Operators and Drivers and Labourers	9	20.9		
Residence	Urban	42	85.8	-	**<0.001**
	Rural/semi-rural	58	14.2		

*Missing values for each characteristic not shown. **The Well-LaD Study included 600 surveys sent in approximately equal numbers to men in each decade aged 40-70 years, plus self-selection from the general population in response to public advertisements and word-of-mouth. [†] This work is based on/includes Statistics New Zealand's data which are licensed by Statistics New Zealand for re-use under the Creative Commons Attribution 3.0 New Zealand licence. [‡‡] Characteristics that are significantly different ($p<0.05$) from the expected national proportions are highlighted in bold.

3.3 Characteristics of the sample population

3.3.1 Vitamin D status

The median 25(OH)D level was 82.5 (24) nmol/L. As shown in Table 5.3, based on the proposed Endocrine Society recommendations [18], 37 men had insufficient vitamin D (<75 nmol/L): eight men had mild-moderately deficient (25-49.9 nmol/L) and 29 had suboptimal (50-74.9 nmol/L) levels. Quartile analysis revealed even the lowest quartile (Q1) was above the 50 nmol/L cut-off for deficiency: Q1 <69.5 nmol/L, Q2 69.5-82.5 nmol/L, Q3 82.5-93 nmol/L, Q4 ≥93 nmol/L. Only 13 men were taking vitamin D supplements and the median intake was 100(300) IU/d. Removing these men from the analysis did not affect the overall 25(OH)D levels (82(24) nmol/L; Q1 <69.5 nmol/L, Q2 69.5-82, nmol/L, Q3 82-93 nmol/L, Q4 ≥93 nmol/L).

Table 5.3. Serum 25(OH)D levels (nmol/L), the prevalence of deficiency and insufficiency according to both the current New Zealand Ministry of Health (MOH) recommendations [17] and the Endocrine Society recommendations [18], and vitamin D supplement intake in study participants (n=100).

		Study prevalence
Vitamin D		**n**
25(OH)D nmol/L*	Median (IQR) nmol/L	82.5 (24)
Vitamin D status	***MOH recommended cut-offs***	
	Severely deficient (<12.5 nmol/L)	0
	Mild-moderately deficient (12.5-24.9 nmol/L)	0
	Insufficient (25-49.9 nmol/L)	8
	Adequate (50-124.9 nmol/L)	89
	High (>125 nmol/L)	3
	Endocrine Society recommended cut-offs	
	Severely deficient (<25 nmol/L)	0
	Mild-moderately deficient (25-49.9 nmol/L)	8
	Insufficient (50-74.9 nmol/L)	29
	Adequate (75-249.9 nmol/L)	63
	High (>250 nmol/L)	0
Vitamin D_3 supplements**	No	87
	Yes	13
	Median (IQR) IU/d	100 (300)

*Serum 25-hydroxyvitamin D (25(OH)D) conversion 2.495 nmol/L = 1 ng/ml, **Vitamin D_3 supplements alone or in combination with other nutrients. IQR, interquartile range.

3.3.2 Erectile function

The median IIEF-5 score was 23(4). Assessed using established IIEF-5 criteria [26], 30 men reported some degree of ED (IIEF-5 score ≤21): 17 mild, six mild-moderate, four moderate and three severe cases (Table 5.4). Similarly, using the single-item self-report, 30 men reported some degree of ED: 16 minimal, 12 moderate and two complete cases. Both the IIEF-5 and single-item self-report categories for ED severity were significantly associated with age ($p<0.001$ and $p=0.013$ respectively): the prevalence of ED increased with increasing age and was highest in men in their 60s (16% in their 40s, 23% in their 50s and 53% in their 60s). There was moderate agreement between the two methods ($\kappa=0.575$, $p<0.001$) when the IIEF-5 categories for mild-moderate and moderate were merged. Furthermore, there was substantial agreement between the two tools when both were used to define ED dichotomously ($\kappa=0.667$, $p<0.001$). However, there were seven men that presented with ED using the IIEF-5 that were not identified by the single-item self-report, and a further seven men that presented with ED using the single-item self-report that were not identified by the IIEF-5: all were cases of mild ED. Of the 30 men with ED (IIEF-5 score ≤21), the majority of men (24) were neither medically diagnosed nor being treated for ED. Five men had been diagnosed of whom three were being treated. One man was not diagnosed but was taking treatment for ED.

Table 5.4. Prevalence (count (n)) of erectile dysfunction (ED) assessed using both the 5-item International Index of Erectile Function (IIEF-5) and the single-item self-assessment tool in study participants (n=100). P-value derived from the Fisher's exact test to compare frequencies between categories.

	Prevalence of erectile dysfunction						
	Measured using the IIEF-5						
Age group (years)	No ED (22-25)	Mild 17-21	Mild-Moderate 12-16	Moderate 8-11	Severe <8	ED (≤21)	p-value*
40-49 (n=37)	31	6	0	0	0	6	**0.013**
50-59 (n=31)	24	3	2	1	1	7	
60-69 (n=32)	15	8	4	3	2	17	
Overall prevalence by IIEF-5	70	17	6	4	3	30	
	Measured using the single-item self-report						
	Not (22-25)	Minimally 17-21		Moderately 8-11	Completely <8	ED (any degree)	p-value*
40-49 (n=37)	34	3	-	0	0	3	**<0.001**
50-59 (n=31)	23	5	-	3	0	8	
60-69 (n=32)	13	8	-	9	2	19	
Overall prevalence by single-item	70	16	-	12	2	30	

3.3.3 Sociodemographic and lifestyle factors

The health profile of participants, including lifestyle and cardiometabolic risk factors, is shown in Table 5.5. Lifestyle factors suggest a high level of healthy behaviours in this group. Only 10 men were current smokers (three regular, five occasional and two had quit within the last 12 months) with a further 31 being former smokers. Although the majority were current drinkers (54 regular and 37 occasional), the median number of standard alcoholic drinks consumed per week was 8 (18) and below the current recommended limit of 15 [72]. The majority (81) self-reported participation in regular vigorous PA and were categorised as active (47) using the Cambridge PA Index. This was reflected in their measured cardiorespiratory fitness with a median estimated VO_2peak of 33.7 (14.1) ml/kg/min and the majority (34) classified as having an "average" level of fitness for their age. Eight participants were classified as having a "very high" level of fitness. However, 13 subjects did not attempt the test (due to lack of time or safety concerns with a resting SBP >160 mmHg), four did not complete the test (due to safety concerns such as signs of distress, irregular HR or abnormal ECG), and nine completed the test but were excluded (due to not meeting the requirement of two stages with a HR >110 bpm). Any men with safety concerns were referred to their General Practitioner. The median handgrip strength was 100 (25) kg with 37 participants categorised as having "excellent" handgrip strength. This further supports the high level of overall physical fitness in this group. However, almost half of participants were taking medication (48) and the majority (28) of those were taking medications to treat one or more of the following: hypertension (i.e. beta-blockers, Ca^{2+}-channel blockers or angiotensin-converting enzyme (ACE) inhibitors), hyperlipidemia (i.e. statins) or blood clotting (i.e. anticoagulants or antiplatelet medication). Over a third (37) were taking one or more dietary supplements: mainly multivitamins or minerals (30) including those containing vitamin D, followed by omega 3 oils (14) and joint health supplements (11).

3.3.4 Anthropometric risk factors

Although all participants self-reported as healthy, results indicate a high level of cardiometabolic risk in this group. The median BMI was 27.11 (4.58) kg/m^2 and the quantification of body fat using DEXA® also indicated a high level of body fatness (29.11±4.67%). Indeed, 81 participants were classified as either overweight or obese (BMI ≥ 25 kg/m^2) and although only 28 were classified as centrally obese using a WC ≥102 cm, 75 were centrally obese using a WHR >0.9 and 74 when using a WHtR of >0.5. Assessment of fat distribution using DEXA® also indicated a high level of central obesity with a median A:G of 1.21(0.26).

3.3.5 Vascular health measurements

Hypertension was present in 27 of the participants. The mean SBP and DBP were 129.32±17.95 mmHg and 79.25±9.10 mmHg respectively. The median AP@HR75 was 5.0(6.7) and the mean AIx@HR75 was 14.42±10.24. The median PWV was 7.8(1.4) m/s and within normal levels for older adults (7-10 m/s with a threshold value of 9.6 m/s [73]).

3.3.6 Biomarkers and health conditions

Although 21 men had dyslipidaemia, there were a further 22 with single lipid abnormalities. Overall, results show that the median levels of TC (5.18±0.99 mmol/L) and LDL-c (3.38±0.90 mmol/L) were above optimal (<5.1 mmol/L and <2.6 mmol/L respectively [65]), but TG (1.10(0.9) mmol/L) and HDL-c (1.20(0.4) mmol/L) were within optimal levels (<1.7 mmol/L and 1.0-1.6 mmol/L respectively [65]). The median TC:HDL-c (4.3(1.5)) and TG:HDL-c (0.90(0.80)) ratios were also within optimal levels (<5.0 and <3.5 respectively) [74].

Fifty-seven participants had raised FPG (≥5.6-6.9 mmol/L) [66] and one participant was an undiagnosed diabetic (FPG >7.0 mmol/L) [66] with a median FPG level of 5.6(0.6) mmol/L and FPI level of 44.5(39.0) pmol/L. The median HOMA1-IR score was 1.9(1.7).

The hormonal profile of participants revealed 26 had undiagnosed hypogonadism; however, overall levels of TT (15.2(6.2) nmol/L) and FT (374.28±132.98 pmol/L) were within the normal range (>12 nmol/L [67] and >225 pmol/L [67] respectively).

Four participants were suffering from major depression.

Twenty-eight men met ≥3 ATPIII criteria and were defined as having MetS; however, a further 28 met two criteria and 24 met one criterion leaving 20 men considered metabolically healthy. This was supported by the Framingham risk assessment scores which showed a high level of cardiovascular risk amongst participants: the median 10-year risk for developing CVD was 8.32(8.92)%.

Table 5.5. Health profile including lifestyle and cardiometabolic health markers of study* participants (n=100) overall and according to the presence of vitamin D insufficiency (serum 25(OH)D level <75 nmol/L) and erectile dysfunction (ED, IIEF-5 score ≤21).

Characteristic/condition**		Study prevalence	Serum 25(OH)D level		p-value***	No ED	ED	p-value***
		mean±SD/ median (IQR)/ count(n)	≥75 nmol/L	<75 nmol/L	T-test, Mann-Whitney, X^2/Fisher's Exact	IIEF-5 >21	IIEF-5 ≤21	T-test, Mann-Whitney, X^2/Fisher's Exact
		n=100	n=63	n=37		n=70	n=30	
SOCIODEMOGRAPHIC AND LIFESTYLE								
Age (years)		54 (16)	52(17)	56(16)	0.203	51(13)	62(13)	**0.001**
Age range	40-49 years	37	26	11	0.331	31	6	**0.002**
	50-59 years	31	20	11	-	24	7	-
	60-69 years	32	17	15	-	15	17	-
Ethnicity	European	93	60	33	0.418	66	27	0.425
	Non-European	7	3	4	-	4	3	-
Income	Low (0-59,999)	25	15	10	0.952	16	9	0.749
	Middle (60,000-	30	19	11	-	21	9	-
	High (100,000+)	44	28	16	-	32	12	-
Smoking status	No	90	59	31	0.166	64	26	0.482
	Yes	10	4	6	-	6	4	-
Alcohol consumption	Never	3	2	1	0.907	3	0	0.569
	Former	6	3	3	-	4	2	-
	Occasionally	37	23	14	-	28	9	-
	Regularly	54	35	19	-	35	19	-
Standard alcoholic drinks per week		8(18)	10(6)	7(20)	0.697	7(15)	13(16)	0.068
Cambridge PA index	Inactive	6	1	5	0.067	3	3	0.639
	Moderately inactive	10	5	5	-	8	2	-
	Moderately active	37	26	11	-	25	12	-
	Active	47	31	16	-	34	13	-

Characteristic/condition**		Study prevalence	Serum 25(OH)D level		p-value***	No ED	ED	p-value***
		mean±SD/ median (IQR)/ count(n)	≥75 nmol/L	<75 nmol/L	T-test, Mann-Whitney, X^2/Fisher's Exact	IIEF-5 >21	IIEF-5 ≤21	T-test, Mann-Whitney, X^2/Fisher's Exact
		n=100	n=63	n=37		n=70	n=30	
VO_2peak (ml/kg/min)		33.7(14.1)	37.2 (12.2)	29.5 (8.2)	**<0.001**	34.3 (13.4)	30.5 (17.9)	0.118
Cardiorespiratory fitness	Low	0	0	0	**0.003**	0	0	0.524
	Fair	4	0	4	-	2	2	-
	Average	34	17	17	-	24	10	-
	Good	19	16	3	-	16	3	-
	High	9	7	2	-	7	2	-
	Very high	8	7	1	-	5	3	-
Handgrip strength (kg)		100(25)	101(72)	97(25)	0.139	102(20)	87(31)	**0.003**
Handgrip strength	Needs improvement	10	7	3	0.570	5	5	**0.014**
	Fair	12	5	7	-	4	8	-
	Good	13	9	4	-	11	2	-
	Very good	27	17	10	-	21	6	-
	Excellent	37	25	12	-	29	8	-
Medications	No	52	34	18	0.607	43	9	**0.005**
	Yes	48	29	19	-	27	21	-
Supplements	No	63	37	26	0.248	43	20	0.658
	Yes	37	26	11	-	27	10	-
ANTHROPOMETRIC								
Height (cm)		177.1±6.3	177.6±5.9	176.2±7.0	0.298	177.2±6.1	176.8±6.9	0.784
Weight (kg)		85.0(15.1)	83.8(13.7)	90.1(16.3)	**0.013**	85.0(16.1)	84.7(15.5)	0.863
BMI (kg/m^2)		27.1(4.6)	26.6(3.3)	29.3(5.4)	**0.001**	27.0(4.8)	27.3(4.0)	0.721
BF% (%)		29.1±4.7	27.5±4.4	31.8±3.8	**<0.001**	28.7±4.6	30.2±4.8	0.132
WC (cm)		96.3±11.1	93.1±10.2	101.8±10.6	**<0.001**	95.2±11.2	98.8±10.7	0.137
WHR		0.95±0.07	0.93±0.06	0.99±0.06	**<0.001**	0.95±0.07	0.97±0.06	0.107
WHtR		0.54±0.06	0.53±0.06	0.58±0.06	**<0.001**	0.54±0.06	0.56±0.06	0.106
A:G		1.21(0.26)	1.17(0.27)	1.27(0.15)	**0.010**	1.19(0.27)	1.25(0.18)	0.060

Characteristic/condition**		Study prevalence	Serum 25(OH)D level		p-value***	No ED	ED	p-value***
		mean±SD/ median (IQR)/ count(n)	≥75 nmol/L	<75 nmol/L	T-test, Mann-Whitney, X^2/Fisher's Exact	IIEF-5 >21	IIEF-5 ≤21	T-test, Mann-Whitney, X^2/Fisher's Exact
		n=100	n=63	n=37		n=70	n=30	
Obesity	BMI <25 kg/m^2	19	17	2	**0.013**	15	4	0.340
	BMI 25-29.9	59	36	23	-	38	21	-
	BMI >30 kg/m^2	22	10	12	-	17	5	-
Central obesity – WC ≥102cm	No	72	53	19	**0.000**	52	20	0.437
	Yes	28	10	18	-	18	10	-
Central obesity – WHR >0.9	No	25	23	2	**0.001**	20	5	0.208
	Yes	75	40	35	-	50	25	-
Central obesity – WHtR >0.5	No	26	22	4	**0.008**	21	5	0.164
	Yes	74	41	33	-	49	25	-
VASCULAR HEALTH								
SBP (mmHg)		129.3±18.0	125.8±17.8	135.4±16.8	**0.009**	128.6±18.9	131.1±15.8	0.519
DBP (mmHg)		79.3±9.1	77.0±8.3	83.1±9.3	**0.001**	79.1±9.7	79.7±7.7	0.748
HR (bpm)		55.5(11)	52(12)	60(12)	**<0.001**	53(11)	59(12)	**0.017**
AP@HR75		5.0(6.7)	3.9(5.9)	7.7(9.3)	**0.005**	4.0(5.7)	7.6(7.0)	0.269
AIx@HR75		14.4±10.2	4.8±4.6	7.5±5.6	**0.043**	13.8±10.4	15.8±10.0	0.424
PWV (m/s)		7.8(1.4)	7.9(1.4)	7.7(1.3)	**0.030**	7.8(1.3)	8.0(1.2)	0.315
Hypertension – >140 and/or 90 mmHg	No	73	51	22	**0.019**	53	20	0.350
	Yes	27	12	15	-	17	10	-
BIOMARKERS AND HEALTH CONDITIONS								
TG (mmol/L)		1.1(0.9)	1.0(0.6)	1.4(0.9)	**0.005**	1.2(0.6)	1.1(1.0)	0.994
TC (mmol/L)		5.2±1.0	5.0±1.0	5.5±1.0	**0.012**	5.1±1.0	5.3±1.0	0.543
LDL-c (mmol/L)		3.4±0.90	3.2±0.9	3.7±0.9	**0.002**	3.4±0.9	3.4±0.9	0.710
HDL-c (mmol/L)		1.2(0.4)	1.2(0.3)	1.1(0.3)	**0.028**	1.2(0.4)	1.2(0.4)	0.982
TC:HDL-c		4.3(1.5)	4.0(1.2)	5.0(1.3)	**<0.001**	4.3(1.6)	4.3(1.3)	0.827
TG:HDL-c		0.9(0.8)	0.8(0.5)	1.2(1.1)	**0.002**	0.9(0.8)	0.9(0.9)	0.919

Characteristic/condition**		Study prevalence	Serum 25(OH)D level		p-value***	No ED	ED	p-value***
		mean±SD/ median (IQR)/ count(n)	≥75 nmol/L	<75 nmol/L	T-test, Mann-Whitney, X^2/Fisher's Exact	IIEF-5 >21	IIEF-5 ≤21	T-test, Mann-Whitney, X^2/Fisher's Exact
		n=100	n=63	n=37		n=70	n=30	
Dyslipidaemia - ≥2 lipid abnormalities	No	79	52	27	0.257	56	23	0.708
	Yes	21	11	10	-	14	7	-
Dyslipidaemia - # of lipid abnormalities	0	57	44	13	**0.003**	42	15	0.727
	1	22	8	14	-	14	8	-
	2	16	9	7	-	11	5	-
	3	4	2	2	-	2	2	-
	4	1	0	1	-	1	0	-
FPG (mmol/L)		5.6(0.6)	5.6(0.5)	5.8(0.5)	0.058	5.6(0.5)	5.6(0.8)	0.913
FPI (pmol/L)		44.5(39)	34(30)	54(53)	**0.001**	43(30)	52(44)	0.059
HOMA1-IR		1.9(1.7)	1.5(1.4)	2.2(2.7)	**0.001**	1.7(1.3)	2.2(2.2)	0.089
Prediabetes/diabetes - FPG >5.6 mmol/L	No	42	29	13	0.286	28	14	0.536
	Yes	58	34	24	-	42	16	-
TT (nmol/L)		15.2(6.2)	15.6(5.7)	13.9(7.9)	0.085	15.3(6.1)	14.9(7.8)	0.761
SHBG (nmol/L)		24(13)	25(15)	23(11)	0.164	22.5(11)	28.5(15)	**0.001**
FT (pmol/L)		374.3±133.0	379.4±123.1	365.6±146.8	0.619	391.9±129.9	333.2±133.	**0.043**
FAI		627.5(343)	613(327)	636(396)	0.568	662.5(330)	479.5(260)	**<0.001**
Hypogonadism - TT <8 nmol/L or <12 and FT <225	No	74	51	23	**0.039**	52	22	0.921
	Yes	26	12	14	-	18	8	-
Depression - PHQ9 ≥10	No	92	59	33	0.147	65	27	1.000
	Yes	4	1	3	-	3	1	-

Characteristic/condition**		Study prevalence	Serum 25(OH)D level		p-value***	No ED	ED	p-value***
		mean±SD/ median (IQR)/ count(n)	≥75 nmol/L	<75 nmol/L	T-test, Mann-Whitney, X^2/Fisher's Exact	IIEF-5 >21	IIEF-5 ≤21	T-test, Mann-Whitney, X^2/Fisher's Exact
		n=100	n=63	n=37		n=70	n=30	
MetS - ≥3 ATPIII criteria	No	72	52	20	**0.002**	52	20	0.437
	Yes	28	11	17	-	18	10	-
MetS - # ATPIII criteria	0	20	19	1	**0.002**	15	5	0.828
	1	24	16	8	-	18	6	-
	2	28	17	11	-	19	9	-
	3	21	9	12	-	14	7	-
	4	6	2	4	-	3	3	-
	5	1	0	1	-	1	0	-
10-year CVD risk (%)		8.3(8.9)	6.1(6.8)	11.7(9.5)	**<0.001**	6.2(6.7)	11.9(9.4)	**0.002**
10-year CHD risk (%)		5.2(5.5)	4.3(3.4)	8.2(4.8)	**<0.001**	4.5(4.9)	7.5(5.5)	**0.004**
10-year MI risk (%)		1.5(2.4)	1.1(1.4)	2.7(3.1)	**<0.001**	1.2(1.9)	2.5(3.4)	**0.008**
10-year Stroke risk (%)		1.0(2.0)	0.8(1.3)	1.6(2.2)	**0.010**	0.8(1.2)	1.9 (2.2)	**0.003**
10-year CVD death risk (%)		0.6(1.9)	0.3(1.0)	1.2(2.9)	**0.001**	0.3(1.0)	1.6(3.4)	**0.001**
10-year CHD death risk (%)		0.3(0.8)	0.1(0.5)	0.6(1.6)	**<0.001**	0.1(0.5)	0.7(1.5)	**0.001**
25(OH)D (nmol/L)		82.5(24)	91(18)	61(20)	**<0.001**	84.5(24)	74.5(34)	0.062
IIEF-5 score		23(4)	24(3)	22(7)	**0.001**	24(2)	18.5(9)	**<0.001**

* Wellness, Lifestyle and Diet (Well-LaD) Study. **Non-responses are not shown. Continuous variables as mean±SD or median(IQR) and categorical variables as absolute frequencies. ***P-values for vitamin D status and ED status were derived from independent samples t-test for comparing means of normally distributed variables, Mann-Whitney U test for comparing distributions of non-normally distributed variables and X^2 or Fisher's exact tests for comparing frequencies between categories of categorical variables. 25(OH)D, 25-hydroxyvitamin D; A:G, android-to-gynoid fat ratio; AIx@HR75, augmentation index adjusted to heart rate 75 bpm; AP@HR75, augmentation pressure adjusted to heart rate 75 bpm; ATP III, adult treatment panel III; BF%, body fat percentage; BMI, Body Mass Index; BP, blood pressure; CHD, coronary heart disease; CVD, cardiovascular disease; DBP, diastolic blood pressure; DEXA, Dual energy x-ray absorptiometry; FAI, free androgen index; FPG, fasting plasma glucose; FPI, fasting plasma insulin; FT, free testosterone; HDL-c, high density lipoprotein cholesterol; HR, heart rate; HOMA1-IR, homeostasis model assessment one- insulin resistance; IIEF-5, 5-item International Index of Erectile Function; IQR, interquartile range; LDL-c, low density lipoprotein cholesterol; MetS, metabolic syndrome; MI, myocardial infarction; PA, physical activity; PHQ-9, 9-Item Patient Health Questionnaire; PWV, pulse wave velocity; SBP, systolic blood pressure; SD, standard deviation; SHBG, sex hormone binding globulin; TC, total cholesterol; TG, triglycerides; TT, total testosterone; VO_2peak, maximal oxygen consumption; WC, waist circumference; WHR, waist-to-hip ratio; WHtR, waist-to-height ratio.

3.4 Association of vitamin D status with health parameters

The associations between vitamin insufficiency (<75 nmol/L) and health parameters are shown in Table 5.5. Insufficiency was not associated with age, ethnicity or household income in this group of men. There was also no association with smoking or alcohol consumption. While there was no association between vitamin D insufficiency and handgrip strength, there was an association with fitness. The median estimated VO_2peak was significantly lower in men with vitamin D insufficiency ($p<0.001$) and they were also significantly more likely to have a lower level of cardiorespiratory fitness than men with sufficient vitamin D ($p=0.003$). This association was supported by the Cambridge PA Index: men with vitamin D insufficiency were observed to have a lower level of PA than men with sufficient vitamin D, although this did not reach statistical significance ($p=0.067$). There was no association with medication ($p=0.607$) or supplement use ($p=0.248$), indicative of the low level of vitamin D supplement use in this group.

Although vitamin D insufficiency was not associated with height ($p=0.298$), it was associated with all other anthropometric measurements assessed (all $p<0.05$) indicating a higher level of obesity, particularly central obesity - an established risk factor for CVD.

Vitamin D insufficiency was also associated with poorer vascular health measurements including higher blood pressure (SBP ($p=0.009$), DBP ($p=0.001$); a higher prevalence of hypertension ($p=0.019$); a higher HR ($p<0.001$); and higher AP@HR75 ($p=0.005$) and AIx@HR75 ($p=0.043$). Contrary to expectation, vitamin D insufficiency was associated with a lower PWV ($p=0.03$).

The lipid profile was worse in men with vitamin D insufficiency, indicated by significantly higher levels of TC, LDL-c, and TG and lower levels of HDL-c. Furthermore, the ratios of TC:HDL-c and TG:HDL-c were significantly higher in men with vitamin D insufficiency. Although there was no significant difference in the number of men with dyslipidemia (defined as ≥2 lipid abnormalities), vitamin D insufficiency was associated with a significant difference in the number of lipid abnormalities.

It was also associated with a significantly higher FPI level and HOMA1-IR score, indicating increased insulin resistance, and a higher FPG level, although this did not reach statistical significance. There was no association between vitamin D insufficiency and the presence of prediabetes or diabetes defined using FPG levels.

Vitamin D insufficiency was not associated with hormone profile or depression. However, hypogonadism was significantly more common in men with vitamin D insufficiency than men with sufficient vitamin D.

Men with 25(OH)D levels <75 nmol/L also had a significantly higher prevalence of MetS (defined as ≥3 ATP III criteria) and met a greater number of MetS criteria - indicating poorer cardiometabolic health. Most importantly, vitamin D insufficiency showed a highly significant association with Framingham risk: it was associated with a higher 10-year % risk of developing CVD including CHD, MI, and stroke, and with death from CVD and CHD events. Overall, these results indicate a significantly higher level of cardiometabolic risk amongst apparently healthy ageing men with a 25(OH)D level <75 nmol/L compared to men with a 25(OH)D level ≥75 nmol/L.

3.5 Association of erectile function with health parameters

The associations between ED (IIEF-5 score ≤21) and health parameters are shown in Table 5.5. ED was strongly associated with both age in years (p=0.001) and age range (p=0.002): the prevalence of ED increased with age. There was no association with ethnicity or income in this group of men (both p>0.05). We observed a higher number of standard alcoholic drinks consumed per week in men with ED, although this did not reach statistical significance (p=0.068). No associations were found between ED and smoking, PA level or cardiorespiratory fitness (all p>0.05), although there was a significant association with handgrip strength (p=0.003): men with ED had lower handgrip strength than men without ED. While the use of supplements was not significantly different in the two groups (p=0.658), men with ED were more likely to be taking some form of medication (p=0.005). Of the 28 subjects on one or more cardiovascular medication (i.e. beta-blockers, Ca^{2+}-channel blockers, ACE inhibitors, statins, anticoagulants or antiplatelets), 14 also had ED; therefore, 47% of men with ED were on cardiovascular medication compared to 20% of men without ED.

ED was not associated with any anthropometric measurements (all p>0.05), although we observed a higher level of A:G fat in men with ED which did not reach statistical significance (p=0.060). ED was not associated with any of the vascular health parameters measured, with the exception of HR (p=0.017): men with ED had a higher HR than those without ED. It was not associated with lipid profile, dyslipidemia or number of lipid abnormalities (all p>0.05). ED was not associated with FPG level (p>0.05); however, men with ED were observed to have a higher insulin level than men without ED (p=0.059) and higher HOMA1-IR scores (p=0.089), although neither reached statistical significance. There was no association with the presence of prediabetes or diabetes defined using FPG levels (p=0.536).

ED was associated with hormonal profile: there was no significant difference in TT between the two groups (p=0.761), however SHBG levels (p=0.001) were significantly higher and FAI (p<0.001) and FT (p=0.043) levels were significantly lower in men with ED compared to men without ED. There was no association with undiagnosed hypogonadism (p=0.921).

Depression (p=1.000) was not associated with ED in this group of men.

ED was not associated with MetS (p=0.437) or the number of MetS criteria met (p=0.823). However, it was strongly associated with a higher Framingham 10 year risk of developing CVD (p=0.002) including CHD (p=0.004), MI (p<0.008), and stroke (p = 0.003), but also death from CVD (p=0.001) and CHD (p=0.001). Overall, this indicates a significantly higher level of cardiovascular risk amongst apparently healthy ageing men with ED.

3.6 Relationship between vitamin D status and erectile function

There was a significant association between vitamin D status and erectile function. There was a weak but significant positive correlation between 25(OH)D level and IIEF-5 score (r_s=0.238, p=0.017, Table 5.6): as 25(OH)D level increased, erectile function improved. Figure 5.1 illustrates this relationship and shows that higher 25(OH)D levels are more likely with a higher IIEF-5 score indicating better erectile function.

Table 5.6. Spearman's correlations between serum 25-hydroxyvitamin D (25(OH)D) concentration and erectile dysfunction (ED, assessed using the 5-item International Index of Erectile Function (IIEF-5) with scores ranging from 5-25 where a higher score indicates better erectile function) in study participants (n=100).

		IIEF-5 score	No ED vs ED[†]
25(OH)D level (nmol/L)	r_s	.238*	-.188
	p-value (2-tailed)	**.017**	.061
Sufficient vs Insufficient**	r_s	-.252*	.176
	p-value (2-tailed)	**.011**	.079

*Correlation is significant at the 0.05 level (2-tailed).**Vitamin D status defined as sufficient (25(OH)D level ≥75 nmol/L) or insufficient (25(OH)D level <75 nmol/L). [†]ED defined as absent (IIEF-5 score >21) or present (IIEF-5 ≤21).

Furthermore, serum 25(OH)D levels were lower in men with ED compared to men without ED (74.5(34) vs 84.5(24) nmol/L respectively, p=0.062, Table 5.5) and there was a very weak correlation observed between 25(OH)D levels and the absence or presence of ED (r_s=-0.188, p=0.061, Table 5.6), however these did not reach statistical significance. Figure 5.2 illustrates the distribution of 25(OH)D levels in men with and without ED. There is no difference in the median 25(OH)D levels between the two groups (p=0.127) and although we observed a greater variation in the distribution of 25(OH)D levels in men with normal erectile function, this did not reach statistical significance (p=0.062). In univariate logistic regression analysis, serum

25(OH)D levels were a significant predictor of ED with every one unit increase associated with a 2% decrease in the risk of having ED (OR=0.98 [0.96-1.00], p=0.030, Table 5.7).

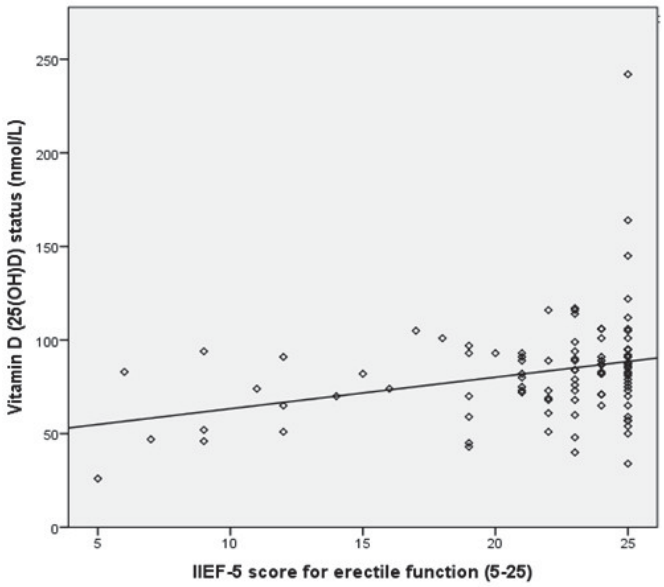

Figure 5.1. Graph of serum 25-hydroxyvitamin D (25(OH)D) concentration versus 5-item International Index of Erectile Function (IIEF-5) score (ranging from 5-25 where a higher score indicates better erectile function) in study participants (n=100): y=46.51 + 1.68(x), r^2=0.088.

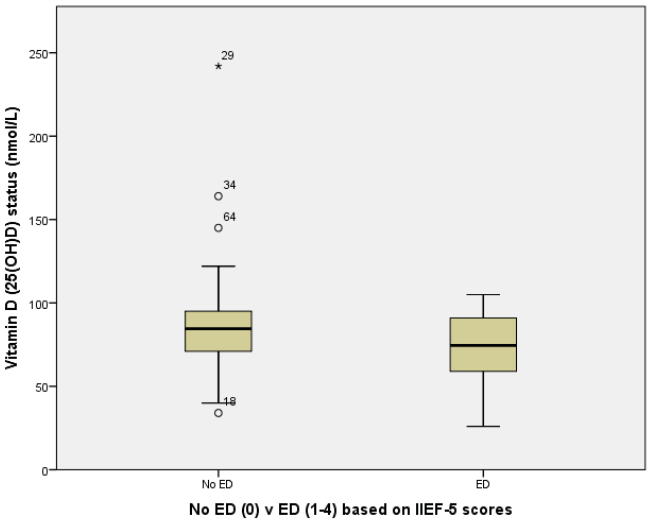

Figure 5.2. Relationship between serum 25-hydroxyvitamin D (25(OH)D) concentration and erectile dysfunction (ED, assessed using the 5-item International Index of Erectile Function (IIEF-5) and defined as a score ≤21) in study participants (n=100). Independent samples Mann-Whitney U test indicates no significant difference between the distributions of the two groups (p=0.062). Independent samples median test indicates no significant difference in the medians in the two groups (p=0.127).

Figure 5.3 shows the serum 25(OH)D levels according to the five established IIEF-5 categories of ED severity and although there is a downwards trend observed with decreasing 25(OH)D levels as ED severity increases, there is no significant difference in the median 25(OH)D levels (p=0.290) or the distributions (p=0.115) between the five categories.

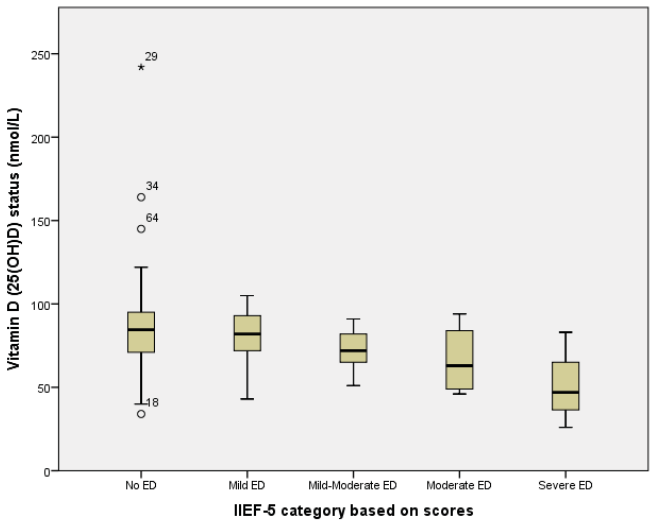

Figure 5.3. Relationship between serum 25-hydroxyvitamin D (25(OH)D) concentration and erectile dysfunction (ED, assessed using the 5-item International Index of Erectile Function (IIEF-5) score and defined according to established cut-off levels) in study participants (n=100). Independent samples Kruskal-Wallis tests indicate no significant difference between the distributions of the 5 groups (p=0.115). Independent samples median tests indicate no significant difference between the medians in the 5 groups (p=0.290).

Men with a serum 25(OH)D level <75 nmol/L had a significantly lower median IIEF-5 score than men with a serum 25(OH)D level ≥75 nmol/L (22(7) vs 24(3), p=0.001, Table 5.5) and there was a weak but significant negative correlation between sufficient and insufficient vitamin D and IIEF-5 score (r_s=-0.252, p=0.011, Table 5.6). This correlation was weaker when sufficient versus insufficient vitamin D status was compared between men with and without ED (r_s=0.176, p=0.079, Table 5.6) and did not reach statistical significance. However, in univariate logistic regression analysis, every one-unit increase in IIEF-5 score was associated with an 11% decrease in the risk of vitamin D insufficiency (OR=0.89 [0.81-0.97], p=0.011, data not shown). There results showed that erectile function was significantly worse in men with a 25(OH)D level <75 nmol/L compared to men with a level ≥75 nmol/L.

3.7 Predictors of erectile dysfunction

Table 5.7 shows the results of logistic regression analysis examining predictors of ED and presents unadjusted and age-adjusted ORs and 95% CI. Age was a significant predictor of ED with every one-unit increase in age (years) associated with a 10% increase in the likelihood of ED (OR=1.10 [1.04-1.16]). While being in the 50th decade was not a significant predictor of increased risk of ED, the risk of ED was over 5-times as high in men in their 60s compared with men in their 40s (OR=5.86 [1.92-17.88]). All other variables were therefore adjusted for age.

The number of standard alcoholic drinks was not a significant predictor of ED. Handgrip strength was found to decrease the risk of ED: every one unit increase in strength (kg) decreased the risk by 4% (OR=0.96 [0.93-0.98]), although this was not a significant predictor after adjusting for age. The use of medication was a significant predictor of an increased likelihood of ED (OR=3.72 [1.49-9.30]) and this remained after adjusting for age (OR=3.16 [1.16-8.61]).

Android obesity was not a significant predictor of ED (p=0.059); however, every one unit increase in A:G fat was observed to confer over 13-times the risk of ED. HR was a significant predictor of ED (OR=1.06 [1.01-1.11]); however, this did not remain after adjusting for age. Fasting insulin and HOMA1-IR scores were not significant predictors of ED. Higher levels of SHBG were found to predict ED with every one unit increase found to increase the likelihood of ED by 7% (OR=1.07 [1.02-1.11]) and this remained a significant predictor after adjusting for age (OR=1.07 [1.02-1.12]). Whereas higher levels of FT were found to predict a slightly lower likelihood of ED (OR=1.00 [0.99-1.00]) although this was not a significant predictor after adjusting for age. However, a higher FAI was associated with a slightly decreased risk of ED (OR=1.00 [0.99-1.00]) and remained a significant predictor after adjusting for age.

Higher Framingham risk scores, with the exception of 10-year stroke risk (OR=1.26 [0.99-1.59]), were all significant predictors of an increased likelihood of ED. For example, a one unit increase in the risk of developing CVD and CVD death over the next 10 years was associated with an 8% increase (OR=1.08 [1.02-1.15]) and a 29% increase (OR=1.29 [1.06-1.57]) in the risk of ED respectively; however they were no longer significant predictors after adjusting for age.

Vitamin D status was a significant predictor of ED. Raising serum 25(OH)D levels lowered the likelihood of ED: every one unit increase (nmol/L) was associated with a 2% decrease in risk (OR=0.98 [0.96-1.00]) and this remained significant predictor after adjusting for age (OR=0.98 [0.96-1.00]). Vitamin D insufficiency (<75 nmol/L) was not a significant predictor (p=0.081) but was observed to more than double the likelihood of ED (OR=2.18 [0.91-5.24]).

Table 5.7. Logistic regression odds ratios (OR) and 95% confidence intervals [95% CI] for lifestyle, metabolic and cardiovascular risk factors predicting erectile dysfunction (ED, IIEF-5 score ≤21) in study* participants (n=100).

		Crude associations		Age-adjusted associations	
Characteristic or condition		**OR [95% CI)**	**p-value****	**OR [95% CI]**	**p-value****
Age (years)		1.10[1.04-1.16]	**0.001**	-	-
Age range (years)	40-49	Referent		-	-
	50-59	1.51[0.45-5.07]	0.508	-	-
	60-69	5.86[1.92-17.88]	**0.002**	-	-
Standard alcoholic drinks per week		1.00[1.00-1.00]	0.727	1.00[1.00-1.00]	0.538
Handgrip strength (kg)		0.96[0.93-0.98]	**0.002**	0.97[0.94-1.01]	0.103
Medications	No	Referent		Referent	
	Yes	3.72[1.49-9.30]	**0.005**	3.16[1.16-8.61]	**0.025**
A:G		13.33[0.91-195.79]	0.059	11.53[0.68-195.301]	0.090
HR (bpm)		1.06[1.01-1.11]	**0.030**	1.04[0.99-1.10]	0.158
FPI (pmol/L)		1.00[1.00-1.01]	0.277	1.00[1.00-1.01]	0.243
HOMA1-IR		1.11[0.94-1.31]	0.211	1.12[0.93-1.34]	0.225
SHBG (nmol/L)		1.07[1.02-1.11]	**0.003**	1.07[1.02-1.12]	**0.005**
FT (pmol/L)		1.00[0.99-1.00]	**0.047**	1.00[0.99-1.00]	0.329
FAI		1.00[0.99-1.00]	**0.002**	1.00[1.00-1.00]	**0.020**
10-year CVD risk (%)		1.08[1.02-1.15]	**0.008**	1.03[0.95-1.11]	0.532
10-year CHD risk (%)		1.13[1.03-1.25]	**0.011**	1.06[0.94-1.19]	0.339
10-year MI risk (%)		1.19[1.01-1.40]	**0.035**	1.09[0.91-1.30]	0.351
10-year Stroke risk (%)		1.26[0.99-1.59]	0.057	0.88[0.63-1.24]	0.472
10-year CVD death risk (%)		1.29[1.06-1.57]	**0.012**	1.04[0.80-1.35]	0.796
10-year CHD death risk (%)		1.50[1.08-2.08]	**0.017**	1.12[0.76-1.66]	0.563
25(OH)D (nmol/L)		0.98[0.96-1.00]	**0.030**	0.98[0.96-1.00]	**0.046**
Vitamin D status	≥75 nmol/L	Referent		Referent	
	<75 nmol/L	2.18[0.91-5.24]	0.081	1.88[0.74-4.78]	0.184

*Wellness, Lifestyle and Diet (Well-LaD) Study. **P-values for unadjusted and age-adjusted associations with erectile dysfunction (ED, IIEF-5 score <21) derived from binary logistic regression. 25(OH)D, 25-hydroxyvitamin D; A:G, android-to-gynoid fat ratio; CHD, coronary heart disease; CVD, cardiovascular disease; FAI, free androgen index; FPI, fasting plasma insulin; FT, free testosterone; HR, heart rate; HOMA1-IR, homeostasis model assessment 1 - insulin resistance; IIEF-5, 5-item International Index of Erectile Function; MI, myocardial infarction; SHBG, sex hormone binding globulin.

ROC analysis of serum 25(OH)D level demonstrated an AUC of 0.618 (95% CI 0.499-0.737, p=0.062) to discriminate ED, indicating a poor diagnostic predictive capability (Figure 5.4). When a <75 nmol/L cut-off level was used the sensitivity and specificity were both very poor (50% and 31% respectively); however a cut-off level of <92 nmol/L showed a fair level of sensitivity and specificity (77% and 70% respectively).

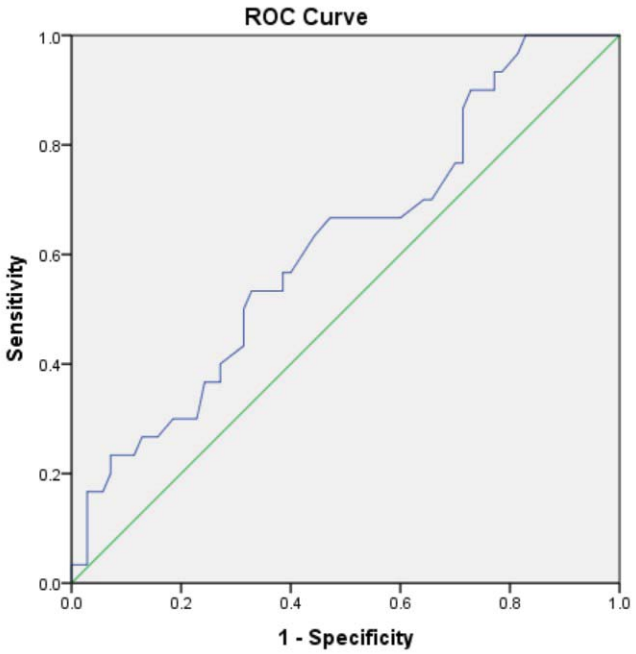

Figure 5.4. Receiver operating curve (ROC) of serum 25-hydroxyvitamin D (25(OH)D) concentrations in discriminating erectile dysfunction (ED, IIEF-5 score ≤21) from normal erectile function. Area under the curve (AUC) is 0.618 (SE=0.061, 95% CI 0.499-0.737, p=0.062). IIEF-5, 5-item International Index of Erectile Function.

4.0 DISCUSSION

The serum 25(OH)D level in our study of men aged 40-70 years is high (82.5 (range 26-242) nmol/L, 8 men <50 nmol/L) compared to the most recent 2008/2009 NZANS of adults aged over 15 years of age [19] (63.6±2 nmol/L, 32% <50 nmol/L) [19]. There were 37 men with vitamin D insufficiency using the Endocrine Society guideline of <75 nmol/L [18]; however, data using this cut-off level have not been reported for the NZANS. Although ageing is a risk factor for vitamin D deficiency due to a combination of decreased levels of the vitamin D precursor 7-dehydrocholesterol in the skin and a reduction in outdoor activity and resultant sun exposure [75], our findings indicate a higher level of serum 25(OH)D in older men. Dennis et al [76] also reported a high level of serum 25(OH)D level in 113 mature (53-94 years) community dwelling men in Dunedin NZ with no history of CVD (99 (range 2-317) nmol/L). This is despite the older age of participants, the majority of the blood samples being taken in autumn/spring and the latitude of Dunedin at 45° 52′ south – three factors that have been shown to result in reduced vitamin D effective sunlight exposure in winter and lower serum 25(OH)D levels [77, 78].

The high level of serum 25(OH)D in our study is likely due to the measurements being taken over the spring-summer period (October 2012 to March 2013) with greater levels of vitamin D effective UV exposure, combined with the lower risk of vitamin D insufficiency due to the predominately European ethnicity of the study population. Maori and Pacific Islanders have significantly lower 25(OH)D levels than NZ Europeans [77]. It may also be due to the largely rural and semi-rural living environment of these men as they were selected from the Manawatu region, not just the urban Palmerston North area. Urban factors such as shade from buildings and pollution can significantly decrease vitamin D effective UV exposure [79] while a rural lifestyle may include high levels of outdoor activity and incidental sunlight exposure. While 13 men consumed vitamin D supplements, the intakes were low (100-1056 IU/d) and removal of these men did not alter the median 25(OH)D level. The high level was affected by the outliers, which were not removed as they were of a priori interest: subject A (242 nmol/L, 65 years), B (164 nmol/L, 65 years) and C (145 nmol/L, 48 years). None of these participants reported taking any supplements and all were currently employed and living in an urban environment. Subject A had recently installed a swimming pool and was spending a lot of time sunbathing but reasons for the high levels in subjects B and C are unclear. The results show however that it is possible for older NZ men to obtain a high level of vitamin D (75-250 nmol/L) without supplementation.

The prevalence of ED (30 cases: 17% mild, 6% mild-moderate, 4% moderate and 3% severe ED) is lower than that found in our nationwide survey of NZ men aged 40-70 years (n = 562, 38% prevalence: 21% mild, 9% mild-moderate, 5% moderate and 3% severe ED, Chapter 3) after adjusting to the age distribution in the NZ population. This is likely to be due to the exclusion criteria which were designed to exclude men with many of the diagnosed medical conditions associated with neurogenic, endocrinological, anatomical and psychogenic ED (e.g., clinical diabetes [27, 80, 81], vascular, pelvic or spinal trauma or surgery [82, 83], endocrine disorders [27, 84-87], penile anatomical conditions [88, 89], and depression [90-92]) leaving predominately ED of vasculogenic aetiology. Despite these exclusion criteria, the proportions of ED cases in the five categories of severity were similar. The prevalence of ED was the same using the IIEF-5 and the single-item self-report question and although there were small differences in the categorisation of severity between the two tools, there remained substantial agreement in defining ED. It is clear however that the majority of men with ED are not medically diagnosed or treated, suggesting that recruitment via medical centres and pharmacy records in future research are not appropriate. These findings highlight the complexity of recruiting otherwise healthy men with ED.

Although self-reported healthy, there was a high level of cardiometabolic risk factors in this group of men (i.e., central adiposity, hypertension, dyslipidemia, prediabetes, hypogonadism and MetS) including a high level of risk of developing CVD over the next 10 years. This was despite a high level of healthful behaviours (i.e. not smoking, moderate alcohol consumption and participation in regular PA), highlighting the need for timely and effective intervention before clinical disease develops.

Our results support the associations between vitamin D insufficiency and CVD risk factors (e.g., obesity [93], hypertension [3, 94], lipid abnormalities [94], insulin resistance [94, 95] and MetS [96, 97]) found in published research [2]. This is further strengthened by the strong association between vitamin D insufficiency and Framingham risk scores showing an increased risk of developing CVD over the next 10 years. The finding of significant associations in such a small sample group highlights the strength of the relationship between vitamin D insufficiency and CVD development and the need for further research in apparently healthy older men.

There were fewer associations found between ED and CVD risk factors. As is consistently shown in epidemiological studies [98-104], we found a highly significant association between age and ED: the prevalence of ED was almost 6-times higher in men in their 60s compared to men in their 40s. After adjusting for age, the only independent predictors of an increased risk of ED in our study were taking medication (including ED medication) and raised SHBG and FAI

levels, while serum 25(OH)D level was the only independent predictor of a decreased risk. While TT levels decrease with age, SHBG levels increase causing a decrease in bioavailable testosterone (albumin-bound and FT) and late-onset hypogonadism [105-107]. ED is highly common amongst men with hypogonadism [108]; however studies have shown inconsistent results regarding the association between ED and TT and FT levels, depending on the cut-off level used [87, 109, 110]. Our results show no significance difference in the TT levels between men with and without ED; however, the SHBG and FT levels are significantly higher amongst men with ED. This suggests that bioavailable testosterone, rather than TT, may be important as a risk factor for ED, particularly in apparently healthy older men.

Contrary to other epidemiological studies, including our nationwide survey of NZ men (Chapter 3), we found no association between ED and sociodemographic (ethnicity [87, 111, 112] or household income [100, 101]) or lifestyle (smoking [46, 113-116], PA level [111, 113, 115, 117, 118] and cardiorespiratory fitness [119]) variables. We did find an association with handgrip strength: men with ED had significantly lower handgrip strength than men with normal erectile function. A simple and effective indicator of overall muscle strength, handgrip strength decreases with increasing age [120]: indeed it was no longer a predictor of ED after adjusting for age in our study. While many studies have shown an association between medical variables (obesity [99, 100, 111, 113, 115-117], hypertension [27, 32, 113], arterial stiffness [121], lipid abnormalities [113, 122-124], insulin resistance [125, 126], hypogonadism [87, 108-110], depression [69, 90] and MetS [127-131]), there was no evidence of this in our study. This was not unexpected given the small sample size and lack of diversity amongst the variables measured. However, the strong associations between ED and increased Framingham risk profile in our study have also been reported in several cross-sectional [132, 133] and longitudinal studies [134, 135] and overall, our results indicate a significantly higher level of cardiovascular risk amongst apparently healthy ageing men with ED. This supports the use of ED as an early marker of CVD.

This is the first study to our knowledge investigating the association between vitamin D status and ED as an early marker of CVD in an apparently healthy group of older men. Our results show a significant correlation between serum 25(OH)D levels and IIEF-5 scores. Furthermore, raising serum 25(OH)D levels is a significant predictor of a decreased likelihood of having ED, an association that is independent of age. This suggests that vitamin D may help protect against ED, revealing an exciting new field for research. However, correlation does not imply causation and further research is required to support our results.

The strengths of our study lie in the criteria used to ensure only men with vasculogenic ED were included, and the comprehensive nature of the health assessments covering a range of cardiovascular risk factors. The study limitations include the small sample size and the reliance on inferior sampling frames. Limited statistical power due to the modest sample size in the present study (n=100) may have played a role in limiting the significance of some of the statistical comparisons conducted. The sample size was limited by a poor response rate and resource constraints and although we attempted to recruit more men with ED through direct advertisement when filling prescriptions for ED medication, this was unsuccessful. The initial use of randomised selection from the Electoral roll, a population-based sampling frame, should have resulted in a cross-sectional sample; however, as the response rate was poor we were forced to rely on inferior sampling frames by advertising the study publicly and recruiting by word-of-mouth. Despite this, the sample was broadly cross-sectional with the age and education profile not significantly different from that reported in the 2013 NZ Census [71]. The Manawatu-Wanganui region is the 6th largest population out of the 16 regions in NZ, containing 5.2% (222,672) of the total NZ population (4,242,048). The sample was selected specifically from the Manawatu and Palmerston North city districts to ensure accessibility to the research unit. The higher response rate from men living in a rural/semi-rural environment (58, Table 5.2) reflects the largely rural nature of the Manawatu district, and therefore it is not surprising that the characteristics of this population differ from national proportions. The poor representation of ethnic minorities and those with lower socioeconomic status is not unexpected as despite meticulous planning there are many well established barriers that can lead to reduced participation amongst these groups (e.g., mistrust of research, fear and perceived harms, inclusion criteria restrictions, direct and indirect costs of participation (time, loss of income, transportation), family considerations, fear, lack of awareness or education and cultural or communication barriers [136]), the effects of which are compounded by the small sample size. Minority groups were reclassified as European or non-European for subsequent analysis. The results are applicable to European men aged 40-70 years and should not be generalised to the NZ population.

5.0 CONCLUSION

Low vitamin D status is associated with ED and other markers and risk factors for CVD amongst self-reported healthy men. A randomised placebo-controlled intervention trial designed to investigate the efficacy of improving serum 25(OH)D levels, in men with both vitamin D deficiency (<50 nmol/L) and vasculogenic ED, in ameliorating ED symptoms is warranted to address the question of causation.

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CHAPTER 6

LITERATURE REVIEW - COMMON POLYMORPHISMS IN THE VITAMIN D RECEPTOR GENE AND THEIR ASSOCIATION WITH VITAMIN D METABOLITES AND CARDIOVASCULAR DISEASE

1.0 INTRODUCTION

Low serum 25-hydroxyvitamin D (25(OH)D) has been associated with an increased risk of a plethora of health conditions, including cardiovascular diseases (CVD); however, the impact of improving vitamin D status remains uncertain [1]. Vitamin D exerts its functions via the binding of its hydroxylated metabolite 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$)) to the vitamin D receptor (VDR). The VDR is a nuclear hormone receptor (also known as NR1I1 or PPP1R163) that is encoded by the VDR gene (*VDR)* and acts as a receptor for both $1,25(OH)_2D_3$ and lithocholic acid, a secondary bile acid. The binding of the ligand $1,25(OH)_2D_3$ to the VDR allows heterodimerisation with the retinoid-X receptor (RXR) and the subsequent binding of the VDR-RXR complex to a vitamin D response element (VDRE) on a vitamin D responsive gene directs the formation of a sizeable transcriptional complex incorporating various co-regulatory molecules which ultimately activates or represses gene transcription [2]. Both the enzymes involved in the regulation of $1,25(OH)_2D_3$ levels (25-hydroxyvitamin D_3 1-alpha-hydroxylase (1α-hydroxylase) and 1,25 dihydroxyvitamin D_3 24-hydroxylase (24-hydroxylase)) and the VDR are expressed almost universally throughout the cells of the human body [3, 4]. Indeed, approximately 3% of all human genes appear to be regulated by vitamin D [5, 6] supporting its diverse biological roles. While it is the entire transcriptional complex (i.e. $1,25(OH)_2D_3$, VDR, RXR, VDRE and co-regulatory molecules) which determines the biological response to vitamin D, genetic variation in the *VDR* is the focus of this review as it may have important structural or functional implications resulting in altered transcriptional complex formation and therefore regulation of target gene expression. The effect of such genetic variation may, at least partially, explain the inconsistency in evidence from epidemiological and intervention studies supporting the association and beneficial effects of vitamin D on CVD outcomes and risk factors in different populations (as outlined in Chapter 4).

This review will focus on the four most well known and commonly studied SNPs located at diverse loci along the length of the *VDR*, hereafter referred to by their restriction enzyme codes: *Cdx*2 (rs11568820 (G/A)), *Fok*I (rs10735810 (C/T)), *Bsm*I (rs1544410 (A/G)) and *Taq*I (rs731236 (T/C or A/G)). This review has four aims: 1) to provide an introduction to the VDR; 2) to examine the prevalence of the *Cdx*2, *Fok*I, *Bsm*I and *Taq*I polymorphisms in the population; 3) to review evidence supporting their relationship with vitamin D metabolites, particularly serum 25(OH)D and; 4) to provide a critical review of studies examining the association between these *VDR* polymorphisms and CVD risk factors including erectile dysfunction (ED).

2.1 BACKGROUND

2.2 The vitamin D receptor gene (*VDR*)

The *VDR* (NCBI Gene ID: 7421) [7] has been mapped to human chromosome 12 at various locations from q12-q22 [8-10]; however, its location is currently referred to as 12q13.11: 47841537- 47905031 base pairs (bp) [7]. It is a large gene at approximately 63500 bp [7] with 9 exons [10, 11]. The various domains of the *VDR* (shown in Figure 6.1) are involved in different functions and consist of the extensive noncoding 5' promoter region (exons 1A to 1F), the DNA binding coding region (exons 2 to 4) and ligand binding coding region (exons 5 to 9) consisting of around 1280 nucleotides [12] and the 3' regulatory untranslated region (UTR) [10, 13-15].

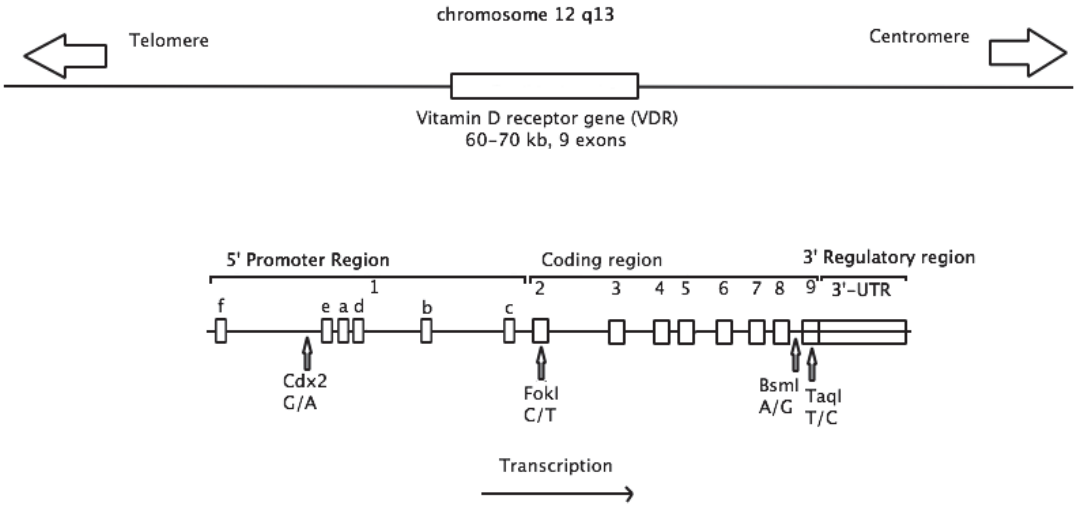

Figure 6.1: The structure of the vitamin D receptor gene (*VDR*) and the location of common polymorphisms. *Fok*I and *Taq*I are polymorphisms in the exon coding sequence (adapted from Uitterlinden et al [11]).

Approximately 64 variants have been described to date (Human Gene Mutation Database (HGMD)® Professional 2016, Qaigen). However, it has been suggested that over 100 polymorphisms are likely to exist in this gene [16], indicating that many are yet to be identified and confirmed. The majority of mutations are polymorphisms, a concept defined by their prevalence in at least 1% of the population [17]. Those identified are predominately synonymous single nucleotide polymorphisms (SNPs) in either the coding or non-coding regions of the gene, the latter of which appear to affect levels of VDR expression, mRNA stability or translational efficiency rather than the amino acid [18]. Several common allelic variants have been widely studied as markers of disease susceptibility: the restriction fragment length polymorphisms (RFLP) detected by the restriction enzymes *Cdx*2 (rs11568820 (G/A)) at the 5'-untranslated region of the gene [19], *Fok*I (rs10735810 (C/T)) at the start codon of exon

2 [20], and *Bsm*I (rs1544410 (A/G)) [21] and *Apa*I (rs7975232 (G/T) [22] at intron 8, and *Taq*I (rs731236 (T/C or A/G)) [23] at exon 9 (as shown in Figure 6.1) [16].

2.3 Functional mechanisms of common *VDR* polymorphisms

The possible functional mechanisms of these polymorphisms are likely to include changes to the structure, expression, or activity of the resultant protein. However, while many polymorphisms have been identified, their functional mechanisms remain unclear.

The exception to this is rs10735810 (also known as rs2228570) detected by the *Fok*I restriction enzyme [20, 24, 25] with a C to a T missense/nonsense mutation. Located at chr12:47879112, this is a start codon polymorphism and is the only SNP thus far that has been shown to alter the amino acid sequence of the translated VDR protein [11]. The ancestral allele is considered to be T with the change ATG-ACG resulting in an amino acid change from methionine to threonine. The T allele (detected as the "f" allele), codes for a longer 427 amino acid protein whereas the minor C allele (detected as the "F" allele) removes the start codon, altering the translation site to further downstream resulting in a shorter 424 amino acid protein [26]. The shorter isoform has been reported to result in more efficient vitamin D signaling by interacting more effectively with transcription factor IIB (TFIIB) [26] and this may result in higher levels of transactivation of the VDRE on target genes [27, 28]; however, the studies are conflicting [29, 30]. It has been reported to have biological implications including altered calcium homeostasis [31] and cell growth [32]. It is therefore a disease-associated polymorphism [31, 33] with supporting functional evidence. Unlike other SNPs such as *Bsm*I, *Apa*I and *Taq*I, there is also currently no evidence to support linkage disequilibrium (LD) with any other VDR gene polymorphisms, therefore *Fok*I is considered an independent genetic marker [11].

The other polymorphisms, such as *Cdx*2 in the 5' promoter region and *Bsm*I, *Tru*9I (rs757343 (G/A)) [34], *ApaI* and *Taq*I near the 3' regulatory region do not appear to have direct structural or functional effects on the VDR protein. Located in the 5'-promoter region, the rs11568820 SNP (identified by the *Cdx*2 restriction enzyme [19]) has been well sequenced. It is a regulatory mutation associated with disease phenotypes (e.g. lower bone mineral density (BMD) [19, 35] and Alzheimer's disease [36]) supported by evidence of functional effects on the level of VDR expression through altered VDR promoter activity [36]. The G allele is associated with a significant 70% decrease in transcriptional activity compared with the A allele [19]. An additional polymorphism located in the promoter region is GATA which has been shown to be in LD with *Cdx*2. The G allele appears to decrease the transcription rate, possibly lowering expression of the VDR and ultimately vitamin D signaling [37].

The other SNPs are all located at the 3'-UTR region of the *VDR* gene with the majority being intronic, with the exception of *Taq*I which is located in exon 9. While none of these SNPs alter the amino acid sequence of the translated VDR protein and their functional effects remain unclear, this may be the result of LD with other functional variants [38] that modify transcription, translation, and processing. They have been reported to be involved in the regulation of mRNA stability [11] and the level of expression [37, 39, 40]. In contrast to *Fok*I, a high level of LD has been reported between the *Bsm*I, *ApaI* and *Taq*I polymorphisms [41] - these alleles are inherited together more often than is suggested by chance alone. The presence of one can predict the presence of another, creating subset of polymorphisms or a haplotype block in that area of the gene. In this case, rather than individual polymorphisms, the haplotype may be responsible for the combined effects on gene expression. The level of LD appears to vary between ethnic groups and is strongest amongst Caucasians [42]. They also appear to be in LD with an additional poly(A) microsatellite repeat in the 3'-UTR that is suggested to affect mRNA stability [11] and local levels of the transcribed VDR protein [42]. It is also possible that the functional effects of these polymorphisms are a result of LD with other functional alleles that are yet to be elucidated [11, 16, 18].

Assessment of the functional effects is complex and can be studied at an *in vitro* or molecular level (i.e. effects of a polymorphism on mRNA, protein expression and cellular activity markers), an *in vivo* level (i.e. effects of a polymorphism on serum biomarkers, health measurements or response to supplementation) and an epidemiological level (i.e. the association between a polymorphism and a disease) [11]. Although there is variation in the prevalence of these common polymorphisms between populations, and inconsistencies in their association with vitamin D metabolites and disease phenotypes, overall there is increasing evidence to support their modulating effect on the biological response to vitamin D.

2.4 Determinination of *VDR* polymorphisms

The Human Genome Project (HGP) (1990-2003) [43] has made a significant contribution to the sequencing of DNA bases and the identification and mapping of genes on the euchromatic human genome. Rapid advancements have since been made in the identification of gene variants and their functional outcomes and disease conditions through studies such as the 1000 Genomes Project (2008-2015) [44] which identified gene variants in 1000 individuals from 5 ethnic populations in 26 countries around the world. Furthermore, advancements in technology such as real-time polymerase chain reaction (PCR) platforms and automated genetic sequencing now support the rapid and accurate identification of the the presence and the amount of a specific sequence of interest [45]. As research in this field progresses,

systematic analysis of the VDR gene may reveal additional polymorphisms with functional consequences that will help elucidate the mechanism behind the plethora of roles for vitamin D in the human body. The individual polymorphisms, their links with other nearby *VDR* polymorphisms and other proximal genes involved in vitamin D metabolism and signaling may be important considerations. Despite rapid advancements in this area, our knowledge of the complex functional gene-gene interactions is in its infancy.

Human genetic analysis requires the extraction and purification of DNA from a tissue sample, usually blood. Accurate genotyping is essential and there are many methods available. While developments in whole genome sequencing offer great potential for the accurate analysis of a range of pre-selected SNPs on multiple genes compared to a reference genome, this method remains expensive, relatively inaccessible and impractical in many clinical and research situations. Gene-specific Sanger sequencing is considered the 'gold standard' for the detection of variants [46] as it provides high sensitivity and specificity and can determine multiple variants within a candidate gene [47]. However, non-sequencing molecular methods with targeted allele-specific variant detection remain the mainstay as they are one of the cheapest and most robust methods available [47].

The most widely used method in *VDR* genetic analysis is PCR-RFLP. This is a second generation technique as the fragment of interest has already been cloned and sequenced, allowing the design of primers to selectively identify, cut and replicate it. Only a small amount of genomic DNA (gDNA) containing the fragment is needed to amplify it *in vitro* and generate a large quantity for qualitative analysis. The PCR products are then incubated with appropriate restriction enzymes and the digestion products analysed using gel electrophoresis to determine the size of restriction fragments compared to a molecular-weight size marker or DNA ladder [45].

Alternatively, the development of high-resolution melting (HRM) compatible real-time PCR machines, such as the LightCycler® 480 with gene scanning software, allows the combining of PCR amplification with HRM analysis in a closed-tube assay. It involves the use of a florescent dye in the PCR reaction mix which flouresces brightly intitially then reduces with the increasing temperature as the double-stranded DNA amplicons melt apart. In real-time, the software automatically measures flouresence and graphs it versus temperature to create a melt curve. The presence of a mutation in the amplified fragment results in a different melt curve, either by temperature (homozygotes) or by shape (heterozygotes). The software can automatically and accurately identify genotypes by analysing differences in the melt profile between no mutation, one allelic mutation, or two allelic mutations compared to controls of a known

genotype. This makes it a simple, fast, powerful, accurate and cost-effective method.

There are three main risks of error associated with PCR-based techniques. Firstly, there is a risk of contamination by exogenous DNA which may lead to false results. This can be monitored through the maintenance of good laboratory technique and the use of negative controls (reagent only) in all reactions. There is also a risk of non-specific amplification resulting from incorrect hybridisation of the primers. The likelihood of this can be reduced by selecting primers that are of sufficient length, purity and have high and identical (if possible) annealing temperatures. Finally, copy infidelity can occur where the enzyme mis-incorporates creating a mutated fragment which is then amplified. This has little effect on subsequent analysis concerned with the length of the fragment of interest but can be an issue for sequencing or quantitative analysis [45]. Finally, the main disadvantage in using these allele-specific PCR methods is that they are unable to determine the presence of any other relevant variants within the candidate gene of interest [47], nor can they offer insights into gene-gene interactions.

2.5 Comparability issues in current literature

A large number of polymorphisms have been identified; however, many have not been confirmed in subsequent studies and it has been suggested that many may not be real [11]. Indeed, this is supported by the merging of previously identified and studied SNPS in the NCBI Database of Single Nucleotide Polymorphisms (dbSNP). For example, rs2228571, rs17777794, rs17880019, rs59730659, rs118037316 and rs386609145 have all subsequently been merged with rs731236 (*Taq*I, (T/C)) [48]. This hinders advancement in this area as studies cannot be reliably compared. The generation of reliable sequence information from multiple individuals is needed to help improve the current databases.

Comparability between studies is further hindered by inconsistencies in allele coding systems, predominately due to the use of the different genetic analysis techniques mentioned above. Classical nomenclature generally applies an uppercase allele where a restriction site is absent and a lowercase allele where a restriction site is present (i.e. “B” for the absence and “b” for the presence of the *Bsm*I restriction site), although some researchers use ACGT alleles with the specific amino acid in the forward strand of the gene sequence used (i.e. the “A” or “G” allele for the *Bsm*I polymorphism), and others use a combination of these two nomenclatures, depending on the polymorphism (i.e. “G” and “A” are typically used to define *Cdx*2 alleles and “B” and “b” for *Bsm*I alleles). This system assumes that the forward strand is known with certainty and that the alleles are defined by the forward strand, rather than the reverse strand. However, there are far too many uncertainties currently in the human genome to

confidently infer the correct forward strand coding. This coding system can lead to rs731236 (*Taq*I) being defined with either T/t alleles, T/C alleles based on the forward strand, or A/G alleles based on the reverse strand [48]. Increasingly researchers are beginning to use other coding systems such as Illuminas A/B, 1/2 or TOP/BOT allele coding systems which removes the need to define a forward strand and can therefore reduce confusion when comparing studies. However, as the majority of published research to date has used classical nomenclature, this review will focus predominately on studies that have used this system of allele coding.

3.1 THE PREVALENCE OF THE *CDX*-2*, FOK*I, *BSM*I AND *TAQ*I POLYMORPHISMS

The determination of ancestral alleles is important to understanding the evolution of the human genome including genomic signatures resulting from selection pressures, the formation of LD patterns and the changing prevalence of disease-associated alleles [49]. The following ancestral alleles are listed on NCBI dbSNP [48] for the common *VDR* SNPs of interest: *Cdx*2 A allele, *Fok*I T allele, *Bsm*I G allele and *Taq*I T allele.

There is large variation in the frequency of common *VDR* polymorphisms in published studies internationally, although much of this apparent geographical variation may be explained by distinct racial and ethnic differences [11, 50, 51]. For example, a large difference in the prevalence of the *Cdx*2 alleles has been reported amongst Americans of different ethnicities [51]. The ancestral A allele was more prevalent amongst African Americans (n=98, 76%), compared to Asian (n=30, 47%), Hispanic (n=144, 22%) and Whites (n=2697, 19%). Data for genotypes were not provided. Similarly, a large difference in the prevalence of the *Taq*I alleles has been reported amongst Africans of different ethnicities. The ancestral T allele and the TT genotype ($p<0.001$) have been shown to be higher amongst Blacks (n=181, T 80%, t 20%, TT 65%, Tt 31%, tt 4%) compared to both Whites (n=238, T 62%, t 38%, TT 36%, Tt 51%, tt 13%) and Indians (n=175, T 62%, t 38%, TT 35%, Tt 54%, tt 11%) in Durban, South Africa [50]. The high proportion of the ancestral alleles in African Americans and Africans is not unexpected given the hypothesised African origin of modern humans [52]. When comparing the frequency of these polymorphisms between geographical locations within a race or ethnic group, such as Caucasian/Europeans, only minor differences are evident (as shown in Table 6.1) and they are generally close to that expected based on the reference minor allele frequency (MAF) reported for British in England and Scotland from the 1000 Genomes Project [53]. The differences in prevalence of these polymorphisms by race and ethnicity may be the result of gene-environment interactions and an adaptive response to environmental conditions. Irrespective of the evolutionary rationale, differences in the frequency of these variants may be a

contributing factor to the differences in disease susceptibility in different populations.

3.2 Prevalence of *VDR* polymorphisms in New Zealand

Data on the prevalence of common *VDR* polymorphisms in New Zealand (NZ) are limited [54-56] (see Table 6.1). In 2012, Jain et al [55] investigated the association between insulin resistance (IR) and the *Cdx*2, *Fok*I, *Bsm*I, *Apa*I, and *Taq*I polymorphisms in 239 South Asian women living in Auckland (mean age = 40.6 ± 10.3 years). They reported the following frequencies: *Cdx*2 AA 18%, AG 55.2%, GG 26.8%; *Fok*I FF 58.2%, Ff 34.3%, ff 7.5%; *Bsm*I BB 19.3%, Bb 49.8%, bb 30.9%; *Apa*I AA 32.3%, Aa 46%, aa 21.8%; and *Taq*I TT 47.3%, Tt 43.1%, tt 9.6%. The MAF were *Cdx2* 45.6%, *Fok*I 24.7%, *BsmI* 44.1%, *Apa*I 21.8% and *Taq*I 31.2%. Also in 2012, Bentley et al [54] investigated the association between *Cdx*2, *Fok*I and *Taq*I polymorphisms and colorectal cancer in 400 Europeans in Canterbury (200 cases, 200 controls, 53% men, mean age = 69.5±0.4 years). The allele coding used was 1/2 and as this was not explained in the paper it has been taken to refer to classical nomenclature for comparability. They reported the following frequencies in the control group: *Cdx*2 AA 3.3%, AG 34.6%, GG 62.1%; *Fok*I FF 41.4%, Ff 41.9%, ff 16.8%; *Taq*I TT 17.6%, Tt 47.3%, tt 35.2%. The MAF were *Cdx*2 20.6%, *Fok*I 37.7%, and *Taq*I 41.2%. Most recently, in 2013, Carvalho et al [56] investigated the association between vitamin D status, Crohn's disease and various genotypes including the *Apa*I and *Taq*I polymorphisms in 608 Caucasians in Auckland (306 controls, 302 Chron's patients, age range 10-91 years) but did not report the frequencies. Despite the small number of available studies, there are clear differences in the frequencies between South Asians [55] and Europeans [54] in NZ. Large epidemiological studies are needed to provide population-based data on the prevalence of these common VDR polymorphisms in NZ, particularly amongst different ethnic groups including Maori and Pacific Islanders.

Table 6.1. A comparison of frequencies of four common polymorphisms of the VDR gene (*Cdx*-2 (rs11568820), *Fok*I (rs10735810), *Bsm*I (rs1544410) and *Taq*I (rs731236)) in different Caucasian/European populations from selected cross-sectional studies or healthy control groups of case-control studies.

First author, year	Country	Sample size (% men)	Age range (yrs)	Genotype frequency (%)			Allele frequency (%)		Ref MAF (%)*
***Cdx*-2 (rs11568820 (A/G)**				**GG**	**GA**	**AA**	**G**	**A**	21
Bentley 2012 [54]	New Zealand	200 (53%)	69.5±0.4	62.1	34.6	3.3	62.1	20.6	
Slattery 2007 [51]	USA	2697 (ND)	30-79	ND	ND	ND	81.0	19.0	
Han 2007 [57]	USA	854 (0%)	ND	64.2	31.5	4.2	80.0	20.0	
Ochs-Balcom 2008 [58]	USA	246 (33%)	58.5±12.1	63.4	32.5	4.1	80.0	20.0	
Randerson-Moor 2009 [59]	England	402 (42%)	ND	62.2	33.3	4.5	78.9	21.1	
Stathopoulou 2011 [60]	Greece	578 (0%)	ND	61.3	33.5	5.2	ND	ND	
Casado-Diaz 2013 [61]	Spain	229 (0%)	57.4±12.8	57.5	34.6	7.9	74.8	25.2	
***Fok*I (rs10735810 (T/C)**				**FF**	**Ff**	**ff**	**F**	**f**	39
Bentley 2012 [54]	New Zealand	200 (53%)	69.5±0.4	41.4	41.9	16.8	62.3	37.7	
Han 2007 [57]	USA	854 (0%)	ND	38.1	49.0	13.0	62.5	37.5	
Li 2008 [62]	USA	841 (67%)	ND	40.9	47.1	12.0	64.4	35.6	
Ochs-Balcom 2008 [58]	USA	246 (33%)	58.5±12.1	36.2	50.4	13.4	61.0	39.0	
Hutchinson 2000 [63]	England	108 (50%)	56 ±20	48.1	40.7	11.1	68.5	31.5	
Barroso 2008 [64]	Spain	245 (50%)	ND	46.2	41.3	12.5	65.9	33.1	
Randerson-Moor 2009 [59]	England	402 (42%)	ND	40.1	43.8	16.2	78.9	21.1	
De Jongh 2011 [65]	The Netherlands	926 (49%)	75.7±6.6	39.0	46.3	14.7	62.1	37.9	
Laczmanski 2013 [66]	Poland	844 (52%)	>65	31.2	49.3	19.5	55.8	44.2	
Jorde 2015 [67]	Norway	5980 (43%)	57.4±9.9	42.5	45.5	12.0	65.2	34.8	
***Bsm*I (rs1544410 (A/G))**				**BB**	**Bb**	**bb**	**B**	**b**	35
Beckett 2014 [68]	Australia	200 (43%)	75.0±0.5	12.0	53.5	34.5	38.7	61.2	
Han 2007 [57]	USA	840 (0%)	ND	15.5	47.4	37.1	39.2	60.8	
Li 2008 [62]	USA	841 (67%)	ND	17.7	50.8	31.5	43.1	56.9	
Ye 2001 [69]	France	143 (50%)	61±16	16.8	45.4	37.8	39.5	60.5	
Tworowska-Bardzinska 2008 [70]	Poland	351 (0%)	50-60	11.7	51.0	37.3	37.2	62.8	
Randerson-Moor 2009 [59]	England	402 (42%)	ND	16.4	50.3	33.3	41.5	58.5	
Stathopoulou 2011 [60]	Greece	578 (0%)	ND	17.0	51.3	31.8	ND	ND	
Laczmanski 2013[66]	Poland	848 (52%)	>65	29.1	49.4	21.5	53.8	46.2	
Jorde 2015 [67]	Norway	5980 (43%)	57.4±9.9	17.7	48.1	34.1	41.7	58.1	

First author, year	Country	Sample size (% men)	Age range (yrs)	Genotype frequency (%)			Allele frequency (%)		Ref MAF (%)*
***Taq*I (rs731236 (T/C))**				**TT**	**Tt**	**tt**	**T**	**t**	
Bentley 2012 [54]	New Zealand	200 (53%)	69.5±0.4	35.2	47.3	17.6	58.8	41.2	
Taylor 1996 [71]	USA	162 (100%)	ND	32.7	45.1	22.2	55.2	44.8	
Ochs-Balcom 2008 [58]	USA	246 (33%)	58.5±12.1	39.4	46.7	13.8	63.0	37.0	
Li 2008 [62]	USA	841 (67%)	ND	32.0	50.2	17.8	57.1	42.9	
Hutchinson 2000 [63]	England	93 (50%)	56±20	41.9	44.1	14.0	64.0	36.0	34
Ye 2001 [69]	France	143 (50%)	61±16	37.8	46.1	16.1	60.8	39.2	
Barroso 2008 [64]	Spain	245 (50%)	ND	38.8	44.3	16.9	61.0	39.0	
Randerson-Moor 2009 [59]	England	402 (42%)	ND	35.8	48.3	15.9	60.0	40.0	
Stathopoulou 2011 [60]	Greece	578 (0%)	ND	34.5	49.7	15.7	ND	ND	
Jorde 2015 [67]	Norway	5980 (43%)	57.4 ± 9.9	34.0	48.2	17.8	58.1	41.9	

*Reference minor allele frequency (MAF) for Caucasian populations taken from 1000 Genomes Project Phase 3 May 2013 call set for British in England and Scotland [53]. The alleles are coded as follows: *Cdx*-2 A/G, *Fok*I F(T)/f(C)), *Bsm*I (rs1544410 B(A)/b(G)) and *Taq*I (T(T)/t(C)). Studies using this coding have been selected where possible. Bentley et al [54] was also included with a 1/2 allele coding system taken to align with absence/presence for restriction sites. Studies where alleles were referred to as minor/major were taken to align with the reference MAF. Where overall prevalence was not provided, it was calculated as the mean of male and female frequencies. Where MAF were not provided they were calculated using allele counts from genotype counts, or frequencies and sample size.

4.0 THE LINK TO VITAMIN D STATUS

The main influence on serum 25(OH)D concentrations is sunlight exposure [72]; however, evidence suggests that genetic factors are also important [73]. Common variants in the *VDR* may influence vitamin D status and also modify the association between serum 25(OH)D concentrations and disease phenotypes.

Many studies investigating the assocation between VDR polymorphisms and disease in Europeans [74] (including those listed in Table 6.1 [51, 54, 58, 68]) have not measured serum 25(OH)D. However, the studies that have, show inconsistent results regarding the association between *Cdx2*, *FokI*, *BsmI* and *TaqI* and serum 25(OH)D levels [75, 76]. In earlier studies, in 2006 Ramos-Lopez et al [77] investigated the assocation between 11 polymorphisms in the *VDR (*including *Fok*I, *Bsm*I and *Taq*I) and serum levels of $25(OH)D_3$ and $1,25(OD)_2D_3$ in 158 German subjects from familes with type 1 diabetes mellitus (T1DM). They found no significant associations with these common SNPs; however, patients with a rare rs3847987 polymorphism CC genotype had a significantly higher median serum 25(OH)D level and this was suggested to infer a genetic basis for vitamin D insufficiency. Also in 2006, Wjst et al [78] reported no association between 14 *VDR* polymorphisms (including *Fok*I, *Bsm*I and *Taq*I) and serum 25(OH)D levels in 872 Caucasian German and Swedish individuals (53% men) in a study of 210 families with asthma.

In contrast to these earlier large studies assessing multiple VDR variants, in 2008 Orton et al [75] reported that mean concentrations of serum 25(OH)D in 198 Canadian twin subjects with the *Fok*I FF genotype (coding for the shorter length VDR) was 64±6 nmol/L compared with 80±4 nmol/L and 100±9 nmol/L in subjects with the Ff and ff genotypes respectively. Similarly, in 2009, Smolders et al [76] reported lower serum 25(OH)D levels with the FF genotype amongst 289 healthy Dutch subjects (50% men); however the $1,25(OH)_2D$/25(OH)D-ratio was higher than in those with the Ff or ff genotypes. This suggests that the 25(OH)D levels may be lower as the F allele results in upregulation of the production of $1,25(OH)_2D$. Indeed, low serum 25(OH)D and raised $1,25(OH)_2D$ levels have been reported in children with *VDR* variants and altered VDR protein function [79] and this has been suggested to be the result of downstream alterations to the activation of genes involved in vitamin D signaling increasing the production and decreasing the catabolism of $1,25(OH)_2D$ [80].

In 2011, De Jongh et al reported that amongst 935 older Dutch adults (49% men, mean age = 75.7±6.6 years), subjects with fewer copies of an AA *Cdx*2-GATA haplotype had higher serum 25(OH)D concentrations (0, 1 and 2 copies: 52.0 ±23.4, 49.7±23.2 and 44.0±21.7 nmol/L respectively, p=0.04). Although data for the individual *Cdx*2 genotypes were not provided, this

suggests that the A allele may be associated with lower serum 25(OH)D levels. In contrast to the earlier Dutch study [76], they reported no significant difference in the levels of serum 25(OH)D by *Fok*I genotype or *Bsm*I-*Apa*I-*Taq*I haplotype.

In other ethnicities the evidence is less clear. In 2011, Jeedigunta et al [81] reported no significant difference in the serum 25(OH)D levels between *Fok*I genotypes in 175 healthy South Indian women (0% men, age range = >25 years). However, in 2012, Li et al [82] reported a significant assocation between *Fok*I and serum 25(OH)D levels in 763 healthy Chinese control subjects, with the lowest levels in those with the FF genotype. There was no association with either *BsmI* or *Taq*I. Most recently, in 2016, Coşkun et al [83] reported no significant association between *Cdx*2, *Bsm*I, *Apa*I and *Taq*I polymorphisms and serum 25(OH)D levels in 85 Turkish children with autism spectrum disorder (age range 2-15 years); however serum 25(OH)D levels were significantly associated with the *Fok*I genotype with levels highest in those with the ff genotype compared to the Ff and FF genotypes (96.23 ± 17.68, 83.78 ± 23.36 and 71.21 ± 28.35 respectively, p=0.041).

The evidence appears to support an association between the *Fok*I polymorphism and serum 25(OH)D levels with the f allele (the minor allele found in approximately 39% of Caucasian populations [53]) associated with better vitamin D status [75, 76]. The implication is that the longer VDR protein resulting from the *Fok*I polymorphism alters the functional properties of the VDR influencing the downstream activation of genes including those involved in vitamin D synthesis and/or hydroxylation. However, there may be ethnic differences [81, 82] which require further investigation. There is little evidence to support an association between the other SNPs and serum 25(OH)D concentrations suggesting that their functional affects, or that of the SNPs with which they are in LD, do not affect the transcription of genes involved in vitamin D synthesis or hydroxylation. However, most studies have focussed on serum 25(OH)D levels and few have also measured 1-25$(OH)_2$D concentrations. Further research is needed to clarify whether these variants affect the transcription of genes involved later in the vitamin D signalling pathway, e.g. altering 1-25$(OH)_2$D degradation.

Although evidence is limited and inconsistent, it does suggest that common *VDR* polymorphisms may interact with 25(OH)D status. Whether this implies personalised recommendations are needed to determine appropriate supplementation levels based on genotype remains unclear. It is possible that serum 25(OH)D levels can exacerbate or ameliorate the impact of *VDR* polymorphisms on health outcomes. For example, TB has been associated with the T allele of the *Taq*I polymorphism in Gujarati Asians living in London, but only in the presence of inadequate vitamin D [84]. It is possible that the optimal 25(OH)D

concentration required to reduce the risk of disease differs depending on VDR genotype and that this is confounding relationships between vitamin D and disease outcomes [85].

Several meta-analyses of genome-wide studies have been conducted, relating different genetic loci involved in vitamin D metabolism and activity [6, 86]. In 2010, Wang et al [6] reported a significant relationship between serum 25(OH)D levels and variants at three genetic loci: a gene involved in the metabolism of 7-dehydrocholesterol (*DHCR7*), and genes encoding the 25-hydroxylase enzyme involved in the metabolism of 25(OH)D (*CYP2R1*) and the vitamin D binding protein (DBP) involved in the transportation of vitamin D (*GC*), in 33,996 Europeans from 15 cohorts. The results of another smaller meta-analysis in 2010 by Ahn et al [86] supported these strong associations in 4501 Europeans from 5 cohorts with replication studies in 2221 individuals. These genome-wide studies highlight the importance of variants in a diverse range of genetic loci to the regulation of vitamin D status. Future studies should consider assessing both serum 25(OH)D and 1-25$(OH)_2$D concentrations and variants in genes involved throughout the vitamin D signaling pathway.

5.1 THE LINK TO DISEASE PHENOTYPES

The wide range of genes regulated by activation of the VDR may explain the varied extra-skeletal roles of vitamin D [87-91]. While substrate availability (1,25$(OH)_2D_3$) determines the activation of the VDR [6, 92], it is possible that the importance of vitamin D in human health may lie in variations in VDR activity, rather than the endogenous and exogenous quantity of vitamin D and its metabolites. The primary role of the VDR is in the regulation of calcium homeostasis with 1,25$(OH)_2D_3$ shown to upregulate intestinal calcium absorption and the expression of calcium transporters in the epithelium [93] and multiple VDRE identified on the gene encoding the intestinal epithelial calcium ion channel [94]. Therefore early studies focused on the association between VDR polymorphisms and skeletal health (e.g., BMD [19, 35]). However, increasingly studies are investigating the potential effects on extra-skeletal health. There is evidence of an association between common *VDR* polymorphisms and overall mortality [65] and increased susceptibility to a diverse range of diseases including osteoporosis [95, 96], autoimmune conditions (e.g., T1DM [97-99], Rheumatoid arthritis [100]), Parkinson's disease [101, 102], Alzheimer's disease [36, 103, 104], cirrhosis of the liver [105], various cancers [18, 106-110], inflammatory bowel disease [111], T2DM [33, 112, 113] and CVD [114-117].

5.2 Cardiovascular disease outcomes

Support for the relationship between VDR polymorphisms and CVD outcomes comes from recent longitudinal cohort studies [118, 119]. In 2012, a community-based prospective cohort in the USA (The Cardiovascular Health Study) [118] (n=1514, 100% Caucasian, 30% men, mean age = 73.9±4.6 years, mean baseline 25(OH)D level = 66.8 nmol/L (26.7 ng/ml), baseline measurements 1992-1993, median follow-up = 11 years) was used to investigate the effect of 141 common variants of six genes (MAF >5%) involved in the vitamin D metabolic pathway on the association between low 25(OH)D levels (below the season specific 20th percentile) and the time until a composite health outcome occurred (incident hip fracture, myocardial infarction (MI), cancer or death). This included variants in the vitamin D binding protein (*GC*), megalin (*LRP2*), cubulin (*CUBN*), 1- α hydroxylase (*CYP27B1*), 24-α hydroxylase (*CYP24A1*) and *VDR* genes. The composite outcome was experienced by 63% of participants and after adjustment for age and sex, low 25(OH)D concentration increased this risk by 32% (HR=1.32 [1.13-1.54], p=0.001). Amongst the five SNPs with significant interactions with the association between low 25(OH)D levels and the composite outcome were two less commonly studied *VDR* SNPs (rs7968585 (MAF 0.48) and rs2239179 (MAF 0.42)). After adjusting for age and sex, each additional minor allele of these SNPs was associated with a 40% increase (HRR=1.4 [1.1-1.7], p=0.002) and a 30% decrease (HRR=0.7 [0.6-0.9], p=0.008) respectively in the risk associated with low 25(OH)D levels. However, the effect on the association with MI alone was not signficant for either SNP. *Bsm*I was also included but did not reach statistical significance. In independent replication meta-analyses of 3 other large international cohort studies, only rs7968585 remained a significant modifier of the relationship between low 25(OH)D and the composite outcome. Similarly, in 2015 a large community-based longitudinal cohort in Norway (The Tromsø Study, n=26956, 47.5% men, mean age = 46.9±15.1 years, mean baseline 25(OH)D level = 52.4 nmol/L (21.0 ng/ml), baseline measurements 1994-1995, median follow-up from birth = 61 years) [119] was used to investigate the association between *VDR* polymorphisms (*Cdx*2, *FokI*, *Bsm*I, *Taq*I, *Apa*I and rs7968585), serum 25(OH)D levels and separate health outcomes including T2DM, MI, cancer, and mortality. Serum 25(O)HD was associated with T2DM, MI and death but not cancer. Those with low 25(OH)D (<20th percentile) had 73% increased risk of T2DM, 20% increased risk of MI and 21% increased risk of death. *Cdx*2, *FokI*, *Bsm*I, *Taq*I and *Apa*I showed no significant associations with serum 25(OH)D levels or any health outcome. After adjusting for age and gender, the rs7968585 minor homozygote genotype was a significant predictor of a 25% increased risk of T2DM and a 14% increased risk in MI. These studies provide strong evidence to support the impact of some

polymorphisms of the *VDR* on the association between 25(OH)D concentrations and T2DM and MI; however they do not support the impact of *Cdx*2, *FokI*, *Bsm*I, *Taq*I, *Apa*I, nor do they support the impact on the association with other CVD markers or risk factors.

Very few studies have explored the association between VDR polymorphisms and ischemic stroke [120]. In 2015, Prabhakar et al [120] investigated the association between *Fok*I, *Bsm*I, *Apa*I and *Taq*I and ischemic stroke in 557 Asian Indians (313 cases, 244 controls). Compared to the *Fok*I FF genotype, the ff genotype was reported to confer almost 3-times the risk (OR=2.97 [1.16-7.63], p=0.02) and this was independent of most traditional risk factors. However, the ff genotype was associated with significantly higher total cholesterol levels (p=0.04) and adjusting for lipid profile diminished the association between *Fok*I and stroke (OR=1.68 [0.75–3.78], *p*=0.21), suggesting that impaired lipid metabolism may be the underlying mechanism.

5.3 Cardiovascular disease clinical signs

Several studies have shown significant associations between *VDR* polymorphisms and the prevalence and severity of CAD [115, 121-125] although there are some inconsistencies evident [126-128]. In 1998, Van Schooten et al [115] reported an association between the *Bsm*I polymorphism and the severity of CAD using angiography to determine coronary stenosis in 41 consecutive patients of European origin undergoing open-heart surgery in the Netherlands. Those with the minor *Bsm*I bb genotype had 4-times the risk of severe coronary artery stenosis compared to the Bb or BB genotype, although it did not reach significance as a predictor (OR=4.2 [0.8-22.5], p=0.09). In contrast, in 2001 Ortlepp et al [121] reported that the frequency of the *Bsm*I B allele was 35% higher (p=0.001) in 100 consecutive elderly German patients with calcific aortic stenosis compared to a control cohort. However, in 2003 Ortlepp et al [126] then reported no association between *Bsm*I and the prevalence or severity of CAD in a large-scale population of 3441 consecutive patients referred for coronary angiography in Germany. As expected, traditional risk factors (T2DM, hypercholesterolaemia, smoking, hypertension, obesity, male gender) were all significantly associated with the presence of CAD (p<0.001); however, the *Bsm*I genotype frequencies showed no significant difference by the prevalence or severity of CAD. Similarly, a 2009 study [127] found no significant difference in the prevalence of either *Fok*I or *Bsm*I in Chinese people with CAD compared to controls. However, in 2014, Hossein-Nezhad et al [122] investigated the association of the *Fok*I polymorphism and serum 25(OH)D concentrations in patients with suspected CAD undergoing angiography. The *Fok*I FF genotype was independently associated with a greater degree of coronary collateralisation - a protective response to maintain blood supply when one vessel cannot supply adequate blood. Moreover, although serum 25(OH)D levels <25nmol/L (10

ng/mL) were significantly more prevalent amongst patients with ≥1 stenoic artery this was not independently associated with collaterisation and was suggested to be a result of the variation in *Fok*I genotype. In 2016 Abu El Maaty et al [123] also suggested that *Fok*I may be a genetic marker for CAD. Their results showed that the ff genotype was expressed to a greater degree in 98 CAD patients compared to 55 controls (68.9% vs 55.5%; p = 0.025), although it was not associated with 25(OH)D concentrations. Moreover, early studies showed the *BsmI* b allele is associated with improved survival amongst hemodialysis patients [129]. In 2010, Testa et al [124] reported that the number of *Bsm*I B alleles were independently associated with left ventricular hypertrophy and its progression over time in end-stage renal disease patients. In 2014, Santoro et al [125] also reported a significant independent relationship between the *Bsm*I B allele and left ventricular hypertrophy in chronic kidney disease patients not on dialysis. They found no relationship with *Fok*I. Overall, these studies support an association between *Fok*I and *Bsm*I and the prevalence and severity of CAD.

5.4 Cardiovascular disease risk factors and markers

Several studies have shown associations between VDR polymorphisms and established CVD risk factors including T2DM [130-132], metabolic syndrome (MetS) and its components [133, 134], IR [132, 135, 136], obesity [130, 137], hypertension [138-140] and dyslipidemia [130, 136], although results are inconsistent [141-143]. In 2001, Ortlepp et al [131] reported that that T2DM (defined as medically treated or an overnight fasting serum glucose >7.8 mmol/L on ≥2 occasions) was significantly more prevalent with an increasing number of *Bsm*I B alleles and that the BB genotype conferred over 3-times the risk of T2DM compared with the bb genotype in a high risk cohort of 293 consecutive German patients (70% men, mean age = 61.5±9.9 years) with hypercholesterolaemia and angina pectoris. However, other studies have found no significant association with T2DM. For example, Malecki et al 2003 [143] found no significant differences in the distribution of genotypes, alleles or haplotypes for *Fok*I, *Bsm*I, *ApaI* or *Taq*I in a Polish case-control study (308 cases, 240 controls). Other studies have found that it is the *Bsm*I b allele that actually increases susceptibility to T2DM. For example, in 2002, Oh et al [132] investigated the association between *Bsm*I, *ApaI* and *Taq*I and T2DM and MetS in non-diabetic Caucasians adults. The frequency of the genotypes did not differ between those with and without T2DM. However, amongst nondiabetics, those with the *Apa*I aa genotype had significantly higher fasting plasma glucose and rates of glucose intolerance than those with the AA genotype and those with the *Bsm*I bb genotype had greater IR (HOMA-IR scores). This suggests that both *Apa*I and *Bsm*I polymorphisms may predispose nondiabetic Caucasians to T2DM.

In 2013, Schuch et al [133] conducted a cross-sectional study investigating the relationship between *Fok*I and *Bsm*I polymorphisms and MetS (defined using the Adult Treatment Panel III (ATPIII) criteria) in Brazilian adults (n=243, 39% men, mean age = 51±15 years). They reported no significant difference in the frequency of the different genotypes between cases and controls. Amongst those with MetS, there were significantly higher HOMA-IR scores in ff carriers compared to Ff carriers, HOMA-β scores in Ff carriers compared to FF carriers and intact parathyroid hormone (PTH) in Ff carriers compared to FF carriers. There were no signficant associations between *Bsm*I genotypes and MetS components. However, in those without MetS, the *Bsm*I bb genotype was associated with significantly lower serum 25(OH)D levels, and the *Fok*I Ff genotype was associated with higher triglyceride (TG) levels and lower high-density lipoprotein cholesterol (HDL-c) than the FF carriers. This suggests that the *Fok*I ff genotype may be associated with IR and an adverse lipid profile in healthy Brazilian adults.

However, in 2014 Zhao et al [134] reported on a 2008-2012 investigation into the association between *Fok*I and *Bsm*I and MetS (defined using the International Diabetes Federation (IDF) 2005 criteria) in a cross-sectional study of 791 Chinese aged 24-75 years (391 cases, 400 controls). There were no signficant differences in the frequency of the *Fok*I genotypes or alleles between cases and controls, although the FF genotype was associated with lower body mass index (BMI) in cases compared to the Ff and ff genotypes (25.1±2.5kg/m2 vs 26.3±3.1 vs 26.4±2.9 respectively, p=0.005). The *Bsm*I BB genotype was more frequent in cases than controls (89% vs 82%, p=0.011) and was a significant predictor of increased risk of MetS (OR=1.77 [1.18-2.66], p=0.006). The b allele was less frequent (5.6% vs 9.4%, p=0.03) and predicted a lower likelihood of having MetS (OR=0.58 [0.39-0.85]). Furthermore, the mean waist circumference (WC) was significantly higher in Chinese adults with MetS and the BB genotype compared to the Bb or bb genotype (90±6.6 cm vs 87.6±6.3 cm, p=0.025). Similarly, the mean WC and BMI were signficantly higher amongst controls with the BB genotype compared to the Bb or bb genotype (79.7±7.6 cm vs 76.3±6.1 cm, p<0.001 and 22.7±2.8 kg/m^2 vs 22±2.4 kg/m^2, p=0.030 respectively). There were no significant differences in other components of the MetS. This study suggests that the *Bsm*I B allele and BB genotype may be associated with increased risk of MetS and central obesity in Chinese adults. These results are in line with Ortlepp et al [131] who reported the BB genotype to increase the risk of T2DM, but in contrast to Oh et al [132] who reported the bb genotype was associated with IR in Caucasians. However, it is clear from these studies that both *Fok*I and *Bsm*I polymorphisms play a role in metabolic dysfunction.

Studies investigating the association with other CVD risk factors such as blood pressure (BP) [70, 138-141] and dyslipidemia [66, 70] are inconsistent. While studies have shown significant associations between *Bsm*I and *FokI* polymorphisms and BP in diverse populations from Asians [138] to Europeans [139, 140], no association was reported between *Cdx*2, *Fok*I or *Bsm*I and BP in a 2014 genome-wide analysis of a large population of 23,294 European women (Women's Genome Health Study) and 69,395 European women and men (International Consortium of Blood Pressure) [141]. In 2008, Tworowska-Bardzinska et al [70] also found no significant differences between *Bsm*I genotypes in BMI, total fat volume, visceral fat, BP, lipid profile (TC, HDL-c, TG), glucose, or fasting insulin in 351 healthy postmenopausal Polish women (mean age = 55.4±2.8 years). Although there was an association with LDL-c which was higher in the BB carriers compared to the other genotypes (p=0.030). In 2013, Laczmanski et al [66] investigated the association between *Fok*I and *Bsm*I polymorphisms and anthropometric (BMI, WC, waist-to-hip ratio (WHR)) and biochemical (glucose, insulin, HOMA scores, serum 25(OH)D, TC, LDL-c, HDL-c, TG) CVD risk factors in Polish adults over 65 years of age (n=881, 51% men). While there were no differences in these risk factors by *Fok*I polymorphism, the b allele of the *Bsm*I polymorphism was associated with significantly higher IR (HOMA scores 3.2±2.2 vs 2.1±1.8 respectively, p=0.049) and marginally higher insulin levels (11.5±10.2 vs 8.3±7.3 µU/ml respectively, p=0.079) and lower HDL-c levels (50.4±13.6 vs 53.9±13.8 mg/100ml respectively, p=0.061) in women, and significantly higher IR (HOMA scores 2.6±1.0 vs 1.8±0.8 respectively, p=0.017) and insulin levels (9.3±8.0 vs 6.9±5.2 µU/ml respectively, p=0.047) but lower BMI (26.4±3.7 vs 27.7±4.6 kg/m^2 respectively, p=0.021) in men. There were no significant differences in glucose, serum 25(OH)D, TC, HDL-c, LDL-c, TG, WC or WHR. These studies suggest that the *Bsm*I polymorphism may be connected to CVD risk factors in older men and women and supports its role in metabolic function. However, it also suggests that there may be gender-specific differences in this association that need further investigation.

Interestingly, in 2009 Wilker et al [144] investigated various genes and markers of systemic inflammation and endothelial dysfunction in an aging population of Americans (n=679, 100% male) including a less commonly studied intronic SNP in the VDR gene (rs2239179). The minor variant was associated with a 7.1% higher fibrinogen level (p=0.006), although no significant differences were found for c-reactive protein (CRP), intercellular adhesion molecule-1 (ICAM-1) or vascular cell adhesion molecule-1 (VCAM-1) [144]. This suggests that this VDR polymorphism may increase the risk of platelet aggregation and atherogenesis, promoting inflammation and endothelial dysfunction. To our knowledge, this is the only study investigating the assocation between *VDR* polymorphisms and endothelial dysfunction.

Furthermore, there have been no studies to date that have investigated the association between any *VDR* polymorphisms and measurements of arterial stiffness such as augmentation pressure, augmentation index or pulse wave velocity.

Recently in 2016 Gussago et al [145] reported an association between *Fok*I, *Bsm*I, *Apa*I and *Taq*I and health and longevity in 102 Italian centenarians (mean age = 102.3±0.3 years) and 163 septuagenarians (mean age 73.0±0.6 years). Centenarians had a lower prevalence of the *Bsm*I bb genotype (bb 24.7% vs 39.9%), the *Apa*I aa genotype (aa 7.5% vs 20.5%) and the a allele (37.8% vs 46.9%) than septuagenarians. They also compared measured risk factors and physician-reported pathologies and found that: the *FokI* FF genotype was associated with significantly higher handgrip strength and cognitive status and lower prevalence of dementia but higher prevalence of hypertension; the *Bsm*I bb genotype was associated with significantly lower BMI and BP but higher prevalence of acute MI, angina and lower cognitive status; the *Apa*I AA genotype was associated with higher BP but a lower prevalence of chronic obstructive pulmonary disease; and the *Taq*I Tt genotype was associated with higher HDL-c and the TT genotype with lower DBP and prevalence of arthrosis. Clearly the associations are quite complex and require further study to elucidate the functional mechanisms behind what appear to be conflicting health and disease phenotypes. However, the *VDR* is evidently an important factor in healthy ageing, providing further support for its importance in cardiometabolic health.

5.5 Effect on the assocation between vitamin D status and cardiovascular disease

Despite the wide range of diseases now associated with suboptimal vitamin D status, very few intervention studies have investigated whether interactions between *VDR* polymorphisms and vitamin D status are confounding these associations [146-149]. In 2014, Vimaleswaran et al [149] investigated the interaction between the rs7968585 and rs2239179 SNPs and 25(OH)D concentrations and cardiometabolic risk factors (obesity, BP, lipid profile, inflammatory markers and metabolic markers) in the 1958 British Birth Cohort (n=5160). They found no evidence of these SNPs modifying the associations between low 25(OH)D levels and cardiometabolic risk factors. Further large cohort studies are needed to determine the modifying effects of the more commonly studied *VDR* polymorphisms on the association between serum 25(OH)D levels and CVD outcomes, clinical signs and risk factors.

Several studies have suggested that *VDR* polymorphisms may affect response to supplementation [147, 148]. In 2011, Elnenaei et al [148] investigated the effect of VDR polymorphisms on response to calcium and vitamin D supplementation in 56 post-menopausal

women (age range = 50-87 years). They found the *Fok*I FF genotype was more frequent in the 36 women with low bone density and, amongst these 36 women, there was a higher frequency of *Bsm*I bb and TaqI tt genotypes amongst non-responders to vitamin D therapy (based on changes in PTH levels over 3 months). This implies that genetic profile analysis could help identify potential non-responders before therapy, helping to target individuals to optimise efficacy of vitamin D supplementation. Indeed, in 2013 Serrano et al [147] reported that the *Bsm*I bb genotype showed a dose-dependent response to 2 months of retinol and 25(OH)D supplemented soy beverage in reduction in lipid profile in comparison with the BB/Bb genotype in 106 healthy Spanish adults (36% men, mean age = 33.7±12.2 years). While there were no baseline differences in vitamin D metabolites or CVD risk factors (BP, lipid profile, inflammatory biomarkers) - with the exception of TG level which was significantly higher in the b allele carriers - after 2 months treatment b allele carriers had a higher response including significant reductions in TC and LDLc compared to the B allele carriers. Furthermore, the change in $1,25(OH)_2D_3$ was significantly greater in b allele carriers after 2 months, suggesting that the benefits can be attributed to increased production or decreased degradation of active vitamin D in the b allele carriers. This partially explains the findings by Schuch et al [133] of significantly lower serum 25(OH)D level with the bb genotype which seemed to go against the findings of increased levels of CVD risk amongst those with the bb genotype in other studies [66, 145]. The *Bsm*I polymorphism may be an important consideration in selecting subjects for vitamin D intervention studies, although further research is needed before it can be advised to use genetic screening as a tool for recruitment.

The complexity of the pathway involved in vitamin D metabolism suggests that individual variation in metabolism, including VDR genotype, may modify the clinical consequences of low 25(OH)D concentrations. Of the commonly studied polymorphisms of the *VDR*, there appears to be evidence of a relationship between *Fok*I and *Bsm*I and an increased risk of CVD, although the results of studies into the association between these VDR and anthropometric and biochemical markers for CVD in different populations are inconsistent. The relationship between *VDR* polymorphisms and disease is likely to be dependent on the environment. For example, the association between specific *VDR* polymorphism and a disease phenotype may only be present in certain ethnic groups, genders, those with low serum 25(OH)D levels or those with certain *VDR* or proximal gene variants. Further genome-wide studies are needed to clarify the relationship between serum 25(OH)D, genetic variants involved in the vitamin D signaling pathway and CVD risk. Furthermore, as most of these SNPs have no known functional effects on the VDR and they appear to be strongly linked to other functional polymorphisms,

the associations do not necessarily imply causation. They do however provide further support for the relationship between vitamin D and cardiovascular health.

6.0 CONCLUSIONS

Current evidence suggests that the *VDR* may be one of the candidate genes implicated in CVD susceptibility. Polymorphisms of the *VDR* are associated with an increased risk of CVD and further research is needed to determine their potential as genetic risk markers, including large studies of all known polymorphisms to determine LD patterns and support haplotype analysis. It is plausible to postulate that *VDR* variants may account, at least partially, for the inconsistent results in intervention studies aimed at improving CVD risk factors and outcomes through vitamin D supplementation. Polymorphisms of the *VDR* including mutant genotypes and alleles or their interrelationship in haplotypes may determine VDR expression and biological activity, modifying target gene expression including those involved in vitamin D synthesis, metabolism, transportation and degradation; predict serum 25(OH)D concentrations and response to supplementation; and impact on the association between serum 25(OH)D and CVD and its risk factors.

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CHAPTER 7

FREQUENCY OF VITAMIN D RECEPTOR GENE POLYMORPHISMS (*BSM*I, *FOK*I, *TAQ*I, *CDX*2) AND THEIR ASSOCIATION WITH CLASSICAL CARDIOVASCULAR RISK FACTORS, 25-HYDROXYVITAMIN D LEVELS AND ERECTILE DYSFUNCTION IN HEALTHY NEW ZEALAND MEN

1.0 INTRODUCTION

Cardiovascular disease (CVD) is the primary cause of death globally, accounting for 31% (17.5 million) of the 56 million deaths worldwide in 2012 [1]. This threatens human health, productivity and economic growth. The cost of CVD is predicted to increase 22% by 2030 [2, 3] and represents a significant burden upon society, particularly the healthcare system. Many of these deaths could be deemed preventable as early detection and effective intervention are both possible with modern medicine. Approximately 50% of sudden cardiac deaths occur in patients with asymptomatic CVD [4]. Early detection relies primarily on cost-effective risk assessment using classical risk factor analysis (e.g., smoking, abdominal obesity, lipid abnormalities, hypertension, diabetes and physical activity (PA)) [5] and the estimation of absolute risk using multivariate risk algorithms (e.g., 10 year Framingham risk scores) [6-8]. A relatively unknown addition to these risk factors is erectile dysfunction (ED).

ED can be measured reliably through self-report using non-invasive well-validated tools such as the 5-item International Index of Erectile Function (IIEF-5, scores range from 5 (poor) to 25 (normal)) [9]. Although a complex multifactorial disease, ED is predominately vasculogenic in aetiology, sharing the same age-related pattern and risk factors as CVD [10, 11]. It is now established as an early sign of asymptomatic CVD [10, 11]: ED symptoms appear to manifest 2-3 years before CVD symptoms [12-15] and the more severe the ED symptoms, the more severe both the risk and the signs of CVD [16-19]. The common denominators are vascular injury, inflammation and endothelial dysfunction [20-22] resulting in impaired smooth muscle cell relaxation and vasodilation [23, 24]. ED is therefore a valuable early marker of systemic vascular disease in asymptomatic men and can be used to identify at risk men at a stage where appropriate interventions may delay or reverse the deterioration towards symptomatic CVD.

Vitamin D insufficiency (assessed via serum 25-hydroxyvitamin D (25(OH)D) concentration) is increasingly associated with a plethora of diseases, including CVD [3, 25-30]; however the results of intervention studies are inconsistent [31-33]. In Chapter 3 it has been shown that ED is highly prevalent in NZ men. In Chapter 5 it was demonstrated that both classical CVD risk factors and ED are associated with vitamin D insufficiency. Men with insufficient (<75 nmol/L) 25(OH)D had poorer erectile function than men with sufficient (≥75 nmol/L) 25(OH)D (median IIEF-5 score 22 vs 24 respectively, p=0.001) and men with ED (IIEF-5 score ≤21) were observed to have poorer vitamin D status than men without ED (IIEF-5 score >21) (median serum 25(OH)D level 74.5 vs 84.5 respectively, p=0.062). This suggests that improving serum 25(OH)D levels in men with ED may improve their erectile function, but also, as ED is an early marker of asymptomatic CVD, lower their risk of developing CVD.

It is well established that it is calcitriol (1,25-hydroyvitamin D or 1,25$(OH)_2$D), which elicits the actions of vitamin D by binding to the vitamin D receptor (VDR). The VDR is a nuclear hormone receptor (also known as NR1I1) that mediates the effects of vitamin D on target gene expression [34]. It is found throughout the tissues and cells of the human body [35, 36], including myocardial tissue [36], vascular smooth muscle [37] and endothelial cells [38]. The binding of 1,25$(OH)_2$D activates the VDR allowing it to form a heterodimer with the retinoid-X receptor (RXR) and subsequently bind to vitamin D response elements (VDREs) in the regulatory regions of target genes to support the transcription of gene products [39]. Approximately 3% of all human genes appear to be regulated by vitamin D via the VDR [40, 41]. However, individuals can have resistance to the 1,25$(OH)_2$D-mediated actions of vitamin D and show variable responsiveness to vitamin D. This may be the result of genetic variation in genes involved in the vitamin D signalling pathway, including the *VDR* leading to altered structure, function, or levels of expression of the VDR protein and diminished 1,25$(OH)_2D_3$-mediated response. These polymorphisms (defined as a prevalence >1% in the population) may explain some inconsistencies in the evidence supporting the beneficial effects of improving 25(OH)D concentrations on CVD risk factors and may affect the relationship between serum 25(OH)D level and ED.

A number of polymorphisms have been identified in the *VDR* detected using restriction enzymes (by which name they are most often referred) and genetic sequencing technology. The most common (generally present in >5% of the population) are the single nucleotide polymorphisms (SNPs) *Cdx*2 (rs11568820 (A/G)) at the 5'-untranslated region of the gene [42], *Fok*I (rs10735810 (C/T)) at the start codon of exon 2 [43], *Bsm*I (rs1544410 (A/G)) at intron 8 [44] and *Taq*I (rs731236 (T/C)) at exon 9 [45]. While many studies have investigated their prevalence in the general population of various countries [46-48] and their association with serum 25(OH)D levels [49-55], few have investigated their association with CVD risk factors [56-64] and to our knowledge none have investigated their association with ED. Given the association between serum 25(OH)D levels and ED reported in our earlier research, it is reasonable to ascertain if this relationship is affected by common *VDR* polymorphisms.

This study had three aims: 1) to examine the frequencies of the *Cdx*2, *Fok*I, *Bsm*I and *Taq*I VDR polymorphism genotypes and alleles in 100 self-reported "healthy" men aged 40-70 years based in the Manawatu region of New Zealand (NZ), 2) to compare CVD risk factors including serum 25(OH)D level and IIEF-5 scores amongst these genotypes and alleles, and 3) to determine the impact of these genotypes and alleles on the association between 25(OH)D level and ED.

2.1 METHODS

2.2 Study population

Venous blood samples and data on classical CVD risk factors (sociodemographic, lifestyle and medical variables) and ED from 100 self-reported "healthy" men aged 40-70 years (median 54 (Interquartile Range (IQR) 16)) living in the Manawatu region of NZ were taken from a previous study: the Wellness, Lifestyle and Diet (Well-LaD) Study. The study protocol and the sociodemographic characteristics of the study group are provided in detail in Chapter 5. The sample was predominately European, in a married/de facto/civil union relationship, with post-secondary school education, currently employed in a highly skilled occupation with a high household income (>$100,000 NZD) and living in a rural or semi-rural area. Although self-reporting as "healthy" there was a high level of cardiometabolic risk in this group, despite a low level of smoking and alcohol consumption and a high level of PA, cardiorespiratory fitness (submaximal oxygen consumption (VO_2peak)) and handgrip strength (Chapter 5). Insufficient vitamin D was defined as <75 nmol/L serum 25(OH)D and was present in 38 men. ED over the past 6 months was defined as an IIEF-5 score ≤21 and was present in 30 men. All subjects provided written informed consent. Ethical approval was granted by the Central Health and Disability Ethics Committee (LRS/10/07/032/AM01).

2.3 *VDR* genotype analysis

Blood samples were collected from the median cubital vein into spray-coated K_2EDTA (anticoagulant) BD Vacutainer® tubes (BD Diagnostics, Auckland, NZ) by a trained phlebotomist and stored at −4°C. Genomic DNA was extracted from whole blood using a Quick-gDNA™ Miniprep kit (Zymo Research Corp., Ngaio Diagnostics Ltd, Nelson, NZ) according to manufacturer instructions to rapidly yield high quality purified DNA (3-7 µg DNA per 100 µL whole blood) suitable for PCR. DNA concentration and purity were validated by verifying the $A_{260/280}$ and $A_{230/280}$ ratios, respectively (Nanodrop ND-1000 V 3.1.0, Thermo Fisher Scientific NZ Pty Ltd, Auckland, NZ). The DNA samples were stored at −20°C.

The genotypes for the four polymorphisms of the VDR gene were determined by means of polymerase chain reaction (PCR)-high resolution amplicon melt (HRM) analysis. The PCR was performed in a 384 well plate with technical duplicates of 1 µL of genomic DNA (gDNA, 2.1-84.3 ng/µL, 1:4 dilution) in 4 µL of reaction mixture containing: 2.5 µL SsoFast™ EvaGreen® Supermix 500 (Bio-Rad Laboratories (NZ) Pty Ltd, Auckland, NZ), 1 µL PCR-grade H_2O and 0.25 µL of each forward (F) and reverse (R) primer (5 µM) for each polymorphism (Table 7.1). A negative control (reagent only) and a positive control (standards of a known genotype (57.1-

128.3 ng/µL, 1:2 dilution) sourced from the Surya Study, Massey University, Albany, NZ) [64] were included in each set of polymorphisms.

Table 7.1. Primers* used in polymerase chain reaction (PCR) assays designed using LightCycler Probe Design Software 2.0.

VDR gene polymorphism	Primers (5'-3')	Amplicon size (bp)
rs11568820 (Cdx2)	F: AGAAAACATTGTAGAACATCTTTTGTATC	104
	R: ATTTTAACTGCAACCCATAATAAGAAAT	
rs10735810 (*Fok*I)	F: GGCCTGCTTGCTGTTCTTA	74
	R: TCCAAGTCTCCAGGGTCA	
rs1544410 (*Bsm*I)	F: GAGGAACTAGATAAGCAGGG	80
	R: TTCACGCAAGAGCAGAG	
rs731236 (*Taq*I)	F: GAGAGCTCCTGTGCCTT	112
	R: ACGTCTGCAGTGTGTTG	

*Primers supplied by Integrated DNA Technologies (NSW, Australia).

Genotypes were determined using LightCycler® 480 Gene Scanning Software (release 1.5.0 SP3, Roche Diagnostics NZ Ltd, Auckland, NZ) according to the known controls. The protocol for all four polymorphisms is shown in Table 7.2. In-group analysis was used, followed by manual discrimination through classical melt curve analysis with visualisation of melting peaks of samples compared to those of the standards. Ambiguous samples were repeated. *Cdx*2 is commonly referred to by its nucleotide substitution (A/G) but the normal nomenclature was applied to the other polymorphisms with an uppercase allele where the restriction site was absent and a lowercase allele where the restriction site was present (F/f, B/b, T/t respectively).

Table 7.2. High resolution amplicon melt (HRM) conditions used in the LightCycler® 480.

	Cycles	Target (°C)	Hold (mm:ss)	Ramp rate (°C/s)	Acquisitions (per °C)
Activation	1	98	02:00	4.40	-
Amplification	45	98 55	00:05 00:05	4.40 2.20	-
Melting	1	95	-	0.02	25
Cooling	1	40	00:30	1.50	-

2.3 Statistical analysis

Genotype and allele frequencies were estimated by gene counting and the distribution of the variants tested against the Hardy-Weinberg (HW) principle. Data were expressed as absolute frequency (count). Normally distributed data (verified using the Kolmogorov–Smirnov criterion) were reported as mean ± standard deviation (SD). No transformations were used. Outliers were not removed. Data not normally distributed were reported as median (interquartile range (IQR)). Statistical differences in the clinical characteristics (CVD risk factors, including serum 25(OH)D concentrations and IIEF-5 score) between the different genotypes and alleles were compared using one-way analysis of variance (ANOVA) or Kruskal-Wallis. Where statistically significant differences were found, post-hoc tests (Tukey HSD or Dunn-

Bonferoni) were applied to locate differences between groups. The frequency of genotypes and alleles of interest were also analysed between groups by the presence and absence of ED (IIEF-5 score ≤21) using the χ^2 test. Genotypes and alleles with a significant univariate association with IIEF-5 scores were further assessed using binomial logistic regression to calculate crude, age-adjusted, and age and serum 25(OH)D adjusted odds ratios (OR) and 95% confidence intervals [95% CI] to determine their effect on the likelihood of having ED and significance as predictors in the model. All tests were two-tailed and p-values ≤0.05 were considered statistically significant. Statistical analysis was conducted using SPSS Statistics version 20.0 (IBM Corp., Armonk, NY, USA).

3.1 RESULTS

3.2 Prevalence of the VDR polymorphisms

The overall frequency of genotypes and alleles for the four polymorphisms are shown in Table 7.3. Conformity with the Hardy-Weinberg principle of all polymorphisms was checked and the distribution found to fit the principle for *Fok*I (χ^2=0.533, p>0.05), *Bsm*I (χ^2=1.0487, p>0.05) and *Taq*I (χ^2=0.9983, p>0.05), but not *Cdx*2 (χ^2=23.594, p<0.05). There may be selective pressure on this SNP in our sample. The prevalences of the alleles were as follows: *Cdx*2 "A" allele p=0.31, "G" allele q=0.69; *Fok*I "F" allele p=0.56, "f" allele q=0.45; *Bsm*I "B" allele p=0.43, "b" allele q=0.57; and *Taq*I "T" allele p=0.51, "t" allele q=0.49.

Table 7.3. Frequency (%) of different genotypes and alleles for four polymorphisms of the vitamin D receptor (*VDR*) gene in study participants (n=100).

		Homozygous genotype	Heterozygous genotype	Homozygous genotype	MAF[a]
rs11568820 (*Cdx*2)		AA	GA	GG	A
	N	20	22	58	62 (31)
rs10735810 (*Fok*I)		FF	Ff	ff	f
	N	29	53	18	89 (45)
rs1544410 (*Bsm*I)		BB	Bb	bb	B
	N	21	44	35	86 (43)
rs731236 (*Taq*I)		TT	Tt	tt	t
	N	28	45	27	99 (49)

[a] MAF = minor allele frequency

3.3 Associations with CVD risk factors

There were no significant differences in the clinical characteristics measured between the rs11568820 (*Cdx*2) polymorphism genotypes (Table 7.4), with the exception of IIEF-5 score, which showed significantly different distributions amongst the three genotypes (p=0.006). Post hoc analysis using Dunn-Bonferoni showed a significantly different distribution amongst men with the AA genotype compared to both the GA and the GG genotypes (test statistic = 3.005, p=0.008 and test statistic = 2.685, p=0.022 respectively); although there was no difference between the GA and GG genotypes. The AA genotype was less prevalent amongst the 30 men with ED (IIEF-5 score ≤21) compared to 70 men without ED (IIEF-5 score >21): 3%(1) AA, 37%(11) GA and 60%(18) GG versus 27%(19) AA, 16%(11) GA and 57%(40) GG respectively (χ^2 = 10.172, p=0.006). The AA genotype was associated with better erectile function in this group of men. Supporting this were significant relationships between several clinical characteristics and the A allele frequency (Table 7.5): better maximal oxygen consumption (VO_2peak, p=0.009), handgrip strength (p=0.040), android-to-gynoid fat distribution (A:G, p=0.014), pulse wave velocity (PWV, p=0.040), and IIEF-5 scores (p=0.008). There were also relationships observed with waist-to-hip ratio (WHR, p=0.066) and body fat percentage (BF%, p=0.079) which did not reach statistical significance. The A allele was associated with better cardiorespiratory fitness and handgrip strength, lower central adiposity, less arterial stiffness and better erectile function in this group of men. This is the first report of the importance of VDR genotype in ED.

There were no significant associations between any of the clinical characteristics measured and the *Fok*I polymorphism genotypes (Table 7.6). There was a difference in the distribution of pulse pressure (PP, p=0.054) that did not reach statistical significance. However, there was a significant difference in the distribution of IIEF-5 scores (p=0.048) between the alleles (Table 7.7). An independent samples median test showed the median IIEF-5 scores were not the same across the two allele categories (23(4) vs 24(3), p=0.037). There were also observed differences in the distribution of A:G (p=0.061), fasting plasma insulin (FPI, p=0.054), and Homeostatic Model Assessment Index of Insulin Resistance (HOMA1-IR, p=0.069) by allele which did not reach statistical significance.

There were several significant associations found between the clinical characteristics measured and the *Bsm*I polymorphism genotypes (Table 7.8) including a difference in mean height (p=0.008), systolic blood pressure (SBP, p=0.043), and statistically significant differences in the distributions of handgrip strength (p=0.048), augmentation pressure adjusted to a heart rate of 75 beats per minute (AP@HR75, p=0.037) and total testosterone (TT, p=0.050). There

were also differences observed in the distribution of PWV (p=0.058) and the mean augmentation index adjusted to a heart rate of 75 beats per minute (AIx@HR75, p=0.063) between genotypes, although neither reached statistical significance. Post hoc analysis with the Tukey HSD test showed a significantly higher mean height in the bb genotype group, compared to the BB genotype group (bb 179.54±6.60 vs BB 174.54±5.14 cm, p=0.010); however the mean height in the Bb genotype lay in the middle. Mean SBP was lower in the group with the bb genotype compared to the BB genotype (124.63±13.67 vs 137.00±21.74 mmHg, p=0.032); although again the mean SBP in the Bb genotype group lay in the middle. Post hoc analysis using Dunn-Bonferoni showed significant differences in the distribution of handgrip strength (test statistic = 2.436, p=0.045) and AP@HR75 (test statistic = -2.565, p=0.031) between bb and BB genotypes but no difference in the distributions between the Bb and bb, or Bb and BB genotype groups. There was also no significant difference in the distribution of TT between the three genotypes. Men with the bb genotype appear to be taller, have lower SBP, greater handgrip strength, lower AP@HR75 (indicative of lower vascular resistance) and marginally higher TT compared to men with the BB genotype. Supporting this were several significant relationships found in allele analysis (Table 7.9). The b allele was associated with greater height (p=0.001), lower SBP (p=0.009), lower AIx@HR75 (p=0.014), total cholesterol (TC, p=0.026) and low-density lipoprotein cholesterol (LDLc, p=0.045) and higher free testosterone (FT, p=0.039) levels. Significant differences in the distribution of age (p=0.022), handgrip strength (p=0.010), PP (p=0.033), AP@HR75 (p=0.007) and HOMA1-IR (p=0.014) are also evident between the b and B alleles. The b allele was associated with greater height and handgrip strength, lower blood pressure and arterial stiffness, a better lipid profile, lower insulin resistance and a healthier hormone profile in this group of men.

There were no significant associations between any of the clinical characteristics measured and the *Taq*I polymorphism genotypes (Table 7.10) or alleles (Table 7.11); although there was a difference in the distribution of PWV between the genotypes which did not reach statistical significance (p=0.068).

Table 7.4. Comparison of clinical characteristics between genotypes in the rs11568820 (*Cdx*2) polymorphism in study* participants (n=100).

Variable	Study prevalence mean±SD / median (IQR)	VDR polymorphism genotype rs11568820 (*Cdx*2) AA N=20	GA N=22	GG N=58	ANOVA F-statistic	ANOVA P-value‡	Kruskal-Wallis P-value‡
Age (years)	54(16)	50.50(12)	54(18)	54.5(17)	-	-	0.495
VO_2peak (ml/kg/min)	33.7(14.1)	43.9(13.0)	34.5(17.9)	32.4(11.2)	-	-	0.070
Handgrip strength (kg)	100(25)	104(27.5)	100(30.8)	98.8(235)	-	-	0.247
Height (cm)	177.06±6.33	177.22±8.16	199.92±6.68	176.69±5.53	0.307	0.737	-
Weight (kg)	84.97 (15.08)	84.81(24.61)	81.72(18.93)	85.90(14.38)	-	-	0.915
BMI (kg/m^2)	27.11 (4.58)	26.72(5.32)	26.06(6.11)	27.17(3.94)	-	-	0.932
WC (cm)	96.30±11.12	93.39±10.01	97.90±15.76	96.70±9.25	0.981	0.385	-
WHR	0.95±0.07	0.93±0.05	0.96±0.09	0.96±0.06	2.218	0.123	-
WHtR	0.54±0.06	0.53±0.06	0.55±0.08	0.55±0.05	0.938	0.395	-
BF%	29.11±4.67	28.11±4.28	28.50±5.45	29.69±4.48	1.091	0.340	-
A:G	1.21(0.26)	1.19(0.21)	1.17(0.33)	1.25(0.23)	-	-	0.126
SBP (mmHg)	129.32±17.95	129.70±15.74	129.73±22.46	129.03±17.05	0.017	0.983	-
DBP (mmHg)	79.25±9.10	79.30±9.40	79.45±9.46	79.16±9.02	0.009	0.991	-
HR (bpm)	55.5(11)	54.0(13)	57(12)	54(10)	-	-	0.434
PP (mmHg)	48.5(16)	48.5(14)	44(20)	49(16)	-	-	0.708
PWV (m/s)	7.8(1.4)	7.6(1.2)	7.8(2.0)	8.0(1.4)	-	-	0.083
AP@HR75	5.0(6.7)	4.0(4.9)	3.7(5.8)	5.0(8.0)	-	-	0.712
AIx@HR75	14.42±10.24	12.39±9.08	15.25±9.80	14.83±10.84	0.449	0.640	-
TC (mmol/L)	5.18±0.99	5.16±0.89	5.14±1.16	5.20±0.96	0.037	0.964	-
TG (mmol/L)	1.1(0.9)	1.1(0.5)	1.1(0.8)	1.2(0.9)	-	-	0.925
HDLc (mmol/L)	1.2(0.4)	1.2(0.3)	1.2(0.4)	1.2(0.4)	-	-	0.632
LDLc (mmol/L)	3.38±0.90	3.38±0.78	3.24±0.95	3.44±0.93	0.368	0.693	-
TC:HDLc	4.3(1.5)	4.3(1.3)	4.3(1.2)	4.2(1.7)	-	-	0.778
TG:HDLc	0.90(0.80)	0.85(0.7)	0.7(0.6)	0.9(0.7)	-	-	0.859
FPG (mmol/L)	5.6(0.6)	5.6(0.6)	5.7(0.8)	5.7(0.7)	-	-	0.898
FPI (pmol/L)	44.5(39)	44.5(30)	45.0(39)	42(45)	-	-	0.304
HOMA1IR	1.9(1.7)	1.75(1.3)	2.0(1.7)	1.75(2.3)	-	-	0.409
TT (nmol/L)	15.2(6.2)	15.75(4.6)	15.0(8.2)	14.55(6.2)	-	-	0.913
SHBG (nmol/L)	24(13)	20(19)	25(17)	24.5(13)	-	-	0.317
FT (pmol/L)	374.28±132.98	397.15±117.91	362.45±140.22	370.88±136.32	0.397	0.674	-
FAI	627.5(343)	713(433)	596(253)	600(308)	-	-	0.139

25(OH)D (nmol/L)	82.5(24)	79.50(38)	89(18)	84(26)	-	-	0.728
IIEF-5 score	23(4)	24.5(2)	23(4)[a]	23(5)[a]	-	-	**0.006**

Continuous variables are shown as mean ± SD or median (IQR). *Wellness, Lifestyle and Diet (Well-LaD) Study. [f]P-values derived from one-way analysis of variance (ANOVA) when data normally distributed data with homogeneity of variance, Welch ANOVA when data normally distributed data with nonhomogeneity of variance, and Kruskal-Wallis when data not normally distributed. [ab]Genotypes sharing the same superscript have distributions that are not significantly different from each other (Dunn-Bonferoni, p>0.05). A:G, android-to-gynoid fat ratio; AIx@HR75, augmentation index adjusted to heart rate 75 bpm; AP@HR75, augmentation pressure adjusted to heart rate 75 bpm; BF%, body fat percentage; BMI, Body Mass Index; DBP, diastolic blood pressure; FAI, free androgen index; FPG, fasting plasma glucose; FPI, fasting plasma insulin; FT, free testosterone; HDL-c, high density lipoprotein cholesterol; HR, heart rate; HOMA1-IR, homeostasis model assessment one- insulin resistance; IIEF-5, 5-item International Index of Erectile Function; IQR, interquartile range; LDL-c, low density lipoprotein cholesterol; PWV, pulse wave velocity; SBP, systolic blood pressure; SD, standard deviation; SHBG, sex hormone binding globulin; TC, total cholesterol; TG, triglycerides; TT, total testosterone; VO_2peak, maximal oxygen consumption; WC, waist circumference; WHR, waist-to-hip ratio; WHtR, waist-to-height ratio.

Table 7.5. Comparison of clinical characteristics between alleles in the rs11568820 (Cdx2) polymorphism in study* participants (n=100).

Variable	VDR polymorphism allele rs11568820 (*Cdx*2)				
	A N=62	**G N=138**	**ANOVA F-statistic**	**ANOVA P-value‡**	**Mann-Whitney U P-value‡**
Age (years)	51(16)	54(17)	-	-	0.597
VO_2peak (ml/kg/min)	38.0(13.3)	32.5(11.8)	-	-	**0.009**
Handgrip strength (kg)	103(27)	99(24.5)	-	-	**0.040**
Height (cm)	177.88±7.57	176.86±5.82	0.363	0.548	-
Weight (kg)	83.96(22.64)	85.07(14.41)	-	-	0.692
BMI (kg/m2)	26.57(4.85)	27.06(4.17)	-	-	0.646
WC (cm)	94.65±11.92	96.44±10.26	1.264	0.262	-
WHR	0.94±0.06	0.95±0.06	3.423	0.066	-
WHtR	0.53±0.07	0.55±0.06	1.960	0.163	-
BF%	28.24±4.57	29.49±4.60	3.110	0.079	-
A:G	1.18(0.22)	1.24(0.27)	-	-	**0.014**
SBP (mmHg)	130.00±18.89	129.12±17.99	0.042	0.837	-
DBP (mmHg)	79.28±9.85	78.86±9.01	0.012	0.913	-
HR (bpm)	55(12)	54(10)	-	-	0.916
PP (mmHg)	48(17)	49(16)	-	-	0.686
PWV (m/s)	7.7(0.9)	7.9(1.3)	-	-	**0.040**
AP@HR75	4.0(4.8)	5.0(7.8)	-	-	0.389
AIx@HR75	13.51±9.37	14.97±10.61	0.827	0.365	-
TC (mmol/L)	5.15±0.99	5.25±0.99	0.071	0.790	-
TG (mmol/L)	1.1(0.5)	1.1(0.8)	-	-	0.723
HDLc (mmol/L)	1.2(0.3)	1.2(0.4)	-	-	0.717
LDLc (mmol/L)	3.36±0.84	3.49±0.90	0.316	0.575	-
TC:HDLc	4.3(1.2)	4.2(1.7)	-	-	0.419
TG:HDLc	0.8(0.6)	0.9(0.7)	-	-	0.654
FPG (mmol/L)	5.6(0.6)	5.7(0.7)	-	-	0.697
FPI (pmol/L)	45(32)	43(43)	-	-	0.862
HOMA1IR	1.8(1.5)	1.8(2.1)	-	-	0.771
TT (nmol/L)	15.7(4.6)	14.6(6.2)	-	-	0.606
SHBG (nmol/L)	24(17)	25(13)	-	-	0.313
FT (pmol/L)	370.59±115.82	366.47±126.39	0.568	0.452	-

FAI	655(356)	596(306)	-	-	0.136
25(OH)D (nmol/L)	82(27)	85(24)	-	-	0.851
IIEF-5 score	24(2)	23(4)	-	-	**0.008**

Continuous variables are shown as mean ± SD or median (IQR). *Wellness, Lifestyle and Diet (Well-LaD) Study. [1]P-values derived from one-way analysis of variance (ANOVA) when data normally distributed and Mann-Whitney U when data not normally distributed. A:G, android-to-gynoid fat ratio; AIx@HR75, augmentation index adjusted to heart rate 75 bpm; AP@HR75, augmentation pressure adjusted to heart rate 75 bpm; BF%, body fat percentage; BMI, Body Mass Index; DBP, diastolic blood pressure; FAI, free androgen index; FPG, fasting plasma glucose; FPI, fasting plasma insulin; FT, free testosterone; HDL-c, high density lipoprotein cholesterol; HR, heart rate; HOMA1-IR, homeostasis model assessment one- insulin resistance; IIEF-5, 5-item International Index of Erectile Function; IQR, interquartile range; LDL-c, low density lipoprotein cholesterol; PWV, pulse wave velocity; SBP, systolic blood pressure; SD, standard deviation; SHBG, sex hormone binding globulin; TC, total cholesterol; TG, triglycerides; TT, total testosterone; VO_2peak, maximal oxygen consumption; WC, waist circumference; WHR, waist-to-hip ratio; WHtR, waist-to-height ratio.

Table 7.6. Comparison of clinical characteristics between genotypes in the rs10735810 (*Fok*I) polymorphism in study* participants (n=100).

Variable	Study prevalence	VDR polymorphism genotype rs10735810 (*Fok*I)			ANOVA F-statistic	ANOVA P-value[ŧ]	Kruskal-Wallis P-value[ŧ]
	mean±SD / median (IQR)	FF N=29	Ff N=53	ff N=18			
Age (years)	54(16)	53(15)	54(21)	50(14)	-	-	0.602
VO_2peak (ml/kg/min)	33.7(14.1)	31.4(12.8)	36.4(12.0)	36.9(17.2)	-	-	0.240
Handgrip strength (kg)	100(25)	102(18)	99(23.5)	97(36)	-	-	0.359
Height (cm)	177.06±6.33	177.07±6.11	176.78±6.35	177.91±6.91	0.213	0.809	-
Weight (kg)	84.97 (15.08)	83.96(15.29)	84.87(13.53)	92.00(24.22)	-	-	0.305
BMI (kg/m2)	27.11 (4.58)	26.59(4.26)	26.77(4.16)	28.28(6.47)	-	-	0.303
WC (cm)	96.30±11.12	95.65±12.98	96.15±10.38	97.82±10.47	0.219	0.804	-
WHR	0.95±0.07	0.95±0.08	0.95±0.06	0.96±0.05	0.086	0.918	-
WHtR	0.54±0.06	0.54±0.07	0.54±0.06	0.55±0.06	0.115	0.892	-
BF%	29.11±4.67	28.33±5.26	29.14±4.23	30.28±4.92	0.963	0.385	-
A:G	1.21(0.26)	1.15(0.27)	1.20(0.28)	1.26(0.21)	-	-	0.151
SBP (mmHg)	129.32±17.95	128.66±21.06	130.89±17.01	125.78±15.44	0.568	0.569	-
DBP (mmHg)	79.25±9.10	80.41±10.58	78.47±8.10	79.67±9.63	0.445	0.642	-
HR (bpm)	55.5(11)	56(14)	54(11)	53(6)	-	-	0.997
PP (mmHg)	48.5(16)	47(18)	50(16)	45(9)	-	-	0.054
PWV (m/s)	7.8(1.4)	7.8(0.9)	7.9(1.4)	8.1(2.2)	-	-	0.192
AP@HR75	5.0(6.7)	4.0(9.0)	5.0(6.3)	4.0(5.7)	-	-	0.879
AIx@HR75	14.42±10.24	15.37±11.96	14.01±9.84	14.18±9.13	0.143	0.867	-
TC (mmol/L)	5.18±0.99	5.04±0.99	5.25±1.05	5.21±0.80	0.417	0.660	-
TG (mmol/L)	1.1(0.9)	1.1(0.6)	1.2(0.8)	1.1(1.0)	-	-	0.908
HDLc (mmol/L)	1.2(0.4)	1.2(0.4)	1.2(0.3)	1.2(0.4)	-	-	0.986
LDLc (mmol/L)	3.38±0.90	3.27±0.92	3.41±0.93	3.48±0.80	0.336	0.716	-
TC:HDLc	4.3(1.5)	4.2(1.7)	4.3(1.3)	4.0(2.0)	-	-	0.947
TG:HDLc	0.9(0.8)	0.8(0.6)	0.9(0.8)	0.9(1.2)	-	-	0.871
FPG (mmol/L)	5.6(0.6)	5.6(0.4)	5.7(0.6)	5.8(0.9)	-	-	0.739
FPI (pmol/L)	44.5(39)	33(18)	48(39)	48(73)	-	-	0.137
HOMA1IR	1.9(1.7)	1.4(0.9)	2.0(1.6)	2.4(3.5)	-	-	0.169
TT (nmol/L)	15.2(6.2)	14.6(4.4)	16.3(6.6)	14.2(7.7)	-	-	0.603
SHBG (nmol/L)	24(13)	22(13)	25(17)	23(18)	-	-	0.209

FT (pmol/L)	374.28±132.98	370.07±129.83	377.87±145.65	370.50±101.20	0.040	0.961	-
FAI	627.5(343)	607(303)	596(380)	650(305)	-	-	0.391
25(OH)D (nmol/L)	82.5(24)	85(30)	88(25)	82(25)	-	-	0.961
IIEF-5 score	23(4)	25(2)	23(4)	23(5)	-	-	0.116

Continuous variables are shown as mean ± SD or median (IQR). *Wellness, Lifestyle and Diet (Well-LaD) Study. †P-values derived from one-way analysis of variance (ANOVA) when data normally distributed data with homogeneity of variance and Kruskal-Wallis when data not normally distributed. A:G, android-to-gynoid fat ratio; AIx@HR75, augmentation index adjusted to heart rate 75 bpm; AP@HR75, augmentation pressure adjusted to heart rate 75 bpm; BF%, body fat percentage; BMI, Body Mass Index; DBP, diastolic blood pressure; FAI, free androgen index; FPG, fasting plasma glucose; FPI, fasting plasma insulin; FT, free testosterone; HDL-c, high density lipoprotein cholesterol; HR, heart rate; HOMA1-IR, homeostasis model assessment one- insulin resistance; IIEF-5, 5-item International Index of Erectile Function; IQR, interquartile range; LDL-c, low density lipoprotein cholesterol; PWV, pulse wave velocity; SBP, systolic blood pressure; SD, standard deviation; SHBG, sex hormone binding globulin; TC, total cholesterol; TG, triglycerides; TT, total testosterone; VO_2peak, maximal oxygen consumption; WC, waist circumference; WHR, waist-to-hip ratio; WHtR, waist-to-height ratio.

Table 7.7. Comparison of clinical characteristics between alleles in the rs10735810 (*Fok*I) polymorphism in study* participants (n=100).

Variable	VDR polymorphism allele rs10735810 (*Fok*I)				
	F N=111	f N=89	ANOVA F-statistic	ANOVA P-value†	Mann-Whitney U P-value†
Age (years)	53(17)	53.5(15)	-	-	0.513
VO_2peak (ml/kg/min)	33.0(12.0)	36.4(15.1)	-	-	0.126
Handgrip strength (kg)	101.5(20.6)	99(27)	-	-	0.169
Height (cm)	176.90±6.27	177.60±6.82	0.117	0.733	-
Weight (kg)	84.62(13.34)	86.15(17.37)	-	-	0.221
BMI (kg/m2)	26.74(4.20)	27.41(4.70)	-	-	0.182
WC (cm)	94.26±10.10	96.51±10.51	0.351	0.554	-
WHR	0.94±0.06	0.95±0.06	0.120	0.729	-
WHtR	0.53±0.06	0.54±0.06	0.209	0.648	-
BF%	28.40±4.50	29.33±4.55	1.775	0.184	-
A:G	1.20(0.25)	1.21(0.25)	-	-	0.061
SBP (mmHg)	130.44±19.55	128.19±16.69	0.124	0.725	-
DBP (mmHg)	73.28±9.84	78.31±8.50	0.169	0.682	-
HR (bpm)	55(12)	53(9)	-	-	0.982
PP (mmHg)	49(17)	48(15)	-	-	0.994
PWV (m/s)	7.8(1.1)	7.9(1.5)	-	-	0.241
AP@HR75	4.6(8.0)	5.0(5.8)	-	-	0.642
AIx@HR75	14.32±10.75	13.84±8.92	0.160	0.690	-
TC (mmol/L)	5.16±1.00	5.19±0.94	0.420	0.518	-
TG (mmol/L)	1.1(0.6)	1.1(0.8)	-	-	0.880
HDLc (mmol/L)	1.2(0.4)	1.2(0.3)	-	-	0.876
LDLc (mmol/L)	3.40±0.88	3.43±0.84	0.606	0.437	-
TC:HDLc	4.3(1.6)	4.2(1.4)	-	-	0.764
TG:HDLc	0.8(0.6)	0.9(0.6)	-	-	0.721
FPG (mmol/L)	5.6(0.5)	5.7(0.8)	-	-	0.454
FPI (pmol/L)	41.5(29)	48(44)	-	-	0.054
HOMA1IR	1.7(1.3)	2.1(2.2)	-	-	0.069
TT (nmol/L)	14.9(5.6)	15.6(6.3)	-	-	0.892
SHBG (nmol/L)	25(16)	24(14)	-	-	0.844
FT (pmol/L)	366.80±114.25	373.49±127.50	0.003	0.954	-

FAI	607(320)	637(380)	-	-	0.768
25(OH)D (nmol/L)	85.5(25)	82(24)	-	-	1.000
IIEF-5 score	24(3)	23(4)	-	-	**0.048**

Continuous variables are shown as mean ± SD or median (IQR). *Wellness, Lifestyle and Diet (Well-LaD) Study. [1]P-values derived from one-way analysis of variance (ANOVA) when data normally distributed and Mann-Whitney U when data not normally distributed. A:G, android-to-gynoid fat ratio; AIx@HR75, augmentation index adjusted to heart rate 75 bpm; AP@HR75, augmentation pressure adjusted to heart rate 75 bpm; BF%, body fat percentage; BMI, Body Mass Index; DBP, diastolic blood pressure; FAI, free androgen index; FPG, fasting plasma glucose; FPI, fasting plasma insulin; FT, free testosterone; HDL-c, high density lipoprotein cholesterol; HR, heart rate; HOMA1-IR, homeostasis model assessment one- insulin resistance; IIEF-5, 5-item International Index of Erectile Function; IQR, interquartile range; LDL-c, low density lipoprotein cholesterol; PWV, pulse wave velocity; SBP, systolic blood pressure; SD, standard deviation; SHBG, sex hormone binding globulin; TC, total cholesterol; TG, triglycerides; TT, total testosterone; VO_2peak, maximal oxygen consumption; WC, waist circumference; WHR, waist-to-hip ratio; WHtR, waist-to-height ratio.

Table 7.8. Comparison of clinical characteristics between genotypes in the rs1544410 (*Bsm*I) polymorphism in study* participants (n=100).

Variable	Study prevalence	VDR polymorphism genotype rs1544410 (*Bsm*I)					
	mean±SD / median (IQR)	BB N=21	Bb N=44	bb N=35	ANOVA F-statistic	ANOVA P-value‡	Kruskal-Wallis P-value‡
Age (years)	54(16)	62.5(20)	52.5(15)	50(12)	-	-	0.082
VO_2peak (ml/kg/min)	33.7(14.1)	31.9(12.7)	33.5(11.5)	34.5(15.0)	-	-	0.936
Handgrip strength (kg)	100(25)	91(32)	102(27.3)	103(17.5)	-	-	**0.048**
Height (cm)	177.06±6.33	174.54±5.14	176.30±6.08	179.54±6.60	5.039	**0.008**	-
Weight (kg)	84.97 (15.08)	82.56(16.24)	87.44(20.70)	84.87(13.03)	-	-	0.217
BMI (kg/m2)	27.11 (4.58)	26.70(3.87)	27.41(5.24)	26.71(3.55)	-	-	0.500
WC (cm)	96.30±11.12	93.87±10.93	98.30±10.70	95.25±11.61	1.381	0.256	-
WHR	0.95±0.07	0.95±0.07	0.96±0.06	0.94±0.07	0.917	0.403	-
WHtR	0.54±0.06	0.54±0.07	0.56±0.06	0.53±0.06	1.932	0.150	-
BF%	29.11±4.67	28.44±4.26	29.73±4.76	28.73±4.83	0.715	0.492	-
A:G	1.21(0.26)	1.13(0.29)	1.25(0.21)	1.15(0.30)	-	-	0.140
SBP (mmHg)	129.32±17.95	137.00±21.74	129.39±18.09	124.63±13.67	3.262	**0.043**	-
DBP (mmHg)	79.25±9.10	82.29±10.30	78.86±9.72	77.91±7.18	1.605	0.206	-
HR (bpm)	55.5(11)	51(15)	57(11)	53(8)	-	-	0.763
PP (mmHg)	48.5(16.0)	48.5(23.0)	49.5(15.0)	45.0(17.0)	-	-	0.120
PWV (m/s)	7.8(1.4)	7.7(0.6)	8.3(1.1)	7.5(1.5)	-	-	0.058
AP@HR75	5.0(6.7)	6.4(10.4)	5.25(5.50)	3.0(5.6)	-	-	**0.037**
AIx@HR75	14.42±10.24	18.65±9.06	14.50±9.59	11.63±11.08	2.850	0.063	-
TC (mmol/L)	5.18±0.99	5.46±1.03	5.27±0.93	4.91±1.00	2.383	0.098	-
TG (mmol/L)	1.1(0.9)	1.1(0.8)	1.25(0.7)	0.9(0.7)	-	-	0.537
HDLc (mmol/L)	1.2(0.4)	1.2(0.4)	1.3(0.4)	1.2(0.3)	-	-	0.654
LDLc (mmol/L)	3.38±0.90	3.63±0.87	3.44±0.88	3.17±0.92	1.892	0.156	-
TC:HDLc	4.3(1.5)	4.45(1.6)	4.4(1.3)	4.1(1.8)	-	-	0.150
TG:HDLc	0.90(0.80)	0.85(0.80)	0.9(0.9)	0.7(0.6)	-	-	0.700
FPG (mmol/L)	5.6(0.6)	5.7(0.7)	5.7(0.7)	5.6(0.5)	-	-	0.085
FPI (pmol/L)	44.5(39.0)	44.5(39.0)	50.0(55.0)	34.0(15.0)	-	-	0.142
HOMA1IR	1.9(1.7)	1.9(1.7)	2.25(2.5)	1.3(0.8)	-	-	0.107
TT (nmol/L)	15.2(6.2)	13.55(6.70)	14.65(4.8)	16.6(8.3)	-	-	**0.050**
SHBG (nmol/L)	24.0(13.0)	24.5(17.0)	23(15.0)	25.0(17.0)	-	-	0.309
FT (pmol/L)	374.28±132.98	344.05±93.65	359.59±136.13	410.89±143.77	2.189	0.118	-
FAI	627.5(343)	603.50(357.0)	583(395)	632(327)	-	-	0.922

25(OH)D (nmol/L)	82.5(24.0)	83.50(17.0)	88.0(27.0)	82.0(32.0)	-	-	0.314
IIEF-5 score	23(4)	24(4)	23(6)	23(3)	-	-	0.864

Continuous variables are shown as mean ± SD or median (IQR). [*]Wellness, Lifestyle and Diet (Well-LaD) Study. [†]P-values derived from one-way analysis of variance (ANOVA) when data normally distributed data with homogeneity of variance and Kruskal-Wallis when data not normally distributed. A:G, android-to-gynoid fat ratio; AIx@HR75, augmentation index adjusted to heart rate 75 bpm; AP@HR75, augmentation pressure adjusted to heart rate 75 bpm; BF%, body fat percentage; BMI, Body Mass Index; DBP, diastolic blood pressure; FAI, free androgen index; FPG, fasting plasma glucose; FPI, fasting plasma insulin; FT, free testosterone; HDL-c, high density lipoprotein cholesterol; HR, heart rate; HOMA1-IR, homeostasis model assessment one- insulin resistance; IIEF-5, 5-item International Index of Erectile Function; IQR, interquartile range; LDL-c, low density lipoprotein cholesterol; PWV, pulse wave velocity; SBP, systolic blood pressure; SD, standard deviation; SHBG, sex hormone binding globulin; TC, total cholesterol; TG, triglycerides; TT, total testosterone; VO_2peak, maximal oxygen consumption; WC, waist circumference; WHR, waist-to-hip ratio; WHtR, waist-to-height ratio.

Table 7.9. Comparison of clinical characteristics between alleles in the rs1544410 (*Bsm*I) polymorphism in study* participants (n=100).

Variable	VDR polymorphism allele rs1544410 (*Bsm*I)				
	B N=86	**b N=114**	**ANOVA F-statistic**	**ANOVA P-value†**	**Mann-Whitney U P-value†**
Age (years)	55(19)	52(12)	-	-	**0.022**
VO_2peak (ml/kg/min)	33.0(10.9)	34.0(14.4)	-	-	0.723
Handgrip strength (kg)	97.0(31.8)	102.5(20.6)	-	-	**0.010**
Height (cm)	175.78±5.98	178.28±6.72	10.431	**0.001**	-
Weight (kg)	83.90(15.22)	85.49(15.16)	-	-	0.165
BMI (kg/m2)	27.04(4.70)	26.89(4.22)	-	-	0.982
WC (cm)	95.11±10.66	95.39±10.11	0.035	0.853	-
WHR	0.95±0.06	0.94±0.06	0.453	0.502	-
WHtR	0.54±0.06	0.54±0.06	0.741	0.390	-
BF%	28.84±4.63	28.85±4.49	0.001	0.981	-
A:G	1.20(0.27)	1.20(0.27)	-	-	0.915
SBP (mmHg)	133.51±21.17	126.38±15.24	6.946	**0.009**	-
DBP (mmHg)	80.43±10.49	77.66±8.05	3.056	0.082	-
HR (bpm)	53.5(12)	54.5(9)	-	-	0.718
PP (mmHg)	49(18)	48(16)	-	-	**0.033**
PWV (m/s)	7.9(1.1)	7.8(1.5)	-	-	0.441
AP@HR75	5.5(7.0)	4.0(6.5)	-	-	**0.007**
AIx@HR75	16.50±9.10	12.32±10.21	6.203	**0.014**	-
TC (mmol/L)	5.29±0.93	5.09±1.00	5.051	**0.026**	-
TG (mmol/L)	1.1(0.6)	1.0(0.7)	-	-	0.332
HDLc (mmol/L)	1.2(0.3)	1.2(0.3)	-	-	0.680
LDLc (mmol/L)	3.52±0.83	3.34±0.88	4.076	**0.045**	-
TC:HDLc	4.3(1.2)	4.2(1.6)	-	-	0.404
TG:HDLc	0.9(0.6)	0.8(0.6)	-	-	0.132
FPG (mmol/L)	5.7(0.6)	5.6(0.7)	-	-	0.092
FPI (pmol/L)	50(44)	38(22)	-	-	0.076
HOMA1IR	2.2(2.1)	1.5(1.1)	-	-	**0.014**
TT (nmol/L)	14.1(6.1)	16.3(5.5)	-	-	0.114
SHBG (nmol/L)	24.5(14)	24.5(16)	-	-	0.799
FT (pmol/L)	355.57±108.86	380.35±127.32	4.328	**0.039**	-

FAI	611(354)	627.5(329)	-	-	0.559
25(OH)D (nmol/L)	84(21(	84(30)	-	-	0.600
IIEF-5 score	24(4)	23(4)	-	-	0.600

Continuous variables are shown as mean ± SD or median (IQR). *Wellness, Lifestyle and Diet (Well-LaD) Study. [1]P-values derived from one-way analysis of variance (ANOVA) when data normally distributed and Mann-Whitney U when data not normally distributed. A:G, android-to-gynoid fat ratio; AIx@HR75, augmentation index adjusted to heart rate 75 bpm; AP@HR75, augmentation pressure adjusted to heart rate 75 bpm; BF%, body fat percentage; BMI, Body Mass Index; DBP, diastolic blood pressure; FAI, free androgen index; FPG, fasting plasma glucose; FPI, fasting plasma insulin; FT, free testosterone; HDL-c, high density lipoprotein cholesterol; HR, heart rate; HOMA1-IR, homeostasis model assessment one- insulin resistance; IIEF-5, 5-item International Index of Erectile Function; IQR, interquartile range; LDL-c, low density lipoprotein cholesterol; PWV, pulse wave velocity; SBP, systolic blood pressure; SD, standard deviation; SHBG, sex hormone binding globulin; TC, total cholesterol; TG, triglycerides; TT, total testosterone; VO_2peak, maximal oxygen consumption; WC, waist circumference; WHR, waist-to-hip ratio; WHtR, waist-to-height ratio.

Table 7.10. Comparison of clinical characteristics between genotypes in the rs731236 (*Taq*I) polymorphism in study* participants (n=100).

Variable	Study prevalence	VDR polymorphism genotype rs731236 (*Taq*I)			ANOVA F-statistic	ANOVA P-value†	Kruskal-Wallis P-value‡
	mean±SD / median (IQR)	TT N=28	Tt N=45	tt N=27			
Age (years)	54(16)	53(10)	52.5(15)	56(22)	-	-	0.252
VO_2peak (ml/kg/min)	33.7(14.1)	33.1(13.7)	33.8(11.7)	37.2(16.4)	-	-	0.977
Handgrip strength (kg)	100(25)	101(21.9)	100(28)	99(26)	-	-	0.588
Height (cm)	177.06±6.33	177.61±3.19	176.19±6.12	177.95±6.87	0.797	0.454	-
Weight (kg)	84.97(15.08)	84.28(12.62)	86.45(20.48)	83.40(14.68)	-	-	0.689
BMI (kg/m2)	27.11(4.58)	27.10(4.54)	27.41(4.89)	26.39(0.26)	-	-	0.358
WC (cm)	96.30±11.12	95.74±12.48	97.93±11.29	94.17±9.13	1.012	0.367	-
WHR	0.95±0.07	0.95±0.07	0.96±0.06	0.95±0.07	0.556	0.575	-
WHtR	0.54±0.06	0.54±0.07	0.56±0.06	0.53±0.05	1.536	0.220	-
BF%	29.11±4.67	29.51±4.63	29.66±5.06	27.79±3.87	1.501	0.228	-
A:G	1.21(0.26)	1.13(0.30)	1.25(0.20)	1.17(0.26)	-	-	0.155
SBP (mmHg)	129.32±17.95	127.00±15.49	129.89±17.67	130.78±20.99	0.341	0.712	-
DBP (mmHg)	79.25±9.10	79.00±9.22	79.47±9.84	79.15±7.94	0.025	0.976	-
HR (bpm)	55.5(11)	55(17)	57(12)	51(10)	-	-	0.445
PP (mmHg)	48.5(16)	46.5(17)	49(15)	47(20)	-	-	0.598
PWV (m/s)	7.8(1.4)	7.5(1.3)	8.2(1.1)	7.7(0.8)	-	-	0.068
AP@HR75	5.0(6.7)	4.6(6.5)	5.3(5.2)	4.0(9.8)	-	-	0.774
AIx@HR75	14.42±10.24	13.72±11.12	14.74±9.44	14.62±11.02	0.081	0.923	-
TC (mmol/L)	5.18±0.99	5.07±1.06	5.24±0.93	5.20±1.02	0.262	0.770	-
TG (mmol/L)	1.1(0.9)	0.9(0.7)	1.3(0.7)	1.1(0.8)	-	-	0.812
HDLc (mmol/L)	1.2(0.4)	1.2(0.4)	1.3(0.4)	1.2(0.2)	-	-	0.641
LDLc (mmol/L)	3.38±0.90	3.34±1.00	3.42±0.97	3.37±0.88	0.068	0.934	-
TC:HDLc	4.3(1.5)	4.15(2.1)	4.4(1.3)	4.2(1.4)	-	-	0.824
TG:HDLc	0.9(0.8)	0.8(0.6)	0.9(0.8)	0.7(0.6)	-	-	0.896
FPG (mmol/L)	5.6(0.6)	5.6(0.5)	5.7(0.8)	5.5(0.6)	-	-	0.113
FPI (pmol/L)	44.5(39)	36(19)	50(53)	43(32)	-	-	0.295
HOMA1IR	1.9(1.7)	1.5(0.9)	2.2(2.5)	1.7(1.6)	-	-	0.245
TT (nmol/L)	15.2(6.2)	16.5(7.2)	14.7(4.8)	15.6(12.0)	-	-	0.342
SHBG (nmol/L)	24(13)	23.5(12)	24(15)	25(18)	-	-	0.730

FT (pmol/L)	374.28±132.98	397.39±122.85	352.42±131.72	386.74±143.98	1.153	0.320	-
FAI	627.5(343)	659(340)	549(389)	611(298)	-	-	0.476
25(OH)D (nmol/L)	82.5(24)	82(31)	86.5(25)	84(19)	-	-	0.351
IIEF-5 score	23(4)	24(3)	23(6)	23(4)	-	-	0.531

Continuous variables are shown as mean ± SD or median (IQR). *Wellness, Lifestyle and Diet (Well-LaD) Study. †P-values derived from one-way analysis of variance (ANOVA) when data normally distributed with homogeneity of variance and Kruskal-Wallis when data not normally distributed. A:G, android-to-gynoid fat ratio; AIx@HR75, augmentation index adjusted to heart rate 75 bpm; AP@HR75, augmentation pressure adjusted to heart rate 75 bpm; BF%, body fat percentage; BMI, Body Mass Index; DBP, diastolic blood pressure; FAI, free androgen index; FPG, fasting plasma glucose; FPI, fasting plasma insulin; FT, free testosterone; HDL-c, high density lipoprotein cholesterol; HR, heart rate; HOMA1-IR, homeostasis model assessment one- insulin resistance; IIEF-5, 5-item International Index of Erectile Function; IQR, interquartile range; LDL-c, low density lipoprotein cholesterol; PWV, pulse wave velocity; SBP, systolic blood pressure; SD, standard deviation; SHBG, sex hormone binding globulin; TC, total cholesterol; TG, triglycerides; TT, total testosterone; VO_2peak, maximal oxygen consumption; WC, waist circumference; WHR, waist-to-hip ratio; WHtR, waist-to-height ratio.

Table 7.11. Comparison of clinical characteristics between alleles in the rs731236 (*Taq*I) polymorphism in study* participants (n=100).

Variable	VDR polymorphism allele rs731236 (*Taq*I)				
	T N=101	t N=99	ANOVA F-statistic	ANOVA P-value[ǂ]	Mann-Whitney U P-value[ǂ]
Age (years)	53(12)	54(20)	-	-	0.081
VO_2peak (ml/kg/min)	33.3(13.2)	35.2(15.6)	-	-	0.815
Handgrip strength (kg)	101.0(23.5)	99.5(24.8)	-	-	0.454
Height (cm)	176.81±6.09	177.66±6.96	0.037	0.849	-
Weight (kg)	84.87(15.23)	85.40(15.10)	-	-	0.689
BMI (kg/m2)	27.28(4.62)	26.82(4.28)	-	-	0.431
WC (cm)	95.50±10.70	95.02±9.94	0.283	0.595	-
WHR	0.95±0.06	0.94±0.05	0.002	0.961	-
WHtR	0.54±0.06	0.52±0.06	0.267	0.606	-
BF%	29.20±4.64	28.45±4.42	2.028	0.156	-
A:G	1.20(0.28)	1.20(0.27)	-	-	0.708
SBP (mmHg)	128.20±16.55	130.78±20.07	0.678	0.411	-
DBP (mmHg)	78.88±9.41	78.79±9.12	0.004	0.947	-
HR (bpm)	55.5(10)	53(12)	-	-	0.313
PP (mmHg)	49(16)	48(16)	-	-	0.404
PWV (m/s)	7.8(1.5)	7.9(1.2)	-	-	0.341
AP@HR75	4.7(5.9)	5.2(7.6)	-	-	0.475
AIx@HR75	13.78±10.01	14.46±9.91	0.106	0.745	-
TC (mmol/L)	5.15±1.00	5.20±0.95	0.294	0.588	-
TG (mmol/L)	1.0(0.7)	1.1(0.6)	-	-	0.992
HDLc (mmol/L)	1.2(0.4)	1.2(0.3)	-	-	0.701
LDLc (mmol/L)	3.41±0.88	3.42±0.84	0.028	0.867	-
TC:HDLc	4.3(1.6)	4.2(1.3)	-	-	0.811
TG:HDLc	0.9(0.6)	0.8(0.6)	-	-	0.757
FPG (mmol/L)	5.6(0.6)	5.7(0.7)	-	-	0.926
FPI (pmol/L)	41.5(38)	44.5(38)	-	-	0.414
HOMA1IR	1.7(1.8)	1.9(1.7)	-	-	0.484
TT (nmol/L)	15.2(5.7)	15.2(6.3)	-	-	0.910
SHBG (nmol/L)	23.5(13)	25(18)	-	-	0.425

FT (pmol/L)	374.60±120.19	364.54±120.50	0.109	0.741	-
FAI	632(353)	611(364)	-	-	0.437
25(OH)D (nmol/L)	83(28)	84.5(22)	-	-	0.417
IIEF-5 score	24(3)	23(4)	-	-	0.237

Continuous variables are shown as mean ± SD or median (IQR). *Wellness, Lifestyle and Diet (Well-LaD) Study. [t]P-values derived from one-way analysis of variance (ANOVA) when data normally distributed and Mann-Whitney U when data not normally distributed. A:G, android-to-gynoid fat ratio; AIx@HR75, augmentation index adjusted to heart rate 75 bpm; AP@HR75, augmentation pressure adjusted to heart rate 75 bpm; BF%, body fat percentage; BMI, Body Mass Index; DBP, diastolic blood pressure; FAI, free androgen index; FPG, fasting plasma glucose; FPI, fasting plasma insulin; FT, free testosterone; HDL-c, high density lipoprotein cholesterol; HR, heart rate; HOMA1-IR, homeostasis model assessment one- insulin resistance; IIEF-5, 5-item International Index of Erectile Function; IQR, interquartile range; LDL-c, low density lipoprotein cholesterol; PWV, pulse wave velocity; SBP, systolic blood pressure; SD, standard deviation; SHBG, sex hormone binding globulin; TC, total cholesterol; TG, triglycerides; TT, total testosterone; VO_2peak, maximal oxygen consumption; WC, waist circumference; WHR, waist-to-hip ratio; WHtR, waist-to-height ratio.

Table 7.12. Logistic regression odds ratios (OR) and 95% confidence intervals (CI) for age, serum 25(OH)D and rs11568820 (*Cdx*2) VDR polymorphism as predictors of erectile dysfunction (IIEF-5 score ≤21) in study* participants (n=100).

		Crude		**Age-adjusted**		**Age and 25(OH)D-adjusted**	
Characteristic or condition		**OR [95% CI]**	**P-value**	**OR [95% CI]**	**P-value***	**OR [95% CI)**	**P-value***
Age	years	1.10[1.06-1.14]	**<0.001**	-	-	1.10[1.04-1.16]	**0.002**
Serum 25(OH)D	nmol/L	0.98[0.96-0.99]	**0.002**	0.98[0.96-0.99]	**0.004**	0.97[0.95-1.00]	**0.032**
rs11568820 (*Cdx*2)**	AA	Referent	-	Referent	-	Referent	-
	AG	19.00[2.15-167.68]	**0.008**	18.78[1.98-178.60]	**0.011**	23.59[2.34-237.53]	**0.007**
	GG	8.55[1.06-68.88]	**0.044**	8.53[1.00-72.73]	**0.050**	9.65[1.08-86.14]	**0.042**
	A	Referent	-	Referent	-	-	-
	G	1.95[0.96-3.94]	0.064	1.92[0.90-4.06]	0.090	-	-

* P-values for multivariate associations with erectile dysfunction derived from logistic regression models including age, or age and serum 25(OH)D concentrations - the full model with *Cdx*2 genotype had a Nagelkerke r^2 value of 0.366 and classified 77% of cases correctly. **Normal nomenclature used for *Cdx*2 (rs11568820) genotypes with A allele where the restriction site was absent and G allele where the restriction site was present. 25(OH)D, 25-hydroxyvitamin D; IIEF-5, 5-item International Index of Erectile Function; VDR, vitamin D receptor.

3.4 Impact of *Cdx*2 on the association between serum 25(OH)D level and ED

Age was a significant predictor of ED (IIEF-5 score ≤21) in this group of men (OR=1.10 [1.06-1.14], p<0.001: Table 7.12). Serum 25(OH)D was also a significant predictor of ED and this remained after adjusting for age: every 1 nmol/L increase predicted a 2% decrease in the risk of ED (age-adjusted OR=0.98 [0.96-0.99], p=0.004). The *Cdx*2 polymorphism genotype was also a significant predictor of ED after age-adjustment with the AG genotype associated with over 18-times (age-adjusted OR=18.78 [1.98-178.60], p=0.011) and the GG genotype associated with over 8-times (age-adjusted OR=8.53 [1.00-72.73], p=0.050) the odds of having ED compared to the AA genotype. However, the G allele was not a significant predictor of increased odds of ED in this analysis (age-adjusted OR=1.92 [0.90-4.06], p=0.090). In the full model, adjusting for age, serum 25(OH)D and *Cdx*2 polymorphism genotype, all three variables remained statistically significant independent predictors of ED. Although the significance of serum 25(OH)D as a predictor in the model was attenuated, it remained significant and the association between serum 25(OH)D and ED was slightly augmented (multi-adjusted OR=0.97 [0.95-1.00], p=0.032). In contrast, both the significance of the *Cdx*2 polymorphism genotype in the model and the effect size of its association with ED were augmented (GA multi-adjusted OR=23.59 [23.59-9.65] p=0.007 and GG multi-adjusted OR=9.65 [1.08-86.14], p=0.042).

4.0 DISCUSSION

The present study compared the genotype and allele distributions of the *Cdx*2, *Fok*I, *Bsm*I and *Taq*I *VDR* polymorphisms and their association with classical cardiovascular risk factors, serum 25(OH)D concentration and ED in healthy NZ men. To our knowledge, this is the first study to investigate the association between *VDR* polymorphisms and ED, an early marker of increased risk of CVD.

It has been well established that the prevalence of *VDR* polymorphisms show marked ethnic and geographical variation [47, 65]. Very few studies have investigated the prevalence of *VDR* polymorphisms in NZ populations [64, 66, 67] and this is the first to investigate this in a healthy male NZ Caucasian population. Of the three available studies, one did not provide prevalence data [66] and one was in NZ women of South Asian origin [64] limiting its comparability. In 2012, Bentley et al [67] investigated the association between *Cdx*2, *Fok*I and *Taq*I polymorphisms and colorectal cancer risk in NZ Caucasians living in Canterbury (n=400, 53% men, 200 cases, 200 controls, mean age = 69.5±0.4 years) and results from the healthy control group were reported to be in HW equilibrium with the following prevalence: *Cdx*2 AA 3.3%, GA 34.6% GG 62.1%; *Fok*I FF 41.4%, Ff 41.9%, ff 16.8%; and *Taq*I TT 17.6%,Tt 47.3%, tt 35.2%. This study did not include assessment of *Bsm*I but the reported frequencies differ widely from the

present study across all three polymorphisms including minor allele frequencies (MAF): *Cdx*2 20.6% vs 31%, *Fok*I 37.7% vs 45% and *Taq*I 41.2% vs 49% respectively. The reasons for this are unclear and future studies need to establish the prevalence of these polymorphisms in the general NZ population to allow comparisons to be made between studies and subpopulations.

The MAF found in the present study appear to differ widely from those listed in the NCBI Database of Single Nucleotide Polymorphisms (dbSNP) [68] (based largely on those reported in the 1000 Genomes Project): 31% vs 46%, 45% vs 33%, 43% vs 30%, and 49% vs 28% for *Cdx*2, *Fok*I*, Bsm*I *and Taq*I respectively. Due to the lack of NZ-based data, it is appropriate to compare the frequencies in the current study to those found in other healthy Caucasian populations in the USA [65], Europe [48, 69, 70] and South Africa [71]. The ancestral *Cdx*2 A allele in the current study is higher than that found in a control group of non-Hispanic Caucasian Americans (n=2697, mixed gender) by Slattery et al [65] (31% vs 19% respectively). The prevalence of the *Fok*I and *Taq*I minor homozygote genotypes are higher than those found in a Caucasian control group in the UK (n=108, mixed gender) reported by Hutchinson et al [72] (FF 48.1%, Ff 40.7%, ff 11.1% and TT 41.9%, Tt 44.1%, tt 14%) and in healthy White South African blood donors (n=238, mixed gender) reported by Ojwang et al [71] (TT 36%, Tt 51%, tt 13%, T 62%, t 38%). A 2015 report on a large longitudinal cohort study [48] in Northern Norway (The Tromsø Study, n=5980, 43% men, mean age = 57.4 ± 9.9 years) found the prevalence of the *Fok*I, *Bsm*I and *Taq*I to be FF 42.5%, Ff 45.5%, ff 12.1%; BB 34.1%, Bb 48.1%, bb 17.1%; and TT 34.0%, Tt 48.2% and tt 17.8%. This indicates a much lower prevalence of the minor homozygote genotypes than in our study with its smaller sample size. However, the prevalence of some polymorphisms in our study is similar to those reported in other predominately Caucasian male populations. For example, in 2013 Laczmanski et al [69] reported a similar prevalence of *Fok*I genotypes in 454 Polish men over 65 years of age (FF 33%, Ff 50%, ff 17%); however the prevalence for *Bsm*I genotypes (BB 32%, Bb 48%, bb 20%) showed much lower levels of the minor homozygote. It is possible that NZ Caucasian men may have a higher prevalence of the minor homozygous genotypes than other Caucasian populations. This could suggest environmental selection pressures in NZ but is more likely to be a random effect due to the small sample size.

Comparison of prevalence data between studies and populations is hindered by the variation in polymorphisms studied (e.g., the majority of studies assess one or two but rarely all common SNPs), the merging of previously identified and studied SNPS (e.g., rs2228571, rs17777794, rs17880019, rs59730659, rs118037316 and rs386609145 have merged with rs731236 (*Taq*I) in the NCBI Database of Single Nucleotide Polymorphisms (dbSNP) [68]), and

differences in the labeling of alleles and the definition of the SNP (e.g., the use of T/t, T/C or even A/G to describe the alleles of the rs731236 (*Taq*I) polymorphism). Furthermore, determining which is the ancestral allele is important to understanding the evolution of the human genome including genomic signatures resulting from selection pressures, the formation of linkage disequilibrium (LD) patterns and the changing prevalence of disease-associated alleles [73]. The following ancestral alleles are listed on NCBI dbSNP [68]: *Cdx*2 A allele, *Fok*I T allele, *Bsm*I G allele and *Taq*I T allele. These data are taken predominately from the 1000 Genomes Project which used multiple sequence alignments from different species to determine the ancestral sequence. However, there appear to be some inconsistencies and without reliable information regarding the ancestral allele it is difficult to explore potential implications of variations in prevalence. Another issue arises from the reporting of MAFs which refer to the least prevalent allele in the population measured, however some studies do not clearly define the alleles [67]. As the minor allele can differ dramatically between ethnic populations, the MAF cannot be used to reliably compare the prevalence of polymorphisms between studies as it may refer to either the ancestral allele or the mutant allele. There are very few comparable studies in healthy male Caucasian populations.

Overall, several studies have shown significant associations between common *VDR* polymorphisms and clinical signs of CVD including left ventricular hypertrophy [74], calcific aortic valve stenosis [75] and Ischemic stroke [76], although there are some inconsistencies evident [77]. Furthermore, some studies have shown associations with biomarkers of cardiometabolic disease including MetS [56], obesity [57], blood pressure [60-62], abnormal lipid profile [57, 59], insulin resistance [58, 59] and T2DM [57]. However the evidence supporting these associations remains equivocal. For example, despite several studies finding an association between some polymorphisms and increased blood pressure [60-62], a large 2014 study involving 23,294 European women (Women's Genome Health Study) and 69,395 European women and men (International Consortium of Blood Pressure) reported no association between *Cdx*2, *Fok*I or *Bsm*I and blood pressure [63].

The current study provides evidence supporting an association between *VDR* polymorphisms and CVD risk factors including ED. The *Cdx*2 G allele, *Fok*I f allele and *Bsm*I B allele all appear to be associated with an adverse CV risk profile, suggesting that they are involved in altered vitamin D signaling resulting in increased CVD susceptibility. Our results show that the AA genotype for the *Cdx*2 polymorphism was less prevalent in men with ED and this may have predictive value for a decreased risk of ED in older age. Further supporting this is the finding that the A allele (the ancestral allele [68]) was associated with greater cardiorespiratory fitness

and handgrip strength, and lower central adiposity and PWV (increasingly considered the gold standard measurement for arterial stiffness [78]), indicative of better cardiovascular health. Nevertheless, the moderate sample size (n=100) suggests approaching these results with caution and the lack of HW equilibrium with this polymorphism suggests there may be some selection pressures occurring in the population. Although, given the sample size, it is surprising that the distribution of alleles for three of the polymorphisms did conform to the HW principle. The additional finding of a significant association between *Fok*I f allele and poorer IIEF-5 scores supports that there is a relationship between the VDR and ED that requires further investigation.

In the current study we found no significant associations between the *Taq*I polymorphism and any of the health variables measured. However, we found several interesting associations with *Bsm*I. The B allele was associated with an adverse cardiovascular risk profile. The association found between both the BB genotype and the B allele and lower height supports earlier studies [79], particularly in healthy Caucasian male children where the BB genotype has been associated with lower height and slower growth in a longitudinal cohort study [80]. This is further strengthened by the finding of lower TT and FT amongst men with the BB genotype and B allele respectively. Testosterone is associated with male secondary sexual characteristics such as growth and muscle mass and studies have shown an association between lower TT and FT levels and poor handgrip strength [81]. Other studies have also shown both low vitamin D status [82] and VDR polymorphisms [83] to be associated with lower handgrip strength, an indicator of overall muscle strength and good health. The BB genotype and B allele were also associated with several markers of vascular health including higher systolic BP, pulse pressure, augmentation pressure and augmentation index (indicative of pulse wave reflectance, resistance and arterial stiffness). Other studies have also shown an association between low vitamin D levels [84], VDR polymorphisms [85], increased blood pressure and hypertension. It is reasonable to postulate that this is due to an effect of vitamin D signaling on endothelial function, inflammation, arterial stiffness and renin–angiotensin–aldosterone system (RAAS) activity. Finally, there was an association between the B allele and lipid abnormalities and insulin resistance. The results of this study support recent research showing a particular VDR genetic profile supporting health status, particularly metabolic and cognitive, into older age in centenarians [85].

The *Cdx*2 polymorphism (A allele), located in the promoter region, has been shown to result in increased transcriptional activity of the *VDR* gene. The *Fok*I polymorphism (f allele), located in the coding region, has an established structural effect on the length of the VDR resulting in a

longer protein with reduced interaction with $1,25(OH)_2D_3$ leading to lower transcriptional activity compared to the longer VDR protein [68, 86]. There is little evidence to support LD between *Fok*I and other VDR polymorphisms, therefore the reduced transcriptional activity of the f allele appears to have a direct functional effect on sexual function. Further research is needed to establish the mechanism behind this association. Finally, the *Bsm*I and *Taq*I polymorphisms, located near the 3' untranslated region (UTR), have not been found to result in structural changes to the protein or functional changes to its level of transcriptional activity, but are in strong LD with other polymorphisms including *Tru9*I, *Apa*I and *poly*(A) in the same region of the gene [87] and have been suggested to interact to affect mRNA stability and levels of expression [88, 89].

The association between serum 25(OH)D and CVD may be modified by VDR polymorphism and this may explain the inconsistent evidence regarding the association between serum 25(OH)D and CVD risk factors in epidemiological studies and the variable responsiveness to supplementation in intervention studies. Contrary to several other studies [51-53, 90], we found no association between any of the VDR polymorphisms assessed and serum 25(OH)D concentrations. The combination of a high level of serum 25(OH)D in the study population and a moderate sample size may have reduced the power to detect such a relationship. However, is it also possible that these VDR polymorphisms are not associated with serum 25(OH)D levels, but may exacerbate or ameliorate the relationship between low vitamin D status and CVD risk factors. However, the inclusion of the *Cdx*2 polymorphism in a model with age and serum 25(OH)D to predict the presence of ED showed no evidence of a modifying effect of this polymorphism on the association between serum 25(OH)D and ED. All three were found to be independent predictors of ED. The modifying effects may be limited to certain polymorphisms and certain disease outcomes. For example, in 2012 Levin et al [91] investigated whether *VDR* polymorphisms modified the associations between serum 25(OH)D and a composite clinical health outcome (hip fracture, myocardial infarction, cancer and mortality) in a longitudinal study (n=1514 white Americans, 1992-2006, median follow-up = 11 years) with replication by meta-analysis of international cohort studies. They reported a significant and association with the VDR rs7968585 which was replicated in the meta-analysis. However, in 2014 Vimaleswaran et al [92] found no evidence to support a modifying effect of either rs7968585 or VDR rs2239179 on the association between 25(OH)D concentrations and cardiometabolic risk factors. Further research is needed to determine the modifying effects of common VDR polymorphisms on disease outcomes and risk factors.

The main strengths of this study were the inclusion of "healthy" men, the comprehensive nature of cardiovascular risk factor assessment and the analysis of four different *VDR* polymorphisms located at different locations along the length of the *VDR* and the use of closed-tube automated HRM analysis for genotyping. However, the power of the study to detect statistical differences is limited by the sample size. Furthermore, the genotyped SNPs did not cover all known variants on the VDR gene region. For example, the assessment of the *Apa*I polymorphism would have supported haplotype block analysis: *Bsm*I, *Apa*I and *Taq*I have been shown to have LD and the analysis of haplotypes may be more relevant to CVD risk prediction than individual polymorphisms.

Over the past decade, genome-wide association studies have identified that polymorphisms at various locations on the human genome are associated with the risk and progression of CVD [93]. However, the evidence to support the *VDR* as a candidate gene affecting cardiovascular health remains inconsistent. It has been suggested that while approximately 64 VDR polymorphisms have been identified, over 100 polymorphisms are likely to exist, indicating that further research is needed to sequence the entire gene in multiple individuals to determine sequence variations and polymorphisms. Once the polymorphisms have been sequenced, population-based studies can determine their prevalence and interactions (i.e. LD and haplotype analysis) in various ethnicities and geographical locations and their associations with CVD risk factors, progression and disease outcomes. Furthermore, the functional effects of these polymorphisms on VDR protein structure, expression, function and signaling remain largely unknown. Much research is needed in the field of functional mechanisms to determine the effects on vitamin D absorption, metabolism, utilisation and signaling [94].

5.0 CONCLUSIONS

Our findings indicate that low serum 25(OH)D level and the rs11568820 (*Cdx*2) polymorphism of the VDR gene are independently associated with ED, an early marker of CVD. This implies that VDR gene polymorphisms may be an important consideration in addition to serum 25(OH)D level when recruiting men with ED for an intervention study as genotypic differences may alter the efficacy, dosage and duration required to see an effect of supplementation on sexual function. Every association found contributes to our understanding of the role of vitamin D in CVD. Larger epidemiological studies are needed to confirm this association and future mechanistic studies are needed to verify the effect of the rs11568820 (*Cdx*2) and other VDR gene polymorphisms on the structure, function and level of expression of the VDR.

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CHAPTER 8

DISCUSSION AND CONCLUSIONS INCLUDING RECOMMENDATIONS FOR FUTURE RESEARCH

1.0 INTRODUCTION

This thesis investigated the link between vitamin D and erectile dysfunction (ED) as an early marker of CVD, including the prevalence of ED and its sociodemographic, lifestyle and medical risk factors in New Zealand (NZ) men; its association with vitamin D status (serum 25-hydroxyvitamin D (25(OH)D) concentrations); and the impact of common vitamin D receptor (VDR) gene (*VDR)* polymorphisms on this relationship. The discussion will summarise the main findings of the various studies, briefly discuss key methodological concerns, examine the implications of the results, draw final conclusions and provide recommendations for future research.

2.0 SUMMARY OF MAIN FINDINGS

The literature review in Chapter 2 highlighted the variation in both the prevalence of ED and its associated risk factors in different countries, populations and studies and identified the lack of NZ data. It also highlighted the evidence supporting ED as an early marker of subclinical cardiovascular disease (CVD) in apparently healthy men.

In the study described in Chapter 3, a self-administered postal survey was developed and used to assess sexual activity and function in conjunction with sociodemographic, lifestyle and medical factors in NZ men aged 40-70 years. The random selection of participants from the NZ Electoral Roll provided the closest possible approximation to a nationally representative sample (599 respondents, 30% response rate). The results showed a 42.2% crude prevalence of ED (21.9% mild, 10.3% mild-moderate, 5.9% moderate, 4.1% severe), 38.4% when adjusted to the age-distribution of the NZ male population. After adjusting for various sociodemographic, lifestyle and medical factors, the independent predictors of an increased risk of ED were older age, non-European ethnicity and current smoking, while a high household income and regular participation in vigorous physical activity (PA) were significant predictors of a lower risk of ED. To our knowledge, this is the first study to determine the prevalence of ED and its risk factors in the NZ male population.

The literature review in Chapter 4 identified a strong level of epidemiological evidence to support an association between low serum 25(OH)D concentrations and CVD and CVD risk factors. It highlighted the inconsistent evidence from intervention studies regarding the benefits of supplementation in improving cardiometabolic health and considered the evidence supporting the hypothesised association between low vitamin D status and ED which has begun to emerge since the commencement of this thesis.

In the study described in Chapter 5, an observational study was conducted to assess ED, serum 25(OH)D levels and a comprehensive range of CVD risk factors in self-reported healthy Manawatu men aged 40-70 years. The study population was a combination of men invited after random selection from the Manawatu Electoral Roll and men self-selected by word-of-mouth and in response to public advertisements (n=100). They were predominately European, well-educated, with a high household income and living in a rural or semi-rural area. From the results, 30 men had ED (IIEF-5 score ≤21), 37 had vitamin D insufficiency (<75 nmol/L 25(OH)D) and there was a high level of cardiometabolic risk in this group. There was a significant but weak correlation between IIEF-5 scores and serum 25(OH)D levels (r_s=0.238, p=0.017). Men with <75 nmol/L 25(OH)D had significantly lower IIEF-5 scores compared to men with ≥75 nmol/L 25(OH)D (median 22(interquartile range 7) vs 24(3) respectively, p=0.001) and men with ED had marginally lower serum 25(OH)D levels compared to men without ED (74.5(34) vs 84.5(24) respectively, p=0.062). After adjusting for age, every 1nmol/L of serum 25(OH)D predicted a 2% decrease in the risk of ED (age-adjusted OR=0.98 [0.96-1.00], p=0.046), although serum 25(OH)D level demonstrated poor predictive capability as a diagnostic tool. To our knowledge this is the first study to investigate an association between ED and vitamin D status in apparently healthy men.

The literature review in Chapter 6 highlighted the importance of the VDR to the vitamin D signalling system and the increasing but inconsistent evidence supporting the association between the common *VDR* polymorphisms (rs11568820 (*Cdx*2), rs10735810 (*Fok*I), rs1544410 (*Bsm*I) and rs731236 (*Taq*I)) and CVD and CVD risk factors.

In the study described in Chapter 7, samples taken from the previous observational study were assessed for the *Cdx*2, *Fok*I, *Bsm*I and *Taq*I polymorphisms using polymerase chain reaction–high-resolution amplicon melt (HRM) analysis. Their associations with CVD risk factors including serum 25(OH)D concentrations and ED were examined. The results showed that *Cdx*2, *Fok*I and *Bsm*I polymorphisms were all significantly associated with elements of an adverse cardiovascular risk profile. In particular, men with the *Cdx*2 G allele had significantly lower IIEF-5 scores (indicating poorer erectile function) compared to the A allele (23(4) vs 24(2), p=0.008) and the GA and GG genotypes were significant predictors of an increased risk of ED, an effect independent of age (age-adjusted OR=18.78 [1.98-178.60], p=0.011 and 8.53 [1.00-72.73], p=0.050 respectively). *Cdx*2 was not found to modify the age-adjusted association between serum 25(OH)D concentration and ED (multi-adjusted OR=0.97 [0.95-1.00], p=0.032). This is the first study to investigate the relationship between *VDR* polymorphisms and ED.

3.0 KEY METHODOLOGICAL CONSIDERATIONS

A comprehensive series of studies was designed to investigate the relationship between vitamin D status and ED as an early marker of CVD in healthy NZ men. However, a number of methodological strengths and limitations must be taken into consideration when examining the validity of the results. These include primarily the study populations, the study designs and the tool used to measure ED. These issues will be discussed separately.

The studies may have inadvertently attracted volunteers who were health conscious or who had previous or current experience of ED. Both studies were marketed as part of a wider male "Wellness, Lifestyle and Diet (Well-LaD)" study, underplaying the importance of the sexual function component. This may have attracted men who were more interested in their health, the research topic, the equipment used, or the high level of individual follow-up information provided as a benefit of participation in the observational study. However, this was intended to avoid biasing recruitment towards men with previous or current experience of ED or other sexual function issues. The respondent profiles for both studies show that predominately well-educated European men volunteered, a characteristic of health research. The moderate response rate to both studies and the mixed recruitment strategy used in the observational study may have compounded this bias. However, as all participation in research is voluntary, the effect of this would be no greater than in any other study and all efforts were made to maximise the chances of obtaining a cross-sectional study population in both studies.

In planning the studies, while the nationwide survey was adequately powered to observe significant results based on similar studies overseas, the observational study was limited by time and resource constraints and the rate at which participants volunteered. From the results of the survey, we expected approximately 38% of volunteers in the observational study to present with ED. Fewer cases volunteered and subsequent to the study, an effort was made to recruit more men with ED. This was via enlisting the help of pharmacists in 14 different pharmacies throughout Palmerston North and Fielding to place discrete advertising cards in bags with ED prescription medication. Only two men volunteered in four months and this method of recruitment was abandoned. This highlights the issues in directly recruiting men with ED from the community for future studies. It would be advisable to consider conducting ED research with the help of specialised staff/health practitioners who have regular direct contact with patients presenting with ED. However, this was not appropriate for the current studies, as their aim was to assess ED in the general male population. This decision was

supported by the results in Chapter 3 which showed that the majority of NZ men with ED do not seek medical diagnosis or treatment.

The study design for both studies was cross-sectional, the benefits of which lie largely in their ability to generate hypotheses and support future research. However, while cross-sectional studies can determine associations, they cannot prove causation, nor can they provide information on the direction of the relationship (i.e. it is not known whether vitamin D insufficiency causes systemic vascular dysfunction and ED, or vice versa). The use of a longitudinal study design would provide stronger evidence to support the determination of independent risk factors for ED and a well-designed randomised controlled trial (RCT) would provide evidence to determine causation. However, these types of studies come with greater resource requirements and can only be successful with highly motivated volunteers. A double-blind placebo-controlled RCT of the benefits of vitamin D_3 supplementation on erectile function and cardiovascular health markers in men with vasculogenic ED (IIEF-5 score ≤21) and hypovitaminosis D (<50 nmol/L 25(OH)D) is warranted based on the preliminary findings in this thesis.

There are several self-administered tools commonly used to assess erectile function in epidemiological research. The IIEF-5 tool is widely considered to be the gold standard of subjective assessment; however, it has several strength and limitations. It is a freely available, brief and private self-administered tool that has been translated into multiple languages and validated in diverse populations worldwide. With high levels of sensitivity and specificity to assess multiple domains of erectile function it is increasingly used internationally. However, it relies on the subject's memory of sexual encounters/attempts over the past 6 months and is not suitable for use in men who are not sexually active (i.e. men not in a relationship or those with severe ED). Men who are not sexually active tend to not attempt to complete it, partially complete the IIEF-5 tool, or complete it by referring to masturbation attempts. This means it is likely to underestimate the prevalence of severe ED in the population. However, limiting the research to only those who are sexually active will also underestimate the prevalence of ED. It is currently the best available option for the subjective assessment of ED.

4.0 IMPLICATIONS OF MAIN RESULTS

The prevalence of ED (38%) and its key risk factors (ageing, non-European ethnicity, low household income, smoking and physical inactivity) in NZ men aged 40-70 years are comparable to overseas populations. Based on the NZ Census population data, this suggests that approximately 295,000 of NZ men in this age group have some degree of ED: 163,000 mild, 68,000 mild-moderate, 39,000 moderate, and 25,000 severe cases. However, only 17% of men with ED are medically diagnosed and only 22% are taking some form of treatment. This indicates a serious missed opportunity not only to improve the sexual function of these men and reduce the negative impact of ED on their lives, but also to identify and intervene at a unique point in the development of CVD.

The association between vitamin D insufficiency and ED in apparently healthy men indicates that it is both a novel marker and potentially a risk factor for ED. As ED is an early marker of subclinical CVD, timely intervention to raise serum 25(OH)D concentrations in men presenting with ED may help delay, halt or reverse the progression to clinical CVD. A randomised controlled trial is warranted. However, vitamin D elicits its effects via the VDR and it appears that common mutations in the gene encoding the VDR protein are also associated with ED. Although there is no evidence in this thesis of an association between common *VDR* polymorphisms and serum 25(OH)D levels or a modifying effect of these mutations on the association between serum 25(OH)D levels and ED, further research is needed to determine whether these polymorphisms modify the transcription of genes involved later in the vitamin D signalling pathway altering $1,25(OH)_2D$-responsiveness. If so, they may affect the potential erectile and cardiovascular benefits resulting from improved serum 25(OH)D concentrations in future intervention studies.

5.0 FINAL CONCLUSIONS

ED is highly prevalent in NZ men and is associated with low serum 25(OH)D concentrations. Therefore, improving vitamin D status via either safe sunlight exposure or supplementation may be a cheap, accessible novel or adjunct treatment for ED. As an early marker of subclinical CVD, ED offers a unique opportunity to intervene and delay, halt or reverse progression towards clinical CVD. A randomised controlled trial is needed to determine whether improving vitamin D status is effective at improving ED and CVD risk in men. With vitamin D insufficiency increasingly viewed as a worldwide pandemic, the results of this thesis suggest that ED is an emerging global health concern. ED is largely an issue in older males and the stereotypical view of an asexual old age may reduce the level of importance placed on this knowledge. However, with the rise in sedentary indoor lifestyles, obesity and diabetes rates, ED is likely to affect increasingly younger men, including those in their prime reproductive years.

6.0 RESEARCH RECOMMENDATIONS

- To investigate the prevalence and severity of ED in NZ using the IIEF-5 in a larger group of men with a wider age range and greater ethnic diversity (particularly Maori, Pacific Island and Asian minorities) and confirm the association with serum 25(OH)D concentrations. To this extent, the IIEF-5 could easily be included in an existing nationwide health survey or large male cohort study; however, a large multi-center male health study with a broader male health focus could provide additional information on a range of risk factors in different age and ethnic groups. Appropriate TV, radio, print and online publicity aimed at different subpopulations could be used to help maximise the response rate. An in-home finger-prick blood spot test could be used for the assessment of serum 25(OH)D concentration.
- To conduct a series of qualitative interviews with general practitioners to explore their attitudes towards ED, how and when they broach the topic of sexual function with male patients, how they diagnose ED, and which treatment options are offered. The outcome would be to create a best practice protocol for the standardised medical assessment of ED in NZ, highlighting the need for assessing underlying etiology including cardiovascular health.
- To examine the experiences of NZ men with ED through qualitative interviews to explore their personal story, knowledge, sources of information, motivation to treat and barriers to treatment using thematic analysis. The outcome of this would be to develop an educational tool such as a printed pamphlet to inform patients in clinical

practice and an online resource to inform the general public about ED including safe scientifically proven treatment options.

- To develop and validate a user-friendly NZ sun exposure assessment tool combining occupational and recreational sun exposure, use of sun protection and clothing, skin colour, body position, weather and ambient UV levels. Sun exposure is the major contributor to vitamin D status in the general population and without this tool it is difficult to assess the contribution of sun exposure to vitamin D status in individuals.
- To develop and validate a user-friendly NZ vitamin D food frequency questionnaire (FFQ) based on natural and fortified foods and supplements currently available on the NZ market. This would require reliable information on the vitamin D levels in these foods and supplements in NZ. Without this tool it is difficult to reliably assess the contribution of dietary intake to vitamin D status in NZ. Due to the narrow range of fortified foods and the low levels of fortification, it is possible that the list of foods in the FFQ could be relatively short (e.g., 10 commonly consumed foods) without substantially lowering its sensitivity and specificity.
- To conduct a well-designed double-blind randomised placebo-controlled intervention trial to investigate the effect of vitamin D_3 supplementation (120,000 IU/m) on ED symptoms and CVD risk factors and markers in men with hypovitaminosis D (<50 nmol/L serum 25(OH)D) and ED (IIEF-5 score ≤21). The outcome of this would be to determine the efficacy of raising serum 25(OH)D levels to ≥75 nmol/L on improving the symptoms of ED and other markers and risk factors for CVD.
- To determine the exact amount of sunlight exposure required for different skin types at different times of the day and year in various locations throughout NZ to attain and maintain a serum 25(OH)D level ≥75 nmol/L. The outcome of this would be to develop guidelines to support safe sun exposure designed to raise and maintain serum 25(OH)D levels ≥75 nmol/L.
- To investigate the potential mechanisms behind the role of vitamin D in CVD. For example, effects on endothelial function could be determined using *in vitro* studies to assess the effects of raising serum vitamin D levels on the health of endothelial cells including the expression of markers of inflammation, oxidation, thrombosis and nitric oxide etc.
- To examine the functional effects of variants in genes involved in the vitamin D micro-endocrine system (i.e. vitamin D synthesis, transportation, metabolism, utilisation and signaling) on the levels of vitamin D metabolites and their association with ED and

other CVD risk factors using genetic sequencing. The outcome would be to determine whether there is a specific genetic profile within vitamin D related genes that predisposes an individual to developing ED as an early marker of CVD.

- To investigate a novel hypothesis generated during this PhD research: that ED is a natural consequence of an extended winter metabolism. It is reasonable to postulate that the modern lifestyle (i.e. mass urbanisation, increasingly sedentary indoor occupations, reduced outdoor physical activity, burgeoning obesity rates and popular sun avoidance practices) mimics a prolonged hibernation and may lead to a sustained altered metabolic state. Vitamin D functions as a photoreceptor with lowered serum 25(OH)D signaling the onset of winter and the metabolic changes required to survive lower temperatures and a scarce food supply: increased blood pressure, altered glucose homeostasis, insulin resistance, increased appetite and weight gain [1]. This winter metabolism may signal the male body to reduce sexual activity and suspend reproduction until the environment is more conducive to survival for both parent and offspring. Examining this hypothesis would require epidemiological assessment of seasonal variation in male sexual activity, sperm quality, conception rates and birth rates in NZ and their association with UV exposure and serum 25(OH)D levels.

7.0 REFERENCES

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APPENDIX 1

CHAPTER 1 - ABSTRACTS

1.0 ABSTRACT FOR CHAPTER 3

Introduction: Erectile dysfunction (ED) is a common condition amongst men worldwide; however, prevalence rates and associated risk factors vary markedly between populations. New Zealand (NZ) specific data are lacking. This is a report of a population-based cross-sectional survey aiming to assess the prevalence of ED in NZ and examine its associated factors. **Methods:** Postal questionnaires were sent, following a modified Dillman method, to a randomly selected computer-generated age-stratified population-based sample of 2000 men aged 40-70 years obtained from the NZ Electoral Roll. Self-reported erectile function was assessed using both the validated 5-item International Index of Erectile Function (IIEF-5) and single-question self-assessment tool. The prevalence of ED is presented as crude, age-adjusted to the distribution of the NZ male population, and standardised to the World Health Organization World Standard Population (WSP). Associations between ED (IIEF-5 ≤21) and sociodemographic, lifestyle and medical factors were analysed using χ^2 and Fisher's exact tests, and binomial logistic regression (odds ratio (OR) [95% CI]) adjusting for age and confounders. **Results:** The response rate was 30% (599) with 28% (562) deemed complete for analysis. The crude prevalence of ED was 42.2%: 21.9% mild, 10.3% mild-moderate, 5.9% moderate and 4.1% severe. The age-adjusted prevalence was 38.4% and the WSP-adjusted prevalence 36.6%. ED affected 24% of men in their 40s, 38% in their 50s and 59% in their 60s. After adjusting for confounders, older age (4.91 [2.75-8.74]), non-European ethnicity (3.50 [1.72-7.13]) and being a current smoker (2.80 [1.41-5.57]) were independent predictors of increased risk. A high household income (0.39 [0.24-0.65]) and regular physical activity (0.58 [0.36-0.92]) were independent predictors of lowered risk. **Conclusions:** The prevalence of ED in NZ is comparable to overseas studies. Although age is the key risk factor, a range of modifiable factors contribute to its prevalence suggesting that lifestyle intervention may benefit NZmen.

2.0 ABSTRACT FOR CHAPTER 5

Introduction: Epidemiological studies support an association between low serum 25-hydroxyvitamin D (25(OH)D) and an increased risk of developing CVD. Erectile dysfunction (ED) is predominately vasculogenic in aetiology and a valuable early marker of CVD. It may also be associated with vitamin D status. This study aimed to investigate the relationship between vitamin D status, erectile function and cardiovascular risk factors in healthy older men. **Method:** An observational study was conducted in 100 self-selected healthy men aged 40-70 years in the Manawatu, New Zealand (NZ). Participants were assessed for serum 25(OH)D level, 5-item International Index of Erectile Function (IIEF-5) score and a comprehensive range of CVD risk factors (sociodemographic, lifestyle, anthropometrical, vascular, biochemical and medical) and 10-year Framingham risk. Submaximal fitness and handgrip strength were assessed. **Results:** The median serum 25(OH)D level was 82.5(24) nmol/L and 37 men were insufficient (<75 nmol/L): 8 deficient (<50 nmol/L) and 29 suboptimal (50-74.9 nmol/L). The median IIEF-5 score was 23(4) and 30 men had ED (IIEF-5 ≤21). A high level of cardiometabolic risk was observed. Vitamin D insufficiency was associated with lower cardiorespiratory fitness, higher central adiposity, poorer vascular health, higher levels of dyslipidaemia, insulin resistance and metabolic syndrome (MetS), and higher Framingham risk (all $p<0.05$). ED was associated with older age, lower handgrip strength, an altered hormone profile, a higher prevalence of medication use and higher Framingham risk (all $p<0.05$). There was a significant correlation between 25(OH)D level and IIEF-5 score ($r_s=0.238$, $p=0.017$). Lower 25(OH)D levels were observed in men with ED (74.5(34) vs 84.5(24) nmol/L, $p=0.062$). Every one unit increase in serum 25(OH)D was associated with a 2% decrease in the likelihood of ED (OR=0.98 [0.96-1.00], $p=0.030$) and this remained after age-adjustment (OR=0.98 [0.96-1.00], $p=0.046$). Area under the curve (AUC) analysis demonstrated poor diagnostic predictive capability. **Conclusions:** Vitamin D insufficiency is associated with both ED and traditional CVD risk factors in apparently healthy men, supporting a potential role for vitamin D in the maintenance of vascular health. A human intervention trial is needed to determine causality.

3.0 ABSTRACT FOR CHAPTER 7

Introduction. Erectile dysfunction (ED) is an early marker of CVD and is associated with low serum 25-hydroxyvitamin D (25(OH)D) concentrations. The activity of vitamin D is mediated by the vitamin D receptor (VDR). Polymorphisms in the VDR gene (*VDR*) may affect the relationship between serum 25(OH)D and ED. This study aimed to determine the frequency of four common *VDR* polymorphisms, their association with cardiovascular disease (CVD) risk factors, and their impact on the association between serum 25(OH)D and ED. **Methods.** One hundred self-selected healthy men aged 40-70 years living in the Manawatu, New Zealand, were studied for the rs11568820 (*Cdx*2, A/G)), rs10735810 (*Fok*I, T/C)), rs1544410 (*Bsm*I, A/G)) and rs731236 (*Taq*I, T/C)) *VDR* polymorphisms using polymerase chain reaction-high resolution amplicon melt (HRM) analysis. Classical CVD risk factors, serum 25(OH)D and 5-item International Index of Erectile Function (IIEF-5) scores were assessed. **Results.** The prevalence of the *Cdx*2 genotypes was: 58% GG, 22% GA, 20% AA. The AA genotype was less prevalent in men with ED (IIEF-5 ≤21) and associated with higher IIEF-5 scores (p=0.006). The A allele was associated with better IIEF-5 scores, cardiorespiratory fitness, handgrip strength, anthropometric and vascular markers (p≤0.05). The prevalence of *Fok*I genotypes was: 29% FF, 53% Ff and 18% ff. The F allele was associated with better IIEF-5 scores (p=0.048). The prevalence of the *Bsm*I genotypes was: 21% BB, 44% Bb and 35% bb. The b allele was associated with younger age, better vascular measurements, lipid and hormone profiles and lower insulin resistance (all p≤0.05). The prevalence of the *Taq*I genotypes was: 28% TT, 45% Tt and 27% tt. No significant associations were found. After age-adjustment, serum 25(OH)D concentration (OR=0.98 [0.96-0.99], p=0.004) and *Cdx*2 genotypes (GA OR=18.78 [1.98-178.60], p=0.011; GG OR=8.53 [1.00-72.73], p=0.050) were significant predictors of ED. The inclusion of *Cdx*2 genotype in the model slightly attenuated the effect size but diminished the significance of the predictive capability of serum 25(OH)D (OR=0.97 [0.95-1.00], p=0.032). **Conclusions.** This is the first study to suggest an association between *VDR* polymorphisms and ED as an early marker of CVD. The *Cdx*2 ancestral A allele confers cardiovascular protection and is associated with better erectile function. Serum 25(OH)D is associated with ED and this is independent of both age and *Cdx*2 genotype. Further studies are needed to confirm these results.

APPENDIX 2

CHAPTER 2 - ADDITIONAL INFORMATION ON ERECTILE DYSFUNCTION

1.1 UROGENITAL EXAMINATION

1.2 Nocturnal penile tumescence testing (NPT)

In a clinical setting, urological examination for erectile dysfunction (ED) often includes two tests: the Nocturnal Penile Tumescence (NPT) test and penile Doppler sonography (PDS). The NPT tests the ability of a patient to achieve normal nocturnal erections. ED in the presence of normal nocturnal erections is considered indicative of psychogenic ED, whilst ED in the absence of normal nocturnal erections signals an underlying organic problem. Two methods are used: 1) the Snap-Gauge method and 2) the Strain-Gauge method or full NPT test. The Snap-Gauge method uses pressure-sensitive bands placed around the penis that break when an erection occurs. In contrast, the full NPT test uses bands which document changes as an erection stretches them, thus it can also support measurements of penile circumference, arterial pulsations and rigidity [1]. Normal is generally defined as ≥1 erection lasting ≥5 minutes with a rigidity >550. However, Allen [1] compared the two methods and reported a poor correlation: full NPT testing was more accurate in determining a clinical diagnosis of ED. Both methods should include additional observer or self-reporting of nocturnal erections.

1.3 Penile Doppler sonography (PDS)

Although there are many imaging techniques now available, PDS has been predominately used in the assessment of penile health since 1985 [2] and can identify issues with penile vascular function [3]. It involves real-time high-resolution ultrasound scanning of the penis after administration of a pharmacological intervention (e.g. Papaverine or prostaglandin E_1 (PGE_1)), to compare and evaluate penile vasculature including the arterial velocity and cavernosal diameter pre and post administration. The response to pharmacological stimulation is lower in ED patients with venous and arterial insufficiency compared with those with normal vasculature [4]. A peak systolic velocity (PSV) <30 cm/s or a cavernosal diameter increase of <60% are generally considered indicative of arteriogenic ED (arterial insufficiency) and an end-diastolic velocity (EDV) >6 cm/s indicative of venogenic ED (venous dysfunction) [5]. Although PSV has been shown to correlate well with full NPT test measurements of axial rigidity and arterial pulsations in men with vasculogenic ED, results need to be treated with caution as the presence of psychogenic ED may suppress the response to pharmacological stimulation giving a false diagnosis of vasculogenic ED [6]. A combination of detailed medical, sexual and psychosocial history, a focused physical exam, baseline laboratory assessment, NPT testing and PDS would be most useful in determining the aetiology of ED in a clinical setting.

2.1 TREATMENT AND PREVENTION OF ERECTILE DYSFUNCTION

2.2 Oral therapies

The first-line therapy for the majority of patients is an oral pharmaceutical agent. The approved PDE_5 inhibitors sildenafil (Viagra®), tadalafil (Cialis®), vardenafil (Levitra®, Staxyn®) and avanafil (Stendra®) all work by preventing the breakdown of cGMP, resulting in higher concentrations and longer duration of this messenger, promoting blood flow and a sustained erection. Sildenafil, approved for use in the treatment of ED in 1998, has been the most extensively reviewed; however these drugs all appear to have similar clinical efficacy and safety profiles [7]. The main differences lie in how quickly they work, how long the effect lasts, and the possible side effects. Sildenafil and vardenafil have a similar onset (30-60 min), plasma half-life (4 hours) and duration of action (up to 12 and 10 hours respectively) [7]. Tadalafil has a longer onset (60-120 min) and plasma half-life (17.5 hours) and therefore a longer duration of action (up to 36 hours) [7]. Avanafil, the newest addition to the drug class, has a shorter onset (15-30 min), plasma half-life (3 hours) and duration of action (up to 6 hours) [8]. Side effects are generally shared and occur as a result of lack of specificity for PDE_5 versus other PDE subtypes. They are considered to be mild and transient and include: headaches, flushing, dyspepsia, nasal congestion, and dizziness. However, users of sildenafil, and to a lesser extent, vardenafil have reported a rare additional visual symptom called 'blue vision' [9, 10] while users of tadalafil have reported back pain and myalgias [11]. The use of all PDE_5 inhibitors must be monitored and treated with caution. There are a number of medications which are contraindicated [12], most importantly nitric oxide donors including any form of organic nitrates (a common vasodilator used in the treatment of angina). They are known to potentiate their hypotensive effects [9] and may cause irreversible and potentially life-threatening hypotension [9]. They are also contraindicated in men with a hypersensitivity to the medication or any components of the tablets. Caution is recommended before use in men: who are taking alpha-blockers; who have resting hypotension (≤ 90/70) or hypertension (≥170/110); who have suffered an MI, stroke or serious arrhythmia in the past 6 months; or who have a history of cardiac failure or CAD resulting in unstable angina. It is important to note that PDE-5 inhibitors do not cure ED, nor can they treat ED in the absence of sexual desire or its transmission to the penis [13]. This makes them ineffective in some men. Other oral therapies are currently undergoing trials but not yet approved for the treatment of ED.

2.3 Vacuum constriction devices

A non-invasive treatment, vacuum constriction devices are manually operated to create a negative atmospheric pressure around the penis [14]. This draws blood into the sinusoidal spaces and creates an erection. It must be maintained by the application of a constriction band at the base of the penis. This treatment is often used in men who do not respond to or prefer not to take oral erectogenic drugs. These have had mixed results with patient satisfaction ranging from 20-90%. The constriction band can be used for up to 30 minutes after which time there may be skin necrosis. Men taking anticoagulants or with bleeding disorders are warned to be cautious with the use of vacuum constriction devices [15].

2.4 Psychosocial counselling

The optimal treatment offers an integrated approach involving not only medical but psychosocial intervention for both the male and their partner Although this thesis focuses on organic ED, psychosocial factors are important in most, if not all, cases of ED. Professional psychosocial counselling may be of benefit to some men in: addressing anxiety and depression; supporting behavioural interventions; educating men on sexual function and alternative forms of intimacy for a satisfactory sexual life [16]; and working through personal and relationship difficulties [17]. It may also maximise treatment efficacy by encouraging compliance, helping to relieve anxiety and maintain realistic expectations surrounding treatment [18].

2.5 Intracavernosal injections and transurethral pellets

Intracavernosal injections and transurethral pellets are considered second-line therapies, if oral agents are unsuccessful. Intracavernosal injections were one of the first discovered treatments for ED in the 1980s. These injections mimic endogenous physiologic mechanism of vasodilation by injecting a vasoactive drug (papaverine, phentolamine or alprostadil) directly into the cavernosum to relax the arterial and sinusoidal smooth muscle. Phentolamine is an α-adrenergic antagonist while papaverine and alprostadil work by increasing cGMP and cAMP concentrations [14]. Alprostadil is approved by the FDA for the treatment of organic ED [19]; however, the technique requires careful dose titration and training of the appropriate self-injection technique. Side effects and complications can include prolonged erections, priapism, penile fibrosis, or haematoma [19]. Transurethral alprostadil pellets requires the placement of a 2 mm pellet of alprostadil directly into the urethra. Urethral absorption allows distribution to the cavernosal tissue, promoting vasodilation and improved erectile function [20]. Although success rates have been found to be approximately 65%, patient satisfaction rates are low at

15-30%. This has been improved with the use of a constriction band to hold the drug and blood in the penis, however in 40% of patients there have been the negative side effects. These include: lowered pain thresholds; pain in the penis, urethra, and testicles; hypotension and vaginal irritation in the partner [18].

2.6 Surgical intervention

The last resort, after first and second line therapies have failed, is surgery. The implantation of a penile prosthesis has been found to be successful and there have been vast improvements in device design since the first inflatable device, released in 1972 [21]. Devices are not visible as they are inside the body. Surgery is generally outpatient and takes 1.5 hours or less, or a brief stay in hospital may be required. Satisfaction rates are reported as 90-98% [22]. Mechanical failure rates over a 10-year period are low at 3-5%. Of the two main types currently available, the most natural but more costly and with increased risk of mechanical failure is the inflatable device. The other is a positional, non-inflatable prosthesis that is less natural but easy to use and reliable. Adverse effects may include infection, autoinflation, and loss of erectile length [18]. Finally, revascularisation surgery is an option for some men with mild-to-moderate venous leakage or where there is arterial trauma due to injury [21], although this is not recommended [22].

2.7 Other therapies

Although many other medications and herbal remedies are commonly used as alternative treatments for ED, there is little evidence to support their use or efficacy. Furthermore, many are contraindicated for common comorbidities and/or have adverse side effects. A recent review by Pavan et al [23] outlined the evidence supporting the following traditional medicines in the treatment of ED: yohimbe (an extract of the bark of the *Corynanthe yohimbe* tree upon which the prescription drug yohimbine hydrochloride is based); Catuama® (an extract from the *Paullinia cupana*, *Trichilia catigua*, *Zingiber officinalis* and *Ptycopetalum olacoides* plants); Berberine (an alkaloid from the *Berberis aristata* and *Berberis vulgaris* plants); Cordyceps (a fungus); Maca (the root of *Lepidium meyenii*); forskolin (a component of the plant *Coleus forskohlii*); Ginseng (Asian or Panax ginseng); and horny goat weed (the Epimedium plants). The evidence came mainly from *in vitro* and rodent studies and suggested potential benefits however further research is needed in higher animal models and human intervention studies. A number of polyphenols (e.g., resveratrol, quercetin and kraussianones) may be beneficial as ED treatments [23] and this may explain the protective effects of increased consumption of fruit and vegetables found in epidemiological studies and the reported beneficial effects of traditional herbal treatments. Some other treatments reportedly used are: L-arginine (an

amino acid); propionyl-L-carnitine (an amino acid); L-citrulline (an amino acid); *Butea superba* (plant root); melanotan-II (a manufactured chemical similar to melanocyte-stimulating hormone); niacin; zinc; pycnogenol®(an extract of *Pinus pinaster atlantica*); saffron (the flower of *Crocus sativus*); Ginkgo (the herb *Ginkgo biloba*); Muira puama (the plant *Ptychopetalum olacoides*); Pomegranate (the juice of the pomegranate fruit); and deer velvet (the dried soft velvet of immature antlers). This area is ripe for further investigation: indeed, as these products are being consumed for the treatment of ED, it is imperative that their safety and efficacy profiles are established scientifically.

3.1 OTHER MEDIAL RISK FACTORS FOR ERECTILE DYSFUNCTION

3.2 Lower urinary tract symptoms (LUTS) and prostate problems

The presence of LUTS - often caused by benign prostatic hypertrophy (BPH) - is common in ageing men [24]. LUTS include both storage (urgency, increased frequency, incontinence and nocturia) and voiding (weak stream, hesitancy, terminal dribble) issues [25]. Its relationship with ED is well established in both cross-sectional and prospective cohort studies [24, 26-32]. There is a high prevalence of LUTS among men with ED: El-Sakka et al [27] found 77% prevalence of LUTS in ED patients. There is also a higher prevalence of ED among men with LUTS and the severity of LUTS is positively correlated with the severity of ED [24]. In the population-based cross-sectional MSAM-7 study, Rosen et al [24] investigated the relationship between LUTS (IPSS score) and ED (IIEF score) in the USA and 6 European countries (n=12815; age range; 50-80 years). They found 90% prevalence of LUTS and ED was reported in 43%, 66%, and 82% of men with mild, moderate and severe LUTS respectively ($P < 0.001$). ED was consistently strongly associated with the severity of LUTS symptoms ($p<0.001$). Men had a 2.0, 3.8 and 7.7-fold higher risk of ED with mild, moderate and severe LUTS respectively adjusting for age and comorbidities in multivariate analysis. Barqawi et al [26] also investigated the relationship between LUTS and ED in men (n=6641) participating in a national multicentre prostate cancer-screening program in 2003 using the IIEF-5. Results showed that after adjusting for all confounding factors, LUTS had a significant negative effect on IIEF-5 score ($p<0.05$). Irwin et al [28] conducted a population-based nested case-control study investigating the prevalence of ED (502 cases; 502 controls) in men with over-active bladder (OAB) from age and country-matched subjects from the EPIC study. Men with OAB were more likely to have ED (OR 1.5 [1.1-2.2]), reduced sexual activity (14% vs 4%, $p<0.05$), decreased enjoyment of sexual activity (15% vs 2%, $p<0.05$) and lower levels of satisfaction with their sex lives (81% vs 90%, $p<0.05$) than controls. Most recently, results from the longitudinal cohort FAMAS [33] showed that voiding LUTS was a significant predictor of incident ED and absence of voiding LUTS was a

predictor of ED remission. Voiding LUTS manifested 5.6 years earlier than ED suggesting a temporal relationship and the importance of LUTS as an early sign of ED. LUTS and BPH are important risk factors for ED.

3.2 Urogenital anatomical disorders

Anatomical disorders affecting the structure and function of the penis can cause ED. Such disorders include: hypospadias, Peyronie's disease and chordee. Hypospadias is characterised by abnormal urethral development; the urethra opens under the penis in the urethral groove instead of the tip of the penis. It is usually a congenital condition but can also occur as a result of injury or surgery in very rare cases. It causes issues with both urination and maintaining an erection [34]. In contrast, Peyronie's disease usually affects older men and is characterised by a benign lump in the erectile tissues that leads to scarring, reduced flexibility and bending of the penis [35] and generally occurs as a result of injury or as a side effect of certain medications (e.g., calcium channel blockers). It can cause severe physical pain, the inability to achieve an erection and the avoidance of sexual intimacy. Chordee is characterised by abnormal penile curvature and the inability to straighten in either the erect or flaccid state. It can be either a congenital condition (associated with hypospadias or not, in which case it results from the development of a short fibrous urethra) or acquired (mainly caused by Peyronie's disease) [36]. It prevents normal erection and successful penetration during intercourse. A simple physical assessment can diagnose these anatomical disorders and all three can generally be corrected with surgery [36]. Anatomical disorders are an important cause of ED and self-reporting and or physical examination should be included in a clinical or research setting.

3.3 Vascular, pelvic or spinal trauma/surgery

Any lifestyle factor, medical condition, trauma or medical intervention that affects the neurological system can result in ED, as sexual desire cannot be transmitted effectively to the penile tissue. This includes peripheral autonomic (cavernous nerve) or somatic (dorsal and pudendal nerve) neuropathy commonly associated with diabetes, alcoholism, vitamin deficiencies, para-infectious diseases, spinal cord injury [37], temporal lobe epilepsy, Parkinson's disease, stroke and multiple sclerosis. Pelvic or spinal surgery comes with a risk of central or peripheral nerve damage. For example, neurogenic ED is highly common after radical prostatectomy. This surgery carries a high risk of damage to pelvic nerves affecting sexual, bladder and bowel function; however, advances in surgical technique over the past decade have resulted in a lower prevalence of post-prostatectomy ED [38]. Vascular, pelvic or spinal trauma or surgery can be an important cause of ED in some men as it can adversely, and

often irreversibly, affect the neurological system. PDE-5 inhibitors are the first line treatment for ED in most men irrespective of the etiology, including after the treatment of prostate cancer (PCa); however, the effectiveness of medications in these circumstances is questionable. Recent research by Pisansky et al [39] reported the results of a 1-year multi-centre placebo-controlled double-blind parallel-design intervention trial in the USA and Canada (n=242). Tadalafil (5 mg daily) was not effective in preserving erectile function (measured using the IIEF), sexual function or marital satisfaction compared to placebo in men who had normal erectile function prior to radiotherapy or brachytherapy for PCa. This can be expected as PDE-5 inhibitors act by inhibiting the breakdown of GMP and neither alter sexual desire nor aid in its transmission to the penis in order to induce an erection. Further research is needed to find alternative treatment options for men with traumatic/surgically induced ED.

3.4 Prescription, non-prescription and recreational drug use

Many prescribed medications are important sources of the manifestation of ED [40] including; antihypertensive medications (beta-blockers, thiazide diuretics, calcium channel blockers), anti-arrhythmics (digoxin, amiodarone, disopyramide), statins (atorvastatin), anti-androgens (gonadotropin-releasing hormone agonists [leuprolide, goserelin, lupron, zoladex], chemotherapy agents [cyclophosphamide, busulfan], flutamide, ketoconazole, spironolactone, cimetidine, H_2 blockers, finasteride, cyproterone), psychotropic drugs (selective serotonin inhibitors, tricyclic antidepressants, lithium, anxiolytics, monoamine oxidase inhibitors, phenothiazines, butyropenones) [41], antiepileptics, opiates, gout medication (allopurinol), glycosides, cholesterol synthesis inhibitors, clofibrate and other fibric acid derivatives and gastrointestinal drugs (cimetidine, omeprazole) [42]. However, much of the evidence supporting this is either from case studies or anecdotal evidence [43]. It has been reported that up to 25% of ED cases may be the result of side effects from medication [44]. Although the mechanism by which these drugs cause ED remains unclear, they appear to interfere with either central neuroendocrine or local neurovascular control of the erectile process. For example, treatments for hypertension are designed to lower blood pressure, making it more difficult to achieve and maintain an erection [12]. These medications are prescribed in the treatment of diseases that contribute to the pathology of ED, yet the treatment itself contributes further to the ED. This highlights the complexity of the disorder. Additionally, the use of recreational drugs such as excessive alcohol consumption [43], nicotine [45] marijuana, opiates and cocaine [41, 46] has also been linked to ED [12, 47], highlighting the need to routinely question patients regarding their past and present use of both prescription and non-prescription drugs.

4.1 DIETARY ASSESSMENT METHODS

The 24-hour recall (24-h recall) is the most cost-effective method of dietary assessment as it imposes the least subject burden. It is used to collect quantitative information on all the foods, beverages and supplements consumed in the previous 24-h or the preceding day (from midnight to midnight). Generally, a trained interviewer is used to conduct a structured interview following a multiple-pass approach with specific probes designed to aid in respondent recall. In contrast, the food record (FR) is completed directly by the respondent, who records all the foods, beverages and supplements consumed over a specified number of days (usually 1-7 consecutive days, including a weekend period), ideally at the time of consumption. Respondents are provided with training to ensure the appropriate level of detail (including the food/brand/product name, preparation, recipes for mixed dishes, and portion sizes). Quantities can be weighed using scales, measured using household measures or estimated with or without the use of portion aids. Despite well-known limitations and imperfections, FRs are considered the gold standard method for dietary assessment. A food frequency questionnaire (FFQ) is generally completed by the respondent who reports their usual frequency of consumption of a range of listed foods over a specified period (usually the past week, month or year). They are generally designed to gather frequency and portion size information but not detailed information on the foods consumed. The frequency and portion size are used alongside a food composition database to calculate a crude estimate of the intake of food groups and nutrients over the specified period. The food list in the FFQ must be appropriate for the specific population in which it is used and cover the breadth of their diet; therefore, many FFQ have been designed and adapted for specific populations and specific research purposes. Although FFQ generally contain over 100 food items, when interested in only one nutrient (especially one that is present in very few foods, such as vitamin D) far fewer foods need to be assessed.

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APPENDIX 3

CHAPTER 3 - SURVEY DOCUMENTS

MASSEY UNIVERSITY
INSTITUTE OF FOOD, NUTRITION AND HUMAN HEALTH
PALMERSTON NORTH, NEW ZEALAND

The Well-LaD Study (Phase 1)

Invitation to take part in a research study investigating sexual function and associated lifestyle patterns in New Zealand men aged 40-70 years:
The Well-LaD Study (Phase I)

Dear

My name is Merrin Quilter. I am a PhD student at Massey University investigating sexual function and associated lifestyle patterns in New Zealand men with the aim of finding an effective alternative natural treatment for erectile dysfunction. We don't know how common erectile dysfunction actually is in New Zealand. This information is urgently needed to support research into nutritional and lifestyle interventions to prevent or improve the symptoms of erectile dysfunction. In order to do this research, I need your help!

Introduction
You are invited to take part in a Massey University research project investigating sexual function and associated lifestyle patterns in New Zealand men aged 40-70 years. You are one of only 2000 men randomly selected from the New Zealand Electoral Roll Database to take part in this health research. You fit our study criteria as you are a male, aged between 40 and 70 years and you live in New Zealand. Before you choose to take part please take your time to read this document and discuss it with others if you wish. If anything is unclear or you would like more information please contact us.

What does taking part involve?

- Completing the enclosed **10 minute anonymous survey** on your background, sexual activity and function, lifestyle and medical history
- Completing the return card
- Placing the completed survey and completed return card in the reply stamped envelope and posting it back to us.

What are the benefits for you?
You will be making a valuable contribution to our understanding of male sexual function in New Zealand. As a sign of our appreciation, you will **go in the draw to win a $250 Mitre 10 voucher.** The return card is your entry into the prize draw. It also allows us to keep the survey anonymous while letting us know that you have completed and returned the survey.

What are the risks for you?
There are no risks for you in taking part. This is a confidential and anonymous survey to ensure your comfort and maintain your privacy. No one will know who you are or your answers to the questions. On the return card, you will be asked if you want to enter your email address to receive a summary of the study results. If you choose to supply your email address it will be stored in a separate database. It will not be linked to your survey responses or used to identify you in any way. It will not be used for any purpose other than to send you a summary of the survey results. It will remain confidential, will not be given out to any third party and will be destroyed at the end of the study. Completion and return of the survey implies consent to participate.

Why is this research being done?
Erectile dysfunction is when a man cannot achieve and maintain an erection sufficient for satisfactory sexual performance. Based on overseas research, it is estimated that about 50% of men over the age of 40 are affected by erectile dysfunction worldwide. Therefore there is a high chance that you, a family member or a friend will suffer from this health issue at some stage in your life. The number of men suffering from erectile dysfunction has increased considerably over the past 10 years but we know very little about why or how to prevent it. Erectile dysfunction can have a major impact on the quality of life of both men and their partners, and has been found to be an early sign of cardiovascular disease in some men, offering the opportunity for early intervention. Its effective prevention or treatment may have a dramatic impact on the health of NZ men. Current medications designed to treat erectile dysfunction help to reduce the symptoms but do not address the cause of the problem. They are often costly and for some men may be inappropriate orineffective.

Your participation is important as we need men both with and without erectile dysfunction to take part. It is just as important for you to be involved if you don't have erectile dysfunction because this will help us to establish patterns in the wider population and allow us to make comparisons between those who do and do not have this health issue.

This research is for the purpose of a PhD and the Research Coordinator is a PhD candidate in Nutritional Science at Massey University.

Research Coordinator:	**Supervisor:**
Merrin Quilter Institute of Food Nutrition and Human Health Massey University, Palmerston North Tel: (06) 356 9099 ext. 81469 Helpline (toll-free): 0800 080 028 Email: well-ladstudy@massey.ac.nz	Assoc. Prof. Jane Coad Institute of Food Nutrition and Human Health Massey University, Palmerston North Tel: (06) 350 5962 Helpline (toll-free): 0800 080 028 Email: J.Coad@massey.ac.nz

Ethics approval
This project has been reviewed and approved by the Massey University Human Ethics Committee: Southern A, Application 10/75. If you have any concerns about the conduct of this research, please contact Dr Brian Finch, Chair, Massey University Human Ethics Committee: Southern A, telephone 06 350 5799 x 8717, email humanethicsoutha@massey.ac.nz

What do you do now?
If you want to take part, please complete the enclosed 10 minute anonymous survey, write your name and email address on the return card and tick the option "I have completed and returned my survey". Finally, post them back to us in the reply stamped envelope.

If you do not want to take part, please write your name on the return card, tick the option "I do not wish to take part in this research" and post it back to us in the reply stamped envelope. Please feel free to include a comment on why you do not wish to take part.

If we do not receive a completed survey and/or return card from you, we will send a reminder after 1 week, a reminder and replacement survey pack after 3 weeks and a final reminder and replacement survey pack after 7 weeks. This is designed to maximise the number of people who respond to the survey.

Please feel free to contact me if you have any questions about this study before you volunteer.

Thank you for your time and help with my research!

Merrin Quilter
Well-LaD Study Research Coordinator

MASSEY UNIVERSITY
INSTITUTE OF FOOD, NUTRITION AND HUMAN HEALTH
PALMERSTON NORTH, NEW ZEALAND

The Well-LaD Study (Phase 1)

An investigation into sexual function and associated lifestyle patterns in New Zealand men aged 40-70 years

The following questionnaire consists of four sections; background information, sexual activity and function, lifestyle and medical history. It should only take about 10 minutes of your time. Please read the questions carefully and tick the one answer that best applies to you, unless otherwise directed.

You must be a male, aged 40-70 years and currently living in New Zealand to complete this questionnaire. Completion and return of the questionnaire implies consent to participate.

Your name is not on the survey and your answers will be completely anonymous. We ask that you write your name on the return card to let us know that you have completed and returned the survey so that we do not send you a reminder. The return card is also your entry into the draw to win a $250 Mitre 10 voucher.

Please answer the questions to the best of your ability and be as honest and open as possible. You have the right to refuse to answer any question. Please complete this questionnaire only once. Your answers are an important part of this research and your participation is much appreciated.

Once you have completed the questionnaire, please fill in the return postcard by writing your name (required), your email address (optional) and ticking the option "I have completed and returned my survey". Place both the questionnaire and the postcard in the enclosed reply stamped envelope, seal the envelope and place it in your nearest post-box.

This project has been reviewed and approved by the **Massey University Human Ethics Committee: Southern A, Application 10/75**. If you have any concerns about the conduct of this research, please contact Dr Brian Finch, Chair, Massey University Human Ethics Committee: Southern A, telephone 06 350 5799 x 8717, email humanethicsoutha@massey.ac.nz.

BACKGROUND INFORMATION

1. **What age are you?**
   - O 40-49
   - O 50-59
   - O 60-69
   - O 70 years or older

2. **Which ethnic group do you belong to?** ***Mark the space or spaces which apply to you.***
   - O New Zealand Maori
   - O New Zealand European *or* Pakeha
   - O Other European such as English, Scottish, Irish, Dutch, Australian
     Please state:
   - O Samoan
   - O Cook Island Maori
   - O Tongan
   - O Niuean
   - O Chinese
   - O Indian
   - O Other such as Fijian, Korean
     Please state:

3. **What is your current employment status?**
   - O I am self-employed
   - O I am a full-time employee
   - O I am a part-time employee
   - O I am not employed but I am seeking work
   - O I am not employed and I am not seeking work – *go to 7*

4. **Under which of the following categories is your main occupation?**
   - O Managers
   - O Professionals
   - O Technicians and Trades Workers
   - O Community and Personal Service Workers
   - O Clerical and Administrative Workers
   - O Sales workers
   - O Machinery Operators and Drivers
   - O Labourers

5. **What is your main occupation?** ***For example, plumber, builder, farmer, teacher, nurse, scientist, computer technician.***
   Please state:

6. **How many hours, to the nearest hour, do you usually work each week in the above occupation?**
   Please state: _____ hrs

7. **What is your average household income per year before tax?**
   - O $0-19,999
   - O $20,000-39,999
   - O $40,000-59,999
   - O $60,000-79,999
   - O $80,000-99,999
   - O $100,000-119,999
   - O $120,000+

8. **What is your highest educational qualification?**
   - O No formal qualifications
   - O NZ School Certificate or overseas equivalent
   - O NZ Sixth Form Certificate or University Entrance before 1986 or overseas equivalent
   - O NZ Higher School Certificate or Higher Leaving Certificate or NZ University Bursary/Scholarship or overseas equivalent
   - O Post secondary school qualification (e.g. Trade Certificate)
   - O Undergraduate qualification (e.g. Certificate or Diploma)
   - O Graduate qualification (e.g. Bachelors or Honors Degree)
   - O Post-graduate qualification (e.g. PG Diploma, Masters Degree, PhD etc.)

9. What region of New Zealand do you live in?

O Northland
O Auckland
O Waikato
O Bay of Plenty
O Gisborne
O Hawkes Bay
O Taranaki
O Manawatu-Whanganui
O Wellington
O Nelson
O Tasman
O Marlborough
O NelsonWest Coast
O Canterbury
O Otago
O Southland

10. Do you live in a rural (country) or urban (city/town) environment?

O Urban
O Rural
O Semi-Rural

Thank you. You have finished this section. Next, you will be asked questions about your SEXUAL ACTIVITY AND FUNCTION. Remember your answers are anonymous, private and confidential and will not be associated with you as an individual. Please be as open and honest as possible.

<u>SEXUAL ACTIVITY AND FUNCTION</u>

11. What is your current relationship status?

O Single – *go to 13*
O Dating
O Living with a de-facto partner
O Married/civil union
O Separated
O Divorced
O Widowed

12. How do you feel about the future of your current relationship?

O I feel confident
O I feel hopeful
O I feel uncertain
O I doubt it will last
O I don't know

13. Have you had sexual intercourse in the past month?

O Yes
O No

14. I usually have sexual intercourse;

O Never
O Less than once a year
O Less than once a month
O Once a month
O A few times a month
O Once a week
O A few times a week
O Once a day
O A few times a day

15. I think about sex;

O Never
O Less than once a year
O Less than once a month
O Once a month
O A few times a month
O Once a week
O A few times a week
O Once a day
O A few times a day
O Every 5 minutes

16. If I were to spend the rest of my life with my sexual function the way it is today, I would feel;

O Dissatisfied
O Somewhat dissatisfied
O Neither satisfied nor dissatisfied
O Somewhat satisfied
O Extremely satisfied

17. Do you suffer from premature ejaculation (when orgasm comes too quickly and reduces sexual satisfaction)?

O Yes
O No

18. Do you suffer from delayed ejaculation (when orgasm is delayed or absent and reduces sexual satisfaction)?

O Yes
O No

19. Erectile dysfunction (sometimes called impotence) means being unable to get and keep an erection that is rigid enough for satisfactory sexual activity. In your opinion are you;

O **Not impotent** *Always* able to get and keep an erection good enough for sexual intercourse

O **Moderately impotent** *Sometimes* able to get and keep an erection good enough for sexual intercourse

O **Completely impotent** *Never* able to get and keep an erection good enough for sexual intercourse

O **Minimally impotent** *Usually* able to get and keep an erection good enough for sexual intercourse

20. Over the past six months:

	1	2	3	4	5
How do you rate your confidence that you can get and keep an erection?	Very low	Low	Moderate	High	Very high
	O	O	O	O	O
With sexual stimulation, how often have your erections been hard enough for penetration (entering your partner)?	Almost never/ never	A few times (much less than half the time)	Sometimes (about half the time)	Most of the time (much more than half the time)	Almost always/ always
	O	O	O	O	O
During sexual intercourse, how often were you able to maintain your erection after penetration?	Almost never/ never	A few times (much less than half the time)	Sometimes (about half the time)	Most of the time (much more than half the time)	Almost always/ always
	O	O	O	O	O
During sexual intercourse, how difficult has it been to maintain your erection until completion of intercourse?	Extremely difficult	Very difficult	Difficult	Slightly difficult	Not difficult
	O	O	O	O	O
When you attempted sexual intercourse, how often was it satisfactory to you?	Almost never/ never	A few times (much less than half the time)	Sometimes (about half the time)	Most of the time (much more than half the time)	Almost always/ always
	O	O	O	O	O

21. Have you ever been diagnosed with erectile dysfunction (the inability to get and keep an erection that is rigid enough for satisfactory sexual activity) by a medical practitioner? Note: Erectile dysfunction does not include premature or delayed ejaculation.

O Yes

O No

22. Are you currently using any of the following treatments for erectile dysfunction? (Please select as many as apply)

O Prescription oral medications such as Viagra, Cialis or Levitra

O Non-prescription oral medications

O Self-injection or penile insertion of a drug

O Psychological counselling

O Vacuum pump devices

O Surgical penile implants

O Testosterone replacement

O Natural or herbal remedies

O None of the above

23. Erectile dysfunction is the inability to achieve or maintain an erection sufficient for satisfactory sexual performance. Please read each item and place a tick in the box opposite the reply which comes closest to how you feel.

	Disagree	Somewhat disagree	Neither agree nor disagree	Somewhat agree	Agree
Erectile dysfunction is an inevitable part of ageing	O	O	O	O	O
Erectile dysfunction is something men just have to accept	O	O	O	O	O
I feel uncomfortable talking about erectile dysfunction	O	O	O	O	O
It would be helpful if men felt more comfortable talking about erectile dysfunction	O	O	O	O	O
If I thought a prescription drug could improve my erectile function, I would take it	O	O	O	O	O
If I thought my diet affected my erectile function, I would change my diet	O	O	O	O	O
If I thought a dietary supplement could improve my erectile function, I would take it	O	O	O	O	O
I am interested in learning more about how to prevent erectile dysfunction / improve my erectile function	O	O	O	O	O

Thank you. You have finished this section. Next, you will be asked questions about your LIFESTYLE. Remember your answers are anonymous and will not be associated with you as an individual. Please be as open and honest as possible.

LIFESTYLE

CAFFEINE

Caffeine is found in many drinks and foods; however the main sources in the New Zealand diet are coffee, tea, caffeinated soft drinks (e.g. Coca Cola, Pepsi, Lift, Mountain Dew etc.) and caffeinated energy drinks (e.g. Demon, Red Bull, V etc.).

24. Which of the following most applies to you?

- O I never drink coffee, tea, caffeinated soft drinks or caffeinated energy drinks – *go to 26*
- O I used to drink coffee, tea, caffeinated soft drinks or caffeinated energy drinks – *go to 26*
- O I occasionally drink coffee, tea, caffeinated soft drinks or caffeinated energy drinks
- O I regularly drink coffee, tea, caffeinated soft drinks or caffeinated energy drinks

25. Which of the following products do you drink and on average how many do you drink <u>each week</u>? (Put '0' if none)

- ☐ Coffee (250ml regular cups)
- ☐ Tea not including green or herbal tea (250ml regular cups)
- ☐ Herbal tea not including green tea (250ml regular cups)
- ☐ Green tea (250ml regular cups)
- ☐ Caffeinated soft drink (300ml standard glasses/cans/bottles)
- ☐ Caffeinated energy drink (300ml standard glasses/cans/bottles)
- ☐ Don't know

SMOKING

26. Which of the following applies to you?

- O I never smoke tobacco – *go to 28*
- O I used to smoke tobacco – *go to 28*
- O I occasionally smoke tobacco
- O I regularly smoke tobacco

27. Which of the following tobacco products do you smoke and on average how many do you smoke <u>each week</u>? (Put '0' if none)

- ☐ Manufactured cigarettes
- ☐ Hand-rolled cigarettes
- ☐ Pipes full of tobacco
- ☐ Cigars, cheroots, cigarillos
- ☐ Don't know

ALCOHOL

28. Which of the following most applies to you?

- O I never drink alcohol – *go to 30*
- O I used to drink alcohol – *go to 30*
- O I occasionally drink alcohol
- O I regularly drink alcohol

29. Which of the following do you drink and on average how many do you drink <u>each week</u>? (Put '0' if none)

- ☐ Beer (330ml standard glasses/cans/bottles)
- ☐ White wine (100ml standard glasses)
- ☐ Red wine (100ml standard glasses)
- ☐ Spirits (30ml standard shot measures)
- ☐ Ready to Drinks (RTDs) or Pre-Mixed Drinks (330ml standard bottles)
- ☐ Don't know

PHYSICAL ACTIVITY

30. We would like to know the type and amount of physical activity involved in your work. Please tick what best corresponds to your present activities from the following four possibilities:

O **Sedentary occupation** - You spend most of your time sitting (such as in an office)

O **Standing occupation** - You spend most of your time standing or walking. However, your work does not require intense physical effort (e.g. shop assistant, hairdresser, guard, etc.)

O **Physical work** - This involves some physical effort including handling of heavy objects and use of tools (e.g. plumber, cleaner, nurse, sports player, electrician, carpenter, etc.)

O **Heavy manual work** - This involves very vigorous physical activity including handling of very heavy objects (e.g. miner, bricklayer, construction worker, etc.)

31. In a typical week during the past 12 months, how many hours did you spend on each of the following activities? (Put '0' if none)

Walking, including walking to work, shopping and leisure

☐ hours per week in summer

☐ hours per week in winter

Cycling, including cycling to work and during leisure time

☐ hours per week in summer

☐ hours per week in winter

Gardening

☐ hours per week in summer

☐ hours per week in winter

Housework such as cleaning, washing, cooking, childcare

☐ hours per week in summer

☐ hours per week in winter

Do-it-yourself (DIY)

☐ hours per week in summer

☐ hours per week in winter

Other physical exercise such as fitness, aerobics, swimming, jogging, tennis, etc.

☐ hours per week in summer

☐ hours per week in winter

32. In a typical week during the past year did you practise any of these activities vigorously enough to cause sweating or a faster heartbeat?

O Yes

O No – *go to 33*

O Don't know

If yes, for how many hours per week in total did you practice such vigorous physical activity? (Put '0' if none)

☐ hours per week

33. In a typical week during the past year, how many flights of stairs did you climb per day?(Put '0' if none)(One flight of stairs consists on average of 20 full steps)

☐ flights of stairs per day

ANXIETY AND DEPRESSION

34. Over the *last two weeks*, how often have you been bothered by the following problems?

	Not at all	Several days	More than half the days	Nearly every day
Little interest or pleasure in doing things	O	O	O	O
Feeling down, depressed, or hopeless	O	O	O	O
Trouble falling/staying asleep, sleeping too much	O	O	O	O
Feeling tired or having little energy	O	O	O	O
Poor appetite or overeating	O	O	O	O
Feeling bad about yourself – or that you are a failure or have let yourself or your family down	O	O	O	O
Trouble concentrating on things, such as reading the newspaper or watching television	O	O	O	O
Moving or speaking so slowly that other people could have noticed. Or the opposite - being so fidgety or restless that you have been moving around a lot more than usual	O	O	O	O
Thought that you would be better off dead or of hurting yourself in some way	O	O	O	O

35. If you checked off any problem in Question 34, how difficult have these problems made it for you to do your work, take care of things at home, or get along with other people?

O Not difficult at all
O Somewhat difficult
O Very difficult
O Extremely difficult

36. Do you believe that you are suffering from anxiety or depression as a result of lack of sexual activity or the inability to perform sexually?

O Yes
O No

SUN EXPOSURE

37. The following questions are about your behaviour towards sun exposure. Please read each of the following statements and respond by selecting the one answer that suits you best.

	Almost never/ never	A few times (much less than half the time)	Sometimes (about half the time)	Most of the time (much more than half the time)	Almost always/ always
When I am outside in summer, I wear sunscreen	O	O	O	O	O
When I am outside in winter, I wear sunscreen	O	O	O	O	O
When I am outside, I wear sunglasses or protective lenses	O	O	O	O	O
When I am outside, I wear a sunhat	O	O	O	O	O
When I am outside, I wear clothing to protect me from the sun	O	O	O	O	O
When I wear sunscreen, I reapply it as recommended on the bottle	O	O	O	O	O
In my occupation (paid or unpaid), I work outside	O	O	O	O	O
When I exercise or play sports, I am outside	O	O	O	O	O
When I do my hobbies, I am outside	O	O	O	O	O

	Not at all	A little	Somewhat	Quite a lot	A great deal
I enjoy being outside in the sun	O	O	O	O	O
I sunbathe	O	O	O	O	O
I use sun beds	O	O	O	O	O
I get sun burnt	O	O	O	O	O
I limit my time in the sun	O	O	O	O	O
I avoid being outside in the sun	O	O	O	O	O

Thank you. You have finished this section. Next, you will be asked questions about your MEDICAL HISTORY. Remember your answers are anonymous and will not be associated with you as an individual. Please be as open and honest as possible.

MEDICAL HISTORY

38. Have you ever been diagnosed with, or do you currently suffer from, any of the following?

	Yes	No
High blood pressure	O	O
High cholesterol	O	O
Atherosclerosis	O	O
Heart disease	O	O
Angina	O	O
Heart attack	O	O
Heart failure	O	O
Stroke	O	O
Type II Diabetes	O	O
Malignant disease (cancer)	O	O
Skin cancer	O	O
Osteoporosis	O	O
Restless Leg Syndrome	O	O
Depression, post-traumatic stress disorder or a psychiatric condition	O	O
Auto-immune disorders such as Type I Diabetes, Lupus, Multiple sclerosis (MS), Myalgic Encephalomyelitis (ME), Rheumatoid arthritis or Psoriasis	O	O
Prostate cancer, Benign prostatic hyperplasia (BPH), Prostatitis, or Peyronie's disease	O	O
Multi-system atrophy (MSA), spinal cord injury or tumors, prolapsed intervertebral discs or tumors, disease to the parasympathetic nerves of the pelvis, pelvic or abdominal surgery	O	O
Chronic renal failure	O	O
High levels of prolactin in the blood (Hyperprolactinaemia)	O	O
Low levels of testosterone in the blood (Hypogonadism)	O	O
Smooth muscle dysfunction	O	O
Recent surgery (within the past year)	O	O
Substance abuse i.e. alcohol, marijuana, opium, heroin	O	O

39. Are you involved in competitive cycling?

O Yes
O No

40. Are you taking <u>any</u> form of medication, including traditional or alternative medicine, and medicine obtained on the internet? (i.e. Ibuprofen, hormone therapy, Warfarin, snake oil etc.)

O Yes
O No – *go to 41*

If so, please list the medications and what they are treating.

Please provide details:

41. **Are you taking <u>any</u> dietary supplements, vitamins, minerals, oils etc.? (i.e. whey protein powder, multivitamin, multimineral, fish oil etc.)**

O Yes

O No – *go to 42*

If so, please list the supplements and what they are treating.

Please provide details:

42. **Would you be interested in taking part in a trial aiming to improve sexual function through nutritional or lifestyle intervention?**

O Yes

O No

You have finished the questionnaire.

Thank you for your participation!

If you have any concerns regarding your sexual function or any aspect of your personal health, please contact your GP or local sexual health clinic.

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The Well-LaD Study

RETURN CARD

402

Please make sure you complete this card, place it with the completed survey in the reply stamped envelope, seal the envelope and place it in your nearest post-office box

First Name*:

Last name*:

* We need your full name to know if you have completed and returned the survey. If you do not complete this card, our system will automatically send out 3 subsequent reminders. If you choose to take part, this is also your entry into the draw to win a $250 Mitre 10 voucher.

Please tick one of the following:

O I have completed and returned my survey

OR

O I do not wish to take part in this research

Email address:**

** This information is optional. If you choose to enter your email address, we will send you a summary of the results from this survey. If you choose not to, you will receive no further contact from us.

THANK YOU FOR YOUR TIME & HELP!

The Well-LaD Research Team

MASSEY UNIVERSITY

INSTITUTE OF FOOD, NUTRITION AND HUMAN HEALTH
PALMERSTON NORTH, NEW ZEALAND

The Well-LaD Study

A REMINDER TO PLEASE COMPLETE OUR SURVEY

404

Invitation to take part in the Well-LaD Study (Phase I)

New Zealand
Permit No. 5

Permit

A week ago we sent you an invitation to take part in an important postal survey investigating the sexual function and associated lifestyle patterns in New Zealand men aged 40-70 years. We are contacting you as we have not yet received a response from you.

If you have not yet completed the survey, we hope that you will do so, seeing the value of this research and the great contribution you can make to our understanding of normal sexual function in New Zealand men. If you need us to send you another copy of the survey or another reply stamped envelope, please call us on 0800 080 028.

If you are receiving this in error as you have already completed and returned the survey, or if you do not wish to take part in this survey, please call us on 0800 080 028 to let us know. We can then remove your name from the follow-up list.

THANK YOU FOR YOUR TIME & HELP!
The Well-LaD Research Team

MASSEY UNIVERSITY

INSTITUTE OF FOOD, NUTRITION AND HUMAN HEALTH
PALMERSTON NORTH, NEW ZEALAND

The Well-LaD Study

A FINAL REMINDER TO PLEASE COMPLETE OUR SURVEY

406

Invitation to take part in the Well-LaD Study (Phase I)

Seven weeks ago we sent you an invitation to take part in an important postal survey investigating the sexual function and associated lifestyle patterns in NZ men aged 40-70 years. We are contacting you as we have not yet received a response from you.

If you have not yet completed the survey, we enclose another copy in the hope that you will do so, seeing the value of this research and the great contribution you can make to our understanding of normal sexual function in NZ men.

In this envelope you will find the answers to the most frequently asked questions. If you have any more questions, or you are receiving this in error as you believe you have already completed and returned the survey, **please call us on 0800 080 028.**

THANK YOU FOR YOUR TIME & HELP!
The Well-LaD Research Team

Please complete this card and return it (with the completed survey) in the envelope provided

First name: _____

Last name: _____

Please tick one of the following:

O I have completed the survey and wish to be entered into the draw to win the $250 Mitre 10 Voucher

O I do not wish to take part in this survey

If you would like to receive a summary of the results when the study is completed please provide your email address:

Email address: 407

Frequently Asked Questions (FAQs)

1. **How did we get your name?**
   We applied for access to the electoral roll data for men aged 40---70 in New Zealand. Under Section 112 of the Electoral Act 1993, the electoral roll data is available for people undertaking health research who are deemed eligible to receive the data by the Chief Registrar of Electors; for the purposes of conducting a Survey; on a topic that relates to a health matter.
2. **How were you selected from all of the men on the electoral roll?**
   We received an electronic file with all of the names on it. We transferred this into a computer programme used for statistical analysis and used its random generator function to randomly select 2000 names.

PTO

1. **Why do you keep contacting me?**
   We are using the Dillman Total Design Method which includes the use of a prize and repeat reminders to those who do not respond. This helps us maximise the number of men who respond. Combined with random sampling, it means we only have to send out a small number of surveys to get a good representation of the population. If you did not return the card, or returned it without your name, we were unable to remove your name from the list, so we sent you another reminder.
2. **How is this study anonymous?**
   You do not need to put your name on the survey. We ask that you put your name on the return card so that we can know if we can take your name off the follow---up list, and to put your name in the prize draw. When we receive a complete survey and a complete return card, we open the envelope, remove the card, check the name and remove it from the follow---up list, then remove the survey and put it in a box with all the other surveys. It is not opened. It is not linked with your name in any way.
   Although your participation in the survey is not anonymous, your survey responses are anonymous.

APPENDIX 4

CHAPTER 4 - ADDITIONAL INFORMATION ON VITAMIN D

1.0 VITAMIN D CONTENT IN FOOD SOURCES

The accurate quantification of dietary intake in New Zealand (NZ) is limited by the lack of reliable data on the vitamin D content in NZ foods. The NZ Food Composition Database (NZFCD) provides information on the vitamin D content in foods [1] and is widely used in NZ to quantify dietary intake, despite well-known issues with the accuracy of the data [2]. For example, the majority of figures have been taken or imputed from overseas data. The vitamin D content of foods can vary widely depending on laboratory practices and the method of analysis, but also due to environmental factors such as ultraviolet (UV) exposure and dietary intake (e.g., farmed salmon has been found to contain only 10-25% of the vitamin D content of wild salmon [3]) and fortification practices [1, 4]. Therefore these values cannot be taken as truly representative of NZ foods.

2.0 SUPPLEMENTATION WITH VITAMIN D

There are little data available on the appropriate dose of vitamin D and the most effective frequency of supplementation. Commercial supplements are generally ≤1000 IU/d and the clinical standard supplement is 50,000 IU/m, however larger doses up to 500,000 IU [5] are available for research purposes and daily, weekly, monthly, quarterly and annual dose regimes are possible. As serum 25(OH)D has a long half-life [6, 7], intermittent dosing is possible and may improve convenience and long-term compliance in research studies. Large single doses (50,000–240,000 IU) have been shown to be clinically useful in raising 25(OH)D to normal levels that remain within the safe zone (i.e. <220 nmol/L (88 ng/ml)) [6-9]. Pharmacokinetic studies have indicated that 25(OH)D levels peak at 7-21 days and thereafter slowly decrease with a half-life of 50-90 days [6, 7]. For example, Ilahi et al [7] (n=40 healthy men and women, aged 27-91 years) found a single large oral dose of 100,000 IU vitamin D_3 caused a prompt increase in mean 25(OH)D from 67.6 nmol/L (27.1 ng/ml) at baseline to 104.8 nmol/L (42.0 ng/ml) at 7 days with a maximum individual level reported of 160 nmol/L (64.2 ng/ml) and a half-life of approximately 50 days. There was no significant change in the control group over the 4 months – indicating no natural seasonal change in vitamin D status over this period [7]. Similarly, a single dose of 50,000 IU vitamin D_3 has been reported to cause a peak in 25(OH)D levels at 7 days with levels remaining elevated at 28 days and a maximum individual level reported of 173 nmol/L (69.2 ng/mL)) [8].

Very large intermittent single doses (500,000 IU) are not considered beneficial [10] and may be harmful. Sanders et al [5] (n=2256 women, aged 70 years and older) found that although annual administration of a single dose of 500,000 IU D_3 increased the median 25(OH)D status to normal levels and this remained raised for 12 months, it was associated with a significant

increased risk of falls and fractures over 5 years. A smaller more frequent dose regime may be more effective. Indeed, in direct contrast to the findings of Sanders et al [5], earlier results from Trivedi et al [11] found that supplementation with 100,000 IU D_3 every four months for five years (n= 2686 men and women, aged 65-85 years) raised the mean 25(OH)D level to 74.3 nmol/L compared to the placebo group (53.4 nmol/L, $p<0.001$) and safely and effectively reduced the risk of falls and fractures by 20% (RR=0.78 [0.61-0.99]). Very few studies have been published with large annual doses and there are no new clinical trials following this dose regime. However, there is one randomised controlled trial (RCT) [12] (n=112 postmenopausal women, 84 with vitamin D deficiency, 28 without) currently underway in the UK designed to study changes in total 25(OH)D and free 25(OH)D (unbound to DBP or albumin) over 3 months in response to a single bolus of one of three different doses of vitamin D_3 (50,000 IU, 150,000 IU and 500,000 IU). The results of this study should provide valuable evidence to help improve our understanding of the optimal dose required.

As humans evolved with regular sunshine exposure, more frequent supplementation regimes (monthly, weekly and daily) are suggested to result in a more physiological and balanced 25(OH)D profile. Daily doses may simplify compliance by coinciding with the taking of other medications and/or supplements, however they appear equally beneficial [13] and again there is no clear consensus on the most effective dose regime. Ish-Shalom et al [13] reported that there was no significant difference in the effect of the same cumulative dose of vitamin D_3 supplied daily (1,500 IU/day), weekly (10,500 IU/week) or monthly (45,000 IU/28 days) on 25(OH)D levels in 48 elderly women (mean age = 81 years) who had undergone hip surgery. The 25(OH)D levels were not significantly different at baseline (37.8±17.3 nmol/L (15.13±6.9 ng/ml), 39.3±25.3 nmol/L (15.7±10.1 ng/ml), and 40.5±25.3 nmol/L (16.2±10.1 ng/ml) respectively ($p>0.05$)) or after 2 months (83.0±21.3 nmol/L (33.2±8.5 ng.ml), 73.0±3.6 nmol/L (29.2±8.9 ng/ml), and 92.8±25.8 (37.1±10.3 ng/ml) respectively ($p>0.05$)). However, a recent RCT [14] (n=100, aged 50 years and older) suggested that although there was no difference in the efficacy of a monthly (25, 000 IU drinkable ampule) versus daily (800 IU chewable tablet) of vitamin D_3 over six months, monthly was preferred by the majority of participants (56.8% vs 18.2%) due to both frequency and ease of use, and resulted in better compliance (100% vs 96.2%). Any dose regime should be based on maximising efficacy and compliance and minimising the risk of adverse effects associated with hypercalcaemia (e.g., reduced appetite, nausea and vomiting, fatigue, confusion, muscle weakness, bone pain, frequent urination and kidney problems). Response to vitamin D supplementation is negatively affected by body fat [15]; therefore, obese people require higher doses of vitamin D to achieve the same increase

in 25(OH)D as a non-obese person. Furthermore, as the increase in total 25(OH)D in response to supplementation is likely to be lower in those already replete prior to supplementation due to saturation of the DBP binding capacity, a variable dose regime would be needed to achieve and maintain optimal vitamin D status in individual participants.

3.0 ASSESSMENT OF DIETARY INTAKE OF VITAMIN D

Although the limited number of rich food sources of vitamin D should simplify the assessment of dietary intake, it is hindered by the high degree of variability in food fortification and rapid changes in fortified food products and supplements available on the market (both domestic and imported). The accuracy of estimating dietary intake of vitamin D is further hindered by the well-established weaknesses of the available dietary assessment methods (24 hour dietary recalls (24-h recalls), 1-7 day food records (FR), food frequency questionnaires (FFQ) and diet histories (DH)) [16] and food composition data on the vitamin D content in foods.

The most frequently used method for the estimation of population-based vitamin D intake is the self-administered FFQ, as it can easily be specifically designed to assess known sources of vitamin D in a given population. However, any dietary assessment method should be validated by comparing it with another deemed superior (i.e. 7-day FRs or multiple 24-h recalls) or a biomarker (i.e. serum 25(OH)D) [17]. Biomarkers are excellent for validation of dietary intake, depending on the nutrient of interest. In general, for estimated vitamin D intake to attain a validation correlation coefficient above 0.5 a FFQ must have ≥100 foods listed [17]; however, in countries with limited fortification, such as NZ, a shorter list may be possible.

An FFQ must be specifically designed to reflect natural and fortified food sources in a given country, which can differ markedly and change rapidly; therefore an FFQ should only be used in its country of origin. They also require an accurate and up-to-date food composition database for the reliable calculation of vitamin D intake. The fast pace of food technology and the vast array of new products on the market makes keeping up to date with these products difficult without a mandatory database listing vitamin D fortified foods. Furthermore, the period covered by the FFQ (generally 1 week to 1 year) is important, especially if it is to be validated to 25(OH)D level which has a long half-life of 50-90 days [6, 7]. Based on this, a minimum period of 3 months appears to be appropriate. It should also include assessment of supplementation as failure to account for this has been suggested to lead to a 65% underestimation of vitamin D intake in some populations [18].

The accuracy of available methods to assess vitamin D intake is highly questionable. There are many published validation studies which differ widely based on the assessment method, the

number and type of foods listed, the reference period for intake, whether they include supplementation and also the reference method used for validation [19-22]. The correlation coefficients between estimated intakes from FFQs and the reference method differ widely: from 0.41-0.53 with FRs to 0.48-0.78 with multiple 24-h recalls [17]. Very few studies have validated intake to plasma 25(OH)D and those that have reported even lower correlation coefficients, ranging from 0.26-0.38 [17]. Jacques et al [23] found a correlation coefficient of 1.35 between a 1 year 116-item FFQ and plasma 25(OH)D in New England, USA. However, even correlations with intakes measured using the gold standard FR are low. Booth et al [24] found a correlation coefficient of 0.32 between four repeated 3-day weighed DRs and plasma 25(OH)D level in the same state. This is probably due to the long half-life of 25(OH)D and variable contribution of diet depending on sunlight exposure. However, even at greater latitudes, with minimal winter UV exposure and greater reliance on dietary intake, the validity of vitamin D FFQs can be low. In Finland, Erkkila et al [25] found a very weak correlation coefficient of 0.19 between vitamin D intakes from an 89-item FFQ and a 3-day FR. In contrast, in Canada with its mandatory fortification policy, Wu et al [26] found that estimated intake from a 37-item FFQ was significantly correlated with both 7-day FR (r=0.529) and 25(OH)D levels (r=0.481). Most recently, Wier et al [27] found a strong correlation between a FFQ covering 13 food groups and both 4-day FR (r=0.562) and serum 25(OH)D levels (r=0.567) validated in winter in England. Furthermore, Kiely et al [28] found a strong association between a FFQ covering 12 food groups and a 14-day DH (r=0.71) and a weaker but significant association with 25(OH)D levels (r=0.31) in winter in Ireland.

It is clear that vitamin D intake assessment tools need to be specifically designed to include the available natural and fortified foods in each country, assess supplementation, and be validated to both another dietary assessment method and serum 25(OH)D. However, the tool also needs to be able to rapidly adapt to new sources as they become available.

4.0 DIETARY INTAKES OF VITAMIN D

Dietary intakes of vitamin D vary between countries, but also within countries by region, ethnicity, gender and age group. Factors affecting dietary intake include availability and consumption of natural foods containing significant amounts of vitamin D, fortified foods and supplements.

Certain dietary patterns are associated with greater vitamin D intakes. Fish consumption is positively associated with 25(OH)D level [29, 30] and in countries with high intakes of fatty fish, dietary vitamin D intake may contribute significantly to vitamin D status (e.g., fish consumption contributes 6.4 μg/d to the dietary vitamin D intake in Norway [31]). Foods rich in vitamin D are generally animal-based foods that may not be a common part of the diet in certain countries and cultures (e.g., those following a vegan diet are reliant on the availability of fortified foods and supplementation, particularly in the absence of adequate sun exposure). Furthermore, they are more expensive and therefore less accessible to lower socioeconomic groups who thus have access to fewer and poorer sources of vitamin D. In countries with fortification of staple foods, consumption of those food groups is significantly correlated with vitamin D status [32-34].

The average estimated dietary intake in Australians ranges from 1.2-2.6 μg/d [35, 36] with a mean of 2.6-3.0 μg/d for adult men and 2.0-2.2 μg/d for adult women [34]. As expected, intakes tend to be lower in countries with limited fortification of food products (i.e. Australia, NZ and Europe) and up to 60% (2-3 μg) higher in countries with mandatory fortification of a wide range of foods (i.e. USA and Canada) [37, 38]. Older people tend to have a lower dietary intake of vitamin D. For example, postmenopausal Spanish women with osteoporosis were found to have a mean vitamin D intake of 4.2 μg/day (167 IU/day) but only 3 μg/d (120 IU/day) in those >75 years [39]. However, in Toronto, Canada, Ginter et al [40] found older adults (n=224, >60 years) had a mean dietary intake of 4.2 μg/day (168 IU/day) and a mean supplemental intake of 22.9 μg/day (917 IU/d) (total mean intake 27.2 μg/day (1086 IU/day) and a mean 25(OH)D level of 82.4 nmol/L with 12.1% <50 nmol/L and 38.8% <75 nmol/L. There was significant correlation between serum 25(OH)D concentrations and supplement use ($p<0.001$). In the same region, Gozdzik et al [41] found that younger adults (n=342) had a mean dietary intake of 4.4 μg/day (176 IU/day), a mean supplemental intake of only 2.9 μg/day (114.9 IU/d) (total mean in take 7.3 μg/day (290.7 IU/day)) and a mean 25(OH)D level of 39.5 nmol/L. The primary factor was differences in supplement use, which was higher in older adults (77%) than younger adults (24%). This highlights the importance of assessing supplementation, particularly amongst older adults.

Obtaining the current estimated dietary requirement for adults is unlikely through consumption of natural sources alone. For example, in order to obtain 400 IU/d (10 μg/d)), a NZ adult (based on Table 4.1) would need to consume: 50 g of cooked salmon, 200 g of canned sardines, or 1.75 L of fortified reduced fat milk every day. Fortification may be successful in maintaining vitamin D status in the general population; however it is still unlikely to be effective in those at greatest risk [35, 42]. In these groups, supplementation and/or increased sunlight exposure must be considered. Furthermore, the proposed optimal serum 25(OH)D concentration of ≥75 nmol/L may be impossible to achieve without supplementation, particularly in high-risk groups. Indeed, in countries with limited vitamin D effective UVR and mandatory vitamin D fortification of foods such as Canada, supplementation has been found to be a significant predictor of vitamin D status and a major contributor to the achievement of a serum 25(OH)D concentration ≥75 nmol/L amongst healthy older adults [43]. Supplementation is likely to be even more important in the elderly in NZ with limited safe opportunities for vitamin D effective UVR and restricted food fortification.

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APPENDIX 5

CHAPTER 5 - OBSERVATIONAL STUDY DOCUMENTS

MASSEY UNIVERSITY
INSTITUTE OF FOOD, NUTRITION AND HUMAN HEALTH
PALMERSTON NORTH, NEW ZEALAND

Invitation to take part in a research study investigating the relationship between wellness, lifestyle and diet in New Zealand men aged 40-70 years; the Well-LaD Study.

Dear

You are invited to take part in a Massey University research project investigating the relationship between wellness, lifestyle and diet in New Zealand men aged 40-70 years: the Well-LaD Study. You are one of only 600 men selected from the Manawatu Electoral Role invited to take part in this health research. We have selected you as you fit our study criteria: you are a male, aged between 40 and 70 years and you live in the Manawatu region. We chose to send out invitations to a random sample of men to ensure everyone has an equal opportunity to take part and can experience the benefits of this study.

Before you choose to take part it is important that you understand why you have been selected, why this research is being done and what it will involve. Please take your time to read the attached information sheet and discuss it with others if you wish. If anything is unclear or you would like more information please contact us.

Introduction
We would like to investigate wellness, lifestyle and dietary patterns in men who are healthy or have lifestyle associated diseases (for example, type II diabetes or disease of the heart or arteries). Please note that if you do not have type II diabetes or disease of the heart or arteries it is just as important for you to be involved to establish patterns in the wider population, allowing us to make comparisons between those who do and do not have these diseases.

What would taking part involve?

- contacting us to complete a confidential telephone screening interview to check you fit the needs of the study
- completing a 2½ -3 hour assessment at the Human Nutrition Unit in the Institute of Food, Nutrition and Human Health at Massey University, Palmerston North.

Why should you take part?
You will be making a valuable contribution to our understanding of male wellness, lifestyle and diet in New Zealand and as a sign of our appreciation you will **go in the draw to win a $250 Mitre 10 voucher.** However, the main benefit of taking part in this study is that you will receive a free personal report on your body composition, diet, fitness and overall health. We will be using equipment and methods that are expensive and cutting edge. You are unlikely to have the chance to experience this again, or get such a detailed report on your personal health. It can

provide valuable information to kick start a life change! There are no risks to you in taking part. Your participation is confidential and we will ensure your comfort and maintain your privacy.

Why is this research being done?

This research is for the purpose of a PhD and the Research Coordinator is a PhD candidate in Nutritional Science at Massey University.

Research Coordinator:	**Supervisor:**
Merrin Quilter Institute of Food Nutrition and Human Health Massey University, Palmerston North Tel: (06) 356 9099 ext. 81469 Email: m.l.quilter@massey.ac.nz	Assoc. Prof. Jane Coad Institute of Food Nutrition and Human Health Massey University, Palmerston North Tel: (06) 350 5962 Email: J.Coad@massey.ac.nz

For more information, please read the enclosed study information sheet**.** Please feel free to contact the Research Coordinator if you have any questions about this study before you volunteer.

If you wish to take part, please contact us **within 2 weeks of receiving this letter** to complete a confidential telephone screening questionnaire to check you fit the needs of the study. If you are eligible to take part, we will then set a convenient date and time for your appointment at the Human Nutrition Research Unit, Massey University.

Thank you for your time and help with our research!

Merrin Quilter
Well-LaD Study Research Coordinator
Email: well-ladstudy@massey.ac.nz
Tel: 0800 MASSEY - ask for The Well-LaD Study (8am-5pm Monday to Friday)

This study has been approved by the **Lower South Regional Ethics Committee** (Reference LRS/10/07/032).

MASSEY UNIVERSITY
INSTITUTE OF FOOD, NUTRITION AND HUMAN HEALTH
PALMERSTON NORTH, NEW ZEALAND

An investigation into the relationship between wellness, lifestyle and diet in New Zealand men aged 40-70 years: the Well-LaD Study (Phase I)

Introduction

You are invited to take part in a Massey University research project investigating the relationship between wellness, lifestyle and diet in New Zealand men aged 40-70 years. Before you choose to take part it is important that you understand why this research is being done and what it will involve. Please take your time to read this document and discuss it with others if you wish. If anything is unclear or you would like more information please contact us. You do not have to take part in this study and if you choose not to participate there will be no negative outcome for you. This document is for your information and you should keep it for your reference.

We are looking for 300 men to participate in this study. To fit in to our study you should be:

- male
- 40-70 years old
- living in the Manawatu region
- either healthy or with lifestyle associated diseases (for example, type II diabetes or disease of the heart or arteries). *Please note that if you do not have type II diabetes or disease of the heart or arteries it is just as important for you to be involved to establish patterns in the wider population, allowing us to make comparisons between those who do and do not have these diseases.*

Taking part in the study involves:

- contacting the research team to complete a confidential telephone screening interview to check you fit the needs of the study
- completing an approximately 2½ -3 hour assessment at the Human Nutrition Unit in the Institute of Food, Nutrition and Human Health at Massey University, Palmerston North
- additionally, you may be invited to complete a 4 day weighed food record when you go home and you may be contacted at a later date to invite you to complete a follow up interview (face-to-face or telephone) on your attitude towards health.

About the study

The Well-LaD Study is intended to find out about wellness, lifestyle and dietary patterns in NZ men. If you fit the above criteria and you would like to take part in our study, please contact the research team to complete the confidential telephone screening interview. We will ask you about your general health and current medications to determine whether you can take part in the study. If you fit the needs of the study, we will invite you to make an appointment to visit the Human Nutrition Unit at Massey University, Palmerston North. You will need to have had no food or drinks other than water for 12 hours before your morning appointment.

This research is for the purpose of a PhD and the Research Coordinator (see details below) is a PhD candidate in Nutritional Science at Massey University.

Research Coordinator:	**Supervisor:**
Merrin Quilter Institute of Food Nutrition and Human Health Massey University, Palmerston North Tel: (06) 356 9099 ext. 81469 Email: M.L.Quilter@massey.ac.nz	Assoc. Prof. Jane Coad Institute of Food Nutrition and Human Health Massey University, Palmerston North Tel: (06) 350 5962 Email: J.Coad@massey.ac.nz

What we will measure

During your appointment…

1) You will be asked to sign an informed consent form and complete a medical history questionnaire.
2) We will measure your height, weight, and waist-hip ratio. These measurements will be taken in private. Body weight will be measured using ordinary weighing scales (you will be asked to remove your shoes and outer clothing). All other measurements will be made using a tape measure.
3) We will ask you to have your body composition measured using another piece of laboratory equipment called the BodPod. This makes a measurement based on air displacement. It involves sitting for 5 minutes in a closed compartment, wearing either a swimming costume or close fitting cycling shorts, which we can provide. The room is private and you can enter the BodPod room in complete privacy. The staff member who will do the measurement is trained to use the BodPod.
4) We will also ask you to have a Total Body Scan wearing surgical scrubs, on our Hologic DXA machine used for estimating body composition. With low dose X-ray beams at 2 different energies, it is able to estimate the difference between lean and fat tissue very accurately. While no dose of radiation is harmless, this dose is very low and unlikely to cause harm. The total effective dose of radiation to which you will be exposed to is 10 microsieverts (µSv), which is much lower than the range normally used in medical diagnostics. To place this in perspective, the amount of radiation you are exposed to during a return flight to the United Kingdom is 100 µSv and from a dental X-ray is 50 µSv. The room is private and you can enter the DXA room in complete privacy. The staff member who will do the measurement is certified to operate the DXA.
5) We will take your blood pressure using an automated sphygmomanometer (blood pressure cuff) and measure arterial stiffness using a SphygmoCor. ECG electrodes will be applied to the bare skin at your chest and hip, then a probe like a blunt pen is pressed onto the skin above an artery in your neck and wrist for a few minutes. The staff member who will do the measurements is trained to use the SphygmoCor.
6) You will then be asked to provide a blood sample (in total about 25 mL which is equivalent to about 5 teaspoons). One of our staff members who is trained to take blood samples will take blood via venipuncture for the measurement of nutrient levels, markers of metabolic and cardiovascular health including: vitamin D, parathyroid hormone, serum calcium and albumin, glucose, insulin, C peptide, haemoglobin A1C (a form of haemoglobin that indicates the average blood glucose level over the last 3 months and may be raised in diabetes), lipid profile, high sensitivity CRP (a marker of inflammation), hormone profile and Vitamin D Receptor (VDR) genotype. (Note that the VDR genotype is an "in-house" research measurement and that the results do not have any clinical credence or insurance implications.)
7) Once you have provided a blood sample, you will be given breakfast.

8) A researcher will interview you about your diet and ask you to complete a 20 minute private online questionnaire (computer provided) about your background information, sexual function, lifestyle and diet. Help will be provided if necessary.
9) You will be asked to complete a strength test. Strength is routinely measured using an isokinetic dynamometer, which measures the force produced by various muscle groups in the body. We will be using a grip strength test. This involves gripping and squeezing a dynamometer as long and hard as possible, first with one hand, then the other.
10) Unless you have been medically diagnosed with disease of the heart or arteries, you will be asked to complete a submaximal aerobic fitness test. This test of cardiovascular fitness involves cycling at a low to moderate intensity. This exercise test consists of three or more consecutive increasing workloads of 3-minutes duration which will raise your heart rate to between 110 bpm and close to 85% of your predicted maximum heart rate. Oxygen uptake and heart rate will be measured simultaneously while you perform this test. This will allow us to determine your cardiovascular fitness.

After your appointment…
You may be asked to complete a 4 day weighed food record when you go home. This involves weighing and recording details of everything you eat and drink over two week days and two weekend days. We will provide full instructions and the equipment needed to do this, and we will collect it from you when you have completed the record.

A random sample of participants will be followed up via email or phone and invited to participate in a 20-40 minute interview on your attitude towards health, to take place at another time. This interview will be held face-to-face in a setting that is convenient for you, or on the telephone. You are free to decline to participate in this interview.

At the end of this phase of the Well-LaD study, we will ask if we can contact you again if you are a suitable subject for the next phase of the study. We will need to look at all of your information and test results to check your eligibility before contacting you and sending you an information sheet about that phase of the study. Giving permission for us to contact you later does not mean you are committed to the next phase of the study.

Risks and benefits
You will be making a valuable contribution to our understanding of the wellness, lifestyle and diet of NZ men in your age group. As a sign of our appreciation, you will **go in the draw to win a $250 Mitre 10 voucher.** There will be no charges made for any of the tests that you undertake and you will receive a detailed report on your body composition, fitness, diet and blood nutrient levels.

There are no personal risks to your health, but the blood tests could potentially identify undiagnosed health problems. If any such problems are identified, we will advise you to contact your General Practitioner (GP) or, with your permission, contact them on your behalf to highlight any concerns.

Please note that this study seeks information about your sexual function as a part of male wellness. It is important that you are aware that during your appointment, the researchers will not see any information about your sexual function. Your answers to these potentially sensitive questions will be given in an online questionnaire and will not be related to you by name. You will be referred to in the study by a unique subject identification number only. Any information on sexual function is provided online, rather than face-to-face, to ensure your comfort and to maintain your privacy. The subject number will link together your answers and test results without identifying you in any way. Your answers and test results will be kept in the strictest of confidence and the data only available to the Research Coordinator and Principal Investigator.

Over recent years, the number of men suffering from sexual dysfunction has greatly increased but we know very little about why or how to prevent it. Sexual dysfunction can have a major impact on the wellness and quality of life of both men and their partners, and erectile dysfunction has been found to be an early sign of impending cardiovascular disease. The effective prevention or treatment may have a dramatic impact on the wellness of NZ men. Current medications designed to treat erectile dysfunction help to reduce the symptoms but do not address the cause of the problem. In some men they have been shown to be ineffective, not tolerated or contra-indicated. Diet and lifestyle modification may have the potential to treat or prevent erectile dysfunction and reduce the risk of cardiovascular disease.

Participation

You are under no obligation to accept this invitation to take part in this research study. If you decide to participate, you have the right to:

- decline to answer any particular question
- withdraw from the study at any time without having to give a reason
- ask any questions about the study at any time during participation
- provide information on the understanding that your name will not be used
- be given access to a summary of the project findings when it is concluded.

If you have any queries or concerns regarding your rights as a participant in this study you may wish to contact a Health and Disability Advocate, telephone 0800 555 050.

General

If you want to discuss any aspect of this study, please contact the Research Coordinator, Merrin Quilter.

At the conclusion of this study we will provide a report of the outcome to those involved in the study. We will also hold a presentation to discuss the results which you can attend if you wish. We anticipate that the anonymous results will be published in an international medical journal.

As samples of human tissue will be taken during this study, there may be cultural issues associated with storing tissue that need to be discussed with your family/whanau. Some Iwi disagree with storage of human tissue citing whakapapa and advise their people to consult prior to participation in research where this occurs. To avoid problems at a later stage, we suggest your family/whanau is involved with you at all stages of the research. However, we also acknowledge that individuals have the right to choose to participate.

Confidentiality

No material which could personally identify you would be used in any reports on this study. All information will be reported in aggregate. Information collected from you in the study will be stored securely in the Department of Nutrition and will be available only to study personnel, unless you request that we release it to some other individual (such as your General Practitioner). When the study is completed, all material will be destroyed.

Compensation for Injury

In the unlikely event of a physical injury as a result of your participation in this study, you will be covered by the accident compensation legislation with its limitations. If you have any questions about ACC please feel free to ask the researcher for more information before you agree to take part in this trial.

Ethics Approval

This study has been approved by the **Lower South Regional Ethics Committee** (Reference LRS/10/07/032).

Please feel free to contact the research coordinator if you would like to take part or if you have any questions about this study.

Merrin Quilter | **Email:** well-ladstudy@massey.ac.nz | **Tel:** 0800 MASSEY - ask for The Well-LaD Study (8am-5pm Monday to Friday) | **Website:** http://www.massey.ac.nz and search "The Well-LaD Study"

Subject ID:

MASSEY UNIVERSITY

INSTITUTE OF FOOD, NUTRITION AND HUMAN HEALTH
PALMERSTON NORTH, NEW ZEALAND

Date: **Time:**

Researchers name:

First name: ……

Family name: ……

Gender Male Female

What is your age? 0-39 40-49 50-59 60-69 70+

What is your date of birth? ……

Do you currently live in the Manawatu region? Yes No

Are you able to come to Massey University in Palmerston North to take part in this study? Yes No

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Street Address: ……

Phone (home): ……

Phone (mobile): ……

Email: ……

Do you currently suffer from any of the following medical conditions?

1. Disease of the heart or arteries? Yes No
   If you answered yes, have you ever been hospitalised for this? Yes No

Please provide details:

2. Type II diabetes or persistent sugar in the urine Yes No
   If you answered yes, have you ever been hospitalised for this? Yes No

Please provide details:

3. Depression, post-traumatic stress disorder or a psychiatric condition Yes No
4. Auto-immune disorders such as Type I Diabetes, Lupus, Multiple Sclerosis (MS), Myalgic encephalomyelitis (ME), Rheumatoid arthritis or Psoriasis Yes No
5. Prostate cancer, Benign prostatic hyperplasia (BPH), Prostatitis, or Peyronie's disease Yes No

6.	Multi-system atrophy (MSE), spinal cord injury or tumors, prolapsed intervertebral discs or tumors, disease to the parasympathetic nerves of the pelvis, pelvic surgery or abdominal surgery	Yes	No
7.	Chronic renal failure	Yes	No
8.	High levels of prolactin in the blood (Hyperprolactinaemia)	Yes	No
9.	High or low levels of testosterone in the blood (Hypergonadism or Hypogonadism)	Yes	No
10.	Smooth muscle dysfunction	Yes	No
11.	Malignant disease (cancer)	Yes	No
12.	Recent surgery (within the past year)	Yes	No
13.	Substance abuse i.e. alcohol, marijuana, opium, heroin	Yes	No

Are you involved in competitive cycling? Yes No

Is there any reason that you know of that you should not give a blood sample, i.e. do you have any blood borne infectious diseases such as hepatitis B or C, HIV, Creutzfeldt–Jakob Disease (CJD), or blood clotting problems such as haemophilia or severe anaemia? Yes No

Are you currently taking any form of prescribed medication? Yes No

Please bring it in with you when you come in for your assessment

If yes, what is it and what is it treating?

Please provide details:

Are you currently taking any form of non-prescribed medication i.e. supplements? Yes No

Please bring it in when you come in for your assessment

If yes, what is it and what is it treating?

Please provide details:

Is the volunteer eligible to take part?[1] Yes No

Appointment for Assessment at Massey: Date: **……...**

Time: **……..**

[1] To be eligible subject must be male, age 40-70, living in the Manawatu and able to come to Massey to take part. Men will be excluded on a case by case basis for response to question 1and 2 if there is evidence of advanced or uncontrolled CVD or T2DM. This includes any incidence of heart attack, heart failure or stroke, recent (in the past 2 years) hospitalisation for coronary heart disease, cardiomyopathy, hypertensive heart disease, cardiac dysrhythmia, inflammatory heart disease, valvular heart disease, cerebrovascular disease or peripheral arterial disease, hypoglycemic seizure or coma. Men will be included if they have been hospitalised in the past but have not been on any associated medication in the past 12 months, or if they have not been hospitalised but are currently on associated medication. Men will be excluded if they provide an affirmative response to any of the questions 3-13, if they are competitive cyclists or are unable to safely give a blood sample.

Subject ID:

MASSEY UNIVERSITY
INSTITUTE OF FOOD, NUTRITION AND HUMAN HEALTH
PALMERSTON NORTH, NEW ZEALAND

The Well-LaD Study (Phase 1)

An investigation into the relationship between wellness, lifestyle and diet in New Zealand men aged 40-70 years

CONSENT FORM

1. I have read and I understand the information sheet dated {insert date} for volunteers taking part in The Well-LaD Study (Phase I). I have had the opportunity to discuss this study. I am satisfied with the answers I have been given.
2. I have had the opportunity to use whānau support or a friend to help me ask questions and understand the study.
3. I understand that taking part in this study is voluntary (my choice), and that I may withdraw from the study at any time, and this will in no way affect my future health care.
4. I understand that my participation in this study is confidential and that no material that could identify me will be used in any reports on this study.
5. I understand that the investigation will be stopped if it should appear harmful to me.
6. I understand that blood samples may be sent overseas for processing and give my permission for this.
7. I have had time to consider whether to take part in the study.
8. I know who to contact if I have any side effects from the study or if I have any questions about the study in general.

Signed: _____

Name: _____

Date: _____

Well-LaD Study Procedures Manual

1. Important Contacts:

Research Coordinator	**Massey Extn**	**Mobile**	**Home**
Merrin Quilter			
Co-investigators			
Jane Coad			
Lynette Hodges			
Pamela von Hurst			
Phlebotomists			
Merrin Quilter			
Jasmine Thomson			
Ying Jin			
Maria-Tine Biersteker			
DEXA Technicians			
Anne Broomfield			
Chris Booth			
Jane Coad			
Facilities			
Chris Booth – *HNRU Manager*			
Facilities Management - *Defibrillator*			
Denise Mist - *First Aid Kit*			

2. Important Documents:

CODE	NAME
WELP1	Study Flow Diagram
WELP2	Study Procedures Manual
WELR1	Letter of Invitation
WELR2	Participant Information Sheet
WELR3	Recruitment Email
WELR4	Poster
WELR5	Flyer/Advertisement
WELR6	Email Invitation
WELR7	Screening Questionnaire
WELR8	Confirmation of Appointment
WELR8a	Map and Directions
WELF1	Consent Form
WELF3	Medical History
WELF4	Data Collection Form
WELF5	24 hour Recall Form
WELF6	Online Questionnaire
WELF7	Weighed Food Record Instruction Booklet
WELSOP1	Anthropometry – Obtaining Basic Anthropometry Measurements
WELSOP2	BodPod – Obtaining a Body Composition Measurements
WELSOP3	DEXA – Obtaining a Hologic Body Composition Measurement
WELSOP4	Omron – Obtaining a Digital BP Measurement
WELSOP5	SphygmoCor – Obtaining a PWA or PWV Measurement
WELSOP6	Venipuncture – Obtaining a Venous Blood Sample
WELSOP7	24 Hour Recall – Obtaining a 24 Hour Dietary Recall
WELSOP8	Handgrip Strength Test – Obtaining a Handgrip Strength Measurement
WELSOP9	YMCA Submaximal Fitness Test

3. Recruitment procedures

3.1. Phase I: Randomly select 600 men aged 40-70 years from the Manawatu electoral list database using SPSS

- Send out letter of invitation (WELR1) and Participant Information Sheet (WELR2)

3.2. Phase II: If response after 2 weeks is insufficient, commence aggressive recruitment strategy

- Send out recruitment email (WELR3) to Massey Staff
- Place posters (WELR4) and flyers (WELR5) in public places i.e. Massey, Public toilets, Gyms, RSA, Medical Centres, Cafes, Supermarket notice boards
- Conduct residential flyer (WELR5) drop within Palmerston North
- Contact local radio stations to push recruitment
- Place Saturday advertisements (WELR5) in the Guardian and the Standard
- Conduct presentations at RSA, Age Concern, Men‟s and Women‟s Health Clinics etc.

4. Screening procedures

4.1. Respond to phone/email expression of interest:

- Explain the study and request full contact details i.e. telephone number, email and postal address
- Email (WELR6) or post a recruitment package consisting of the Participant Information Form (WELR2)
- Explain that once they have read the information sheet they will need to ring/email to complete a telephone screening to check they are eligible
- Advise them that you will follow up the email/letter after 3 business days with a phone call/email

4.2. When the participant contacts you:

- Check for subject suitability by completing the screening check list on the Health Screening Questionnaire (WELR7)

4.2.1. If not eligible to participate:

- Inform subject that they are unfortunately not eligible to participate
- Thank them for volunteering and their interest in the study

4.2.2. If eligible to participate:

- Make an appointment to come to the Human Nutrition Unit
- Email/post a Confirmation of Appointment (WELR8) confirming appointment date and time, containing a map of the campus (WELR8a), information on parking, explaining the need for fasting (food and all liquids except water), close fitting clothing for the BodPod (togs/speedos/bike pants) and ask them to bring any medication/supplements they may be taking

5. Two days before the appointment

- Confirm appointment by phone or email
- Provide subject with directions to IFNHH and parking information
- Assign subject number and enter subject details into master spreadsheet
- Set up subject folder with:
    - Participant Information Form (WELR2)
    - Health Screening Questionnaire (WELR7)
    - Consent Form (WELF1)
    - Medical History Form (WELF3)
    - Data Collection Form (WELF4)
    - 24 Hour Recall Form (WELF5)
    - 4 Day Weighed Food Record Form (WELF7)

6. On the day of the appointment

6.1. Before participant arrives

- BodPod Room:
  - Prepare equipment: stadiometer, measuring tape, calculator, BodPod, tubes, togs/skins, robes, beard guards, bags for personal belongings
  - Turn on the BodPod and leave it to **warm up for 30 minutes**
  - Place the clean BodPod clothing and bags in the changing room
- DEXA room:
  - Prepare equipment: DEXA
  - Turn on and calibrate the DEXA
- Phlebotomy Area:
  - Prepare equipment: Omron BP monitor, cuffs, SphygmoCor, laptop, ECG pads, disposable razors, hand sanitizer, gloves, blood tray, tourniquet, aseptic wipes, needle, needle holder, Vacutainers, rack, gauze, Micropore tape, plasters, scissors, sharps bin, MedLab forms and blood packets, beaker with TriGene, spray bottle with TriGene, spray bottle with 70% alcohol, paper towels, medical kit, tube labels
  - Set up the BP monitor, connect the SphygmoCor to the laptop and turn it on
  - Set up the blood tray for each subject, label the required Vacutainers and place in a rack
- Working Lab:
  - Prepare equipment: centrifuge, blanks, vacutainer rack, microtube racks, sterile microtubes (1 ml), pipette (1 ml), sterile pipette tips (1 ml), container for contaminated tips, biohazard bag, plastic ziplock bags, plastic freezer box, gloves, spray bottle with TriGene, paper towels, medical kit, tube labels
  - Run a set of blanks in the centrifuge to ensure temperature is at 4°C when blood samples are ready to be spun
  - Label all microtubes to be used and lay in microtube rack
  - Fill the polystyrene blood bin with ice
- Fitness Area:
  - Prepare equipment: metabolic cart, cycle ergometer, Polar monitor, fan, mouth pieces and tubes, ECG monitor, ECG pads, dynamometer
  - Metabolic cart: Switch on the PARVO using the orange switch at the back of the unit, turn on the computer, ensure the correct software is open (TrueOne32 Exercise) and leave to **warm up for 60 minutes.** Connect mouth piece to headset and breathing tube to filter.
  - Ergometer**:** Connect batteries and press function until the mode shows RPM
  - Polar monitor: Connect polar monitor to strap, check battery is full, place on bike
  - ECG monitor: Get out sufficient ECG pads (3 per subject), connect ECG monitor to laptop and turn on laptop. Open PowerLab software.
- Questionnaire Area:
  - Prepare equipment: laptop, food modelling book, online questionnaire, food record presentation. Place on desk in Break Out Room.
- Breakfast Area:
  - Prepare equipment: tray, plates, bowls, cutlery, mugs, electric jug, tea, coffee, sugar, milk, toaster, bread, butter, margarine, jam, vegemite, peanut butter, cereal. Place tray on table in HNRU or FQA lab.
- Calibrate the BodPod
- Calibrate Metabolic Cart

6.2. RECEPTION : Greeting - MQ (1 min)

- Greet subject and take them to the B/O room

6.3. B/O ROOM: Introduction -MQ (5 min)

- Check they have read the Participant Information Form (WELR2), if not provide a copy
- Check name and Health Screening Questionnaire (WELR7) for eligibility
- Complete and check the Consent Form (WELF1)
- Explain procedure for the morning and the layout of the labs
- Ask subject if they have fasted (no food OR drink except water) since 9 pm the night before, check if they have exercised that morning, if they have bought any medications/supplements with them and remind them to use the bathroom if needed

6.4. B/O ROOM: Medical History –MQ (5 minutes)

- Briefly explain procedure
- Participant completes the Medical History Form (WELF3) and is interviewed to confirm details, complete the medications/supplement section using those supplied and check back to Health Screening Questionnaire (WELR7) responses
- Check if the participant wishes to receive a copy of their individual results and/or a summary of the study results, record on Medical History Form (WELF3) and ensure the appropriate email/postal address has been provided
- Ask subject to move to the HNRU and change into appropriate attire for the BodPod (togs/bike pants/skin suit, robe, cap and bread guard if necessary) in changing room

6.5. BODPOD ROOM: Anthropometry and BodPod– MQ (15 minutes)

- Briefly explain procedure
- Ensure subject has removed shoes and all jewellery and metal items (make a note if they can‟t take them off)
- Measure height in duplicate/triplicate following the **SOP**
- Measure weight in duplicate/triplicate using BODPOD – F2 Practice, then F1 Weight
- Measure waist and hip circumference in duplicate/triplicate following the **SOP**
- Calculate average height, weight, BMI, waist and hip circumference
- Calibrate BodPod and measure body composition following the **SOP**
- Print BodPod form, place in folder, record all measurements including % fat and % fat free mass in the appropriate box on the data collection form (WELF4)
- Ask subject to put on a robe or if necessary change into scrubs in changing room

6.6. DEXA ROOM: DEXA scan– JC/AB/CB (10 minutes)

- Briefly explain procedure
- Ask subject to lay on the DEXA table
- Ensure subject as removed all jewellery, glasses, and metal items including any piercings (make a note if they can‟t take them off)
- Enter patient information into computer
- Measure body composition following the **SOP**
- Record participants DEXA record number on the data collection form (WELF4)
- Move to the B/O Room

6.7. B/O ROOM: BP and arterial stiffness– MQ (15 minutes)

- Briefly explain the BP and SphygmoCor procedure
- Ask the subject to lie down and ensure they are relaxed and comfortable
- Take BP using the left arm in triplicate following the **SOP**
- Record BP in the appropriate box on the data collection form (WELF4)) and calculate the mean of the last two measurements
- Ask if they know what their BP normally is (make a note), if they are on any blood pressure or heart medications including aspirin
- Check if they have any diagnosed conditions of the heart or arteries including arrhythmia or arterial plaques that may rupture upon massage
- Enter patient information into computer
- Measure PWV in triplicate following the **SOP**
- Record values in the appropriate boxes on the data collection form (WELF4)
- Print detailed reports and place in subject folder

**Note: Once daily export full text file, copy into excel spread sheet and save to H drive*

6.8. B/O ROOM: Phlebotomy – MQ (15 minutes)

- Briefly explain the procedure
- A set of labelled Vacutainer tubes will be set out for each subject. Please ask subjects name and check code (Study_ID_Test_Date) against subject number
- Take a venipuncture blood sample following the **SOP**
- Fill tubes in the following order: 2 gold, 3 lavender, 1 grey
- Invert according to blood processing protocol and place tubes in a rack
- Wait 5 minutes for subject site to clot. While doing so, complete the appropriate section of the data collection form (WELF4) including phlebotomist, time taken, arm, tubes filled, and comments. Check you have: a full set of labelled Vacutainers, safely disposed of sharps and biohazards and sanitised blood tray and hands, prepared for next subject
- Ensure subject is happy to move on to breakfast and escort them to the Main Room
- Take rack of bloods into Working lab
- Put Lavender tube labelled "VDR" into rack in fridge, place 2 lavender tubes and grey tube into the centrifuge with appropriate blanks opposite the blood samples and centrifuge at 3500 rpm, 4°C for 10 minutes

** Note: Any available research team member is to process blood in the Working Lab*

6.9. MAIN ROOM: Breakfast – (10 minutes)

- Participant eats breakfast consisting of: tea, coffee, milo and toast with spreads. If necessary cereal, milk, bananas, Up and Go, muesli bars, yoghurt and canned fruit may be provided.
- Move to the B/O Room

** Note: Any available research team member is to process blood in the Working Lab*

6.10. B/O ROOM: Online Questionnaire and 24 Hour Recall – MQ (30 minutes)

- Briefly explain the 24 Hour Recall
- Conduct the 24 Hour Recall following the **SOP**
- Complete the 24 Hour Recall Form (WELF5) and place the completed form in the folder
- Briefly explain the online questionnaire
- Ensure subject number is completed accurately and that the subject is able to read and complete the questionnaire. Provide assistance if necessary.

** Note: Any available research team member is to process blood in the Working Lab*

6.11. B/O ROOM: Handgrip Strength Test – JT/LH/MQ (5 minutes)

- Check if subject is eligible and capable of completing the test safely (exclude those who suffer from arthritis, musculoskeletal impairment that will result in pain if they complete the test)
- Briefly explain the procedure
- Measure grip strength in each hand in triplicate following the **SOP**
- Record grip strength measurements and hand dominance on the data collection form (WELF4) and calculate overall grip strength (max L + max R)
- Ask the subject to change into their own clothes in the HNRU changing room and return to the B/O room

6.12. B/O ROOM: Fitness Test – JT/LH/MQ (15 minutes)

- Check if subject is eligible and capable of completing the test safely (exclude those medically diagnosed with disease of the heart or arteries).
- Briefly explain the procedure
- Measure VO_2 and calculate VO_2max following the **SOP**
- Record all of the information required in the data collection form (WELF4)
- When subject has had a 5 minute monitored recovery period, recovered and breathing has returned to normal, check they are ready to continue

6.13. B/O ROOM: Weighed Food Record – JT/MQ (15 minutes)

- Check if the subject is capable of completing the 3 day weighed food record (exclude those who would not be able to record the data or who obviously lack the motivation to complete it)
- Briefly explain the procedure and play the presentation on the computer
- Give the subject the Weighed Food Record Form (WELF7), specify the dates for completion, provide digital scales, standardised measuring cups and spoons (if necessary) to complete at home
- Organise to collect the record and equipment from them when complete

6.14. B/O ROOM: Departure – MQ (5 minutes)

- Check folder is complete; Health Screening Questionnaire (WELR7), Consent Form (WELF1), Medical History Form (WELF3), Data Collection Form (WELF4) and 24 Hour Recall Form (WELF5) and print outs (BodPod, Fitness Test) place folder in desk drawer for later filing
- Remind them that;
  - you will call/email to arrange the return of the food record and equipment
  - they may be contacted to confirm details of their food record information
  - they may be one of 50 participants randomly selected to take part in a telephone interview on their health behaviours but that they may decline to participant if they choose to
- Thank the subject for their time, effort and commitment to the study
- Collect their belongings and escort them to reception.

7. Blood processing

5.1 Blood processing protocol: (MQ/JT/JC)

TUBE DETAILS	VARIABLES & AMOUNT OF SERUM/PLASMA	PROCEDURE FOR THE PREPARATION OF SAMPLES
Gold lid: 2x 5 mL tubes (Clot activator and gel of serum separation for serum analysis)	**SERUM** **Tube 1:** • 1 mL lipid profile (TC, TG, HDL-C) • 0.5 mL Ca • 0.5 mL albumin TOTAL: 2.0 mL min **Tube 2:** • 0.5 mL vit D (Canterbury Health Labs) TOTAL: 0.5 mL min	**Invert 8 times** Protect blood from light Leave blood for ±30 minutes to clot Centrifuge* within 2 hours to yield serum **Tube 1** to be delivered on ice to Massey Medical Centre before 11 am or 1 pm for analysis of; lipid profile (TC, TG, HDL-C), Ca and albumin. Place blood sample into MedLab bag and complete form (see below). Place entire bag on ice for later delivery to Massey Medical Centre for MedLab collection or direct to MedLab **Tube 2** dispense multiple aliquots of serum into labelled (yellow) plastic micro tubes as follows: • 0.5ml vitamin D (to be sent to CHL) YELLOW **#Freeze as soon as possible**
Lavender lid: 3x 6.0 mL tubes (Spray-dried K_2EDTA for whole blood haematology analysis)	**PLASMA** **Tube 1:** DNA analysis (K. Stowell) TOTAL: 0.1 mL min **Tube 2:** **Plasma:** • 0.5 mL l insulin • 0.5 mL for hsCRP • 0.5 mL PTH • 0.5 mL C-peptide • 0.1 mL HbA1c • 0.3 mL testosterone • 0.5 mL SHBG TOTAL: 2.9 mL min	**Invert 8 times** **Tube 1** immediately place into rack in fridge. DO NOT SPIN. To be delivered to K. Stowell (Massey IMB) on ice every Friday afternoon, for DNA analysis (VDR Genotyping – 0.1ml plasma required at minimum **Tube 2 & 3** place in the centrifuge* (with grey tube), insert appropriate blanks to balance samples and start centrifuge. Dispense (multiple if sample permits) aliquots into coloured, labelled plastic micro tubes as follows: **Plasma:** • 0.5 mL l insulin BLUE • 0.5 mL for CRP BLUE • 0.5 mL PTH RED • 0.5 mL C-peptide GREEN • 0.5 mL HbA1c (0.1ml if limited) GREEN • 0.5 mL testosterone (0.3ml if limited) PINK • 0.5 mL SHBG PINK **#Freeze as soon as possible**
Grey lid: 1x 4.0 mL tube (Potassium oxalate & sodium fluoride for glucose analysis)	**PLASMA** **Tube 1:** • 0.5ml glucose TOTAL:0.5 mL min	**Invert 8 times** Centrifuge* asap with lavender tube 2 **GLUCOSE** Dispense multiple aliquots of plasma immediately into clear plastic micro tubes as follows: • 0.5 mL glucose (3 x if possible) CLEAR **#Freeze as soon as possible.**
Total blood: 26 mL max	**Total blood: 6.0 mL min**	*Centrifuge for 10 minutes at 3500 rpm (2000 g) and at 4°C. #Serum and plasma samples should be stored short-term in -20°C freezer and transferred to -80°C freezer for long term storage

1) Follow appropriate working lab safety procedures

2) **Check tubes:** a rack containing 2x gold, 3x lavender and 1x grey tubes should have been delivered from the Phlebotomy Room to the Working Lab. Note that the tubes should have been inverted by the phlebotomist before delivery and the time and arm recorded on the data collection form (WELF4)

3) **Immediately** take the lavender tubes and the grey tube
- Place lavender tube 1 (labelled VDR) in the fridge for later delivery to K. Stowell. DO NOT SPIN!
- Place lavender tube 2 & 3 and grey tube in the centrifuge with appropriate blanks
- Centrifuge at 2000 g (3500 rpm) 4°C for 10 minutes
- Once centrifuge is finished, dispense multiple aliquots (if sample volume permits) of plasma from lavender tube 2 into coloured, labelled plastic micro tubes as follows;
  - 0.5 mL l insulin BLUE
  - 0.5 mL for CRP BLUE
  - 0.5 mL PTH RED
  - 0.5 mL C-peptide RED
  - 0.5 mL SHBG GREEN
  - 0.3 mL testosterone GREEN
  - 0.1 mL HbA1c PINK
- Dispense multiple aliquots (if sample volume permits) of plasma from the grey tube into clear labelled plastic microtubes as follows;
  - 0.5 mL glucose PINK
- Pack samples in box/rack, clearly label, bag and store in the Working Lab freezer

4) **Within 2 hours (having left ±30min to clot)** take the Gold tubes:
- Centrifuge both tubes at 2000 g (3500 rpm) and 4°C for 10 minutes
- Place tube 1 (labelled LCA) in the polystyrene container on ice for later delivery to Massey Medical Centre or MedLab either before 11 am or 1 pm.
- Dispense multiple aliquots (if sample volume permits) of serum from tube 2 into plastic micro tubes as follows;
  - 0.5 mL vitamin D – to be sent to CHL YELLOW
- Pack samples in box/rack, clearly label, bag and store in the Working Lab freezer

5) Once processing is complete;
- Ensure all empty tubes, contaminated tips and gloves are disposed of appropriately
- Place MedLab Gold Tube in plastic MedLab bag, fill out MedLab form (found on blood trolley) and place in the front pocket of the bag. Put bag into ice container ready for transport to MedLab. Form should be filled out as follows:
  Name: WL###_mmyy *Address*: C/- IFNHH, Massey *Date taken:* /01/12
  *Time bloods taken:*_____ *Taken by:* IFNHH *Fasting:* Yes
  DOB: dd.mm.yy *Sex:* M
  Tick calcium, lipids (fasting), and „other tests" and write in albumin.
- Disinfect the bench with Trigene and wash hands before leaving the Working Lab

6) Every day;
- Take MedLab gold tubes (#1) to Massey Medical Centre for collection at 11am by MedLab. If late, take to MedLab Reception in the PN Hospital (main entrance, 2nd floor)

7) Every Friday;
- Take accumulated lavender tubes (#1) to IFS for DNA extraction. It will take approximately 20-30 minutes for about 16 blood samples
- Move all the previous days samples from the Working Lab freezer to standing -80°C freezer on the 3rd floor. Place samples in the box labelled "Well-LaD Study" inside the appropriate bags to be stored for later analysis.

Subject ID:

MASSEY UNIVERSITY
INSTITUTE OF FOOD, NUTRITION AND HUMAN HEALTH
PALMERSTON NORTH, NEW ZEALAND

The Well-LaD Study (Phase 1)

An investigation into the relationship between wellness, lifestyle and diet in New Zealand men aged 40-70 years

Participant details

Date of birth: ……

General Practitioner (GP): …..……

GP's Address (if known): …….

GP's Phone (if known): …..……

Medical History

Have you ever been diagnosed with any of the following?			**Comments**
High blood pressure	Yes	No	……..
High cholesterol	Yes	No	…….
Atherosclerosis	Yes	No	…….
Heart disease	Yes	No	……..
Angina	Yes	No	……..
Heart attack	Yes	No	……..
Heart failure	Yes	No	……..
Stroke	Yes	No	……..
Type II Diabetes	Yes	No	……..
Skin cancer	Yes	No	……..
Osteoporosis	Yes	No	……..
Restless Leg Syndrome	Yes	No	……..

Other …….

Do you have any blood borne infectious diseases?	Yes	No
Do you have clotting problems?	Yes	No
Are you allergic to plasters or antiseptic wipes?	Yes	No
Are you uncomfortable with needles or having blood taken?	Yes	No

---

Medication

Are you taking any form of medication, including traditional or homeopathic medicine, and medicine obtained on the internet that is not included on your screening questionnaire? If so, please list the medications and what they are treating.

…….

…….

…….

…….

Hormone therapy …….

Supplements

Are you taking any dietary supplements, vitamins, minerals, oils etc? If so, please list the supplements and what they are treating.

……

……

…….

…….

Vitamin D supplements ……..

If eligible, would you be interested in taking part in Phase II of the Well-LaD Study, a nutritional intervention trial aiming to improve sexual function through nutritional intervention?

	Yes	No
Would you like to receive a summary of your personal results?	Yes	No
Would you like to receive a summary of the overall study results?	Yes	No

---

THANK YOU for your participation

Please return this form to the researcher

Data Collection Form

Subject ID:

Date: _____

Participant DOB: _____

1. ANTHROPOMETRY

	Participant Results			Mean*
Age (yrs)				
Height (cm)				
Weight (kg)				
BMI (kg/m²)				
Waist (cm)				
Hip (cm)				

*Calculate the mean of the three height, weight, waist and hip circumference readings

Comments

2. BODPOD

	Participant Results
Percent Fat (%)	
Percent Lean (%)	
Fat weight (kg)	
Lean weight (kg)	
Total weight (kg)	

Comments

3. DEXA

DEXA unique record number: _____

Comments

4. BLOOD PRESSURE AND ARTERIAL STIFFNESS

	Participant Results			Mean*
Systolic BP (mmHg)				
Diastolic BP (mmHg)				
Augmentation Index (AI)				
Augmentation Index @HR75				
PWV (m/s)				

*Calculate the mean of the final two BP readings to use for PWV analysis, and the mean of the three AI, AI@75 and PWV readings

Comments

5. PHLEBOTOMY

Comments

	Participant Results/Checklist	
Time taken		
Arm used		
	Complete Y/N	**Inverted Y/N**
2 x Gold vacutainers		
2 x Lavender vacutainers		
1 x Grey vacutainer		

6. QUESTIONNAIRES

Comments

	Complete Y/N
Online questionnaire	
24 Hour recall	

7. STRENGTH TEST

Comments

Grip size:		**cm**
Dominant hand:		**Left Right**
Reading		**Strength (kg)***
Left hand	**1**	
	2	
	3	
Right hand	**1**	
	2	
	3	

*Only the highest reading is used for analysis of handgrip strength

8. FITNESS TEST

Resting HR:	**bpm**	**Resting BP:**	**mmHg**
Age:	**yrs**	**Weight:**	**kg**
HRmax (220-age):	**bpm**	**85% HRmax:**	**bpm**
85% HRmax – 10 bpm:	**bpm**	**Seat Height:**	**cm**

Time (min)		**Resistance (W)**	**Cadence (rpm)**	**HR (bpm)**	**VO_2 (ml/kg/min)**
First workload	**1**				
	2				
	3				
	4*				
Second workload	**1**				
	2				
	3				
	4*				
Third workload	**1**				
	2				
	3				
	4*				
Fourth workload	**1**				
	2				
	3				
	4*				
Recovery[#] (reduce resistance)	**1**				
	2				
	3				
	4*				
Mean of second-last workload[ǂ]				**HR1=**	**SM1=**
Mean of last workload[ǂ]				**HR2=**	**SM2=**

[*] The fourth minute is only required if the HR during the 2nd and 3rd minutes are not at a steady state (within 5-6 bpm)

[#] An active recovery period of 3 minutes should follow this test before proceeding to post-test HR and BP measurements which can be taken in a seated position.

[ǂ] Calculate and record the mean HR and VO_2 using the ssHR and VO_2 in the last and second-last minute of that workload.

Post-test Measurements:

Time (min)		**HR (bpm)**	**BP (mmHg)**
Post-test recovery	**1**		
	2		
	5		

Calculations:

Formulae	**Working**	**Value**
$b = \frac{(SM2-SM1)}{(HR2-HR1)}$	$b = \frac{(\quad - \quad)}{(\quad - \quad)}$	b=
$VO_2peak = b\,(HRmax-HR2) + SM2$	$VO_2peak = (\quad - \quad) +$	VO_2peak=

9. FOOD RECORD

	Completed Y/N
Verbal instructions	
Video instructions	
Booklet and equipment provided	

Comments

10.DEPARTURE CHECKLIST

	✓
Consent form	
Health Screening Questionnaire	
Medical History Form	
Complete Data Collection Form	
Bod Pod Printout	
Sphygmocor Printout	
Fitness Test Printout	
Blood Processing complete	
Complete 24 Hour Recall Form	
Equipment and Food Record booklet	
Asked about study/personal results	
Asked about Database	
Reminded about future contact	
Collected personal belongings	

Comments

Subject ID:

Date: _____

Participant DOB: _____

What day of the week are you describing?

☐ Monday ☐ Tuesday ☐ Wednesday ☐ Thursday ☐ Friday ☐ Saturday ☐ Sunday

Would you describe the food you ate yesterday as typical? Yes/No

Time eaten/ name of eating occasion	**Place eaten**	**Detailed description of food eaten (type, brand, low-fat/not, method of preparation, combination of foods)** Ask about beverages, sauces, condiments, chewing gum, alcohol, and ingredients of recipes (herbs/spices).	**Amount eaten**	**Code**

WELL-LAD STUDY ONLINE QUESTIONNAIRE

INSTRUCTIONS: The following questionnaire consists of four sections: lifestyle, sexual function, diet and background information with a total of 71 questions. The questions are designed to provide information on your usual behaviour over the past 12 months. This questionnaire should take around 20 minutes to complete. Please read the questions carefully and tick the one answer that best applies to you, unless instructed otherwise.

Please be as honest and open as possible. Your answers are an important part of this research and your participation is much appreciated.

You have been given a three digit unique subject ID. This allows the computer to link all of your information together without identifying you as an individual by name. Your answers will feed through to our database automatically. In this way, confidentiality and privacy will be maintained.

Before completing the questionnaire, please enter your subject ID number here:

LIFESTYLE

CAFFEINE INTAKE

Caffeine is found in many drinks and foods; however the main sources in the New Zealand diet are coffee, tea, caffeinated soft drinks and caffeinated energy drinks.

1. Which of the following most applies to you?

- o I never drink coffee, tea, caffeinated soft drinks or caffeinated energy drinks
- o I used to drink coffee, tea, caffeinated soft drinks or caffeinated energy drinks
- o I occasionally drink coffee, tea, caffeinated soft drinks or caffeinated energy drinks
- o I regularly drink coffee, tea, caffeinated soft drinks or caffeinated energy drinks

2. Do you currently drink coffee, tea, caffeinated soft drinks or caffeinated energy drinks daily?

- o Not applicable
- o Yes
- o No

3. Which of the following products do you drink and on average how many do you drink each week? (Put '0' if none)

- o Not applicable
- o Coffee ⌊__⌋⌊__⌋ 250ml regular cups
- o Tea *not including green or herbal tea* ⌊__⌋⌊__⌋ 250ml regular cups
- o Green tea ⌊__⌋⌊__⌋ 250ml regular cups
- o Herbal tea *not including green tea* ⌊__⌋⌊__⌋ 250ml regular cups
- o Caffeinated soft drink ⌊__⌋⌊__⌋ 300ml standard glasses, cans or bottles *(e.g. Coca Cola, Pepsi, Lift, Mountain Dew etc)*
- o Caffeinated energy drink ⌊__⌋⌊__⌋ 300ml standard glasses, cans or bottles (*e.g. Demon, Red Bull, V etc)*
- o Don't know

TOBACCO USE

4. Which of the following applies to you?

- o I never smoke tobacco
- o I used to smoke tobacco
- o I occasionally smoke tobacco
- o I regularly smoke tobacco

5. Do you currently smoke tobacco products daily?

- o Not applicable
- o Yes
- o No

6. Which of the following tobacco products do you smoke and on average how many do you smoke each week? (Put '0' if none)

- o Not applicable
- o Manufactured cigarettes └__┘__┘
- o Hand-rolled cigarettes └__┘__┘
- o Pipes full of tobacco └__┘__┘
- o Cigars, cheroots, cigarillos └__┘__┘
- o Don't know

ALCOHOL CONSUMPTION

7. Which of the following most applies to you?

- o I never drink alcohol
- o I used to drink alcohol
- o I occasionally drink alcohol
- o I regularly drink alcohol

8. Do you currently drink alcohol daily?

- o Not applicable
- o Yes
- o No

9. How many standard alcoholic drinks do you drink each week? (Put '0' if none) (One 300ml glass of beer, one 80ml glass of wine, one 25ml shot measure of spirits)

- o Not applicable
- o Number of drinks └__┘__┘
- o Don't know

10. Which of the following do you drink and on average how many do you drink each week? (Put '0' if none)

- o Not applicable
- o Beer └__┘__┘ 300ml standard glasses/cans/bottles
- o White wine └__┘__┘ 80ml standard glasses
- o Red wine └__┘__┘ 80ml standard glasses
- o Spirits └__┘__┘ 25ml standard shot measure
- o RTDs └__┘__┘ 300ml standard bottles
- o Don't know

PHYSICAL ACTIVITY

11. We would like to know the type and amount of physical activity involved in your work. Please tick what best corresponds to your present activities from the following four possibilities:

- o Sedentary occupation - You spend most of your time sitting (such as in an office)
- o Standing occupation - You spend most of your time standing and walking. However, your work does not require intense physical effort (e.g. shop assistant, hairdresser, guard, etc.)
- o Manual work - This involves some physical effort including handling of heavy objects and use of tools (e.g. plumber, cleaner, nurse, sports player, electrician, carpenter, etc.)
- o Heavy manual work - This involves very vigorous physical activity including handling of very heavy objects (e.g. miner, bricklayer, construction worker, etc.)

12. In a typical week during the past year, how many hours did you spend per week on each of the following activities? (Put '0' if none)

Walking, including walking to work, shopping and leisure

In summer ☐ hours per week

In winter ☐ hours per week

Cycling, including cycling to work, shopping and leisure time

In summer ☐ hours per week

In winter ☐ hours per week

Gardening

In summer ☐ hours per week

In winter ☐ hours per week

Do-it-yourself

In summer ☐ hours per week

In winter ☐ hours per week

Physical exercise such as fitness, aerobics, swimming, jogging, tennis, etc.

In summer ☐ hours per week

In winter ☐ hours per week

Housework such as cleaning, washing, cooking, childcare, etc.

In summer ☐ hours per week

In winter ☐ hours per week

13. In a typical week during the past year did you practice any of these activities vigorously enough to cause sweating or a faster heartbeat?

- o Yes
- o No

If yes, for how many hours per week in total did you perform vigorous physical activity? (Put '0' if none)

☐ hours per week

14. In a typical week during the past year, how many flights of stairs* did you climb per day? (Put '0' if none)

☐ floors per day

***Note that a single flight of stairs consists on average of 20 full steps.**

ANXIETY AND DEPRESSION

15. Over the *last two weeks*, how often have you been bothered by the following problems?

	Not at all 1	***Several days 2***	***More than half the days 3***	***Nearly every day 4***
Little interest or pleasure in doing things	○	○	○	○
Feeling down, depressed, or hopeless	○	○	○	○
Trouble falling/staying asleep, sleeping too much	○	○	○	○
Feeling tired or having little energy	○	○	○	○
Poor appetite or overeating	○	○	○	○
Feeling bad about yourself – or that you are a failure or have let yourself or your family down	○	○	○	○
Trouble concentrating on things, such as reading the newspaper or watching television	○	○	○	○
Moving or speaking so slowly that other people could have noticed. Or the opposite - being so fidgety or restless that you have been moving around a lot more than usual	○	○	○	○
Thought that you would be better off dead or of hurting yourself in some way	○	○	○	○

16. If you checked off <u>any</u> problem on this questionnaire so far, how <u>difficult</u> have these problems made it for you to do your work, take care of things at home, or get along with other people?

- o Not difficult at all
- o Somewhat difficult
- o Very difficult
- o Extremely difficult

17. Do you believe that you are suffering from anxiety or depression as a result of lack of sexual activity or the inability to perform sexually?

- o Yes
- o No

18. Have you experienced stress over the past 12 months and if so, was it as a result of any of the following (please select as many as apply):

- o Not applicable
- o Separation
- o Divorce
- o Bereavement
- o Financial hardship
- o Post-traumatic stress
- o Other

ATTITUDE TOWARDS SUN EXPOSURE

19. The following questions are about your beliefs and behaviour towards sun exposure. Please read each of the following statements and respond by selecting the one answer that suits you best.

	1	2	3	4	5
When I am outside in summer, I wear sunscreen	o Almost never/Never	o A few times (Much less than half the time)	o Sometimes (About half the time)	o Most of the time (Much more than half the time)	o Almost always/Always
When I am outside in winter, I wear sunscreen	o Almost never/Never	o A few times (Much less than half the time)	o Sometimes (About half the time)	o Most of the time (Much more than half the time)	o Almost always/Always
When I am outside, I wear sunglasses or protective lenses	o Almost never/Never	o A few times (Much less than half the time)	o Sometimes (About half the time)	o Most of the time (Much more than half the time)	o Almost always/Always
When I am outside, I wear a sunhat	o Almost never/Never	o A few times (Much less than half the time)	o Sometimes (About half the time)	o Most of the time (Much more than half the time)	o Almost always/Always
When I am outside, I wear clothing to protect me from the sun	o Almost never/Never	o A few times (Much less than half the time)	o Sometimes (About half the time)	o Most of the time (Much more than half the time)	o Almost always/Always

When I wear sunscreen, I reapply it as recommended on the bottle	o Almost never/Never	o A few times (Much less than half the time)	o Sometimes (About half the time)	o Most of the time (Much more than half the time)	o Almost always/Always
In my occupation (paid or unpaid), I work outside	o Almost never/Never	o A few times (Much less than half the time)	o Sometimes (About half the time)	o Most of the time (Much more than half the time)	o Almost always/Always
When I exercise or play sports, I am outside	o Almost never/Never	o A few times (Much less than half the time)	o Sometimes (About half the time)	o Most of the time (Much more than half the time)	o Almost always/Always
When I do my hobbies, I am outside	o Almost never/Never	o A few times (Much less than half the time)	o Sometimes (About half the time)	o Most of the time (Much more than half the time)	o Almost always/Always
I enjoy spending time outside in the sun	o Not at all	o A little	o Somewhat	o Quite a lot	o A great deal
During summer I sunbathe	o Not at all	o A little	o Somewhat	o Quite a lot	o A great deal
I use sun beds	o Not at all	o A little	o Somewhat	o Quite a lot	o A great deal
I avoid the sun	o Not at all	o A little	o Somewhat	o Quite a lot	o A great deal
I limit my time in the sun	o Not at all	o A little	o Somewhat	o Quite a lot	o A great deal
I get sun burnt	o Not at all	o A little	o Somewhat	o Quite a lot	o A great deal
It is important to get some sunshine everyday	o Disagree	o Somewhat disagree	o Neither agree nor disagree	o Somewhat agree	o Agree
I feel better if I spend some time in the sun	o Disagree	o Somewhat disagree	o Neither agree nor disagree	o Somewhat agree	o Agree
I believe I look better with a sun tan	o Disagree	o Somewhat disagree	o Neither agree nor disagree	o Somewhat agree	o Agree
I believe sun exposure is bad for your health	o Disagree	o Somewhat disagree	o Neither agree nor disagree	o Somewhat agree	o Agree
I believe sun exposure is good for your health	o Disagree	o Somewhat disagree	o Neither agree nor disagree	o Somewhat agree	o Agree
My main reasons for avoiding the sun are	o I don't avoid the sun	o Public health messages say to avoid the sun	o Specific health reasons	o I don't want darker skin	o Religious or cultural reasons
I would spend more time in the sun if	o I wouldn't spend more time in the sun	o I wasn't worried about skin cancer	o I had more time	o I had somewhere private to sunbathe	o I was not in New Zealand

ATTITUDE TOWARDS HEALTH

20. Please circle the response that best describes your current feelings and try to avoid thinking too long about your answer.

1=STRONGLY DISAGREE **(SD)** 2=MODERATELY DISAGREE **(MD)** 3=SLIGHTLY DISAGREE **(D)**	4=SLIGHTLY AGREE **(A)** 5=MODERATELY AGREE **(MA)** 6=STRONGLY AGREE **(SA)**					
	SD	**MD**	**D**	**A**	**MA**	**SA**
If I become sick, I have the power to make myself well again.	1	2	3	4	5	6
Often I feel that no matter what I do, if I am going to get sick, I will get sick.	1	2	3	4	5	6
If I see an excellent doctor regularly, I am less likely to have health problems.	1	2	3	4	5	6
It seems that my health is greatly influenced by accidental happenings.	1	2	3	4	5	6
I can only maintain my health by consulting health professionals.	1	2	3	4	5	6
I am directly responsible for my health.	1	2	3	4	5	6
Other people play a big part in whether I stay healthy or become sick.	1	2	3	4	5	6
Whatever goes wrong with my health is my own fault.	1	2	3	4	5	6
When I am sick, I just have to let nature run its course.	1	2	3	4	5	6
Health professionals keep me healthy.	1	2	3	4	5	6
When I stay healthy, I'm just plain lucky.	1	2	3	4	5	6
My physical well-being depends on how well I take care of myself.	1	2	3	4	5	6
When I feel ill, I know it is because I have not been taking care of myself properly.	1	2	3	4	5	6
The type of care I receive from other people is what is responsible for how well I recover from an illness.	1	2	3	4	5	6
Even when I take care of myself, it's easy to get sick.	1	2	3	4	5	6
When I become ill, it's a matter of fate.	1	2	3	4	5	6
I can pretty much stay healthy by taking good care of myself.	1	2	3	4	5	6
Following doctor's orders to the letter is the best way for me to stay healthy.	1	2	3	4	5	6
I believe that food and nutrition play a great role in maintaining or improving my overall health.	1	2	3	4	5	6
I believe that eating certain foods reduces the risk of disease.	1	2	3	4	5	6

21	I eat at least one food because of the health benefits I believe it provides.	1	2	3	4	5	6
22	I regularly take nutrient supplements for my health.	1	2	3	4	5	6
23	People become ill regardless of what they eat.	1	2	3	4	5	6
24	I look at the nutrition panel on a food pack to help decide whether to buy a product.	1	2	3	4	5	6
25	I eat a well-balanced diet.	1	2	3	4	5	6

Thank you. You have finished this section. Next, you will be asked questions about your SEXUAL ACTIVITY AND FUNCTION. Remember your answers are anonymous, private and confidential and will not be associated with you as an individual. Please be as open and honest as possible.

SEXUAL ACTIVITY AND FUNCTION

21. What is your current relationship status?

- o Single
- o Dating
- o Living with a de-facto partner
- o Married/civil union
- o Separated
- o Divorced
- o Widowed

22. How do you feel about the future of your current relationship?

- o Not applicable
- o I feel confident
- o I feel hopeful
- o I feel uncertain
- o I doubt it will last
- o I don’t know

23. Have you had sexual intercourse in the past month?

- o Yes
- o No

24. I usually have sexual intercourse:

- o Never
- o Less than once a year
- o Less than once a month
- o Once a month
- o A few times a month
- o Once a week
- o A few times a week
- o Once a day
- o A few times a day
- o I don’t know

25. I think about sex:

- o Never
- o Less than once a year
- o Less than once a month
- o Once a month
- o A few times a month
- o Once a week
- o A few times a week
- o Once a day
- o A few times a day
- o Every 5 minutes
- o I don't know

26. If I were to spend the rest of my life with my sexual function the way it is today, I would feel:

- o Dissatisfied
- o Somewhat dissatisfied
- o Neither satisfied nor dissatisfied
- o Somewhat satisfied
- o Extremely satisfied

27. Do you suffer from premature ejaculation (when orgasm comes too quickly and reduces sexual satisfaction)?

- o Yes
- o No

28. Do you suffer from delayed ejaculation (when orgasm is delayed or absent and reduces sexual satisfaction) ?

- o Yes
- o No

29. Have you ever been diagnosed with erectile dysfunction (the inability to achieve or maintain an erection sufficient for satisfactory sexual performance) by a medical practitioner? Note: Erectile dysfunction does not include premature ejaculation.

- o Yes
- o No

30. Are you currently using any of the following treatments for erectile dysfunction? (Please select as many as apply)

- o Prescription oral medications such as Viagra, Cialis or Levitra
- o Non-prescription oral medications
- o **S**elf-injection or penile insertion of a drug
- o Psychological counselling
- o Vacuum pump devices
- o Rigid or inflatable surgical penile implants
- o Testosterone replacement
- o Natural or herbal remedies
- o None of the above

31. Erectile dysfunction (sometimes called impotence) means being unable to get and keep an erection that is rigid enough for satisfactory sexual activity. In your opinion are you:

- o Not impotent - *Always* able to get and keep an erection good enough for sexual intercourse
- o Minimally impotent - *Usually* able to get and keep an erection good enough for sexual intercourse
- o Moderately impotent - *Sometimes* able to get and keep an erection good enough for sexual intercourse
- o Completely impotent - *Never* able to get and keep an erection good enough for sexual intercourse

32. Over the past six months:

	1	2	3	4	5
How do you rate your confidence that you can get and keep an erection?	Very low	Low	Moderate	High	Very high
With sexual stimulation, how often have your erections been hard enough for penetration (entering your partner)?	Almost never/Never	A few times (Much less than half the time)	Sometimes (About half the time)	Most of the time (Much more than half the time)	Almost always/Always
During sexual intercourse, how often were you able to maintain your erection after penetration?	Almost never/Never	A few times (Much less than half the time)	Sometimes (About half the time)	Most of the time (Much more than half the time)	Almost always/Always
During sexual intercourse, how difficult has it been to maintain your erection until completion of intercourse?	Extremely difficult	Very difficult	Difficult	Slightly difficult	Not difficult
When you attempted sexual intercourse, how often was it satisfactory to you?	Almost never/Never	A few times (Much less than half the time)	Sometimes (About half the time)	Most of the time (Much more than half the time)	Almost always/Always

33. Erectile dysfunction is the inability to achieve or maintain an erection sufficient for satisfactory sexual performance. Please read each item and place a tick in the box opposite the reply which comes closest to how you feel.

	1	2	3	4	5
Erectile dysfunction is an inevitable part of ageing	o Disagree	o Somewhat disagree	o Neither agree nor disagree	o Somewhat agree	o Agree
Erectile dysfunction is something men just have to accept	o Disagree	o Somewhat disagree	o Neither agree nor disagree	o Somewhat agree	o Agree
I feel uncomfortable talking about erectile dysfunction	o Disagree	o Somewhat disagree	o Neither agree nor disagree	o Somewhat agree	o Agree
It would be helpful if men felt more comfortable talking about erectile dysfunction	o Disagree	o Somewhat disagree	o Neither agree nor disagree	o Somewhat agree	o Agree
If I thought a prescription drug could improve my erectile function, I would take it	o Disagree	o Somewhat disagree	o Neither agree nor disagree	o Somewhat agree	o Agree
If I thought my diet affected my erectile function, I would change my diet	o Disagree	o Somewhat disagree	o Neither agree nor disagree	o Somewhat agree	o Agree
If I thought a dietary supplement could improve my erectile function, I would take it	o Disagree	o Somewhat disagree	o Neither agree nor disagree	o Somewhat agree	o Agree
I am interested in learning more about how to prevent erectile dysfunction/improve my erectile function	o Disagree	o Somewhat disagree	o Neither agree nor disagree	o Somewhat agree	o Agree

Thank you. You have finished this section. Next, you will be asked questions about your DIET over the last 3 months. Remember your answers are anonymous, private and confidential and will not be associated with you as an individual. Please be as open and honest as possible.

DIET

MILK

34. How often do you consume milk? (Include milk as a drink, added to tea, coffee, cereal, pudding, milk based sauces, milk puddings etc.)

- o Never
- o Less than once per month
- o 1 to 3 times per month
- o Once per week
- o Twice per week
- o 3 to 4 times per week
- o 5 to 6 times per week
- o Once a day or more

35. When you do consume milk, how much do you usually consume per day? (Include milk as a drink, added to tea, coffee, cereal, pudding, milk based sauces, milk puddings etc.)

- o I don't consume milk
- o 1 tablespoon
- o 2 tablespoons
- o 62.5 ml (1/4 cup)
- o 125 ml (1/2 cup)
- o 250 ml (1 cup)
- o Between 250 and 500 ml (1-2 cups)
- o Between 500 and 750 ml (2-3 cups)
- o 750 ml (3 cups) or more

36. When you do consume milk, what type do you usually use?

- o I don't consume milk
- o Regular milk – please specify brand and type:

OPTIONS: Full cream/Farmhouse/Silver top/Grey top, Full fat/Standard/Blue top, Reduced fat/Lite/Light blue top, Fully skimmed/Trim/ Extra trim/Super trim/Green top

- o Vitamin and mineral enriched milk. Please specify brand:

OPTIONS: Anchor Mega Milk/Orange top, Anchor Xtra milk/Yellow top, Anchor Vital Milk UHT with Omega, Anchor Pre-Bio Milk, Meadow Fresh Calci-trim/Yellow top, Meadow Fresh Calci Strong, Meadow Fresh Smart Choice, Meadow Fresh Pre-Bio Milk, Sun Latte Milk, Pams Calci Smart, Anlene Milk Drink

- o Organic Milk
- o Soy Milk
- o Vitamin enriched Soy Milk. Please specify brand:

OPTIONS: Homebrand, Signature Range, Get Natural, Sanatarium So Good, Vitasoy

- o Rice Milk
- o Vitamin enriched Rice Milk. Please specify brand:

OPTIONS: Get Natural, Imagine Rice Dream, Sanatarium So Good, Vitasoy

- o Butter Milk
- o Flavoured Milk
- o Vitamin enriched flavoured milk i.e. Calcistrong, Mega milk
- o Other *please specify brand and type:*

YOGHURT

37. How often do you consume yoghurt? (Include yoghurt eaten with cereal, yoghurt smoothies etc.)

- o Never
- o Less than once per month
- o 1 to 3 times per month
- o Once per week
- o Twice per week
- o 3 to 4 times per week
- o 5 to 6 times per week
- o Once a day or more

38. When you do consume yoghurt, how much do you usually consume per day? (Include yoghurt eaten with cereal, yoghurt smoothies etc.)

- o I don't consume yoghurt
- o 1 tablespoon
- o 2 tablespoons
- o 62.5 g (1/4 cup)
- o 125 g (1/2 cup)
- o 250 g (1 cup)
- o Between 250 and 500 g (1-2 cups)
- o Between 500 and 750 g (2-3 cups)
- o 750 g (3 cups) or more

39. When you do consume yoghurt, what type do you usually use?

- o I don't consume yoghurt
- o Fresh n Fruity Superfruits, Lite, Simply Strawberry or Vanilla and Hazelnut
- o Meadowfresh Live Lite
- o Symbio
- o Petit Miam or Calci-Yum
- o Other *please specify brand and flavour*:

CHEESE

40. How often do you consume cheese? (Include cheese eaten alone, with crackers, grilled or included in a pie, pizza etc.)

- **o** Never
- **o** Less than once per month
- **o** 1 to 3 times per month
- **o** Once per week
- **o** Twice per week
- **o** 3 to 4 times per week
- **o** 5 to 6 times per week
- **o** Once a day or more

41. When you do consume cheese, how much do you usually consume per day? (Include cheese eaten alone, with crackers, grilled or included in a pie, pizza etc.)

- o I don't eat cheese
- o 1 tablespoon
- o 2 tablespoons
- o 62.5 g (1/4 cup)
- o 125 g (1/2 cup)
- o 250 g (1 cup)
- o Between 250 and 500 g (1-2 cups)
- o Between 500 and 750 g (2-3 cups)
- o 750 g (3 cups) or more

42. When you do consume cheese, what type do you usually use?

- o I don't eat cheese
- o Kraft Cheesy Pops
- o Kraft Singles (processed cheese slices)
- o Other *please specify brand and type:*

MARGARINE/SPREAD

43. How often do you consume margarine/spread?

- o Never
- o Less than once per month
- o 1 to 3 times per month
- o Once per week
- o Twice per week
- o 3 to 4 times per week
- o 5 to 6 times per week
- o Once a day or more

44. When you do consume margarine/spread, how much do you usually consume per day?

- o I don't use margarine/spread
- o 1/2 tablespoon
- o 1 tablespoon
- o 2 tablespoons
- o 3 tablespoons
- o 4 tablespoons
- o 5-7 tablespoons
- o 8 or more tablespoons

45. When you do consume margarine/spread, what type do you usually use?

- o I don't use margarine/spread
- o Butter
- o Gold n Canola spread (canola, canola lite)
- o Logicol
- o Flora Buttery Taste
- o Flora spread (original, light, reduced salt, ultra-light, pro-activ, olive oil)
- o Homebrand Table Spread
- o Weight watchers spread (canola)
- o Meadowlea spread (original, canola, light, logical, logical lite, low salt)
- o Olivio spread (bertolli, virgin bertolli, light bertolli)
- o Olivani spread (avocado, extra virgin, lite)
- o Alfa one spread rice bran oil spread
- o Anchor spreadable spread lite
- o Constantia spread garlic margarine
- o Tararua spread (semi soft lite, supersoft)
- o Countrysoft blend 50/50 butter and margarine
- o Sunrise spread
- o Ceres butter (almond, cashew)
- o Olive or Flaxseed/Linseed Oil
- o Other *please specify brand and type:*

FATS AND OILS

46. How often do you consume oil/butter/lard? (Include oil/butter/lard used in cooking etc. but not used as a spread)

- o Never
- o Less than once per month
- o 1 to 3 times per month
- o Once per week
- o Twice per week
- o 3 to 4 times per week
- o 5 to 6 times per week
- o Once a day or more

47. When you do consume oil/butter/lard, how much do you usually consume per day? (Include oil/butter/lard used in cooking etc. but not used as a spread)

- o I don’t use oil/butter/lard
- o 1/2 tablespoon
- o 1 tablespoon
- o 2 tablespoons
- o 3 tablespoons
- o 4 tablespoons
- o 5-7 tablespoons
- o 8 or more tablespoons

48. When you do consume oil/butter/lard, what type do you usually use?

- o I don't use oil/butter/lard
- o Butter
- o Ghee
- o Lard, dripping, shortening, kremelta
- o Olive oil
- o Flaxseed/linseed oil
- o Canola oil
- o Soybean oil
- o Salad and cooking oil
- o Sunflower, Safflower, Corn, Cottonseed or Grapeseed Oil
- o Rice bran oil
- o Avocado oil
- o Almond, peanut, macadamia or sesame seed oil
- o Vegetable oil
- o Other *please specify brand and type:*

EGGS

49. How often do you consume eggs? (Include whole eggs, omelettes, slices of quiche etc.)

- o Never
- o Less than once per month
- o 1 to 3 times per month
- o Once per week
- o Twice per week
- o 3 to 4 times per week
- o 5 to 6 times per week
- o Once a day or more

50. When you do consume eggs, how many eggs do you usually eat in a day (Include whole eggs, omelettes, slices of quiche etc)

- o I don't eat eggs
- o 1 egg per day
- o 1 to 2 eggs per day
- o 2 eggs per day
- o 3 to 4 eggs per day
- o 5 or more eggs per day

51. When you do consume eggs, what type do you usually use?

- o I don't eat eggs
- o Normal chicken eggs
- o Free range chicken eggs
- o Organic chicken eggs
- o Duck eggs
- o Other *please specify:*

CANNED FISH

52. How often do you eat canned fish? (Include tuna, salmon, sardines, mackerel, herring, anchovies etc.)

- o Never
- o Less than once per month
- o 1 to 3 times per month
- o Once per week
- o Twice per week
- o 3 to 4 times per week
- o 5 to 6 times per week
- o Once a day or more

53. When you eat canned fish, how much do you usually eat per day? (Include tuna, salmon, sardines, mackerel, herring, anchovies etc.)

- o I don't eat canned fish
- o Less than a small can (95g)
- o A small can (95g)
- o Between a small can and a medium can
- o A medium can (125g)
- o Between a medium can and a large can
- o A large can (210g)
- o More than a large can

54. When you consume canned fish, what type do you usually eat?

- o I don't eat canned fish
- o Tuna (flavoured, in brine, in oil, in spring water)
- o Pink Salmon
- o Red Salmon
- o Sardines (flavoured, in oil, spring water)
- o Mackerel (flavoured, in oil)
- o Kippered Herring
- o Fish Fillets Smoked
- o Anchovies
- o Other *please specify:*

FRESH & FROZEN FISH

55. How often do you eat fresh or frozen fish? (Include fish meals, takeaway fish, sushi, fishcakes etc.)

- o Never
- o Less than once per month
- o 1 to 3 times per month
- o Once per week
- o Twice per week
- o 3 to 4 times per week
- o 5 to 6 times per week
- o Once a day or more

56. When you eat fresh or frozen fish, how much do you usually eat? (Include fish meals, takeaway fish, sushi, fishcakes etc.)

- o I don't eat fresh or frozen fish
- o Less than one fillet (<100g)
- o One fillet (100g)
- o One to two fillets (100-200g)
- o Two fillets (200g)
- o Two to three fillets (200-300g)
- o More than three fillets (>300g)

57. When you consume fresh or frozen fish, what type do you usually eat?

- o I don't eat fresh or frozen fish
- o Gurnard
- o Snapper
- o Lemon Fish
- o Kippers
- o Hoki
- o Hapuka
- o John dory
- o Cod
- o Terakihi
- o Basa
- o Flounder
- o Salmon
- o Tuna
- o Trout
- o Whitebait
- o Eel
- o Processed fish (unknown)
- o Other *please specify:*

LIVER

58. How often do you eat liver?

- o Never
- o Less than once per month
- o 1 to 3 times per month
- o Once per week
- o Twice per week
- o 3 to 4 times per week
- o 5 to 6 times per week
- o Once a day or more

59. Please specify the number of slices usually eaten on each occasion:

_____ (1 slice = 20g)

60. When you consume liver, what type do you usually eat?

- o Beef liver
- o Lamb liver
- o Chicken liver
- o Other *please specify:*

OTHER FORTIFIED PRODUCTS

61. Do you take any vitamin D supplements? If so, please provide the name of the supplement, the amount of vitamin D (IU) contained in it, how many capsules you take and how often in the space provided. If not, please leave blank.

62. Do you consume any other vitamin D enriched food that is not covered in this questionnaire? If so, please provide the detail, the amount in grams and how often you consume this product in the space provided. If not, please leave blank

CHANGE OF DIET

62. Have you changed your diet in any significant way over the past year?

- o Yes
- o No

If so, please explain the change and the reasons for making this change:

Thank you. You have finished the section on your dietary habits. Finally I will ask you about your BACKGROUND. Remember your answers are anonymous, private and confidential and will not be associated with you as an individual. Please be as open and honest as possible.

BACKGROUND INFORMATION

63. What age are you?

- o 40-49 years
- o 50-59 years
- o 60-69 years
- o 70 years or older

64.* Which ethnic group do you belong to? *Mark the space or spaces which apply to you.

- o New Zealand Maori
- o New Zealand European *or* Pakeha
- o Other European such as English, Scottish, Irish, Dutch, Australian
- o Please state:
- o Samoan
- o Cook Island Maori
- o Tongan
- o Niuean
- o Chinese
- o Indian
- o Other such as Fijian, Korean
- o Please state:

65. What is your highest educational qualification?

- o No formal qualifications
- o NZ School Certificate *or* overseas equivalent
- o NZ Sixth Form Certificate *or* University Entrance before 1986 *or* overseas equivalent
- o NZ Higher School Certificate *or* Higher Leaving Certificate *or* NZ University Bursary/Scholarship *or* overseas equivalent
- o Post secondary school qualification (e.g. Trade Certificate)
- o Undergraduate qualification (e.g. Certificate or Diploma)
- o Graduate qualification (e.g. Bachelors or Honors Degree)
- o Post-graduate qualification (e.g. PG Diploma or Master's Degree)

66. What is your current employment status?

- o I am self-employed
- o I am a full-time employee
- o I am a part-time employee
- o I am not employed but I am seeking work – *go to question 69*
- o I am not employed and I am not seeking work – *go to question 69*

67. Under which of the following categories is your main occupation?

- o Managers
- o Professionals
- o Technicians and Trades Workers
- o Community and Personal Service Workers
- o Clerical and Administrative Workers
- o Sales workers
- o Machinery Operators and Drivers
- o Labourers

68. **What is your main occupation?** ***For example, plumber, builder, farmer, teacher, nurse, scientist, computer technician.***

Please state:

69. How many hours, to the nearest hour, do you usually work each week in the above occupation?

Please state: hrs

70. What is your average household income per year before tax?

- o $0-19,999
- o $20,000-39,999
- o $40,000-59,999
- o $60,000-79,999
- o $80,000-99,999
- o $100,000-$119,999
- o $120,000+

71. Do you live in a rural (country) or urban (city/town) environment?

- o Urban
- o Rural
- o Semi-rural

You have finished the questionnaire. Thank you for your time!
If you have any concerns regarding your sexual function or any aspect of your personal health, please contact your GP or local sexual health clinic.