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The role of vitamin D in metabolism and bone health

A thesis presented in partial fulfillment of the
requirements for the degree of

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Abstract

Background

Hypovitaminosis D is becoming recognised as an emerging threat to health, even in countries like New Zealand which enjoy plentiful sunshine. The evidence for a role for vitamin D deficiency in the aetiology of a plethora of diseases continues to accumulate, including type 2 diabetes, and the preceding insulin resistance.

Objectives

The primary objective of the Surya Study was to investigate the effect of improved vitamin D status (through supplementation) on insulin resistance. The secondary objectives were to investigate the vitamin D status and bone mineral density of South Asian women living in New Zealand, and to investigate the effect of vitamin D supplementation on bone turnover as measured by biochemical markers of bone resorption and formation.

Method

Women of South Asian origin, ≥ 20 years old, living in Auckland ($n = 235$) were recruited for the study. All were asked to complete a 4-day food diary, invited to have a bone scan, and were screened for entry into the intervention phase which required insulin resistance (HOMA-IR > 1.93) and serum 25(OH)D < 50 nmol/L.

Eighty-one completed a 6-month randomised controlled trial with 4000 IU vitamin D3 ($n = 42$) or placebo ($n = 39$). Primary endpoint measures included insulin resistance, insulin sensitivity (HOMA2%S), fasting C-peptide and markers of bone turnover, osteocalcin (OC) and collagen C-telopeptide (CTX). Ninety-one of the 239 had a bone scan and bone mineral density (BMD) was measured in the proximal femur and lumbar spine.

Results

Adequate serum 25(OH)D concentrations (> 50 nmol/L) were observed in only 16% of subjects screened. Median (25th, 75th percentile) serum 25(OH)D increased significantly from 21 (11,40) to 75 (55,84) nmol/L with supplementation. Significant improvements were seen in insulin sensitivity and insulin resistance ($P = 0.003$, $P = 0.02$ respectively), and circulating serum insulin decreased ($P = 0.02$) with supplementation compared to placebo. There was no change in C-peptide with supplementation. Insulin resistance was most improved when endpoint serum

25(OH)D \geq 80 nmol/L. In post-menopausal women OC and CTX levels increased in the placebo arm but CTX decreased from 0.39 ± 0.15 to 0.36 ± 0.17 ($P = 0.012$) with supplementation. Osteoporosis (T score < -2.5) was present in 32% of postmenopausal, and 3% of premenopausal women. Women 20 – 29 years ($n=10$) had very low BMD, calcium intake and serum 25(OH)D

Conclusions

Improving vitamin D status in insulin resistant women resulted in improved insulin resistance and sensitivity but no change in insulin secretion. Optimal 25(OH)D concentrations for reducing insulin resistance were shown to be ≥ 80 nmol/L. The prevalence of low 25(OH)D concentrations in this population was alarmingly high, especially in younger women. In post-menopausal women, vitamin D supplementation appeared to ameliorate increased bone turnover attributed to oestrogen deficiency.

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Abbreviations

1 α OHase	1 α -hydroxylase
1,25(OH) ₂ D ₃	1 α ,25-dihydroxyvitamin D ₃ or calcitriol
24OHase	24-hydroxylase
25(OH)D ₃	25-hydroxyvitamin D ₃
25OHase	25-hydroxylase
ALP	Alkaline phosphatase
ATP	Adult Treatment Panel
BMD	Bone mineral density
BMI	Body mass index
BTM	Bone turnover markers
CaSR	Calcium sensing receptors
CMDHB	Counties Manukau District Health Board
CRP	C-reactive protein
CTX	Cross-linked telopeptide
CVD	Cardiovascular disease
DBP	Vitamin D-binding protein
DXA	Dual energy x-ray absorptiometry
EDTA	Ethylene diamine tetraacetic acid
ER	Endoplasmic reticulum
FPG	Fasting plasma glucose
FPI	Fasting plasma insulin
HDL	High-density lipoprotein
HGO	Hepatic Glucose Output
HOMA	Homeostasis assessment model
HOMA2-IR	HOMA2-Insulin Resistance
IDF	International Diabetes Institute
IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
IL-6	Interleukin 6
IR	Insulin receptor
IRS-proteins	Insulin receptor substrate proteins
IVGTT	Intra-venous glucose tolerance test

K _{ATP}	Potassium channels
KO	Knock out
MED	Minimal erythematous dose
MoH	Ministry of Health
NCEP	National Cholesterol Education Programme
NCX-1	Na ⁺ /Ca ²⁺ exchanger
NEFAs	Non-esterified fatty acids
NGT	Normal glucose tolerance
NHANES	National health and Nutrition Examination Survey
NIDDM	Non-insulin dependant diabetes
OC	Osteocalcin
OGIS	Oral glucose insulin sensitivity
OGTT	Oral glucose tolerance test
OPG	Osteoprotegerin
PI3-kinase	Phosphatidylinositol 3-kinase
PICP	Procollagen type 1 C-terminal
PMCA	Plasma membrane calcium ATPase
PTH	Parathyroid hormone
PTHR	PTH receptor
PTHrP	PTH related protein
RANK	Receptor activator nuclear factor- κ B
RANKL	Receptor activator nuclear factor- κ B ligand
RCT	Randomised controlled trial
RDI	Recommended daily intake
RXR	Retinoid X receptor
TNF	Tumour necrosis factor
TRP	Transient receptor potential
UV	Ultraviolet
UVB	Ultraviolet beta radiation
UVR	Ultraviolet radiation
VDR	Vitamin D receptor
VDRE	Vitamin D response element
WHO	World Health Organization

CHAPTER 1



Introduction
Justification for study
Study objectives



Introduction

The sun is the source of life on Earth. It provides us with heat, light, and food via photosynthesis of energy in plants. It is also the source of vitamin D, an essential hormone, the production of which begins when ultra violet- β radiation from the sun penetrates our skin.

The migration of early man from the equatorial regions of his origin to the cooler climates of Northern Europe resulted in the devolution of melanin, the dark pigment in the skin which acts as a natural sunscreen. Those with lighter skin were better able to synthesise vitamin D in conditions of lower ultraviolet radiation (UVR), thus avoiding the pelvic deformities associated with vitamin D deficiency and the subsequent poor birth outcomes (Jablonski et al. 2000; Hollis 2005). Meanwhile, the high concentration of melanin in the dark skin of humans at low latitudes provided protection against the strong UVR in that environment. This reduced the risk of cutaneous damage and prevented the photo-degradation of important micronutrients such as folate, low levels of which are implicated in neural tube defects (Jablonski et al. 2000).

In recent centuries, as mankind has become more transient, these well-tuned evolutionary adjustments have become less advantageous. The slave trade initiated the relocation of dark-skinned Africans from an environment of high UVR, warm temperatures and few clothes to the higher latitudes of North America with lower UVR, cooler temperatures and a requirement for modesty of dress. Now black Americans are exhibiting serum 25(OH)D levels 40 – 50% lower than their white countrymen in both winter and summer (Harris et al. 1998; Bodnar et al. 2007). Meanwhile, recent immigration from Sub-Saharan Africa and the Middle East is resulting in populations of people with very dark skins or covering for religious reasons in Europe (Carling 2007), Britain and Australia (Pettifor 2008). It has been estimated that to achieve optimum vitamin D levels through sun exposure, a deeply-pigmented African can take up to 10 times longer than a Caucasian (Hollis 2005).

European colonialism began a continuing trend of fair-skinned families from Britain and northern Europe immigrating to southern hemisphere countries such as New

Zealand and Australia. Their fair skin has little in-built protection from the high summer UVR and now these two countries have the highest rates of skin cancer in the world (Salmon et al. 2007). Nearly 100 years ago Australian dermatologist Norman Paul noted that, “the common occurrence of these cancerous diseases . . . is to be regarded as one of the penalties to be paid for inhabiting a country normally destined (in geographical location) to be occupied by a coloured race” (Albert et al. 2002). The modern response has been the development, over the past 2 decades, of public education campaigns which advocate avoidance of sun exposure altogether (Albert et al. 2003). As the public pays more attention to these messages, so the potential for population-wide hypovitaminosis D increases.

Concurrent with the increasing incidence of hypovitaminosis D has been an exponential growth in our knowledge of the importance of vitamin D in many aspects of health including its traditional role in bone health. Recent research has shown relationships between vitamin D status and cancer (Grant 2002), auto-immune diseases including type 1 diabetes (Harris 2005), cardiovascular disease (Wang et al. 2008) and type 2 diabetes (Scragg et al. 1995b).

In New Zealand the Maori and Pacific people with their light to medium brown skin are exhibiting a greater incidence of vitamin D deficiency than the Caucasian population (Rockell et al. 2006), and the rapidly expanding populations of migrants from Asia and the Indian subcontinent (Statistics New Zealand 2006) are also likely to be at risk due in part to their skin colour, and also to sun-avoidance behaviours. To date there is no evidence of this, apart from anecdotal descriptions from Indian General Practitioners in Auckland of vitamin D deficiency in their patients and an over-representation of South Asian children being diagnosed with rickets (Blok et al. 2000).

In 2006 the New Zealand Ministry of Health (MoH) published a study reporting the health status of Asian and South Asian people living in New Zealand (Ministry of Health 2006). The incidence of type 2 diabetes and cardiovascular disease in South Asian Indians was markedly higher (3 times and 2 times respectively) than the general population. Nine percent of South Asian people who were surveyed reported having been diagnosed with type 2 diabetes. The MoH predicts that only half of the total number of cases of type 2 diabetes in the New Zealand population

have been diagnosed (Ministry of Health 2009). If this is true, it is possible that 18% of the South Asian adult population in New Zealand has the disease.

Since the identification of the vitamin D receptor in chick pancreatic tissue (Christakos et al. 1979) and subsequently in human pancreatic β -cells (Johnson et al. 1994), the role of vitamin D in the development of type 2 diabetes has stimulated scientific interest. One of the earlier studies to find a relationship between glucose intolerance and vitamin D deficiency was conducted in South Asian populations in Britain (Boucher et al. 1995). Meanwhile, in New Zealand, Scragg et al (1995b) found that cases of newly diagnosed type 2 diabetes and impaired glucose tolerance had significantly lower serum 25(OH)D than matched controls. Subsequently evidence has continued to accumulate and is discussed in greater depth in the following chapter.

Immigration from the Indian sub-continent and Sri Lanka almost doubled between the 2001 and 2006 censuses, and numbered 113,000 in 2006. Unless there is a major change in New Zealand's immigration policy in the near future, the number of New Zealand residents of South Asian origin will probably continue to increase at a similar rate to that which we have seen over the past 5 years. These new residents are already demonstrating a tendency toward health problems which are going to impose high costs on the New Zealand health budget.

Sufficient evidence now exists to justify investigations into both the vitamin D status of this group, and the possibility that poor vitamin D status is increasing the risk of diseases to which they are already genetically predisposed. If an improvement in vitamin D status can be shown to ameliorate the risk of diabetes, cardiovascular disease or osteoporosis in this population, even to a small degree, it is important that this be investigated. When compared to the cost of treating diabetes or osteoporosis, supplementation with vitamin D is a very cost-effective prophylactic.

Study objectives

The ***Surya Study*** commenced in Auckland, New Zealand in February 2007. The name, *Surya*, is the Sanskrit and Hindu name for the Sun Goddess.

The primary objective of this study was to conduct a double-blind, randomised controlled trial (RCT) with vitamin D supplementation in South Asian women who are vitamin D deficient and insulin resistant. We aimed to assess the effect of improved vitamin D status on insulin resistance and other markers of metabolic syndrome which are associated with increased risk of type 2 diabetes and CVD. At the time of conception of this study, there had been no trials of this nature reported. Subsequently one RCT with supplemental vitamin D has been published. Coincidentally the subject group were Indian men, but they were not insulin resistant and vitamin D deficiency was not an inclusion criteria (Nagpal et al. 2009). Therefore, the Surya Study remains the first RCT with vitamin D supplementation in subjects who are insulin resistant and vitamin D deficient.

Because vitamin D plays such an important role in bone health and the maintenance of bone mineral density, secondary objectives were developed to a) investigate the bone mineral density of the group of women recruited for the study, and b) to investigate the effect of vitamin D supplementation on markers for bone formation and resorption. To date only one other RCT has supplemented with vitamin D alone and measured bone markers – the subjects were 11 year-old girls.

The screening phase of the study provided the opportunity to investigate the vitamin D status, as well as a number of other health and lifestyle factors, in a group of 235 South Asian women living in Auckland. This was the first descriptive study of this nature to be conducted in this relatively new migrant population and although participants were self-selected the study has provided some important insights.

Primary Outcome

- Changes in vitamin D status, insulin resistance (homeostasis model assessment), lipid profiles including total cholesterol, high-density lipoprotein (HDL) cholesterol and triglycerides, in response to vitamin D supplementation. Chapter 5.

Secondary outcomes

- Investigation of the screening population including vitamin D status, lipid profiles, diet and physical activity, attitudes and behaviours around sun exposure. Chapter 4.
- Changes in markers of bone turnover (osteocalcin and c-telopeptide), parathyroid hormone and serum calcium in response to vitamin D supplementation. Chapter 6.
- Investigation of the bone mineral density (BMD) of the study population, together with vitamin D status and calcium intake as assessed by dietary analysis. Chapter 7.

Hypotheses

Hypothesis 1: That women of South Asian origin living in Auckland, New Zealand will have low vitamin D status

Hypothesis 2: That supplemental vitamin D will improve the vitamin D status, and subsequently reduce insulin resistance, in women who are insulin resistant and have low vitamin D

Hypothesis 3: That increasing serum 25(OH)D will reduce bone turnover and bone resorption, and increase bone formation in women who have low vitamin D status

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CHAPTER 2



Review of the Literature



Vitamin D

History of vitamin D

We have known for over 100 years that sunlight is required for bone health. In 1822, Sniadecki hypothesised that the devastating childhood bone disease, rickets, was associated with deprivation of sunlight following observations that urban children in Warsaw developed the disease but children living in rural areas did not (Holick 2004). In the early 20th century, Sir Edward Mellanby cured rickets in dogs by giving them cod liver oil (Oxbury 1985). In 1919, Huldschinski demonstrated that exposing children to a mercury arc lamp for one hour 3 times a week was an effective treatment for rickets (Holick 2006).

Around the same time, in New York, Hess and Unger (1922) exposed children and experimental rats with rickets to sunshine and demonstrated a cure. Eventually, vitamin D was isolated and identified as an essential micronutrient, but it was not until 1963 that the most biologically active metabolite of vitamin D, 1 α ,25-dihydroxyvitamin D₃ (calcitriol or 1,25(OH)₂D₃) was discovered (Brown et al. 1999), and vitamin D was subsequently established as a secosteroid with the classic role of regulating bone metabolism and calcium homeostasis.

Metabolism of vitamin D

Calcitriol is generated from sequential hydroxylations of vitamin D₃ (fig. 1B), a micronutrient which is synthesised when unprotected skin is exposed to sunlight (Holick et al. 1980). The ultraviolet- β (UVB) radiation in sunlight penetrates the skin and causes the conversion of 7-dehydrocholesterol to pre-vitamin D (fig. 1A). The sterol 7-dehydrocholesterol is always present in the skin and is found throughout the layers of the epidermis and, to a lesser extent, in the dermis as well (Holick et al. 1980). Once formed, pre-vitamin D immediately begins to isomerise into vitamin D₃ in a temperature-dependent manner which can continue for up to three days following one exposure of UVB (Holick et al. 1980). The newly formed vitamin D₃ diffuses into the circulatory system and is bound to vitamin-D binding protein (DBP).

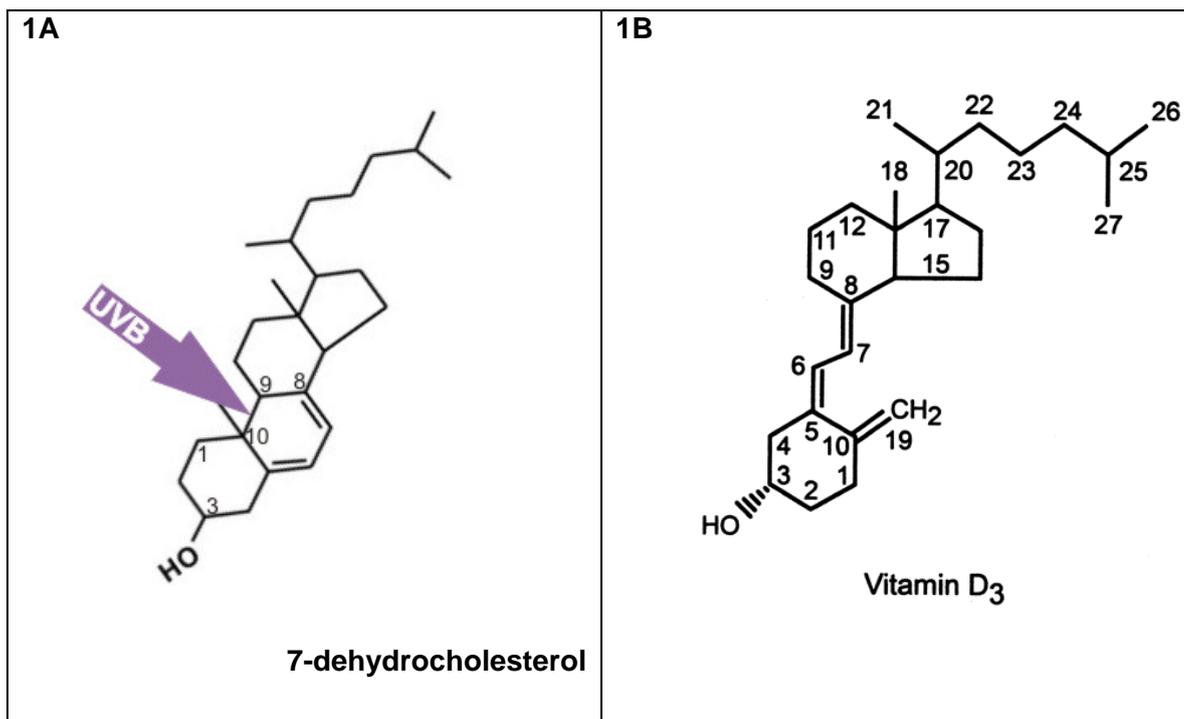


Figure 1A: Ultra violet- β radiation cleaves 7-dehydrocholesterol between carbon 9 and 10, forming previtamin D₃. Heat-induced isomerisation occurs almost immediately, and vitamin D₃ is formed.

Figure 1B: The structure of vitamin D₃ and its numbering system (DeLuca 2004)

The first hydroxylation takes place in the liver (fig. 2), catalysed by vitamin D-25-hydroxylase, producing the major circulating form, 25-hydroxyvitamin D₃ (25(OH)D₃) (DeLuca 2004). The second hydroxylation was originally thought to take place only in the kidney. Here the activity of the second hydroxylase, renal 25(OH)D₃-1 α -hydroxylase (1 α OHase), is stimulated by parathyroid hormone (PTH) resulting in the production of the active metabolite 1 α ,25(OH)₂D₃ or calcitriol.

Serum levels of calcitriol are controlled by a negative feedback system which reduces the expression of 1 α OHase, in response to high local levels of calcitriol. At the same time, the high calcitriol levels promote the expression of the hydroxylase enzyme 1,24-hydroxylase which is responsible for commencing the degradation of calcitriol. Thus the hormone has the intrinsic ability to programme its own destruction via a positive feedback loop which results in increased transcription of 1,24-hydroxylase, as well as a negative feedback system (Jones et al. 1998).

Calcitriol is so tightly regulated that measurement of circulating calcitriol is not a good measure of vitamin D status. Rather 25(OH)D (includes 25(OH)D₃ and 25(OH)D₂ as most assays do not differentiate between the two) which is the major circulating form and is more subject to variability of sun exposure or dietary intake, is a better indicator of vitamin D status.

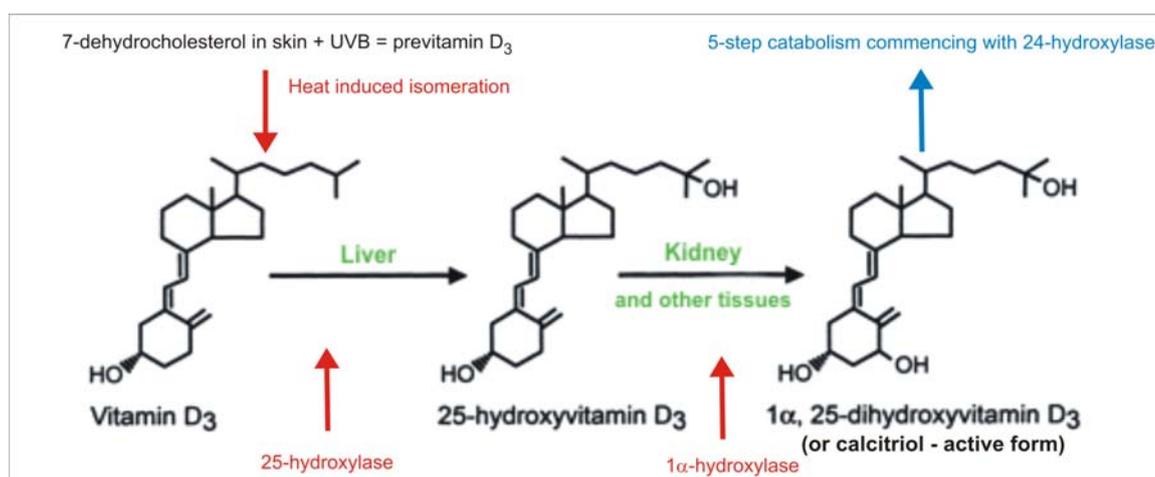


Figure 2: The metabolic activation of vitamin D₃ to the bioactive metabolite 1,25-dihydroxyvitamin D₃ or calcitriol involves the hydroxylation of carbon 25, then carbon 1 (see fig.1B for numbering) (adapted from DeLuca (2004))

Endogenous synthesis of pre-vitamin D and vitamin D₃ in response to UVB exposure does have in-built controls. There appears to be an equilibrium reaction in the conversion of pre-vitamin D to vitamin D₃, allowing the skin to act as a storage facility for up to 3 days following exposure to sunlight (Holick et al. 1980). Excessive exposure to UVB radiation results in the conversion of pre-vitamin D to biologically inactive lumesterol or tachysterol, reducing the amount of vitamin D₃ produced (Hollis 2005), and excess sunlight also results in the photodegradation of vitamin D₃ into inert photoproducts (Holick 2007). Thus a number of mechanisms exist to ensure the prevention of 25(OH)D₃ toxicity as a result of sun exposure.

It is well recognised that a number of variables are involved in determining the amount of vitamin D₃ that will be generated in human skin due to sun exposure. Skin pigmentation, age, season, latitude and protection from clothes or sun screen all play a role, making a determination of a level of required exposure very difficult. The concept of the minimal erythral dose or MED is a useful guide (Clemens et al. 1982). This is the least amount of exposure required to cause an erythral response (slight pinkening of the skin). A whole-body MED has been shown to

release 10,000 to 20,000 IU of vitamin D₃ into the circulation within 24 hours of exposure, and circulating levels of 25(OH)D in people receiving unlimited sun exposure range from 165 nmol/L to 225 nmol/L (Hollis 2005). When considered in light of the above, the currently accepted level of adequacy in New Zealand of 50 nmol/L (Working Group of the Australian and New Zealand Bone and Mineral Society et al. 2005), and even the 75-80 nmol/L minimum advocated by a number of scientists working in vitamin D research (Vieth et al. 2007; Melamed et al. 2008) could be considered potentially inadequate.

In the absence of sun exposure and endogenous synthesis of vitamin D, humans rely on dietary sources or supplementation. In New Zealand the recommended daily intake (RDI) for vitamin D is 200 IU from infancy through to adulthood, 400 IU for 51 – 70 year olds, and 600 IU for 70+ years (Commonwealth Department of Health and Ageing et al. 2006). It has been clearly demonstrated that this level of supplementation, if not augmented by sun exposure, will have little if any effect on circulating 25(OH)D concentrations (Heaney et al. 2003). Whilst there had been historical concerns about safe upper levels of supplementation, and although there is some evidence in mice that vitamin D toxicity can occur with high doses of calcitriol (>0.25 µg/kg/day) (Zittermann et al. 2008), strong evidence now exists to support supplemental doses of vitamin D, 2000 – 4000 IU/day, in the absence of adequate endogenous synthesis (Hollis 2005; Hathcock et al. 2007).

There are very few sources of dietary vitamin D₃, and none which provide other than minimal amounts (table 1). A second dietary form is ergocalciferol or vitamin D₂ which originates from plants and yeast and has been used in vitamin supplements and food fortification for a number of years (Holick et al. 2008). As with vitamin D₃, vitamin D₂ is metabolised first in the liver then in the kidneys to the active form. In North America, vitamin D₂ is available as a pharmaceutical preparation, but is not commonly used in New Zealand, where only vitamin D₃ is used in pharmaceutical preparations (Medsafe New Zealand 2006), and there is little or no food fortification. A range of supplements containing vitamin D₃ are available in New Zealand either by prescription or “over the counter” (table 2).

Table 1: Vitamin D content of the main food sources in the New Zealand diet (New Zealand Foods database Version 8, Foodworks 2007; Xyris Software).

Food	vitamin D ₃ µg/100gm	vitamin D ₃ IU/100gm
Eggs	1.65	66
Canned red salmon	4.00	160
Fresh King salmon	5.83	233
Butter	0.93	37
Sardines	11.00	440
Canned tuna	5.80	232
Kahawai	5.00	200

Table 2: Examples of the dose and form of vitamin D supplements available in New Zealand.

Supplemental form – vitamin D ₃ (cholecalciferol)	Dose
Prescription only	1.25 mg (50,000 IU)
Cal D Forte - 1/month for 3 months* (PSM Healthcare) (Medsafe New Zealand 2006)	
“Over the counter” supplements	12.5 µg (500 IU)
Elevit – Pregnancy multi-vitamin - Baxters Healthcare (Medsafe New Zealand 2006)	
Vitamin D and fish oil - Thompsons (www.thompsons.co.nz)	25 µg (1000 IU)
Women’s Multi Advantage – Thompsons (www.thompsons.co.nz)	2.5 µg (100 IU)
Vitamin D3 – Blackmores (www.Blackmores.co.nz)	25 µg (1000 IU)
Cod Liver Oil 1000mg capsules - Blackmores (www.Blackmores.co.nz)	2.5 µg (100 IU)
Calcium and Vitamin D3 - Healtheries (www.healtheries.co.nz)	2.5 µg (100 IU)

**In cases of moderate/Severe Vitamin D insufficiency (< 25 nmol/L of serum 25 hydroxy Vitamin D concentration) Dosage = 1 x Cholecalciferol tablet a day for 10 days (loading).*

It has been a common convention in nutritional science to link a nutrient to a disease (when a deficiency disease has been identified) in terms of defining deficiency - vitamin C and scurvy, iodine and goitre for example. In this way, vitamin D has been linked to rickets as its index disease, and deficiency levels are still defined as those which fail to prevent the occurrence of rickets. However, over recent years calcitriol

has been shown to be involved in a diverse range of physiological processes, both genomic and non-genomic. Vitamin D is now the subject of intense research interest, and long-held beliefs about recommended daily intake and levels of adequacy are being questioned.

The vitamin D receptor - VDR

The actions of calcitriol are mediated through a nuclear receptor called the Vitamin D Receptor (VDR) (reviewed in Jones et al, 1998). The discovery of this receptor in the cells of many different tissues supported the earlier hypothesis which emerged from epidemiological studies that vitamin D might have roles other than calcium homeostasis (Garland et al. 1990; Lefkowitz et al. 1994). Subsequently, such hypotheses have been further reinforced by the discovery of 1α OHase in tissues other than renal cell mitochondria. During the past decade, a multitude of epidemiological and observational studies have emerged linking vitamin D deficiency with a range of disease conditions including age-related muscle loss and the development of sarcopenia (Visser et al. 2003; Inderjeeth et al. 2007), cancer (Grant 2002; Krishnan et al. 2003; Harris et al. 2004; Valrance et al. 2004), cardiovascular disease (Wang et al. 2008), auto-immune conditions (Hyppönen et al. 2001; Munger et al. 2004; Harris 2005), metabolic syndrome and type 2 diabetes (Boucher et al. 1995; Scragg et al. 1995b; Boucher 1998; Borissova et al. 2003; Scragg et al. 2004).

As a lipid-soluble hormone, calcitriol is able to cross the cell membrane and enter the cytoplasm where it encounters the VDR. The human VDR is a 427-amino acid peptide with a DNA-binding domain that acts through vitamin D-response elements (VDREs) which are located near the start site of the target gene (DeLuca 2004). Once calcitriol binds to the VDR, the conformation of the receptor changes and the heterodimerisation with retinoid X receptor (RXR) is promoted. The heterodimer of VDR/RXR binds with high affinity to the VDREs in the promoter sections of the target genes (Sutton et al. 2003).

It would appear that other proteins also bind with this complex as co-activators. To date at least four of these have been identified (Sutton et al. 2003), and it is thought that these co-activators may confer selectivity to which gene is expressed, as there appears to be only one VDR (Kimmel-Jehan et al. 1999). However, one receptor is responsible for the activation or suppression of a number of gene transcription events and, as a consequence, is involved in ensuring homeostasis in a number of physiological systems.

In the epithelial cells of the small intestine, increased concentrations of calcitriol have been demonstrated to have an immediate and positive effect on the rate of Ca^{2+} transcellular transport. It has been shown that this up regulation is modulated by VDR content in the cell, and results in increased transcription of the influx and efflux channel proteins, and calbindins (DeLuca 2004).

The discovery of the VDR and VDREs in the cells of the immune system suggest that vitamin D plays a direct role in the regulation of immune defence (Wang et al. 2004) and immune tolerance (Meehan et al. 2002; Harris 2005; Mathieu et al. 2005). Meanwhile, evidence mounts for a multifactorial role for vitamin D in the pathogenesis of type 2 diabetes. A role for vitamin D in insulin secretion was first suggested by Norman et al (1980) from results of studies in vitamin D deficient rats. Following that, the presence of the VDR in the pancreatic β -cells established that calcitriol can influence insulin secretion (Gedik et al. 1986; Kumar et al. 1994; Ogunkolande et al. 2002) and more recently, a VDRE has been found on the insulin receptor gene (Maestro et al. 2003) suggesting a mechanism for the effect of vitamin D deficiency on insulin sensitivity.

Insulin resistance and type 2 diabetes

Glucose Homeostasis

The task of maintaining glucose homeostasis is often seen as the responsibility of the endocrine pancreas, and the appropriate, efficient secretion of insulin from the pancreatic islet cells. In reality, the regulation of blood glucose levels is the work of a complex interaction between organs, hormonal and neuronal control systems and intra-cellular signalling, although there is no doubt that insulin has the leading role (fig. 3).

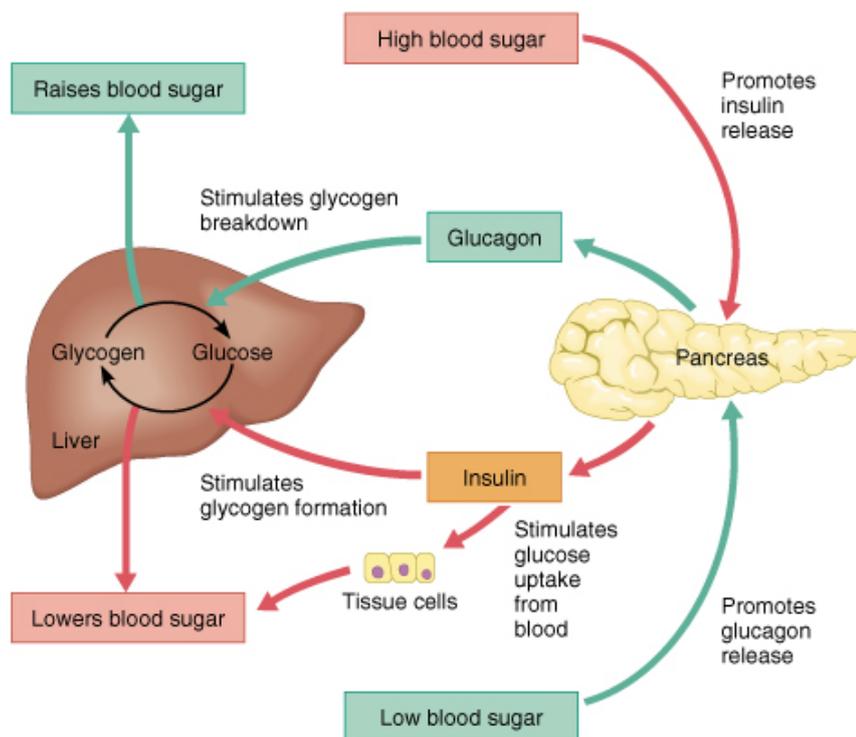


Figure 3. Diagram of the complex interaction of organs, hormones, metabolites and tissues involved in the maintenance of glucose homeostasis. In response to increases in plasma glucose, insulin is secreted from the pancreatic β -cells. Glucagon secretion is suppressed, as is hepatic glucose output. Glucose uptake is stimulated in the muscle and adipose tissue, and glycogen synthesis and glycolysis increase in the liver. Diagram from Marieb (2004).

In the normoglycaemic individual, blood glucose levels vary within a narrow range (4 – 7 mmol/L) during periods of fasting and feeding. During fasting periods, when

blood glucose would otherwise drop to dangerously low levels, glucagon is released from the pancreas, stimulating glycogenolysis and gluconeogenesis in the liver. The subsequent release of glucose into circulation ensures maintenance of plasma glucose levels between feeds, and provision of adequate glucose for the central nervous system which uses approximately two-thirds, and the skeletal muscle (Home et al. 2008) .

Following feeding, insulin release is stimulated by glucose together with other nutrients (Marieb 2004). Uptake of glucose into the muscle and adipose cells is dependent on the insulin-stimulated translocation of GLUT4 glucose transporters (fig. 4) from the cytosol to the plasma membrane (Klip et al. 1990). Approximately 75% of insulin-dependent glucose disposal occurs in the muscle cells, with a small amount of uptake in the adipose tissue (Klip et al. 1990). The liver is not dependent on insulin for glucose uptake. Glucose transport in hepatocytes is mainly via the GLUT2 glucose transporters, although a small sub-set of perivenular hepatocytes express GLUT1 transporters (Weinstein et al. 1994). Insulin suppresses hepatic glucose output (HGO) and stimulates glycogen synthesis. Insulin suppresses lipolysis in adipose tissue, thereby also reducing HGO indirectly by influencing substrate availability (Saltiel et al. 2001).

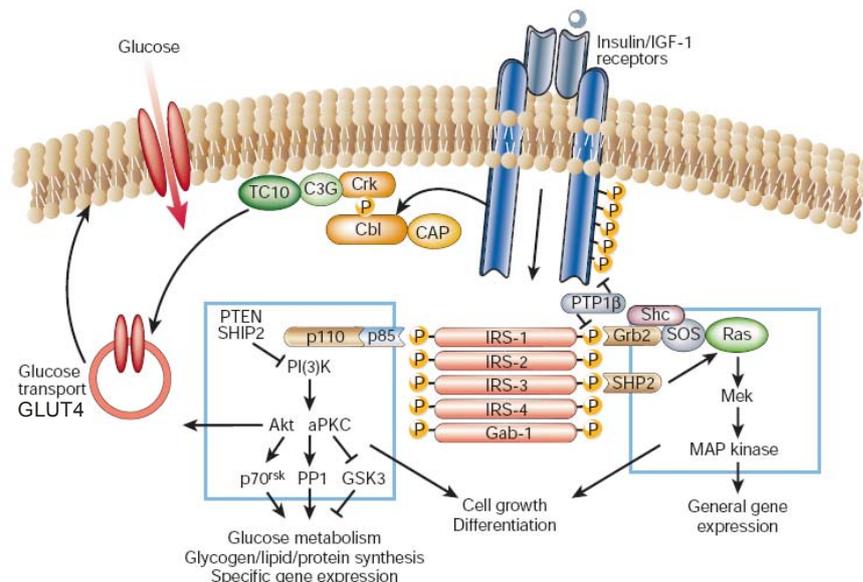


Figure 4. The insulin receptor is a tyrosine kinase which, on binding of its ligand, undergoes autophosphorylation and catalyses a number of diverse downstream

signalling pathways. These pathways regulate and coordinate vesicle trafficking, including the translocation of the GLUT4 transporter proteins (Saltiel et al. 2001).

Factors influencing the production, secretion and clearance of insulin

The primary translation product of the insulin gene (INS) is an inactive protein, 110 amino acids in length, called preproinsulin. A signal peptide, 24 amino acids in length, is cleaved off in the endoplasmic reticulum (ER), creating proinsulin which consists of an amino-terminal B chain, a connecting peptide (C-peptide) and a carboxyl-terminal A chain. The proinsulin is packaged into secretory granules where C-peptide is cleaved, resulting in equimolar amounts of insulin and C-peptide (Trout et al. 2007). The vesicles are stored in the cytoplasm of the β -cell ready for secretion by exocytosis (Rutter 2004).

The secretion of insulin is biphasic. There are essentially two pools of insulin-filled vesicles; one pool is closely related to, or even docked to, the cell membrane and is ready for immediate release which occurs within seconds of glucose entering the cell (first phase) (Rutter 2004). A reserve pool which needs to be mobilised to the cell membrane to be available for secretion forms the second phase, releasing insulin within 5 – 10 minutes (Rutter 2004). As glucose stimulation continues, new secretory vesicles are formed and moved to the cell membrane for docking and exocytosis as a continuation of the second phase (Cobelli et al. 2007).

Glucose is the primary secretagogue for insulin, although other regulating factors such as amino acids, peptides, fatty acids and integrins are involved (Tengholm et al. 2009). Glucose enters the β -cell via GLUT2 glucose transporters and its metabolism results in the production of ATP in the mitochondria (fig. 5). The increase in ATP/ADP ratio closes ATP-gated potassium channels, causing depolarisation and opening voltage-gated calcium channels. The subsequent increase in intracellular calcium concentration triggers the first phase release of insulin by exocytosis of the docked vesicles, and stimulates the movement of the stored vesicles toward the plasma membrane (Rutter 2004). This oscillatory pattern of insulin secretion requires less insulin to maintain glucose homeostasis than insulin infused at a constant rate, and the better efficiency is probably due to a higher expression of insulin receptors (Tengholm et al. 2009).

Approximately 50% of insulin is taken up by the liver during first-pass transit and consequently, mean insulin concentrations in the portal vein are around twice those

in the periphery (Home et al. 2008). Most of this uptake is receptor-mediated, and insulin clearance and sensitivity have been shown to be well correlated, with timing which suggests that the clearance is predominantly via the liver (Ahren et al. 2003). Therefore, it is likely that in the initial stages of insulin resistance it is reduced hepatic sensitivity, rather than peripheral resistance, which is responsible for increased concentrations of circulating insulin.

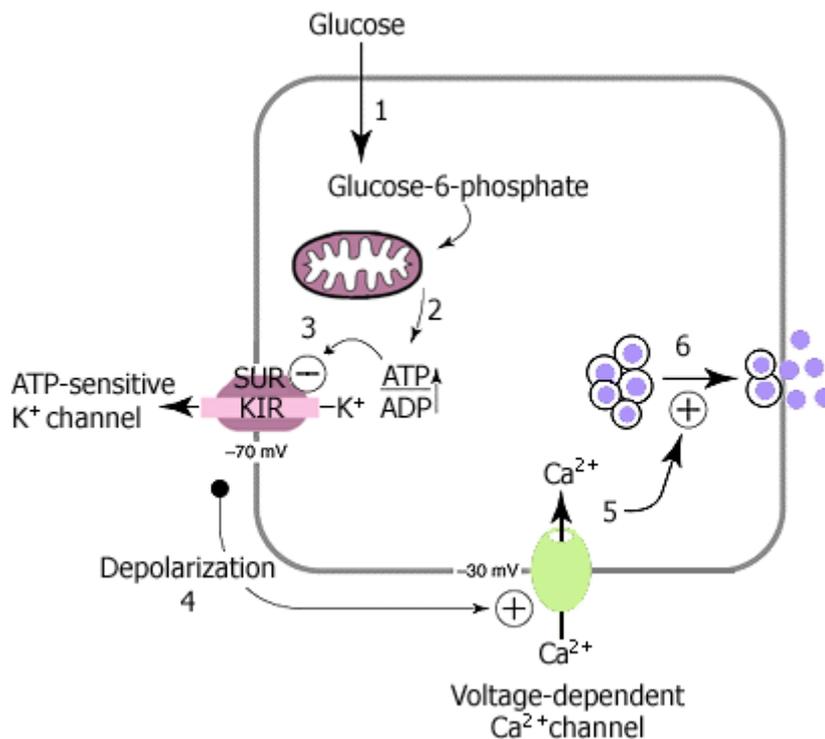


Figure 5. Glucose enters the cell via GLUT2 transport proteins (1). Metabolism of glucose results in elevated ATP (2). The ATP-sensitive potassium channels (K_{ATP}) close (3), the cell depolarises (4) calcium floods in (5) and insulin vesicles are released (6). Diagram from Dean et al (2004).

Insulin resistance

Insulin resistance occurs when the cells of the body fail to respond appropriately to a given dose of insulin. At a whole body level, glucose oxidation and glycogen synthesis are reduced, whilst hepatic glucose output continues unsuppressed (Ferrannini 2006). The cells exhibit decreased responsiveness or sensitivity to the actions of insulin and glucose homeostasis becomes dysregulated, with increased insulin secretion compensating for hyperglycaemia (Muniyappa et al. 2007). Insulin resistance heralds the potential development not only of type 2 diabetes, but also cardiovascular disease, dyslipidaemia, hypertension and a cluster of other metabolic abnormalities (Ferrannini 2006) and therefore diagnosis and progression of insulin resistance is of importance to clinicians and researchers. A number of tools have been developed to assess insulin resistance in a variety of settings, and some of the more commonly used are described below.

Most widely used for diagnosis of diabetes and glucose intolerance in clinical practice is the oral glucose tolerance test / meal tolerance test (OGTT). The OGTT involves the administration of a standard oral glucose load (75g) following an overnight fast. If the glucose is administered intravenously, the test is called the intravenous glucose tolerance test (IVGTT). Blood samples are taken at 0, 30, 60, and 120 minutes and glucose levels monitored. With normal glucose tolerance, glucose concentrations should be < 7.8 mmol/L at the end of 2 hours. The meal tolerance test follows the same pattern, and is possibly superior to the OGTT as it contains a mix of macro-nutrients and therefore mirrors more closely the normal physiological response to feeding (Cobelli et al. 2007). The standard OGTT or meal can provide useful information about glucose tolerance, but cannot accurately describe β -cell function or insulin sensitivity. However mathematical models have been developed to allow the derivation of further information from the OGTT. The recently developed and validated Oral Minimal Model using an OGTT or meal can simultaneously and quantitatively provide predictions of β -cell function, insulin sensitivity and hepatic extraction over a 2 hour test with 7 samples (Cobelli et al. 2007). Similarly, the oral glucose sensitivity index (OGIS) utilises the 2-hour OGTT measurements to predict insulin sensitivity which correlate well with the glucose clamp (Mari et al. 2001).

The original Homeostasis model assessment (HOMA1) model was developed in 1985, and utilises a simple linear equation based on pairing fasting serum glucose

(FSG) and fasting serum insulin (FSI) to establish a measure for insulin resistance: $\text{HOMA1-IR} = (\text{FSI} \times \text{FSG}) / 22.5$ (Matthews et al. 1985). The revised HOMA2 model is a computer model consisting of nonlinear empirical equations which, when solved, allow the determination of insulin sensitivity (HOMA2%S) from FSG and FSI, and beta cell function (HOMA2%B) from paired FSG and C-peptide. C-peptide is a reliable marker for insulin secretion as, unlike insulin, it is unaffected by first pass hepatic uptake (Wallace et al. 2004). Both β -cell function and insulin sensitivity are reported as a percentage, where 100% is normal (Levy et al. 1998). Insulin resistance (IR) is the reciprocal of % sensitivity, and 1.0 is normal with values >1.0 indicating increasing insulin resistance.

The technique is simple and inexpensive with relatively low subject burden and, as such, is often suitable for use in large studies (Wallace et al. 2004). HOMA1 has been shown to correlate well with the euglycaemic clamp in predicting insulin resistance but does have certain limitations (Bonora et al. 2000). HOMA is a measure of basal state insulin secretion and insulin sensitivity, whereas OGTT measures the dynamic state and clamp techniques (below) measure the maximally stimulated states (Wallace et al. 2004).

Quantitative insulin sensitivity check index (QUICKI) is another simple model based on FSG and FSI and assesses insulin sensitivity. It has been shown to correlate well with the glucose clamp in lean and obese adults, and adults with diabetes (Katz et al. 2000). Like HOMA, QUICKI requires just one fasting blood sample and is very practical for use in large studies, but shares the same limitations.

The euglycaemic hyperinsulinaemic clamp involves the infusion of insulin to maintain a steady-state insulin level above fasting and a glucose analyser which monitors blood glucose levels at 5 – 10 minute intervals over a number of hours (2 – 4) (Trout et al. 2007). Dextrose is infused to keep the subject euglycaemic and whole body glucose disposal can be determined at a certain level of hyperinsulinaemia (Muniyappa et al. 2007). Whilst the euglycaemic clamp technique can measure whole body glucose disposal, it cannot assess β -cell function (Cobelli et al. 2007). It is also time consuming, expensive, and labour intensive requiring highly skilled personnel to ensure subject safety (Muniyappa et al. 2007), and thus is not appropriate for use in large clinical studies or routine clinical investigations.

The frequently sampled intra-venous glucose tolerance test (FSIVGTT) minimal model is a mathematical model which, when applied to the intravenous glucose tolerance test, allows the derivation of an insulin sensitivity index, an acute response to glucose (AIR) which assesses insulin secretion, and a disposition index (DI) (Bergman et al. 1987). The model was developed by Richard Bergman in 1979 and is often referred to as the “Bergman Minimal Model”. It is less labour-intensive than the euglycaemic clamp as it does not require continuous intravenous infusions, but does require continuous blood sampling for three hours. Subjects are given an intravenous glucose load following an overnight fast and blood samples to establish fasting status of glucose and insulin. Blood samples are then taken every minute for the first ten minutes, then every two minutes up to 30 minutes, and every ten minutes up to 180 minutes, for glucose and insulin measurements (Muniyappa et al. 2007). The minimal model correlates well with the clamp for estimates of insulin sensitivity (Steil et al. 1994).

Type 2 diabetes

Type 2 diabetes (previously referred to as non-insulin dependant diabetes, NIDDM or adult-onset diabetes) is a slow onset disease. It appears to be strongly linked to visceral fat mass or obesity and now accounts for 90 – 95% of all of those with some type of diabetes (Expert Committee on the Diagnosis and Classification of Diabetes 2008). A diagnosis of type 2 diabetes (see table 3 for diagnostic criteria) is usually preceded by a long period during which underlying changes are taking place in glycaemic control but of which there are no overt symptoms.

Table 3 Criteria for the diagnosis of diabetes (Expert Committee on the Diagnosis and Classification of Diabetes 2008)

1	FPG \geq 126 mg/dL (7.0 mmol/L). Fasting is defined as no caloric intake for at least 8 hours OR
2	Symptoms of hyperglycaemia and a casual plasma glucose \geq 200 mg/dl (11.1 mmol/L). Casual is defined as any time of day without regard to time since last meal. The classic symptoms of hyperglycaemia include polyuria, polydipsia and unexplained weight loss. OR
3	2-hour plasma glucose \geq 200mg/dl (11.1 mmol/L) during an OGTT. The test should be performed as described by the World Health Organization, using a glucose load containing the equivalent of 75g anhydrous glucose dissolved in water.

Abbreviations: FPG – fasting plasma glucose; OGTT – oral glucose tolerance test

In the absence of unequivocal hyperglycaemia, these criteria should be confirmed by repeat testing on a different day. Diagnosis criteria for type 2 diabetes does not currently include measurement of HBA1c although there is increasing international discussion about the advantages and disadvantages of doing so (Zimmet 2009)

This period of glycaemic abnormalities up to the time of fulfilment of the diagnostic criteria for diabetes can be usefully called “prediabetes” indicating that there is a relatively high risk of the patient developing diabetes (Expert Committee on the Diagnosis and Classification of Diabetes 2008). The degree of such risk and the time period involved appears to vary widely and will be influenced by the presence of other metabolic abnormalities such as obesity (Depres 2006). The proportion of total cases that progress to diabetes varies from as few as 2.7% with impaired fasting glucose (IFG) over a period of 30 months, to as many as 66% with previous IFG and/or impaired glucose tolerance (IGT) over 5 years (see table 4). Nichols et al

(2007) concluded that patients were progressing from newly identified IFG to diabetes in less than 3 years.

Impaired fasting glucose and impaired glucose tolerance are separable conditions with different clinical diagnostic criteria. They provide cut-off points which can be used diagnostically in a clinical or research setting, but they are really points on the continuum towards fully developed diabetes. IFG can be present without IGT but the two usually co-exist in the period preceding the diagnosis of diabetes.

Table 4. The rate of progression from prediabetes (IFG or IFG and IGT) to diabetes

Subjects and setting	Duration of study	Proportion of progression from IFG	Proportion of progression from IFG and IGT	Reference
Men and women, 25 – 74 years. Mixed race incl. South Asian Indians, Chinese, African/Creole <i>Mauritius</i>	5 years	Men 24% Women 26%	Men 37% Women 66%	(Shaw et al. 1999)
Italian men and women, 40 – 59 years at baseline <i>Naples</i>	11.5 years	9.1%	44.4%	(Vaccaro et al. 1999)
Pima Indians, men and women > 15 years <i>Arizona, USA</i>	5 years		41.2%	(Gabor et al. 2000)
Caucasian men, 44-55 years <i>Paris</i>	30 months	2.7% (129 from 4744 with NGT or IFG)	14%	(Eschwege et al. 2001)
Men and women, mean age at follow up 57.9 years <i>Portland, Oregon</i>	3 years	11.3%		(Nichols et al. 2007)

Abbreviations: IFG – impaired fasting glucose; IGT – impaired glucose tolerance; NGT – normal glucose tolerance.

The pathophysiology of prediabetes is characterised by impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) (Nathan et al. 2007). IFG is defined as an elevated fasting plasma glucose but normal 2-hour glucose following an OGTT (table 5). In this early stage, glucose is elevated in the fasting state probably because endogenous glucose production from the liver is occurring at a faster rate than glucose is being cleared from the blood (Bock et al. 2006). This indicates

reduced insulin sensitivity in the liver resulting in poor control of hepatic glucose release (Abdul-Ghani et al. 2006).

Table 5. Definition of stages of glycaemic status

	Fasting glucose	OGTT glucose
Normal Glucose Tolerance (NGT)	< 5.6 mmol/L	< 7.8 mmol/L (140 mg/dL)
Impaired Fasting Glucose (IFG)	5.6 to 6.9 mmol/L (100 mg/dL to 125 mg/dL)	<7.8mmol/l (140 mg/dL)
Impaired Glucose Tolerance (IGT)	<7.0 mmol/L (126 mg/dL)	≥7.8 and <11.1 mmol/L (140 mg/dL and 200 mg/dL)
Diabetes	≥7.0 mmol/L (126mg/dL)	≥11.1 mmol/L (200 mg/dL)

Diagnostic criteria for stages of glucose tolerance through to diagnosis of type 2 diabetes (Expert Committee on the Diagnosis and Classification of Diabetes 2008). Note that WHO defines IFG as 6.1 – 6.9 mmol/L, and does not provide a definition for NGT other than: “Since there are insufficient data to accurately define normal glucose levels, the term ‘normoglycaemia’ should be used for glucose levels associated with low risk of developing diabetes or cardiovascular disease, that is levels below those used to define intermediate hyperglycaemia.” (World Health Organization 2006)

During a 2-hour oral glucose tolerance test (OGTT), people with IFG show a rise in blood glucose which is only slightly higher than that in NGT because they have little or no insulin resistance in the muscle cells (Abdul-Ghani et al. 2006). Insulin secretion is only mildly impaired if at all, and seemingly it is the early-phase (first 30 minutes) response which is most affected. The late phase response during an OGTT is close to normal, resulting in a return of blood glucose concentration to normal levels by the end of a 2-hour test (fig. 6) (Abdul-Ghani et al. 2006). Faerch et al (2009) found a stationary reduced insulin secretion in subjects with NGT who then later developed IFG, suggesting that IFG is primarily caused by an inherent dysfunction in insulin secretion followed by hepatic insulin resistance. The subjects in this study did not show any further deterioration in insulin secretion as they progressed to IFG which suggests that the β -cell impairment is not a major feature in IFG (Faerch et al. 2009). However, fasting hyperinsulinaemia has been well

documented in individuals with IFG (Weyer et al. 1999; Tripathy et al. 2000; Abdul-Ghani et al. 2006).

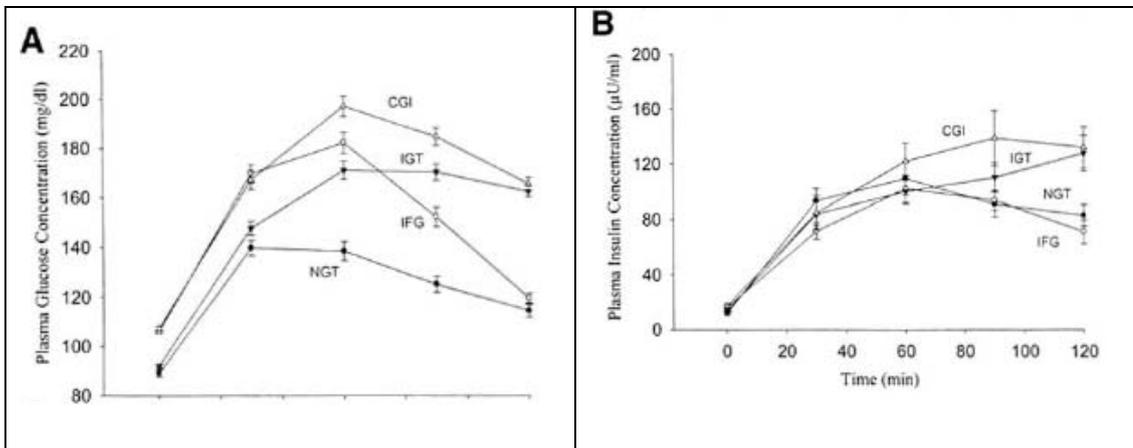


Figure 6. Glucose and insulin response during an OGTT in subjects with NGT, IFG, IGT and combined IFG and IGT (CGI). (Abdul-Ghani et al. 2006)

Conversely, IGT appears to be characterised by moderate to severe peripheral insulin resistance, normal to mild hepatic insulin resistance, followed by a decline in β -cell function (Nathan et al. 2007; Faerch et al. 2009) seen in both reduced early phase response and deficient late-phase insulin secretion (Festa et al. 2004). By the time fasting hyperglycaemia is present, it is estimated that β -cell function is reduced by 75% (Kahn et al. 2006), which would explain the much higher rate of progression to diabetes from IGT, because β -cell function must be impaired for type 2 diabetes to develop (Kahn 2001).

IGT will often co-exist with IFG, but sometimes does not, indicating different pathophysiological mechanisms contributing to the disturbed glucose homeostasis (Shaw et al. 1999; Nichols et al. 2007; Faerch et al. 2009). It is becoming apparent that although both conditions represent a state of insulin resistance, the site of the resistance differs and there is also a different pattern in the impairment of insulin secretion (Nathan et al. 2007). Where both conditions exist, naturally there will be insulin resistance in both the liver and muscle (Abdul-Ghani et al. 2006), together with reduced β -cell function which increases in its severity until blood glucose levels can no longer be controlled by endogenous insulin secretion (Weyer et al. 1999).

If diagnosed in the early stages, the progression from prediabetes to diabetes can be potentially halted or even reversed with lifestyle changes or medication. Physical

activity and weight loss programmes have been shown to be especially effective, demonstrating reduction in the development of diabetes of 25 – 60% (Tuomilehto et al. 2001; Diabetes Prevention Program Research Group 2002; Payne et al. 2008; Shaw et al. 2009). These programmes primarily targeted weight loss, as their subjects were obese or overweight, and even with very modest weight loss they saw marked improvements in insulin sensitivity (Schäfer et al. 2007; Payne et al. 2008; Thompson et al. 2008; Shaw et al. 2009). There does not appear to be any evidence for lifestyle changes having any effect on insulin secretion or impaired β -cell function.

Metformin, a drug which improves insulin action at peripheral sites (MedSafe New Zealand 1999), was the first of the pharmacological interventions to prove effective. It is inexpensive and has minimal side effects (Nathan et al. 2007). However, its effectiveness, at least in the short term, has been shown to be only half that of successful lifestyle interventions (Knowler et al. 2002).

Metabolic syndrome

The metabolic syndrome is a collection of metabolic abnormalities which, together or in part, are associated with increased risk of diabetes and cardiovascular disease. (Hanson et al. 2002; Bonora 2006).

These abnormalities include abdominal obesity, impaired glucose regulation, raised triglycerides, decreased high-density lipoprotein (HDL) cholesterol, elevated blood pressure and hyperinsulinaemia with underlying insulin resistance (Magliano et al. 2006). The presence of 3 or more of the metabolic syndrome abnormalities, in particular hyperinsulinaemia, dyslipidaemia and body size, are associated with a greatly increased risk of the eventual development of type 2 diabetes (Wannamethee et al. 2005). Meanwhile, hypertension, hyperinsulinaemia and dyslipidaemia are important risk factors for cardiovascular disease, increasing the risk of developing CVD 2 – 4 fold compared to individuals without any metabolic syndrome abnormalities (Bonora 2006).

Definitions of the syndrome have varied over the past 3 decades, sometimes with more emphasis on risk factors for diabetes, sometimes more on cardiovascular disease. In 1988, Reaven coined the name “Syndrome X” but also called the collection of symptoms “insulin resistance syndrome” (Reaven 2005). Reaven’s definition however, did not include central adiposity and this omission was corrected in 1999 in a definition proposed by the World Health Organization (WHO) Diabetes Group (Alberti et al. 1998). In 2001 the National Cholesterol Education Program (NCEP): Adult Treatment Panel III (ATP III) produced a definition more focused on diagnosis of cardiovascular disease risk. It required the presence of any three of five components: central obesity, raised blood pressure, raised triglycerides, low HDL-cholesterol, and fasting hyperglycaemia (Adult Treatment Panel III 2001).

In 2005 the International Diabetes Federation (IDF) proposed a new definition which places abdominal obesity in a central position, and allows for different cut-off points for abdominal obesity in different population groups. The IDF definition (table 6) (Zimmet et al. 2005), also allows for the further recognition of other components as yet not included in the definition due to lack of sufficient evidence. These include aspects of body composition, inflammatory markers, thrombotic markers, additional lipid-based factors, insulin resistance and others (Alberti et al. 2005; International Diabetes Federation 2005).

Table 6. The 2005 International Diabetes Federation definition of the metabolic syndrome (International Diabetes Federation 2005)

According to the International Diabetes Federation definition, for a person to be defined as having the metabolic syndrome, they must have:

- Central obesity (defined as waist circumference ≥ 94 cm for European men and ≥ 80 cm for European women, with ethnicity specific values for other groups*)

Plus any two of the following four factors:

- Raised serum triglyceride level (≥ 1.7 mmol/L)
- Reduced serum HDL-cholesterol level (< 1.03 mmol/L in males and < 1.29 mmol/L in females), or specific treatment for these abnormalities.
- Raised blood pressure (systolic blood pressure ≥ 130 mmHg or diastolic blood pressure ≥ 85 mmHg) or treatment of previously diagnosed hypertension.
- Impaired fasting glycaemia (fasting plasma glucose ≥ 5.6 mmol/L) or previously diagnosed type 2 diabetes.

*South Asian and South-East Asian men ≥ 90 cm, women ≥ 80 cm; Japanese men ≥ 85 cm, women ≥ 90 cm.

Putting central obesity in a priority position in the list of diagnostic criteria for metabolic syndrome seems appropriate. The secretions of adipose tissue, especially the more metabolically active visceral adipocytes, induce insulin resistance along with other metabolic abnormalities such as inflammation, atherosclerosis and even cancer (Scherer 2006).

However, in a joint interim statement issued weeks prior to this thesis being submitted, the International Diabetes Federation Task Force on Epidemiology and prevention, The National Heart, Lung and Blood Institute, The American Heart Association, The World Heart Federation, The International Atherosclerosis Society, and the International Association for the Study of Obesity, changes were made to the above definition. Central obesity is no longer an obligatory component, although it is suggested that waist measurement be used as a screening tool. Three abnormal findings out of the five listed above would qualify a person for metabolic syndrome (Alberti et al. 2009).

Genetic susceptibility to β -cell dysfunction and insulin resistance

The healthy β -cell responds to increased plasma glucose, which reflects insulin resistance, by increasing both insulin secretion and β -cell mass to ensure that glucose homeostasis is maintained (fig. 7). Thus an individual with insulin resistance might exhibit hyperinsulinaemia, but plasma glucose will be normal (Kahn 2001). However, if the β -cell is genetically susceptible to dysfunction, it is unable to increase insulin secretion, and impaired glucose tolerance and, ultimately, type 2 diabetes will develop (Kahn 2001).

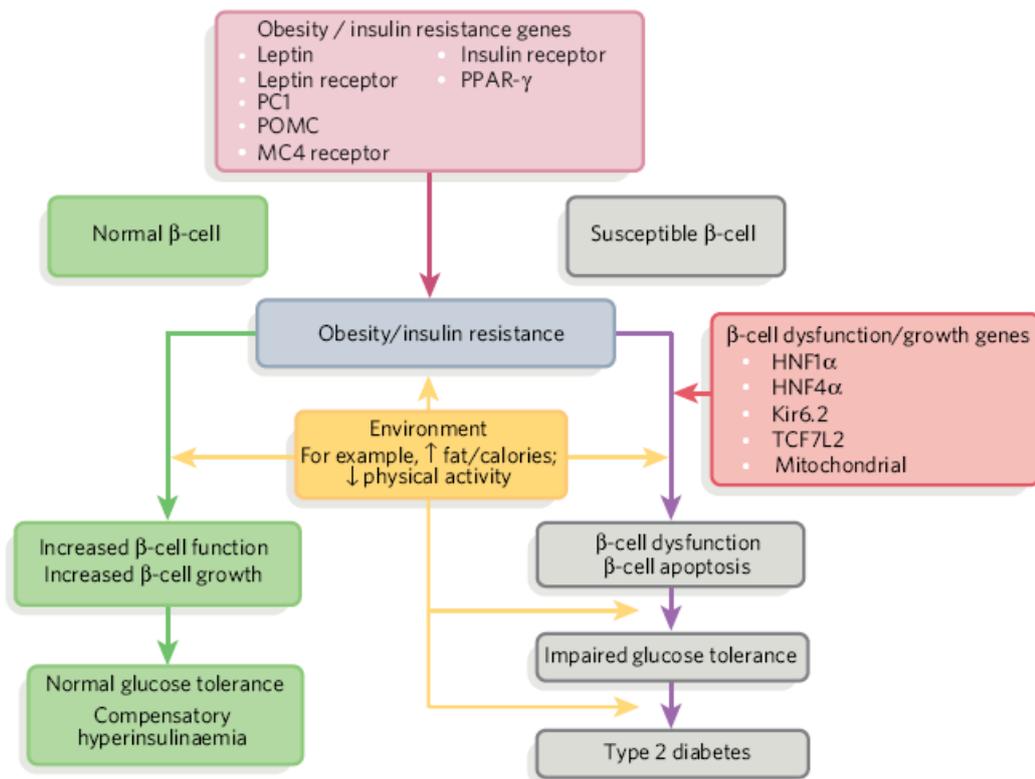


Figure 7. Genetic predisposition for β -cell dysfunction, plus obesity, plus environmental factors progress to impaired glucose tolerance and type 2 diabetes. Diagram from Kahn et al. (2006).

Along with lifestyle factors (McAuley et al. 2002; Corpeineijn et al. 2007) and increased adiposity (Hotamisligil et al. 1993; Hu et al. 2004; Scherer 2006), family history is one of the main risk factors for the development of type 2 diabetes (Pontiroli et al. 2000; Jensen et al. 2002; Knowles et al. 2002).

Genetic susceptibility to β -cell dysfunction and the development of type 1 diabetes has been identified in people carrying certain VDR haplotypes (Ramos-Lopez et al. 2006 ; Mimbacas et al. 2007; Garcia et al. 2007). One VDR polymorphism has been identified in both Indian and Bangladeshi populations and correlates with increased risk of type 1 diabetes (McDermott et al. 1997).

A connection between genetic susceptibility for type 2 diabetes and vitamin D has also been seen in polymorphisms in the vitamin D receptor which are thought to modify susceptibility to insulin resistance (Filus et al. 2008).

Hypovitaminosis D and type 2 diabetes

Correlation not causation

The identification of the VDR in the pancreas of chickens provided the first clue that calcitriol may play a role in the regulation of pancreatic endocrine function, including the secretion of insulin (Christakos et al. 1979 ; Norman et al. 1980). Studies in rat pancreatic cells *in vitro* and *in vivo* indicated that vitamin D or its metabolites were essential for the efficient secretion of insulin and control of glucose homeostasis (Kadowaki et al. 1984; Cade et al. 1986). The presence of the VDR was then confirmed in human pancreatic islet β -cells (Johnson et al. 1994). Subsequently, the links between vitamin D insufficiency and aspects of diabetes risk have been demonstrated in a number of epidemiological studies.

Analysis of the Third National Health and Nutrition Examination Survey (NHANES III) data by Scragg et al (2004) found an inverse relationship between serum 25(OH)D concentration, and diabetes risk and insulin resistance (HOMA-IR) but not in β -cell function. In New Zealand, a case control comparison nested within a cross-sectional study which compared newly detected cases of IGT or type 2 diabetes with matched controls, found that the controls had higher serum 25(OH)D levels than the cases (Scragg et al. 1995b). The mean difference was only 7 nmol/L (2.8 ng/mL), but was highly significant ($P = 0.0016$). Serum 25(OH)D levels were also significantly lower in elderly Italian women with type 2 diabetes compared to controls (11 ± 9.8 vs 9 ± 11.3 ng/mL, $P < 0.008$) (Isaia et al. 2001).

More recently, a prospective study from Finland followed two cohorts for 17 and 24 years and found that high serum 25(OH)D at baseline was protective against future development of type 2 diabetes, with men in the highest quartile of 25(OH)D (mean 75, range 58-148 nmol/L) having an 82% lower risk compared to the lowest quartile (mean 24, range 11-32 nmol/L) (Knekt et al. 2008). Another 10-year prospective study in the United Kingdom had similar findings, with baseline 25(OH)D inversely correlated with risk of hyperglycaemia, insulin resistance and metabolic syndrome (Forouhi et al. 2008). Hypponen et al (2008) reported an inverse association between 25(OH)D levels and metabolic syndrome prevalence, levels of glycosylated

haemoglobin, blood pressure and triglycerides in the 1958 birth cohort of 6,810 white British adults at the age of 45 years.

Vitamin D supplementation

There have been a number of intervention trials assessing the effect of improved vitamin D status on glucose handling and various components of metabolic syndrome (table 7). In general, these studies have been small intervention studies administering varying doses of either vitamin D or calcitriol, and measuring different outcomes.

The most commonly measured outcome has been β -cell function as measured by change in insulin secretion. Gedik et al (1986) supplemented 4 severely vitamin D deficient women with 2000 IU vitamin D₃ for 6 months and compared them with healthy controls. Mean serum 25(OH)D levels prior to supplementation were 29.7 ± 3.3 pg/mL increasing to 70.0 ± 10.3 pg/mL post treatment. Insulin secretion, which had been significantly lower than in the healthy controls, increased to a slightly higher level than the normal subjects, and the insulinogenic index (Δ Insulin/ Δ Glucose) increased from 1.71 ± 0.4 pre-treatment to 2.48 ± 0.3 post treatment. Changes in glucagon did not change significantly, and levels were not significantly different to those in the control group, thus indicating that glucagon secretion is not affected by vitamin D status.

A similar observation was made in a single vitamin D deficient patient who was given 2000 IU vitamin D₃ per day for one month (Kumar et al. 1994) and in 44 vitamin D deficient adults following a single 100,000 IU intra-muscular injection of vitamin D₃ (Boucher et al. 1995). Improving vitamin D status with 1332 IU vitamin D₃ per day for one month was shown to improve first-phase insulin secretion in 10 women with type 2 diabetes compared to healthy controls (Borissova et al. 2003). The subjects in these studies were all vitamin D deficient, but did not share baseline glycaemic status, neither was there any commonality in the amount, duration or method of administration of the vitamin D.

Despite the wide variation in study methodology, supplementation with vitamin D has mostly shown some kind of improvement in either insulin secretion or, in fewer cases, glucose tolerance. Even in the Pittas (2007) study, with vitamin D replete subjects (baseline 25(OH)D 71.2 ± 5.2 nmol/L) and vitamin D₃ supplementation of

700 IU/day, there was an ameliorating effect of the supplementation compared with placebo on subjects with IFG. Over the three years of the study, serum 25(OH)D levels increased in the supplemented group by 31.2 ± 4.4 nmol/L. Meanwhile, HOMA-IR and FPG both increased significantly more in the placebo group compared to the supplemented group.

There have, however, been two studies where no improvement was observed in the pre-existing condition: Taylor (1998) administered a single intra-muscular (IM) dose of 300,000 IU vitamin D₂ to three Asian subjects with type 2 diabetes and observed increased insulin resistance, glycosylated haemoglobin and triglycerides. The authors report measuring serum 25(OH)D₃ at baseline and 3 months after treatment and serum levels increased from 13, 13 and 8 nmol/L to 51, 47 and 33 nmol/L. Fasting insulin increased, suggesting that β -cell function could have been improved by supplementation, but FPG also increased. Meanwhile Tai et al (2008) gave two oral doses of 100,000 IU each, two weeks apart to 33 non-diabetic adults and failed to see any change in measures of glucose tolerance or insulin sensitivity. Mean serum 25-hydroxyvitamin D increased from 39.9 ± 1.5 (SEM) at baseline to 90.3 ± 4.3 nmol/L 4 weeks later, but there were no changes in FPG or FPI or 2-hour assessments following an OGTT.

Only very recently has there been a randomised placebo-controlled trial to test the effect of vitamin D₃ supplementation on measures of glucose tolerance and insulin secretion. Nagpal et al (2009) gave three doses of 120,000IU vitamin D₃ (n = 35) or placebo (n = 36), 2 weeks apart to Indian men. These subjects were centrally obese, but were otherwise healthy and not selected for vitamin D deficiency although mean baseline 25(OH)D concentration was 36.5 ± 14.6 nmol/L, increasing by 35.1 ± 27.3 nmol/L in the supplemented group. There was a small improvement in oral glucose insulin sensitivity (OGIS), but not insulin secretion or HOMA2IR. Greater central adiposity and lower baseline vitamin D levels were significant predictors of a greater improvement in OGIS in response to supplementation. In both this study and the Tai (2008) study samples for endpoint measures were taken two weeks after administration of the final dose, therefore times from baseline to endpoint measures were 6 and 4 weeks respectively.

The administration of large doses either orally or by IM injection features throughout vitamin D supplementation trials, but very little research has been done on the

efficacy of this type of supplementation. In New Zealand the only large dose available for prescribed delivery is a 50,000 IU tablet of vitamin D₃ (Medsafe New Zealand 2006). In cases of diagnosed deficiency, a course of one tablet per day for 10 days may be prescribed, thus delivering 500,000 IU in a short course. The efficacy of this dose has been measured against a single 300,000 IU IM injection and with both treatments serum 25(OH)D peaked within 13-21 days and then declined over a half-life of 90 days (Wu et al. 2003). Conversely, Vicchio et al (1993) report a half life for serum 25(OH)D of 19 days which raises a question about the length of treatment period required to reach a steady-state. From the limited evidence available, it does appear that the greatest increase in serum 25(OH)D occurs in the early part of treatment with supplementation (Barger-Lux et al. 1998; Chel et al. 2008; Tai et al. 2008; Nagpal et al. 2009). Whilst the larger, less frequent doses may be more convenient to administer, daily doses have been shown to be more effective than weekly or monthly doses as measured by serum 25(OH)D, PTH and bone markers (Chel et al. 2008), although this was not supported by Ish-Shalom et al (Ish-Shalom et al. 2008). Daily doses have also been shown to maintain a steady state of serum 25(OH)D concentration over the longer term (Barger-Lux et al. 1998).

Table 7. Vitamin D intervention studies investigating the effect of supplementation on aspects of the metabolic syndrome

Subjects	Intervention	Subject number	Outcome	Reference
Vitamin D deficient adults	Vitamin D ₃ 2000 IU/day (50 µg) for 6 months	4 vs 10	Improved insulin secretion	(Gedik et al. 1986)
Vitamin D deficient, middle aged men with IGT	Alphacalcidol (2 µg/day) for 18 months	14	Transient increase in insulin response. No change in glucose tolerance. Decrease in BP.	(Lind et al. 1989)
Diabetic subjects, early spring (25(OH)D 35 ± 7 mmol/l)	Alphacalcidol (1 µg/day) for 4 days	RCT n = 10 + 10	No significant effect	(Orwoll et al. 1994)
Vitamin D deficient woman	Vitamin D ₂ 2000 IU/day (50 µg) for 1 month	1	Improved glucose tolerance and improved beta cell function	(Kumar et al. 1994)
Vitamin D deficient adults	Vitamin D via single IM injection 100,000 IU	44 vs 15	Improved insulin secretion (C-peptide + specific insulin), but not glucose tolerance after 8-12 weeks	(Boucher et al. 1995)
Healthy young men, normal calcitriol levels	Calcitriol 1.5 µg/day for 7 days or placebo	RCT (n = 9 + 9)	25 (OH)D levels not measured, no change in glucose tolerance (clamp)	(Fliser et al. 1997)
Type 2 diabetic adults	Vitamin D ₂ supplementation via single IM injection 300,000 IU	3	Increased insulin resistance, increased fasting insulin and glucose after 3 months	(Taylor et al. 1998)
Vitamin D deficient elderly women	Vitamin D ₃ 800 IU/day (20 µg) plus Calcium or Calcium alone for 8 weeks	74 vs 74	72% increase in serum 25(OH)D, decrease in systolic BP and heart rate	(Pfeifer et al. 2001)

Table continued overleaf

Non-diabetic Bangladeshi adults, mostly Vit D deficient	3-monthly injections of Vitamin D ₃ IM at 'high' (50,000 IU) or 'low' (500 IU) dosage over one year	41	Decreased Mean MMP9 and CRP levels. CRP reductions greater in "high" dose group	(Timms et al. 2002)
Type 2 diabetic women	Vitamin D supplementation 1,332 IU/day for 1 month	10 vs 17	Improved first-phase insulin secretion	(Borissova et al. 2003)
Men, 50 – 63 years with congestive heart failure	Supplement: 500mg Calcium, 200 IU Vitamin D ₃ Placebo: 500 mg Calcium	RCT	Anti-inflammatory IL10 increased, PTH decreased, TNF- α suppressed in treatment group.	(Schleithoff et al. 2006)
Caucasian adults. 70 years at baseline. Non-diabetic, 92 with IFG, 222 with NFG	Primarily bone study. Supplement – 500 mg calcium citrate and 700 IU Vitamin D ₃ daily for 3 years. Baseline 25(OH)D 70 – 80 nmol/L	RCT Suppl. n = 143 Placebo n = 161	Suppl. IFG subjects had less increase in FPG and HOMA-IR than placebo IFG group. No difference in NFG groups.	(Pittas et al. 2007)
Vitamin D deficient, healthy, non-diabetic adults 19 – 75 years	Vitamin D ₃ 2 x 100,000 IU 2 weeks apart	33	No change in any measures of glucose tolerance or insulin sensitivity	(Tai et al. 2008)
Indian men, healthy, not vitamin D deficient, centrally obese, >35yrs	Vitamin D ₃ 3 x 120,000IU doses 2 weeks apart	RCT Suppl. n = 35 Placebo n = 36	Improved OGIS, not HOMA2-IR, not insulin secretion	(Nagpal et al. 2009)

Abbreviations: RCT – randomised controlled trial; BP – blood pressure; IM – intramuscular; MMP9 – matrix metalloproteinase 9; CRP – C-reactive protein; IL10 – interleukin-10; PTH – parathyroid hormone; TNF- α - tumour necrosis factor- alpha; IFG – impaired fasting glucose; FPG – fasting plasma glucose; NFG – normal fasting glucose; HOMA-IR – homeostasis model assessment – insulin resistance; OGIS – oral glucose insulin sensitivity

Insulin secretion

As discussed above, there is clearly a role for calcitriol in the regulation of insulin secretion. The presence of the VDR in pancreatic β -cells appears to confirm that, but to date there has been no evidence that calcitriol influences the expression of the insulin gene. It appears more likely that calcitriol is involved in the regulation of the calcium flux which initiates the release of insulin (Lee et al. 2006).

The identification and localisation of the vitamin D-dependant calcium-binding protein, calbindin- D_{28K} in pancreatic islet cells provided a clear target for the action of calcitriol in insulin secretion (Johnson et al. 1994). Calbindin- D_{28K} is expressed in β -cells and has been shown to modulate depolarisation-stimulated insulin release (Sooy et al. 1999; Lee et al. 2006). In the absence of calbindin- D_{28K} (as demonstrated in calbindin- D_{28K} KO mice) there is an increase in sustained insulin release and loss of the oscillatory pattern of release. Conversely, when calbindin- D_{28K} is over expressed, insulin release is decreased or inhibited (Sooy et al. 1999). Interestingly, it has been observed that the characteristic oscillatory pattern of insulin release deteriorates in the very early stages of the development of type 2 diabetes (Tengholm et al. 2009).

A protective role for calbindin- D_{28K} has been established also, in the prevention of apoptotic cell death in a number of cell types including the β -cell. This is considered to be of more importance in the prevention of autoimmune destruction in type 1 diabetes, however Rabinovitch et al (2001) demonstrated a protective effect of calbindin- D_{28K} against inflammatory cytokine-induced β -cell apoptosis. It is known (see below) that proinflammatory cytokines are often increased in the conditions which precede the development of type 2 diabetes.

Insulin sensitivity

Although there is very little evidence to date that vitamin D deficiency has a deleterious effect on insulin sensitivity, two mechanisms have been postulated for an increase in insulin sensitivity in response to improved vitamin D status – suppression of chronic inflammation and increased expression of the insulin receptor and/or proteins of the insulin signalling cascade.

A mild inflammatory state, marked by the presence of proinflammatory cytokines, is associated with obesity, insulin resistance, hyperglycaemia and hyperlipidaemia.

These cytokines, predominantly TNF- α and IL-6, are both known to be released from adipose tissue, and by the macrophages which infiltrate adipose tissue (Weisberg et al. 2003). The increased serum concentration of TNF- α and IL-6 decreases insulin sensitivity in the adipocytes, resulting in increased serum non-esterified fatty acids (NEFAs) which further induce insulin resistance in multiple tissues including muscle and liver (Kershaw et al. 2004). Neutralisation of TNF- α has demonstrated that its effect is in the inhibition of peripheral glucose uptake (i.e. insulin sensitivity in muscle and adipose tissue) rather than reducing the suppressive effect of insulin on hepatic glucose output (Hotamisligil et al. 1993). In contrast, IL-6 exerts a greater effect on hepatic insulin resistance while possibly enhancing insulin action in muscle tissue. IL-6 appears to induce insulin resistance in the liver by inhibition of insulin receptor signal transduction (Senn et al. 2003; Steensberg et al. 2003).

Vitamin D has recognised anti-inflammatory actions: it has been shown to dose-dependently suppress the release of TNF- α and IL-6 (Muller et al. 1992; Schleithoff et al. 2006; Zittermann et al. 2009) and CRP (Timms et al. 2002) whilst up-regulating synthesis of the anti-inflammatory cytokine IL-10 (Canning et al. 2001; Schleithoff et al. 2006), thus potentially counteracting the inflammatory consequences of increased adiposity.

TNF- α induces expression of IL-6 in the liver, and they both induce synthesis of CRP (Senn et al. 2003). Because TNF- α and IL-6 are strong inducers of hepatic CRP production, CRP is considered to be a useful biomarker for the presence of the other cytokines. Also, CRP has been shown to have a stronger and more robust relationship with diabetes and elements of the metabolic syndrome than either TNF- α or IL-6 before and after adjustment for BMI (Duncan et al. 2003; Hu et al. 2004).

A second potential mechanism for the influence of vitamin D on insulin sensitivity is the hormone's role in the expression of the insulin receptor gene. A vitamin D response element (VDRE) has been identified on the human insulin receptor (IR) gene promoter (Maestro et al. 2003) and treatment of U-937 human promonocytic cells with calcitriol in vitro resulted in increased transcription of the insulin receptor gene, together with improved insulin-dependent glucose transport (Maestro et al. 2000; Maestro et al. 2002). Additionally, calcitriol appears to stimulate glucose oxidation either via the activation of IR transcription or by a direct regulation of

phosphatidylinositol 3-kinase (PI₃-kinase) activity (Maestro et al. 2002). PI₃-kinase, together with the insulin receptor substrate proteins (IRS-proteins), has been shown in murine studies to be an important coordinator of insulin regulation (Withers et al. 2000). Insulin-stimulated activity of PI₃-kinase and other proteins downstream of PI₃-kinase such as protein kinase C (PKC) has been shown to be impaired in obese and diabetic humans, and improved in obese subjects following weight loss (Kim et al. 2003).

It appears that there are many potential roles for vitamin D in the regulation of glucose homeostasis, from the calbindin-D_{28K} mediated modulation of insulin secretion to the expression of the insulin receptor and/or the downstream signalling proteins. Also to be considered are the systemic effects of vitamin D status on other metabolic abnormalities including inflammation.

Obesity and bioavailability of vitamin D

Despite any controversy which may exist about obesity occupying the central role in the metabolic syndrome, there is no doubt that obesity does play a pivotal part in the development of type 2 diabetes. Obesity has been directly linked to increased insulin resistance (Kershaw et al. 2004) and as discussed above, even modest weight loss has been shown to reduce the risk of subsequent development of diabetes.

Vitamin D is a fat soluble vitamin and is stored in adipose tissue as protection against times of deprivation (Arunabh et al. 2003). The vitamin D endocrine system is affected by obesity. Measured doses of supplementation have been shown to induce serum 25(OH)D concentrations inversely correlated to body mass (Barger-Lux et al. 1998). Levels of serum intact PTH have been shown to be elevated in morbidly obese subjects (Arunabh et al. 2003), whilst serum levels of 25(OH)D have been clearly demonstrated to be inversely correlated with fat mass or percentage body fat (Wortsman et al. 2000; Arunabh et al. 2003; Parikh et al. 2004; Looker 2005; Lucas et al. 2005; Seidell et al. 2005).

Studies identifying this correlation have utilised the dual energy x-ray absorptiometry (DXA) technique to achieve a sensitive assessment of body fat percentage. However, all have found a less significant relationship between 25(OH)D and body mass index (BMI) or other anthropometric techniques for assessing body composition. In a study of a large cohort of New Zealand workers, Scragg et al (1995) failed to find a relationship between obesity and serum levels of 25(OH)D using BMI as the measurement for obesity. These findings suggest that adiposity needs to be carefully measured, and that BMI is not a sufficiently sensitive measurement tool.

There have been many theories to explain the increased risk of hypovitaminosis D in obese individuals. It has been postulated that obese people spend less time outside involved in physical activity, and subsequently, have less exposure to UVB radiation (Wortsman et al. 2000). As natural dietary sources of the vitamin are minimal, sunlight is a particularly relevant source in countries such as New Zealand where food is not routinely fortified with vitamin D (Olsen et al. 2000).

It is also possible that, as a fat soluble vitamin, 25(OH)D is sequestered in adipose tissue. In a case control study of obese subjects and subjects with normal body weight. Wortsman et al (2000) found that 24 hours after UVB radiation the obese subjects had an increase in circulating 25(OH)D that was less than half that of the normal weight controls. Following the UVB exposure it was determined that there was no significant difference between the obese and non-obese subjects in the capacity of the skin to produce 25(OH)D₃, but it appeared that the increased mass of subcutaneous fat sequestered more of the 25(OH)D₃ in the obese subjects and therefore less of the vitamin was released into circulation. There was no significant difference between obese and non-obese subjects in serum 25(OH)D when they were given an oral dose of 50,000 IU vitamin D₂. However, an inverse correlation was detected between peak blood vitamin D₂ and BMI. These results suggest that in the obese subjects, the orally administered vitamin D₂ was more bioavailable than the 25(OH)D₃ synthesised cutaneously, but is still likely to be sequestered in body fat.

Vitamin D and bone

Calcium plays a crucial role in a multitude of processes from mineralisation of the skeleton to highly specific actions in cell signalling. New Zealand adults consume an average of 820mg of calcium per day (Russell et al. 1999), and the degree to which this is absorbed is managed by a complex control system in which vitamin D and parathyroid hormone play a prominent part (Hoenderop et al. 2005).

Vitamin D and Calcium absorption

The absorption of dietary calcium can vary greatly depending on vitamin D status: in a state of vitamin D deficiency, only 10 – 15% of dietary calcium is absorbed, but with adequate levels of vitamin D dietary calcium absorption increases to 30 – 40% (Holick 2007). During pregnancy, lactation and periods of growth absorption can be as high as 60 – 80% if vitamin D is adequate (Holick 2004). The optimum concentration of serum 25(OH)D to ensure maximum absorption of available dietary calcium is not necessarily that which is currently regarded as “adequate”. In New Zealand the current level considered to be adequate is 50 nmol/L (Working Group of the Australian and New Zealand Bone and Mineral Society et al. 2005). Figure 8 presents data from 3 studies suggesting that calcium absorption increases proportionally with 25(OH)D concentrations to a plateau at 80 nmol/L (Barger-Lux et al. 2002; Bischoff et al. 2003; Heaney et al. 2003).

The role of calcitriol in bone metabolism and control of the calcium economy is multifaceted. It is critical for the absorption of dietary calcium, the movement of calcium through the cytosol of intestinal epithelial cells and the reabsorption of filtered calcium from the renal tubule cells (DeLuca 2004). When serum calcium levels drop calcitriol, together with parathyroid hormone, up-regulates bone resorption by activating osteoclasts and stimulating osteoclastogenesis (Holick 2004).

The main calcium influx pathways are via the epithelia of the small intestine and the tubules of the kidney. Absorption can occur by both paracellular and transcellular transport (fig. 9), with the former being dependant on the electrochemical gradient for calcium and the latter being largely mediated by calcitriol and its involvement in gene transcription (Nijenhuis et al. 2005). High dietary calcium intake results in greater paracellular transport, whilst low intake, and therefore a lower calcium gradient, means that the transcellular transport system is more active and more critical (Hoenderop et al. 2005).

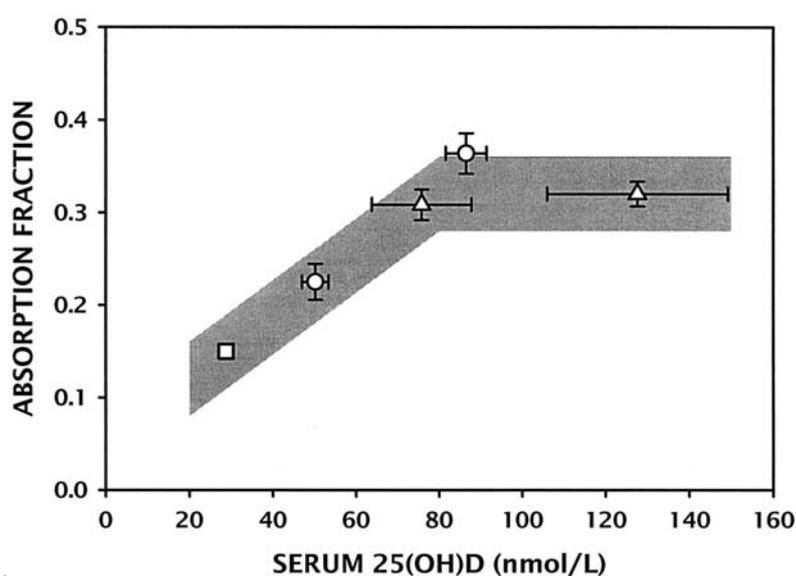


Figure 8: Calcium absorption fraction as a function of serum 25(OH)D concentrations, from 3 published reports: square - study by Bischoff et al (2003); circle - study by Heaney et al (2003); triangle - study by Barger-Lux et al (2002). Figure from Heaney (2004)

Transcellular calcium (Ca^{2+}) transport consists of a number of steps which involve the transfer of the Ca^{2+} through the apical membrane of the enterocyte or renal epithelial cell, its translocation across the cell to the basolateral membrane, and then transport through that membrane and into the circulatory system (Hoenderop et al. 2005). Influx channels on the apical membrane act as gatekeepers, protecting the cytosol from the steep concentration gradient of free Ca^{2+} between the intestinal lumen at 1 mMol and the cytosol at 0.0001 mMol (Nijenhuis et al. 2005). The channel proteins which have been identified are members of the transient receptor potential (TRP) super family, TRP vanilloid TRPV5 and TRPV6, and are found, respectively, on the apical membrane of the renal and intestinal epithelia (Nijenhuis

et al. 2005). Both of these calcium influx channel proteins have been shown to be very sensitive to calcitriol. A number of VDREs have been found in the TRPV6 promoter region, (Pike et al. 2007) suggesting a direct action for calcitriol on the TRPV6 locus. Also, expression of TRPV6 and TRPV5, together with calbindin_{9K} (see below) was considerably reduced in VDR knock out (KO) mice compared with wild-type littermates (Kim et al. 2009). Transcription of calbindin has also been shown to be regulated by calcitriol (Choi et al. 2008).

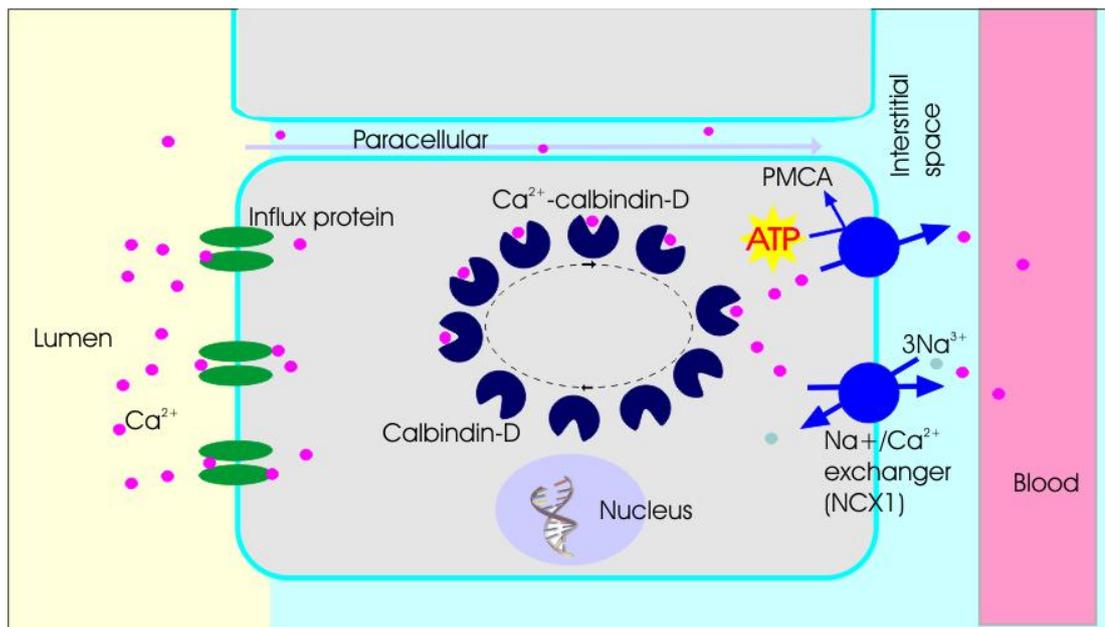


Figure 9: Transport of Ca^{2+} from the lumen of the small intestine (or the renal tubules), either by paracellular transport via tight junctions between the epithelial cells, or by transcellular transport. (Adapted from Hoenderop et al. 2005). Abbreviations: PMCA, Plasma Membrane Ca^{2+} ATPase efflux pump; $\text{Na}^{+}/\text{Ca}^{2+}$ exchanger, Sodium/Calcium exchanger; ATP, adenosine triphosphate.

The diffusion of Ca^{2+} through the cytoplasm requires that the concentration of cytosolic Ca^{2+} must be maintained at low levels so as to not interfere with intracellular signalling (Nijenhuis et al. 2005). Calbindin_{9K}, a vitamin D-dependant calcium binding protein has been proposed as the means of sequestering and transporting Ca^{2+} across the cytosol to the basal membrane (Hoenderop et al. 2005). Recent studies with TRPV6 KO and calbindin_{9K} KO mice suggest that these proteins are not unique in their roles, and there may be other calcitriol-dependent mechanisms involved in the intestinal transport of calcium (Ben et al. 2008).

Active transport across a substantial electrochemical gradient is required to transfer Ca^{2+} across the basal membrane and into the circulation. Two mechanisms have been identified as being responsible for this function - a plasma membrane Ca^{2+} -ATPase pump (PMCA) and a $\text{Na}^+/\text{Ca}^{2+}$ exchanger (NCX-1) (Tissandie et al. 2007). It appears that whilst NCX-1 is the primary extrusion mechanism in the renal epithelia, PMCA seems to be of greater importance in the enterocytes (Hoenderop et al. 2005). Interruption of vitamin D metabolism has been shown to decrease mRNA of NCX-1 in the kidney, suggesting that this transporter is also regulated by calcitriol (Tissandie et al. 2007).

Bone remodelling

Bone remodelling consists of a cycle of resorption followed by rebuilding and results in the reconstruction of bone for growth, maintenance and repair throughout life, and bone-thinning during older age (fig. 10).

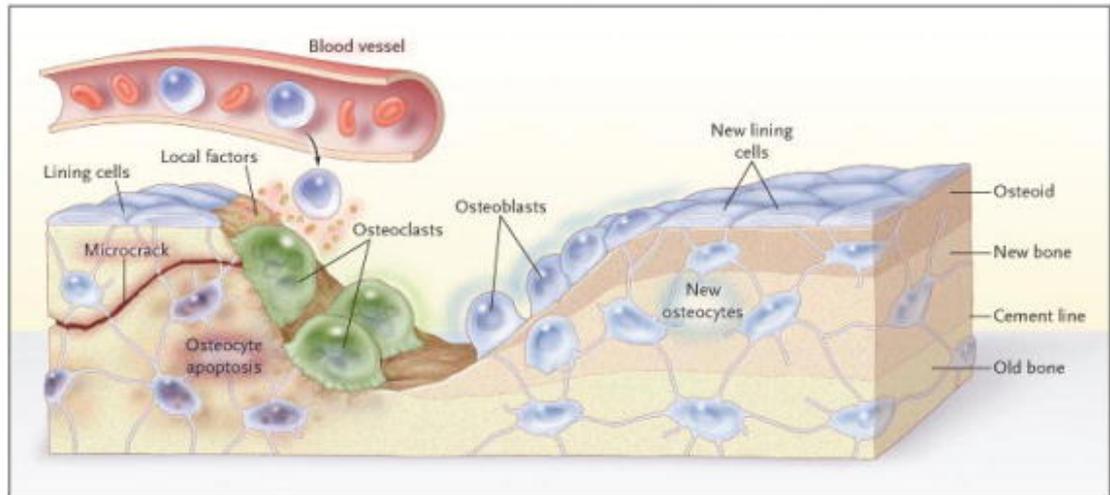


Figure 10: The remodelling cycle during maintenance and repair. A microcrack severs canaliculi causing apoptosis of the osteocytes. The lining cells and osteocytes release local factors that attract osteoclasts and macrophages from the blood, and osteoblasts from the bone marrow. The osteoclasts resorb the bone matrix and the microcrack, and the macrophages clean the newly exposed surface. Osteoblast precursors arise from mesenchymal stem cells, mature into osteoid-forming osteoblasts and begin to rebuild the bone. During the synthesis of the new bone matrix, some osteoblasts become entombed, undergo a morphological change and become osteocytes, while others become lining cells on the new bone surface. Figure from Seeman et al (2006).

During periods of growth, when the skeleton is changing in shape and size, bone is constantly being remodelled with the aim of establishing maximum strength and mineralisation. At this time there is an imbalance in favour of rebuilding, influenced by the presence of growth hormones and, later, sex hormones which sees bone grow first in length, and then in volume and mineral content (Parfitt et al. 2000; Riggs et al. 2002). During adulthood the purpose of remodelling is to maintain strength and repair the damage which occurs due to load-induced fatigue (Seeman et al. 2006).

The maintenance of bone strength and structure requires that the resorption and rebuilding cycles are balanced to ensure that the volume of bone removed is matched by the new bone replacing it (fig 10). However, in times of hormonal or nutritional deficiencies, or in older age, this balance will shift in favour of bone resorption, and bone integrity is threatened (Golder et al. 2003).

Vitamin D, parathyroid hormone and calcium homeostasis

The endocrine regulation of calcium concentration in the blood begins with highly sensitive calcium sensing receptors (CaSR) located in the parathyroid gland (fig. 11). Upon the detection of even slightly lowered calcium levels, these receptors, by way of a G-protein signalling system, stimulate the secretion of parathyroid hormone (PTH) into the circulation.

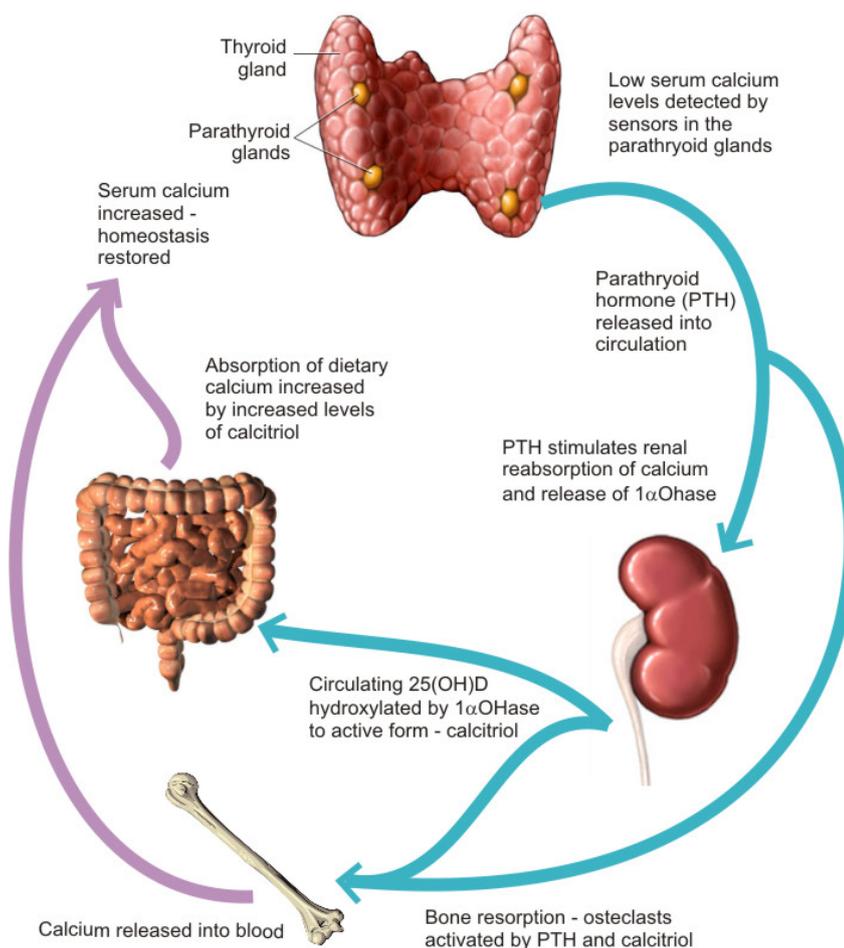


Figure 11: Overview of calcium homeostasis.

Abbreviations: 1 α OHase, 25(OH) D₃-1 α -hydroxylase. Figure created from information from (Yamamoto et al. 1984; Suda et al. 2002; Holick 2004)

This in turn activates the PTH/PTHrP (PTH/PTH related protein) receptor in the kidney and stimulates the renal proximal tubule cells to secrete 1α OHase (Suda et al. 2002). This enzyme completes the metabolism of calcitriol which is then released into circulation. PTH also stimulates the reabsorption of calcium from the filtrate passing through the kidneys (Holick 2004). Calcitriol's main task at this point is to increase absorption of dietary calcium, but it also plays a role in the reabsorption of the last 1% of filtered calcium from the distal renal tubule (Yamamoto et al. 1984).

If there is insufficient dietary calcium to maintain homeostasis, calcitriol acts together with parathyroid hormone on the osteoblasts to stimulate osteoclastogenesis and bone resorption (fig. 12). If calcitriol levels are too low to maintain serum calcium and phosphate, PTH compensates by mobilising more bone and releasing calcium into the blood (Wolpowitz et al. 2006).

Although calcitriol acts in concert with PTH on the immature osteoblasts to activate osteoclastogenesis, it also appears to have a regulatory effect, influencing the mature osteoblasts to inhibit bone resorption via a change in the ratio between osteoprotegerin (OPG) and receptor activator nuclear factor- κ B ligand (RANKL) (Hofbauer et al. 1998; Baldock et al. 2006).

OPG, which is a member of the TNF receptor family, competes with the receptor nuclear factor- κ B (RANK) for binding with RANKL. In this way it interrupts the RANK-RANKL signalling system and suppresses osteoclast formation and maturation (Simonet et al. 1997; Baldock et al. 2006). Calcitriol appears to stimulate mature osteoblasts to produce more OPG and less RANKL, thus slowing bone resorption in preparation for the osteoblasts to commence rebuilding the bone (Baldock et al. 2006).

Oestrogen also influences OPG secretion. Hofbauer et al (1999) showed that human osteoblast cells *in vitro* produced OPG mRNA proportional to their oestrogen receptor levels. Also, OGP secretion was increased, in a dose-dependent way, by oestrogen, suggesting that the protective effect of oestrogen on bone may be mediated by OPG.

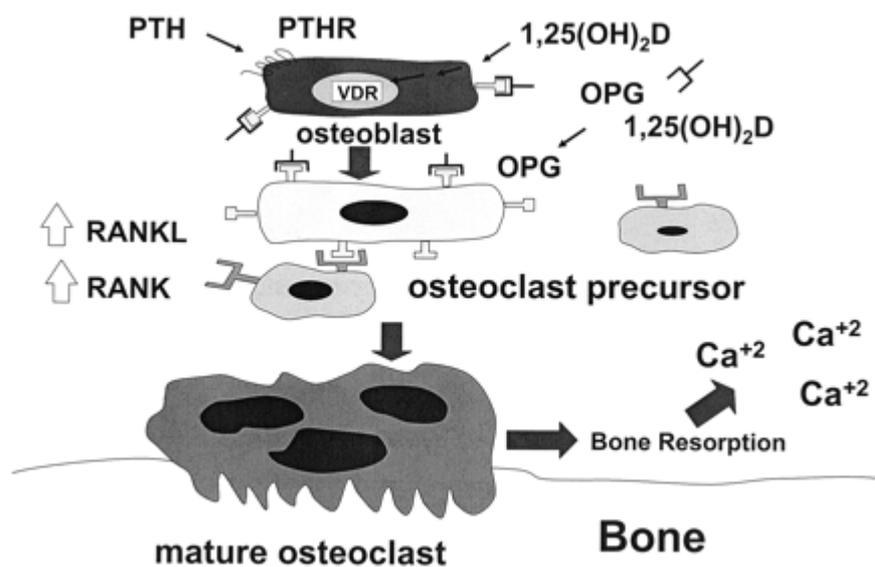


Figure 12: The role of calcitriol and PTH in bone resorption. When dietary calcium intake is insufficient to maintain serum calcium homeostasis calcitriol binds with the VDR, and PTH binds with the PTH receptor (PTHR), both of which are present in osteoblasts. This stimulates the osteoblasts to produce receptor activator nuclear factor- κ B ligand (RANKL). RANKL then binds to its receptor (RANK) on immature osteoclasts, inducing maturity, and also activates resting osteoclasts (Khosla 2001). The mature, multi-nucleated osteoclasts then release more calcium into circulation from the skeleton (Holick 2004). Abbreviations: OPG, osteoprotegerin; PTH, parathyroid hormone.

The compensatory action of PTH in the case of calcitriol deficiency principally results in an elevation of PTH levels generally known as secondary hyperparathyroidism (PTH levels >65pg/mL) (Lips et al. 2001). The high levels of PTH stimulate bone remodelling, acting on both the osteoblasts and the osteoclasts, but initially the consequence of increased levels of PTH is the increase in both numbers and activity of the osteoclasts (Hoenderop et al. 2005). The subsequent resorption of bone together with the inadequate supplies of calcium and phosphorous for bone deposition leads to deficient mineralisation of the bone matrix and ultimately the development of rickets in children and osteomalacia in adults (Wolpowitz et al. 2006).

Rickets and osteomalacia

Rickets has long been known as the disease of vitamin D-deficient children. It was initially medically described in the mid-17th century and probably coincided with the growth of urban dwelling and air pollution (Wharton et al. 2003). The discovery of the therapeutic benefit of cod-liver oil in the early 20th century (Oxbury 1985) and the subsequent elucidation of the role of sunshine and vitamin D (Hess et al. 1922) should have banished rickets to the Dickensian realms of early industrial Britain. However, rickets is reappearing, not just in the sunshine-impooverished climes of northern Europe and the nutritionally challenged developing countries, but in sub-tropical climates such as Australia and New Zealand (Blok et al. 2000; Nozza et al. 2001). Infants who are at high risk in developed countries appear to be most often dark-skinned and breast-fed (Blok et al. 2000; Kreiter et al. 2000; Nozza et al. 2001; Weisberg et al. 2004; Robinson et al. 2006). Neonates who are exclusively breast-fed are reliant on the vitamin D status of their mother. The vitamin D activity in human breast milk is approximately 40-50 IU/L when the mother has adequate levels herself (Reeve et al. 1982). This will not supply the suggested requirement of 200 - 400 IU/day to the infant (Kreiter et al. 2000; Holick 2004). Therefore, if the infant is not receiving sufficient UV exposure to synthesise vitamin D or receiving vitamin D supplementation, he will be deficient or at least insufficient in circulating 25(OH)D (Kreiter et al. 2000).

Studies in nutritionally impoverished children in parts of Africa where the diet is low in calcium and high in phytate, have demonstrated a role for low dietary calcium (<200 mg/day) in combination with moderately low (<25 nmol/L) serum 25(OH)D in the development of rickets (Pettifor 2008). However, in most cases of infantile rickets, the disease is resolved with vitamin D supplementation alone (Smith 2001).

Rickets is a disease of infancy and childhood, which occurs prior to the fusion of the epiphyses. During the rapid process of bone remodelling, which occurs during early growth, under-mineralised bone known as osteoid is formed. The failure to mineralise the newly formed osteoid results in rickets. The bone is soft and once the child begins to walk, deformities such as bowed legs and occasionally spinal curvature occur (fig. 13). The metaphyses of the long bones become cupped, splayed and frayed, and closure of the fontanelle is delayed as is tooth eruption (Wharton et al. 2003). Other skeletal symptoms include rachitic rosary of the rib cage and epyphyseal enlargement of the wrists and ankles (Weisberg et al. 2004).



Figure 13. Two children with rickets. The child in the middle is normal, while the children on either side exhibit the typical signs of rickets – bowed legs (right) and knock-knees (left) (as shown in Holick, 2004).

Once the epiphyses have fused, in late adolescence and adulthood, poor vitamin D status and low dietary calcium intake results in osteomalacia. The symptoms are similar to those of rickets, with generalised bone pain and tenderness, poorly mineralised osteoid, soft bones with the long bones of leg prone to bowing and other bone deformities including kyphosis and scoliosis (Smith 2001; Golder et al. 2003). Osteomalacia is often misdiagnosed as fibromyalgia; Plotnikoff et al (2003) reported 93% incidence of severe vitamin D deficiency in 150 patients reporting to a medical centre in the U.S.A. with generalised musculoskeletal pain. Similarly, 83% of 299 patients with idiopathic low back pain in Saudi Arabia were also found to have abnormally low levels of vitamin D. Supplementation with vitamin D was followed by an improvement in 95% of those treated (Al Faraj et al. 2003).

Osteoporosis

Osteoporosis is a disease of increasing bone porosity and fragility, predisposing to fracture. It is defined diagnostically by a bone mineral density (BMD) value ≥ 2.5 standard deviations below the mean for a healthy, young adult (World Health Organization 1994). Unlike osteomalacia and rickets, osteoporosis is not associated with any symptomatic pain (Holick 2007). It is a loss of fully-formed bone, mineral and matrix, rather than the failure to mineralise the collagen matrix, and the remaining bone is normal (Vieth 2005).

Osteoporosis is also a disease of older age, and is most often associated with peri- and post-menopausal women (Riggs et al. 2002). Oestrogen deficiency in postmenopausal women results in a small imbalance between bone formation and resorption, which favours bone loss. This is partly because a decline in oestrogen results in a reduced life span of osteoblasts and prolonged life of osteoclasts (Manolagas 2000), and a decreased secretion of OPG (Hofbauer et al. 1999). At the same time, the rate of bone turnover increases resulting in more excavated resorption sites and the subsequent replacement of well mineralised bone with new, less dense bone (Boivin et al. 2002). Because the amount of bone lost in each cycle is small, it is probably this increased rate of turnover which dictates the volume of calcium released into the extra-cellular pool and subsequently excreted (Young et al. 1967; Seeman et al. 2006).

Osteoporosis has been described as having two distinct types: Type 1 occurs in women 5 - 10 years around and immediately following the onset of menopause, whereas type 2 can occur at any age, but is most prevalent in men and women over the age of 70 years (Golder et al. 2003; Riggs et al. 2003). In type 1 osteoporosis, the loss of mineral density occurs more rapidly in bones which have a high proportion of trabecular bone, with approximately 20 - 30% loss in trabecular bone over the period of a decade, but only 5 – 10% loss of cortical bone (Golder et al. 2003). The associated “crush” fractures are observed in the vertebrae and wrist. Bone loss in the spine is rapid at $>3\%$ per year for the first 3 years following menopause and then slows with increasing years (Guthrie et al. 1998; Zhai et al. 2008). Bone loss in type 2 osteoporosis appears to affect both trabecular and cortical bone, and is characterised by fractures of the hip and pelvis (Riggs et al. 2003).

Whilst type 1 osteoporosis is largely driven by the sudden decline in oestrogen, type 2 osteoporosis seems to be more closely associated with age-related secondary hyperparathyroidism (Riggs et al. 2002). It has been shown that elevated PTH in older women can be reduced with calcium supplementation and by improving vitamin D status to levels > 75 nmol/L (Ledger et al. 1994; Need et al. 2004; Sakhaee et al. 2005; Steingrimsdottir et al. 2005; Dawson-Hughes et al. 2007; Bischoff-Ferrari et al. 2008). There is also clear evidence for vitamin D supplementation in the reduction of fracture risk in older people. A meta-analysis by Bischoff-Ferrari et al (2005) of 7 vitamin D intervention trials indicated that when the supplemented dose was 700 – 800 IU/day, with or without calcium supplementation, hip fracture risk was reduced by 26% in older adults. The authors subsequently reported a similar finding for hip and non-vertebral fractures in a meta-analysis of 20 RCTs (Bischoff-Ferrari et al. 2009).

As well as decreasing the loss of bone in older age, improving vitamin D status appears to influence muscle strength and maintenance of muscle mass. The VDR has been identified in muscle tissue (Bischoff et al. 2001) and VDR expression in muscle decreases with advancing age (Bischoff-Ferrari et al. 2004). Increased serum 25(OH)D concentrations have been associated with better muscle function in older people (Bischoff-Ferrari et al. 2004), and has been shown to reduce falls (Visser et al. 2003; Inderjeeth et al. 2007), thus reducing the risk of fracture.

Bone mineral density

As mentioned above, the diagnosis of osteoporosis centres around the assessment of BMD, although the clinical significance of osteoporosis from the patient's viewpoint is the risk of fracture. Low bone mass contributes significantly to the risk of fracture, but so too do other non-skeletal factors such as those which contribute to the risk of falling (Kanis 2002).

BMD is a value derived from the measurement of bone mineral content in a specific site, divided by the area measured. Bone mineral content is assessed using dual X-ray absorptiometry (DXA) (Kanis 2002), which is generally considered the gold standard for clinical measurement of BMD. DXA technology demonstrates a very low precision error, and in posterior-anterior spine measurement, the coefficient of variation is close to 0.5% (Genant et al. 1996). However DXA scanners can differ in terms of the way they produce, deliver and detect the X-rays. The size and

orientation of the DXA beam can also differ with some machine manufacturers using a pencil beam and some using a fan beam. In general, the latter are more modern, have shorter scan acquisition times and higher image resolution (Genant et al. 1996). For these reasons, it is important that when considering patient progress or the results of a trial, the same scanner is used for all measurements.

Biochemical indicators of bone turnover

Bone turnover markers (BTMs) are analytes which can be measured in the serum or urine to provide a clinical picture of bone turnover by measuring levels of bone resorption or formation (table 8). Because these two stages of bone metabolism are closely linked (fig. 9), markers of either provide an indication of the rate of bone turnover. Increased rate of bone turnover is associated with low bone mass (Ravn et al. 1996) and increased risk of fragility and fracture (Seeman et al. 2006).

Markers of bone formation are essentially by-products of active osteoblast activity and collagen synthesis, whilst bone resorption markers are products of collagen degradation or enzymes released by osteoclasts (Delmas et al. 2000; Eastell et al. 2008).

Table 8: Most commonly used biochemical markers of bone turnover (Eastell et al. 2008)

Markers of bone formation	Markers of bone resorption
By-products of collagen synthesis: Procollagen type 1 C-terminal propeptide Procollagen type 1 N-terminal propeptide Matrix protein: Osteocalcin - a small hydroxyapatite-binding protein produced by osteoblasts Osteoblast enzymes: Total alkaline phosphatase Bone alkaline phosphatase	Collagen degradation products: Hydroxyproline Pyridinoline Deoxypyridinoline Cross-linked telopeptides of type 1 collagen N-terminal cross-linked telopeptide C-terminal cross-linked telopeptide C-terminal cross-linked telopeptide generated by matrix metalloproteinases Osteoclast enzymes: Tartrate-resistant acid phosphatase Cathepsin K

The rate of bone turnover varies during the life time. During the second and third decade BTMs are elevated. This is when the final growth spurt takes place in the long bones, followed by the fusing of the epiphyses. Then a period of mineralisation occurs resulting in peak bone mass being achieved in the mid to late twenties. It

appears that in women BTM levels remain elevated into the early thirties, reaching and maintaining a plateau from around 35 years to pre-menopause (Glover et al. 2008). Bone turnover increases markedly at time of menopause, although it is possible that activity starts to increase before menstruation has ceased. Premenopausal women over the age of 40 have been shown to have higher levels of osteocalcin (OC) and C-terminal cross-linked telopeptides (CTX) than younger women. In post-menopausal women, OC has been seen to increase by 50 – 100% (Eastell et al. 2008). Although it is not certain how long this elevated rate of bone turnover persists for, high concentrations of BTMs have been found in women > 70 years (Eastell et al. 1988).

Vitamin D deficiency (serum 25(OH)D < 12.5 nmol/L) causes an increase in bone resorption which is thought to be due to the subsequent rise in PTH in response to hypocalcaemia induced by hypovitaminosis D (fig. 7). Increased levels of serum alkaline phosphatase (ALP) are a recognised feature of osteomalacia (Need 2006) and have been observed in vitamin D insufficient older women (Sahota et al. 1999). However, there is also evidence for increased bone turnover in both young (Jones et al. 2005; Viljakainen et al. 2006; Cashman et al. 2008) and older people with vitamin D insufficiency (13 – 50 nmol/L) (Lips et al. 2001; Kuchuk et al. 2009).

There have been very few studies investigating the effect of vitamin D supplementation (alone or with calcium) on bone markers, and the results have mostly been inconclusive.

Gram et al (1996) saw an immediate increase in procollagen type 1 C-terminal propeptide (PICP) and OC, both markers of bone formation, following short term supplementation (7 days) with calcitriol in 36 healthy males aged 21 – 54 years in Denmark. This suggests that osteoblast activity increased in response to the increase in calcitriol, but both the bone markers and serum calcitriol had returned to baseline levels in less than 28 days after supplementation stopped. However, there were no observed changes in markers of bone resorption. The authors speculate that this could be the protective effect of a high calcium diet, although calcium intake was not measured. It is also likely that calcitriol levels returned very quickly to baseline levels due to the tight regulatory systems that exist for calcitriol, and that possibly supplementation with vitamin D₃ would be more appropriate and have longer lasting effects.

Viljakainen et al (2006) supplemented 11 year old Finnish girls for one year with either 5 µg (200 IU) (n = 65) or 10 µg (400 IU) (n = 74) vitamin D₃ or a placebo (n = 73). The girls were calcium replete with a daily intake of ~1200mg. The mean serum 25(OH)D was 47 nmol/L. Although bone mineral content increases (as measured by DXA) were proportional to supplemental dose, there were no significant differences observed in any of the bone markers measured.

Barnes et al (2006) gave calcium (1500 mg) with vitamin D₃ (600 IU), or calcium (1500 mg) alone to students aged 18 – 27 years for 8 weeks. The mean vitamin D levels for both groups at baseline were ~ 50 nmol/L, and dietary calcium intake was assessed at ~ 700 mg per day. No difference was seen in bone markers or PTH, despite a significant increase in serum 25(OH)D (Barnes et al. 2006). It is possible that the high calcium intake together with an adequate vitamin D status at baseline blunted the effect of the vitamin D supplementation.

In a double-blind, randomised controlled trial, fracture patients, mean age 70 ± 11 years, were given either 3000 mg calcium carbonate and 1400 IU vitamin D₃ or a placebo containing just 200 IU vitamin D₃. The results of this trial were confounded by the recent fracture, immobility of some subjects coupled with some at a very old age (Hitz et al. 2007). However, there was a measurable increase in BMD (using DXA) and an immediate increase in markers of bone turnover was followed by a decrease to levels below baseline at 3 and 12 months in those subjects < 70 years.

Bone markers provide a way in which short term changes in bone metabolism may be monitored. This is useful for measuring the response to pharmacological treatment, lifestyle or environmental interventions. As more is learnt about the relationships of the various markers with reduced BMD, bone markers could become a reliable method for predicting risk of fracture in post-menopausal women.

South Asian population in New Zealand

Immigration

Over the past decade, a burgeoning middle class in India has seen a substantial increase in the number of Indian Asians immigrating to countries such as the United States of America and Australia (U.S. Census Bureau 2005; Australian Bureau of Statistics 2006), as well as to New Zealand. Since 2001, immigration to New Zealand from the Indian sub-continent and Sri Lanka has almost doubled. According to the 2006 census, the number of people of South Asian origin now living in New Zealand is over 113,000 and the majority (70%) of these are resident in Auckland (Statistics New Zealand 2006).

There is no reason to expect that the rapid growth in immigration from South Asia over the past decade should subside, and population predictions by Statistics New Zealand forecast an “all Asian” population of 788,000 by 2026, compared to 818,000 predicted for Maori and 482,000 for Pacific. In 2006, people who described themselves as Asian Indian or South Asian comprised one third of the “Asian” population (Statistics New Zealand 2006). Simple math, therefore, would predict that by 2026 the South Asian population in New Zealand will number at least 262,000.

Vitamin D status

Given the number of sunshine hours and the geographical latitude of New Zealand, it would be reasonable to assume that people living in New Zealand obtain sufficient UVR exposure to synthesise adequate vitamin D. However there is increasing evidence that this may not be the case, especially in some ethnic groups who, because of a darker skin, appear to be at higher risk of deficiency (Rockell et al. 2005; Judkins et al. 2006; Rockell et al. 2006). Infantile rickets is re-emerging in both New Zealand and Australia with a high proportion of South Asian children represented (Blok et al. 2000; Nozza et al. 2001; Robinson et al. 2006), and anecdotal evidence from physicians working with the South Asian population in Auckland suggests high levels of vitamin D deficiency.

To date there has been no assessment of the vitamin D status of South Asians living in New Zealand, but studies in the USA, Britain and Europe indicate that this immigrant population is at very high risk of vitamin D deficiency (Sachan et al. 2005;

Roy et al. 2007; Andersen et al. 2007 ; Meyer et al. 2008). Similarly, reports are emerging from the Indian sub-continent documenting poor vitamin D status in people across the spectrum of age, occupation and geographic location (Agarwal et al. 2002; Harinarayan et al. 2004; Marwaha et al. 2005; Goswami et al. 2008; Goswami et al. 2008; Islam et al. 2008; Puri et al. 2008).

A number of studies suggest that South Asian migrants in New Zealand, Australia, U.S.A. and the U.K. may have a higher risk of disease states associated with low vitamin D status such as infantile rickets, osteoporosis and both type 1 and type 2 diabetes (Boucher et al. 1995; Solanki et al. 1995; McDermott et al. 1997; Blok et al. 2000; Venkataraman et al. 2004; Robinson et al. 2006). Reasons for low vitamin D status are various and mostly unconfirmed. They include low incidental exposure to sunlight, deliberate avoidance of sunlight, limited dietary intake of vitamin D (there is no routine fortification of the New Zealand food supply with vitamin D) and possibly genetic factors. Polymorphisms in the vitamin D receptor, which are thought to modify susceptibility to diabetes for instance, have been identified in Indian families in Madras, Southern India (McDermott et al. 1997).

Metabolic syndrome and diabetes

In New Zealand, the prevalence of type 2 diabetes and cardiovascular disease in South Asians is considerably higher than that of the general population. In 2006 the NZ Ministry of Health (MoH) published the Asian Health Chart Book (Ministry of Health 2006), the first comprehensive survey of the health of Asian people living in New Zealand. It documents self-reported incidence of type 2 diabetes in the South Asian population as being 3 times the national average, and hospitalisation and mortality due to cardiovascular disease or ischaemic heart disease as approximately double. A report published by the Counties Manukau District Health Board (CMDHB), in whose area a large proportion of the New Zealand South Asian community reside, shows statistics that are similar to or worse than the MoH report (Gala 2008). Gala suggests that in 2007 between 2300 and 2500 adult Indians in the CMDHB area had diagnosed diabetes and another 600 - 800 were undiagnosed.

South Asian Indians have been shown to have a high susceptibility to insulin resistance and type 2 diabetes, with family history more than doubling an already increased risk (Chandalia et al. 1999; Ramachandran et al. 2001). A polymorphism in the gene coding for plasma cell membrane glycoprotein (PC)-1 K121Q has been

found to contribute to increased insulin resistance, and has been found at a higher frequency in Asian Indians than in Caucasians (Abate et al. 2003).

Bone health

More studies are emerging describing the bone health of people of South Asian origin, both from within the Indian sub-continent and in migrant populations around the world, including New Zealand (table 9). Due to differences in study design and variables measured it is difficult to draw firm conclusions, but there is some evidence for Indians living in India, as well as South Asian immigrants around the world, having low BMD which correlates strongly with poor vitamin D status (Solanki et al. 1995; Arya et al. 2004; Harinarayan et al. 2007; Andersen et al. 2008). The outcome of this relationship is exacerbated in India when lower socio-economic status or rural living results in low dietary calcium and high phytate consumption (Shatrugna et al. 2005; Harinarayan et al. 2007; Shatrugna et al. 2008), and in urban centres air pollution is sufficiently severe to impact on vitamin D status (Agarwal et al. 2002; Harinarayan et al. 2008).

Table 9: Bone studies in South Asian people

Subject group	Type of study	Findings	Reference
Published studies from the Indian sub-continent			
Men (n = 40) and women (n = 50), 20 – 30 years, Indian Paramilitary Force	Observational	Low BMD despite optimal diet, sun exposure and physical activity	(Tandon et al. 2003)
Women (n = 289) 30 – 60 years Indian, Low SES, Hyderabad	Observational	BMD had strong positive correlation with BMI and dietary calcium	(Shatrugna et al. 2005)
Children (n = 555) 10 – 18 years Indian, Low SES and Upper SES, New Delhi	Observational	High prevalence of hypovitaminosis D, but no correlation with BMD. Low BMD related to SES, poor nutrition including low dietary calcium	(Marwaha et al. 2005)
Men and women (n=92), 24 – 53 years Indian, Lucknow, North India	Observational	High prevalence of hypovitaminosis D: 21% severe, 27% moderate, 19% mild deficiency. Positively correlated with BMD at Ward's Triangle and femoral neck	(Arya et al. 2004)
Women (n = 200), 18-36 years. Factory workers Bangladeshi Dhaka, Bangladesh	Observational	High prevalence of vitamin D insufficiency, associated with reduced BMD at femoral neck and lumbar spine	(Islam et al. 2008)
Women (n = 150), ≥ 50 years, postmenopausal. Indian Tamil Nadu, India	Observational	48% osteoporosis in lumbar spine, 16.7% at femoral neck (FN). FN BMD correlated with vitamin D, Spine and FN correlated with BMI	(Paul et al. 2008)
Females (n = 858), males (n = 683), 5-70 years Indian, Pune, India	Observational	32% osteoporosis and 43% osteopenia in women aged >60 years. BMD higher in females than males < 15 years. Marginally higher in males 16 – 50 years (p = 0.1).	(Kadam et al. 2009)

Table continued overleaf

Published studies on South Asian immigrants			
Women (n = 78), 18 – 36 years. Pakistani Manchester, UK	Observational	Serum 25(OH)D low, associated with reduced BMD at hip and wrist	(Roy et al. 2007)
Women (n = 50), mean age 31.3 ±8.2 years. Indian, Auckland, New Zealand	Observational	No significant difference between Chinese, Indian and Caucasian women once corrected for skeletal size	(Cundy et al. 1995)
Women (n = 107) and Men (n = 117) Indian, Auckland, New Zealand	Observational	Bone mineral content significantly lower in Indian men and women compared to European, Maori and Pacific	(Rush et al. 2009)
Girls (n = 37), women (n = 115), men (n = 95). Pakistani Copenhagen, Denmark	Observational	Severely low 25(OH)D, high prevalence of osteopenia, no correlation with vitamin D status	(Andersen et al. 2007)

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CHAPTER 3



Study Protocol – Metabolic syndrome, vitamin D and bone status in South Asian women living in Auckland, New Zealand: a randomised, placebo-controlled, double-blind vitamin D intervention.



**The following chapter describes the study protocol.
It was published in 2008 in “BMC Public Health”**

Abstract

Background: The identification of the vitamin D receptor in the endocrine pancreas suggests a role for vitamin D in insulin secretion. There is also some limited evidence that vitamin D influences insulin resistance, and thus the early stages of the development of type 2 diabetes.

Methods: Eighty-four women of South Asian origin, living in Auckland, New Zealand, were randomised to receive either a supplement (4000IU vitamin D₃ per day) or a placebo for 6 months. At baseline, all participants were vitamin D deficient (serum 25(OH)D <50nmol/L), insulin resistant (HOMA-IR>1.93) and/or hyperinsulaemic, hyperglycaemic or had clinical signs of dyslipidaemia. Changes in HOMA-IR, lipids, parathyroid hormone, calcium and bone markers were monitored at 3 months and 6 months.

Discussion: This randomised, controlled trial will be the first to investigate the effect of vitamin D supplementation on insulin resistance in non-diabetic subjects. It will subsequently contribute to the growing body of evidence about the role of vitamin D in metabolic syndrome.

Registered clinical trial – Registration No. ACTRN12607000642482

Background

Hypovitaminosis D is becoming recognised as a worldwide problem, and exists even in countries such as New Zealand and Australia which enjoy plentiful sunshine and latitudes sufficiently moderate to allow some endogenous synthesis throughout the year (Grover et al. 2001; Skeaff et al. 2004; Lucas et al. 2005; Working Group of the Australian and New Zealand Bone and Mineral Society et al. 2005; Holick 2006). Once thought to impact only on bone health, there is now evidence to implicate vitamin D deficiency in a plethora of adverse health conditions such as cancer and auto-immune diseases. There is mounting interest in the role of vitamin D in the constellation of metabolic abnormalities grouped under the term “metabolic syndrome”, including hypertension, dyslipidaemia, abdominal obesity, glucose intolerance and type 2 diabetes.

Low serum vitamin D has been shown to correlate with impaired glucose tolerance (Boucher et al. 1995; Scragg et al. 1995b; Isaia et al. 2001; Lucas et al. 2005), whilst administration of supplemental vitamin D to subjects with elevated blood glucose levels has resulted in an improvement in insulin secretion (Kumar et al. 1994; Borissova et al. 2003). Similar improvements have been observed in vitamin D-deficient subjects following supplementation (Gedik et al. 1986; Boucher et al. 1995).

The role, if any, that vitamin D deficiency plays in insulin resistance has had little investigation. Whilst a correlation between hypovitaminosis D and insulin resistance has been identified in pregnant women (Maghbooli et al. 2008) and obese adolescents (Alemzadeha et al. 2008) randomised, controlled trials with vitamin D supplementation are sparse, especially in non-diabetic, insulin resistant, vitamin D deficient subjects. Borissova et al (2003) measured a 29% (but not significant) decrease in HOMA-IR following supplementation of 1332IU vitamin D₃ /day for one month in a group of 10 diabetic women, 70% of whom were vitamin D deficient (<50nmol/L). Pittas et.al. (2007) saw a significantly lower increase in HOMA-IR in vitamin D/calcium supplemented subjects with impaired fasting glucose, compared to those receiving calcium only, but subjects were not vitamin D deficient. Meanwhile, Taylor (1998) reported an increase in insulin resistance in 3 diabetic patients following a single intramuscular dose of 300,000 of vitamin D₂.

The importance of vitamin D in the maintenance of the calcium economy and bone health is well known. A significant positive correlation has been found between serum vitamin D levels and bone mineral density in South Asians living in India (Arya et al. 2004), Britain (Roy et al. 2007) and Bangladesh (Islam et al. 2008). An inverse relationship has been identified between vitamin D status and OC, a marker of bone mineralisation (Saadi et al. 2006). Bone markers are useful for identifying changes in bone metabolism, particularly when monitoring intervention or therapy (Delmas et al. 2000).

The population of interest in this study is women of South Asian origin living in Auckland, New Zealand. There is a high incidence of type 2 diabetes in South Asians living in New Zealand; self-reported diabetes is over three times higher for this group compared to national incidence (Ministry of Health 2006). The possibility of an ethnic or genetic predisposition to develop diabetes in this population is supported by epidemiological evidence from around the world (Venkataraman et al. 2004) and India itself where there are over 33 million people diagnosed with diabetes (Ramachandran 2005).

Between 1991 and 2001 the numbers of Asian Indians living in NZ doubled. This trend has continued exponentially with a current population of over 113,000 South Asians living predominantly in the Auckland area (Statistics New Zealand 2006). The projections for future growth indicate that the Asian population of which Indians are the second largest group will increase from 272,000 in 2001 to 667,000 by 2021, an increase of 395,000 (145%) (Statistics New Zealand 2006).

Very little is known about vitamin D status of this population. However, a number of studies suggest that South Asian migrants may have a higher risk of disease states associated with low vitamin D status such as infantile rickets, osteoporosis and both type-1 and type-2 diabetes (Blok et al. 2000; Venkataraman et al. 2004; Robinson et al. 2006). Reasons for low vitamin D status are various and mostly unconfirmed. They include low incidental exposure to sunlight, deliberate avoidance of sunlight, limited dietary intake of vitamin D (there is no routine fortification of the New Zealand food supply with vitamin D) and possibly genetic factors. Polymorphisms in the vitamin D receptor, which are thought to modify susceptibility to diabetes, have been identified in both Indian and Bangladeshi populations (McDermott et al. 1997).

Hypotheses

- There is a high prevalence of hypovitaminosis D in South Asian women living in Auckland, New Zealand.
- Supplementation of vitamin D in subjects who have demonstrated insulin resistance and vitamin D deficiency will result in an improvement in markers for metabolic syndrome.
- Supplementation of vitamin D in subjects who have demonstrated vitamin D deficiency will result in an improvement in bone marker ratios in favour of bone mineralisation.

Aims

- To establish the vitamin D status of South Asian women living in Auckland, NZ.
- To investigate the effectiveness of vitamin D supplementation in improving insulin sensitivity in women with hypovitaminosis D who have demonstrated insulin resistance, and/or lipid profiles in those with dyslipidaemia.
- To investigate the effect of vitamin D supplementation on bone marker ratios in women who have hypovitaminosis D.

Method

The study consists of two phases: Phase one included initial recruitment and screening, phase two included recruitment of selected individuals into the vitamin D intervention trial (fig. 1).

Phase one provided the opportunity for a comprehensive description of the population group with respect to general health, anthropometry, diet and other lifestyle factors. The majority of the phase one data were collected during one visit of the participant to either the Massey Human Nutrition Research Unit or Mount Roskill Surgical and Medical Centre. Participants were weighed and measured, blood samples taken, blood pressure and aural temperature measured, then the participant was given breakfast. During breakfast the medical history form was completed and checked and the diet diary explained.

Participants

It was calculated that 42 subjects would be required for each arm of the trial to demonstrate a significant difference at 80% power and 5% significance. Power

Women were invited to participate in the study through advertisements in suburban and Auckland newspapers, and Indian media such as television, radio and newspapers. Posters and leaflets were distributed in a number of venues around Auckland such as General Medical Practices with high numbers of South Asian patients, clubs, and temples. A number of South Asian social and community groups were given presentations about the study by the researchers.

A short television documentary on a channel popular with Indian viewers elicited the greatest response of all the methods used. However, the most effective form of promotion was word-of-mouth. A relatively small group of supportive women from the South Asian community promoted the study and were instrumental in the successful recruitment of 250 women.

Inclusion/exclusion criteria

Women had to be 20+ years and of South Asian origin; either the subject, both parents or all grandparents must have been born on the Indian sub-continent i.e. India, Pakistan, Bangladesh, Sri Lanka – generally referred to as South Asia.

Volunteers were excluded if suffering from significant renal dysfunction, major systemic illness, or diabetes requiring medication. Use of vitamin D supplements exceeding 1000IU/day (i.e. prescription dose), or any form of calcitriol (1, 25(OH)₂D₃) were also exclusion criteria.

Additional inclusion criteria for the intervention trial were low vitamin D (<50nmol/L) and a HOMA-IR (insulin resistance) measurement of >1.93 or hyperinsulinaemia (FSI >13 mIU/L) or hyperglycaemia (FSG 5.6 – 7.2 mmol/L) or a triglyceride/HDL-C ratio >3.0. HOMA-IR >1.93 was based on the upper quartile determined in the Chennai Urban

Population Study-7 (Deepa et al. 2002). A triglyceride/HDL ratio >3.0 has been shown to be a surrogate marker for early insulin resistance in overweight/obese people (Reaven 2005).

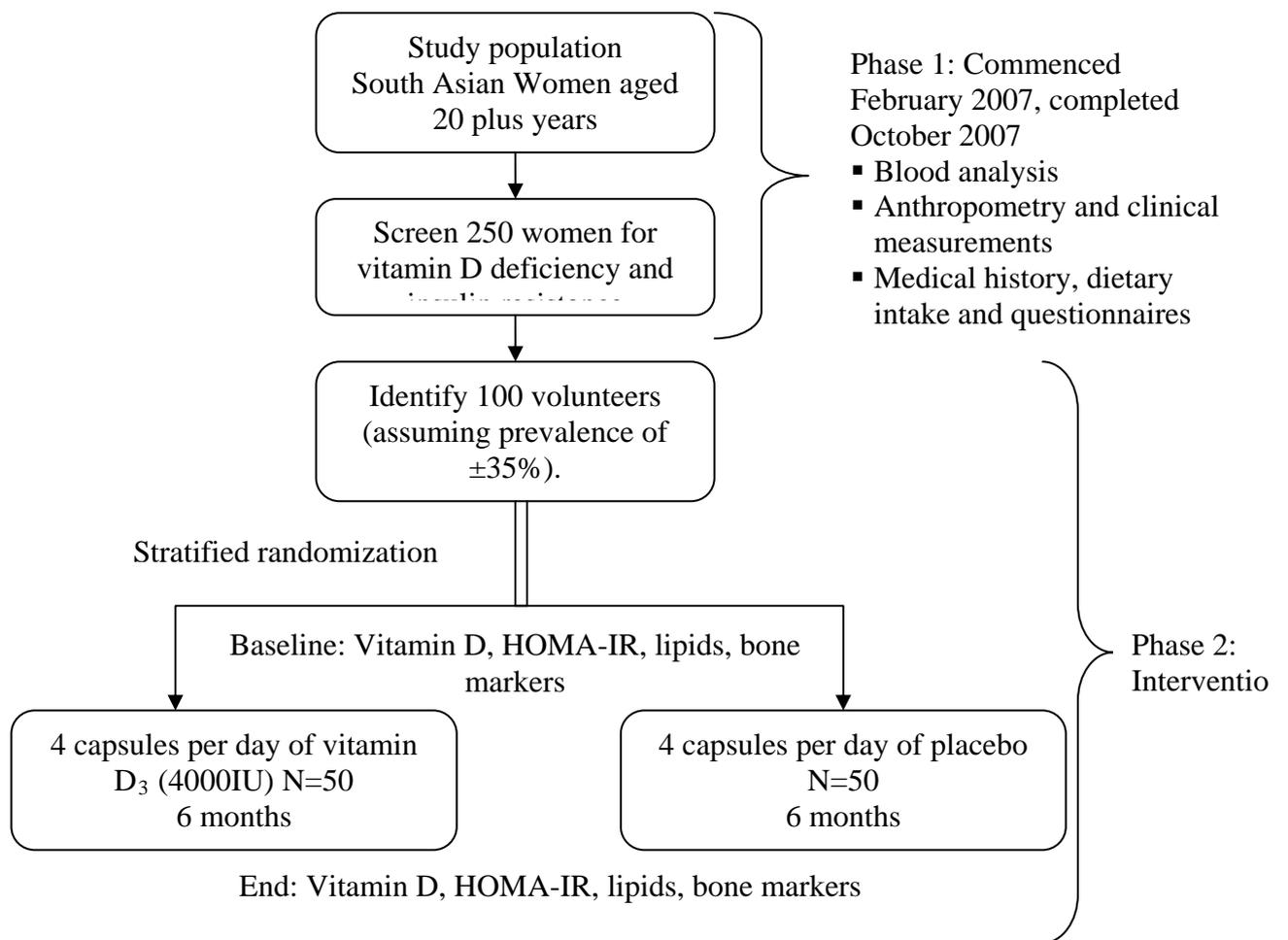


Figure 1: Study design of the Surya Study

Funding and ethics

Funding for the study was provided by the New Zealand Lottery Board (Lottery Health Grant), New Zealand Department of Internal Affairs. Additional support was provided by Blackmores Pty Ltd, Australia, who supplied the active supplement and the placebo.

Ethical approval was granted by the Massey University Human Ethics Committee (Southern A), Reference No. 06/67 and the subjects signed an informed consent form for participation in the study.

Setting

The study took place in Auckland, New Zealand. Auckland is New Zealand's largest city with a population of just over 1 million. The majority of South Asian immigrants settle in Auckland (Statistics New Zealand 2006).

Vitamin D intervention

Initial screening (phase 1 of the study) took place over a period of 9 months, commencing February 2007. See Table 1 for outcome measures and testing methods for phase 1. From the 235 women screened in phase 1, 116 qualified for phase 2, with the inclusion criteria described above. Women with FSG >7.2 mmol/L were offered a second blood test, and then withdrawn from the study and referred to their medical practitioner if the second blood test yielded similar results.

One hundred and six women were recruited for phase 2, but a number were lost to the study due to becoming pregnant (n=3), moving overseas (n=4) perceived side-effects (n=2) and medical practitioner prescribing vitamin D (n=3).

The intervention consisted of a vitamin D₃ supplement, 4000 international units (IU) per day, or placebo, in the form of 4 oral capsules, for 6 months. The consumption of 4000IU vitamin D₃ has been shown to be a safe and effective dose (Vieth et al. 2001; Heaney et al. 2003). The active supplement was tested for vitamin D₃ content by the Massey University Nutrition laboratory. The average content per dose was 4226 IU, no vitamin D was detected in the placebo. The vitamin D content will be tested again at the completion of the study to determine any degradation during storage over the period of the study.

Subjects were matched into pairs by age and BMI. Randomisation of the active/placebo capsules and allocation to pairs was performed by Blackmores using nQuery Advisor[®], version 6.0, (Statistical Solutions, Cork, Ireland). Randomisation and allocation were fully concealed from the researchers.

Table 1: Outcome measures and testing methods used for phase one .

Measure	Method
Blood analysis	All analysed by LabPlus, Auckland City Hospital, Auckland
Fasting serum insulin	Micro-particle enzyme immunoassay technology (MEIA), Abbott Diagnostics
Fasting serum glucose	An enzymatic colourimetric assay; Roche Glucose reagent kit (Cat. No. 1876899)
Total Cholesterol	Enzymatic colourimetric method (Roeschlau and Allain). Roche Cholesterol reagent kit (Cat. No. 1491458)
Triglycerides	Enzymatic conversion. Roche Triglycerides reagent kit (Cat. No. 1727711)
HDL-C	Homogenous enzymatic colourimetric assay. Roche HDL-Cholesterol Plus reagent kit (Cat. No. 04713214)
LDL-C	Calculated
Serum Calcium	Colourimetric assay. Roche Calcium reagent kit (Cat. No. 1730240)
Serum Albumin	Colourimetric assay. Roche Albumin Plus reagent kit (Cat. No. 1970909)
Serum High sensitivity CRP	Particle enhanced immunoturbidimetric assay. Roche CRPLX reagent kit (Cat. No. 03002039)
Serum Parathyroid hormone	“ECLIA” electrochemiluminescence immunoassay. PTH Reagent Pack (Cat. No. 11972103122)
Serum 25 OH vitamin D	Radioimmunoassay, DiaSorin RIA kit (Cat. No. 68100E)
Anthropometric and clinical assessments:	
Blood pressure	Omron HEM-907 Digital Automatic Blood Pressure Monitor
Temperature	Braun ThermoScan Pro 3000 aural thermometer
Height, weight, waist and hip circumference	ISAK anthropometry methods – ISAK level one accredited anthropometrist, Tanita electronic scales, stadiometer, Lufkin tape
Questionnaires:	
Dietary assessment	4-day food diary. Completed by subjects. Analysed using Foodworks 2007 (Xyris Software). Additional recipes and food lists provided by Indian dietitian
Medical and family history	Questionnaire administered following blood tests
Demographics	

Once recruited into phase two, participants were recalled for a baseline blood test prior to being given their supply of supplements. After 3 months another blood sample was taken to check for hypercalcaemia. Once the intervention was completed (6 months) participants provided one more blood sample and were weighed again. See Table 2 for outcome measures and testing methods for the intervention phase of the study.

Blood sampling and processing

Venous blood samples were taken by registered phlebotomists, using a sterile Vacutainer Flashback needle and needle holder between 8.30am and 9.30am. There is considerable circadian variability in bone markers; OC levels are increased by 20% at peak (very early morning) and CTX levels at the nadir may be half those at the nocturnal peak (Delmas et al. 2000). Accordingly, all blood samples were obtained within a consistent time period. The subjects were asked to fast overnight (at least 8 hours with no food or beverage, excluding water). Serum was used for the analysis of lipid profiles, CRP, calcium, albumin, parathyroid hormone, glucose, insulin, vitamin D. The blood was protected from light, allowed to clot for ± 30 minutes and centrifuged for 10 minutes at 2000g at 4°C within 2 hours. For the preparation of plasma, the blood was collected in vacutainers buffered with EDTA anticoagulant, and centrifuged for 10 minutes at 2000g at 4°C. For OC and CTX, plasma was dispensed and frozen within 2 hours of collection and flown to Christchurch, New Zealand on dry ice for analysis at conclusion of the study. Aliquots of serum and plasma were stored at -80°C whilst awaiting analysis.

Table 2: Outcome measures and testing method for phase two (measured at baseline, 3 months and 6 months)

Test	Method
Blood tests As listed in table 1, plus:	
Collagen C-telopeptide (CTX) Osteocalcin (OC)	Roche Elecsys beta cross-laps Roche Elecsys 2010 Canterbury District Health Board Laboratory
Weight at baseline and 6 months	Tanita electronic scales

Questionnaires

The medical history form was completed by participants during their visit for blood tests and anthropometry. Before the participant departed the researcher checked all answers and probed any responses requiring clarification. The questionnaire included basic demographics, country of birth, length of time in New Zealand and number of years of education since age 5 years. All medication and dietary supplement use was recorded, together with tobacco, betel nut and alcohol use, menstrual status, birth control and /or HRT use, and dental history. Family history of diabetes, CVD and osteoporosis was also investigated.

The 4-day estimated dietary record was presented in an open entry booklet form with pages for recording each day's food intake and extra pages for recipes. Participants were asked to record at least one weekend or feast day. Measuring cups and spoons were provided if required. Instructions for the completion of the diary were given verbally when the participant received the diary and a list of written instructions, including examples, were provided on the first 2 pages of the booklet. The women were asked to give a detailed description of the foods eaten, if possible to give a brand name and to estimate the amounts using natural measures (e.g., pieces, slices) or household measures (e.g., coffee spoon, cup).

Experienced dietitians used a standardised protocol, including a manual on household weights and measures to convert the estimated amounts into weights. All dietary records were checked for quality and completeness prior to data entry into the Foodworks 2007 programme. Additional new foods and recipes were added as required, with food composition data taken from an Indian food composition reference (Gopalan C et al. 1999). The recipes were adapted for individual subjects based on the ingredients that are being used in New Zealand. After data entry, each record was verified by a nutrition researcher and corrections were made if misinterpretation or errors in data entry were found. To ensure the best possible quality of dietary data, a subset of subjects were recalled for a qualitative, in-depth interview with a dietitian assessing their 4-day food records for dietary content (quality and quantity), food types, additions to foods and drinks and recipe composition (to further clarify fat and sugar intake.)

In the present study, a 4-day estimated dietary record was chosen because of the high respondent burden and time-consuming characteristics of 7-day dietary food records. However, Nelson *et al* (Nelson et al. 1996) stated that a 4-day record, randomised to cover weekday variations, seems to be the optimum. To obtain a highly accurate estimate of individual intake, a longer period would be recommended for many nutrients, especially those with a high within-person variation, but this was not feasible due to practical considerations (Willett 1998).

Participants were given a free-post, pre-addressed envelope for the return of the booklet.

Provision of results to participants

Following the receipt and analysis of the 4-day diet diary and the completion of the biochemical assays done during the screening phase, each participant received a feedback form. This contained an overview of the analysis of the diet, with particular reference to macronutrients, iron and calcium. Anthropometric measurements, blood pressure and blood results (fasting glucose, fasting insulin and lipids) were also included. Once recruitment into the trial was completed, participants not involved in the trial received notification of their 25(OH)D levels together with advice about supplementation where applicable.

On completion of the trial, participants will be informed of their current vitamin D status, their baseline status and if they were taking the active or placebo dose. All trial participants will be given 6 months supply of vitamin D supplements (courtesy of Blackmores), regardless of which arm of the trial they were on, upon completion of the 6-month intervention.

Newsletters were sent to all participants at intervals during the study with the goal of keeping them interested and informed.

Data Handling and statistical analysis

Name and address details were maintained in Microsoft Access. Check boxes recorded the progress of a participant through the study and thus allowed personalised letters to be sent to groups of participants as required throughout the study. All other data were entered into a single Microsoft Excel spreadsheet with participants identified only by their unique Subject Number. All entries were double-checked by another member of the research team.

Before commencement of statistical analysis the data was cleaned and checked for coding errors. Descriptive statistics were used for the baseline population characteristics: mean (SD), median, mode and range summary statistics, independent t-tests and paired t-tests for numerical normally distributed data, and Mann Whitney U and Wilcoxon Paired Rank Test (for parnormal distribution) and Chi-Squared tests (for categorical data). Spearman (checked with Pearson) correlations and ANOVA were calculated to test for associations between variables.

The primary analysis is a comparison of the change of the primary outcome (vitamin D status) between the intervention and the control group following the 'intention to treat' principle at three months and six months. In order to assess whether protocol deviations have caused bias, the results of the intention-to-treat analyses were compared to 'per protocol' analyses. Furthermore, secondary analyses were performed to explore intervention effects on the insulin resistance, HDL/triglycerides and bone markers. Analysis of covariance was used to analyse the effects of treatment on changes in variables while controlling for the effects of possible confounding factors for example different baseline conditions (e.g. BMI). Statistical analyses were performed using SPSS software (version 15).

Discussion

The information that was obtained from the screening phase of this study provided a valuable and unique insight into this rapidly increasing group of migrants entering New Zealand. It was not the intention during this phase to conduct a cross-sectional study and there was no attempt to recruit in a fully random way. Indeed, such recruitment in any specific, ethnic minority within a large population would be highly problematic. However, the 235 women who participated in phase one of the Surya Study represent approximately 0.5% of the total number of South Asian women living in New Zealand. Distribution in terms of country of origin reflected that of the entire population as reported in the 2006 census (Statistics New Zealand 2006). Level of education, although higher than the New Zealand national average, was as expected of a newly migrant population given New Zealand's immigration requirements for high educational standards (Immigration New Zealand 2008).

This trial, if successful, will provide another clue about the aetiology of type 2 diabetes, and the opportunity to reduce one of the many risk factors.

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CHAPTER 4



Vitamin D status and attitudes towards sun exposure in South Asian women living in Auckland, New Zealand



For the randomised-controlled trial, it was necessary to find 100 women who were insulin resistant and had low vitamin D status. This involved recruiting and screening a much larger number of women from the Auckland South Asian community and provided the opportunity to gather some valuable information about the health and lifestyle of this rapidly growing population.

This chapter describes the vitamin D status and attitudes towards sun exposure. It was published in “Public Health Nutrition” epub ahead of print, August 2009.

Abstract

Objective: To determine the vitamin D status of women of South Asian origin living in Auckland, New Zealand, and to investigate their attitudes and behaviours with regard to sun exposure.

Design: Cross-sectional study

Subjects: Women of South Asian origin (n=235) aged 20+ years, living in Auckland, New Zealand were tested for serum 25(OH)D, and 228 were included in these analyses. Of these, 140 completed a questionnaire about attitudes and behaviours to sun exposure, and health motivation. Exclusion criteria included high dose (>1000IU/day) supplementation with vitamin D, or any supplementation with 1,25(OH)₂D₃.

Results: As serum vitamin D concentrations were not normally distributed data are reported as median (25th, 75th percentile). Median serum 25(OH)D was 27.5 (18.0, 41.0) nmol/L. Adequate concentrations (>50 nmol/L) were observed in only 16% of subjects. Concern about skin cancer and the strength of the New Zealand sun were the most prevalent reasons given for sun avoidance, with 65 % saying they did avoid the sun. However a seasonal variation was observed, with concentrations reducing significantly (P<0.001) from summer through to early spring by 19.5 nmol/L.

Conclusions: The results of this study suggest that South Asian women are at high risk of hypovitaminosis D, due in part to deliberate sun-avoidance and an indoor lifestyle, and that they are especially vulnerable in winter and spring.

Introduction

Worldwide, there have been increasing concerns about hypovitaminosis D with high rates of deficiency reported in both developing and developed countries (Holick et al. 2008). Infantile rickets and osteomalacia are re-emerging throughout the United Kingdom, Northern Europe, USA, Saudi Arabia (Allgrove 2004) (Al Faraj et al. 2003; Plotnikoff et al. 2003) and even in temperate and sub-tropical countries like New Zealand and Australia (Blok et al. 2000; Nozza et al. 2001; Robinson et al. 2006).

New Zealand lies in the South Pacific between the latitudes of 34°S and 47°S (Bradley 1999), and during summer has very high levels of solar radiation (World Health Organisation 2002). However, there is increasing evidence of widespread hypovitaminosis D, with some ethnic groups appearing to be at higher risk (Rockell et al. 2005; Judkins et al. 2006; Rockell et al. 2006).

Over the last decade, immigration to New Zealand from the Indian sub-continent and Sri Lanka, generally referred to as South Asia, has more than doubled. According to the 2006 census the number of people of South Asian origin now living in New Zealand is over 113,000 and the majority (70%) are resident in Auckland (Statistics New Zealand 2006). There have been similar patterns of migration from India into Australia (Australian Bureau of Statistics 2006). To date there has been no assessment of the vitamin D status of South Asians living in New Zealand.

The potential consequences of inadequate vitamin D are now known to extend beyond poor bone health. Vitamin D deficiency has been implicated in age-related muscle loss and the development of sarcopenia (Visser et al. 2003), cancer (Grant 2002), cardiovascular disease (Wang et al. 2008), auto-immune conditions (Hyppönen et al. 2001; Munger et al. 2004) and type 2 diabetes (Borissova et al. 2003). In New Zealand, the prevalence of some of these diseases in South Asians is considerably higher than that of the general population. The 2006 Asian Health Chart Book (Ministry of Health 2006), the first comprehensive survey of the health of Asian people living in New Zealand, documents self-reported incidence of type 2 diabetes in the South Asian population as being 3 times the national average, and hospitalisation and mortality due to cardiovascular disease or ischaemic heart disease as approximately double.

Aims

The aim of this study was to determine the vitamin D status of women of South Asian origin living in Auckland, New Zealand, and to investigate their attitudes and behaviours with regard to sun exposure.

Method

The methodology is reported in greater detail elsewhere, both in chapter 3 of this thesis and von Hurst et al (2008). In brief, the study was promoted in media intended for an Indian and South Asian audience, as well as through community groups, temples and local medical centres. Exclusion criteria included taking a prescription dose of cholecalciferol (>1000IU/day) or any supplementation with 1,25(OH)₂D₃, major systemic illness, chronic kidney disease, diabetes, <20 years of age. Ethnicity was confirmed with a questionnaire which established country of birth for subject, her parents and all grandparents. Demographic information, medical history, nutritional supplement and medication use was obtained by interviewer-based questionnaires.

Screening commenced in February 2007 (NZ summer) and continued through the winter into early spring. To test the effect of seasonal variation on vitamin D status the screening period was divided into seasons: February and March were deemed to be summer, April and May autumn, June to August winter, and September to November spring. Serum 25(OH)D was measured using a DiaSorin double antibody radio-immunoassay. Participants were asked to complete a web-based questionnaire about attitudes and behaviours relating to sun exposure and health motivation. The 6 health motivation questions were a sub-set of a larger questionnaire, the Osteoporosis Health Belief Survey, which is subject to copyright and was used with permission of the developers (Kim et al. 1991). The sun exposure questions were developed for this study, and consisted of 7 statements with pre-set response options plus a section for free comments. These questions were tested in a focus group of South Asian women to ensure that all options were covered, that the questions were understood, and none caused offence.

Subjects were also asked to complete a 4-day food diary. These were analysed using Foodworks 2007 (Xyris Software), New Zealand Foods Data Base.

Statistical analysis was performed using SPSS software (version 15, SPSS Inc, Chicago IL.) 25(OH)D was not normally distributed, so non-parametric tests were used where applicable. The data are reported as frequencies, median (25th, 75th percentile) or mean (SD). Mann-Whitney U and Kruskal-Wallis H tests were used to compare groups, and Spearman's for correlations.

Results

The majority of participants (91%) were from India, with 6% from Sri Lanka and 3% from Pakistan. These proportions reflect those reported in the 2006 census for the South Asian population (Statistics New Zealand 2006). Seventy-nine percent were recent migrants, having been in New Zealand for ≤ 10 years. The mean age was 40.6 (10.3) years. There was a high level of education reported, with 75% having ≥ 15 years of education from age 5 years as expected from a newly migrant population given New Zealand's immigration requirements for high educational standards. Of the 250 women screened for entry into the study, 5 were excluded for use of the 50,000IU supplement which is only available on doctor's prescription, 3 due to illness or use of other medication, and 7 due to haemolysed blood sample.

Over a period of 9 months, serum 25(OH)D samples were obtained from 235 women. Serum 25(OH)D concentrations in 7 women were over 72nmol/L and, upon further investigation, it was discovered that all these women had been taking the 50,000 IU supplement, 1-2 months prior to recruitment. The exclusion criteria precluded anyone currently taking supplements > 1000 IU/day, but due to the long half-life (~ 90 days) of 25(OH)D (Wu et al. 2003), the 50,000IU supplements have a longer-lasting effect than anticipated. Consequently, these 7 women have not been included in the analysis below. An additional 33 women reported either very recent or current use of some form of vitamin D supplementation including cod liver oil and multivitamins. The median 25(OH)D concentrations of these women was 38 (24, 59) nmol/L, significantly higher than those taking no supplements 27 (17, 40) nmol/L ($P < 0.001$). The dose available in the dietary supplements reported ranged from 1.2 μ g (48IU) in cod-liver oil capsules, to 10 μ g (400IU) in multi-vitamins.

The median serum 25(OH)D concentration of the study group ($n=228$) was 27.5 (18.0, 41.0) nmol/L. Only 16% of participants were vitamin D sufficient according to the current reference concentration of ≥ 50 nmol/L. Forty-three percent had concentrations less than 25nmol/L which are associated with reduced bone mineral

density and increased risk of fracture (Working Group of the Australian and New Zealand Bone and Mineral Society et al. 2005). Vitamin D stores were at their peak in summer and reached the nadir by spring (fig 1). Median levels by season were: summer 33.5 (25.7, 46.2) nmol/L, autumn 27.0 (18.0, 41.0) nmol/L, winter 18.0 (11.0, 37.0) nmol/L and spring 13.0 (8.5, 30.0) nmol/L. Significant differences were observed in median 25(OH)D concentration between summer and winter, and autumn and winter (Mann Whitney test, Bonferroni adjusted; p value for multiple comparisons: *significance* = $P < 0.01$). There was a significant inverse correlation between PTH and serum 25(OH)D₃ concentration ($r = -0.372$, $P < 0.001$), mean PTH 5.0 (2.2) pmol/L.

Despite very determined follow-up, we were able to retrieve completed food diaries from only 139 subjects. These were analysed for the dietary contribution of vitamin D. Median dietary intake of vitamin D was 0.84µg/day (0.31µg, 1.89µg) or 33.6(12.4,75.6) IU/day. When dietary supplements were reported they were included in the assessment of dietary intake of vitamin D, and the maximum mean daily intake (including supplements) was 8.41µg.

Responses from the 141 women who answered the sun exposure questions are shown in Table 1. With the exception of question 3, there was no correlation between the responses to these questions and vitamin D status. The serum vitamin D concentrations were significantly higher ($p = 0.013$) in the respondents who said that they “enjoyed spending time in the sun” (35 [22.0, 53.7] nmol/L) compared to those who said they rarely (26.5 [18.0, 35.7] nmol/L) or never (19.5 [16.5, 27.7] nmol/L) “enjoyed spending time in the sun”.

Of the 69 women who chose to make further comments, 42% remarked on the strength of the sun in New Zealand, the ozone hole and cancer risk. Five women said that they found New Zealand to be too cold, and were always covered by clothing when they went outside.

Responses to the Health Motivation section are shown below in Table 2. Only statement 5, regarding having regular health check-ups, attracted a high level of disagreement (41.9%). Internal reliability of this subsection was acceptable with a Cronbach’s alpha value of 0.7.

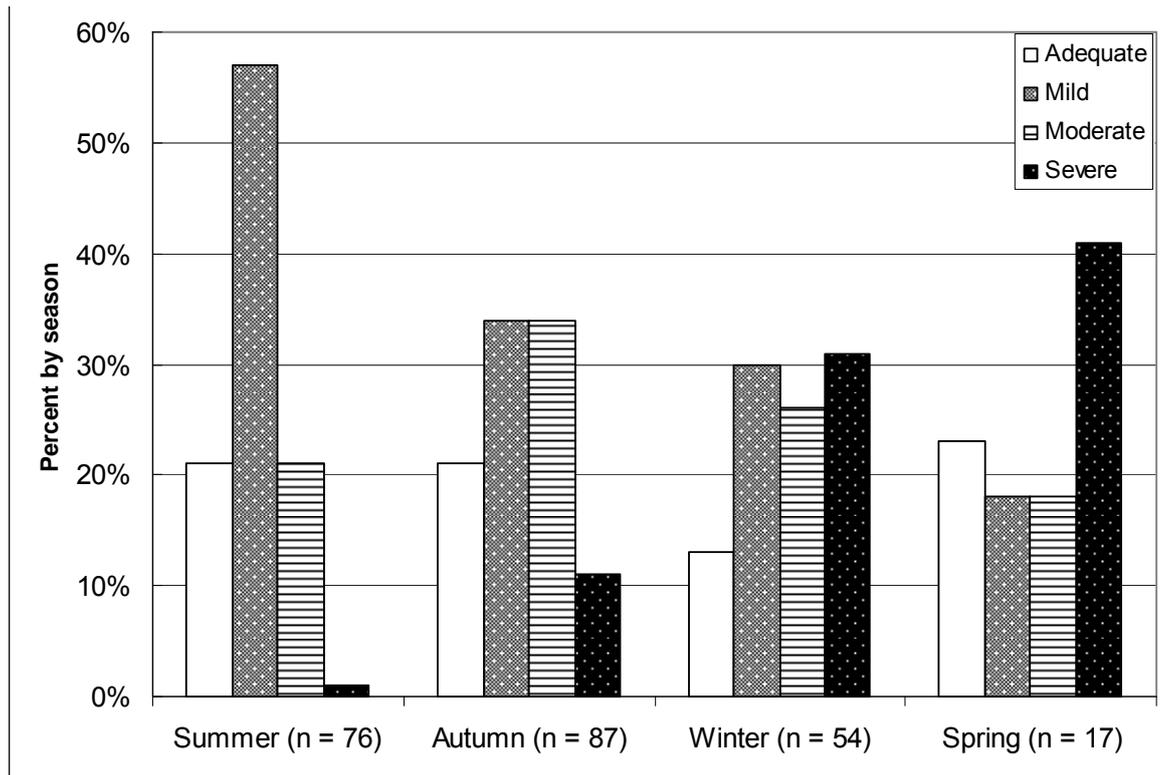


Figure 1. Levels of deficiency or adequacy by season. Bars indicate percentage of subjects tested in that season. Classification of serum 25(OH)D₃ levels of deficiency/sufficiency as per cut-offs from the Working Group of the Australian and New Zealand Bone and Mineral Society et.al. (2005): Severe deficiency <12.5 nmol/L, moderate deficiency 12.5-24.9 nmol/L, mild deficiency 25.0 – 50nmol/L, adequate >50 nmol/L.

Table 1: Responses to the questionnaire on attitudes and behaviour regarding sun exposure (n=140)

Sun exposure questions		Response	
1	I believe that sunlight can be good for your health	Always	38%
		Sometimes	62%
2	I believe that sunlight is bad for your health	Always	1%
		Sometimes	66%
		Never	29%
		Don't know	4%
3	I enjoy spending time outside in the sun	Often	56%
		Rarely	39%
		Never	4%
		Often	4%
4	I sunbathe	Never	69%
		Rarely	25%
		Often	4%
		Never	12%
5	I use sunscreen when going outside	Always	24%
		Sometimes	44%
		Rarely	20%
		Never	12%
6	My primary reason for avoiding the sun is:	Custom or religion	1%
		Public health messages	30%
		Specific health reasons	15%
		To avoid dark skin	19%
		Do not avoid the sun	35%
7	I would spend more time in the sun if . . .	I wasn't worried about skin cancer	49%
		I had more time	24%
		I had more privacy	9%
		I would not spend more time in the sun	29%

Table 2: Responses to the Health Motivation statements (n=140)

Health Motivation statement		% Agree/Strongly agree	% Neutral
1.	I eat a well-balanced diet	39.7	41.9
2.	I look for new information related to health	82.4	13.2
3.	Keeping healthy is very important for me	94.1	2.2
4.	I try to discover health problems early	75.0	19.1
5.	I have a regular health check-up even when I'm not sick	33.1	25.0
6.	I follow recommendations to keep healthy	70.6	22.8

Discussion

Due to the method of participant recruitment, this is not a random sample. However, that 84% were found to have less than the currently recommended level of vitamin D, suggests a problem in this particular ethnic group. There is an increased risk, not only of bone disease later in life, but also a range of other disease conditions now being linked to vitamin D deficiency. It is already acknowledged that this population group is particularly vulnerable to at least two of these diseases – type 2 diabetes and cardiovascular disease (Ministry of Health 2006).

As there is no fortification of the food supply with vitamin D in New Zealand, and dietary contribution of vitamin D is minimal, endogenous synthesis or supplemental vitamin D are the only realistic sources for this population. The participants who reported taking dietary supplements, either cod liver oil, multivitamins or vitamin D packaged with calcium, were not achieving recommended serum levels. It has been shown that a supplemental dose of at least 25µg (1000IU)/day is required to increase serum 25(OH)D from levels of moderate or severe deficiency to 50 nmol/L (Vieth et al. 2001) .

It would seem that South Asian women living in New Zealand are not getting sufficient sun exposure to synthesise adequate levels of vitamin D. Concerns about the strength of the New Zealand sun and the risk of skin cancer were the most prevalent reasons for avoiding sun. Although 29% of respondents said that they would not spend more time in the sun, 49% indicated they would spend more time in sun if they were not afraid of skin cancer.

In a small, qualitative study in a group of South Asian women (n=23) living in Auckland, Pettit (2007) (Pettit 2007) also found a high awareness of the risks of sun exposure in New Zealand, and of the sun safety messages which flood the media throughout spring and summer. She argues that this high level of awareness has resulted in a heightened perception of personal risk. The responses to the Health Motivation questionnaire suggest that considerable importance is placed on being healthy and obtaining information about health risks, with 82.4% saying that they look for new information related to health, and over 70% following recommendations to keep healthy. Few of these women, however, indulge in pre-emptive health checks and are therefore unlikely to have their vitamin D status checked.

There is reality behind the perceptions of the stronger New Zealand sun. The global solar ultraviolet index (UVI) has been designed as a simple measure of UVR at the earth's surface (World Health Organisation 2002). The UVI is a scale which predicts the maximum daily level of UVR with values of 8-10 regarded as very high, and ≥ 11 as extreme. Peak UVI values in the New Zealand summer can reach 14, up to 40% higher than those at corresponding latitudes in North America. This is due in part to the elliptical orbit of the earth which brings it closer to the sun during the southern hemisphere summer (McKenzie et al. 2006). Lower levels of air pollution also contribute, as does the thinning of the ozone layer over New Zealand in spring (Liley et al. 2006). Although no direct comparison can be made with India, all these factors would potentiate a lower UVI in many Indian locations, especially urban areas where high levels of pollution have been shown to impact on vitamin D status in children (Agarwal et al. 2002).

In 2005, the SunsmartTM Partnership published a position statement on the Risks and Benefits of Sun Exposure in New Zealand. This document acknowledges the health implications of too little sun exposure, but states, "There is no evidence that the sun protection messages promoted in New Zealand have affected vitamin D levels" (The Sunsmart Partnership 2005). More recently the Cancer Society of New Zealand (2008) has announced plans to develop a range of healthy sun exposure messages, that take into account season, skin type, time of day and related research findings as they emerge (Cancer Society of New Zealand 2008). Since late 2007 there has been an information sheet on the Cancer Society web site about sun exposure for people with dark skin (Cancer Society of New Zealand 2008).

To a lesser degree lifestyle, cultural and personal appearance reasons also featured as reasons to avoid the sun. A number of women commented on long work hours, working in an office, and driving to and from work. Twenty-four percent of respondents said that they would spend more time in the sun if they had more time. Pettit also found that amongst the married women in her study, time and opportunity were important constraints (Pettit 2007).

Despite the seasonal variation, the majority were still failing to reach concentrations >50 nmol/L, even those tested at the end of summer. An assessment of serum 25(OH)D concentrations in the New Zealand population also showed seasonal variation with a difference (in women) of 31 nmol/L between summer and spring

(Rockell et al. 2006). Above latitudes of 40° it is not possible to make vitamin D during winter and 25(OH)D levels in summer can be 20 – 120% higher (Porojnicu et al. 2007).

Low levels of vitamin D have been seen in other migrant South Asian populations in Denmark and Britain (Roy et al. 2007; Andersen et al. 2008), however these populations were subject to much lower UVI than this study population in Auckland, New Zealand. It is probably more useful to compare the vitamin D levels of these South Asian women to other brown-skinned ethnic groups in New Zealand. Rockell et al (2006) found the mean 25(OH)D concentration in New Zealand European and other women (adjusted for age, ethnicity, season, region and BMI) to be 49 nmol/L, compared to Maori women at 38 nmol/L and Pacific women at 33 nmol/L. Mean serum vitamin D concentration in participants of this study was 32 (19) nmol/L.

There is currently much debate around the use of 50nmol/L as the cut off for sufficiency, and scientists working in vitamin D research have recently called for this minimum to be raised to 80nmol/L (Vieth et al. 2007). None of the participants in this study met that criterion for adequacy.

In conclusion, this study shows that we can no longer afford to assume that vitamin D levels in the population will be concurrent with environmental UVR. Some groups, such as South Asians, are at higher risk of hypovitaminosis D, due in part to deliberate avoidance of sun exposure, a lifestyle which allows little or no incidental sun exposure, and lack of fortification of the food supply. Such groups are especially vulnerable during winter and spring.

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CHAPTER 5



Vitamin D supplementation reduces insulin resistance in women who are insulin resistant and vitamin D deficient – a randomised, placebo-controlled trial



The primary objective of the Surya Study was to investigate the effect of vitamin D supplementation on insulin resistance in women who were insulin resistant and had low vitamin D status. The following chapter describes the results of the trial and discusses the possible mechanisms for action of $1\alpha,25$ -dihydroxyvitamin D₃ in ameliorating insulin resistance.

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Abstract

Introduction: Low serum 25(OH)D has been shown to correlate with increased risk of type 2 diabetes. Small, observational studies suggest an action for vitamin D in improving insulin sensitivity and/or insulin secretion.

Objective: To investigate the effect of improved vitamin D status on insulin resistance.

Research design and methods: Randomised controlled double-blind intervention administering 4000 IU vitamin D₃ (n=42) or placebo (n=39) daily for 6 months to South Asian women, aged 23 - 68 years, living in Auckland, New Zealand. Subjects were insulin resistant (HOMA-IR >1.93) and had serum 25(OH)D concentration < 50 nmol/L. Exclusion criteria included diabetes medication and vitamin D supplementation > 1000 IU/day. The HOMA2 computer model was used to calculate outcomes.

Results: Median (25th, 75th percentile) serum 25(OH)D increased significantly from 21 (11, 40) to 75 (55,84) nmol/L with supplementation. Significant improvements were seen in insulin sensitivity and insulin resistance ($P = 0.003$, $P = 0.02$ respectively), and circulating serum insulin decreased ($P = 0.02$) with supplementation compared to placebo. There was no change in C-peptide with supplementation. Insulin resistance was most improved when endpoint serum 25(OH)D 80 - 119 nmol/L. Secondary outcome variables (serum lipid profile and CRP) were not affected by supplementation.

Conclusion: Improving vitamin D status in insulin resistant women resulted in improved insulin resistance and sensitivity but no change in insulin secretion. Optimal 25(OH)D concentrations for reducing insulin resistance were shown to be ≥ 80 nmol/L, providing further evidence for an increase in the recommended adequate levels.

Registered Trial No. ACTRN12607000642482

Introduction

There is mounting interest in the role of vitamin D in the aetiology of type 2 diabetes, and the most commonly preceding conditions, reduced insulin sensitivity and compromised beta-cell function.

Low serum 25(OH)D has been shown to correlate with impaired glucose tolerance and an increased risk of type 2 diabetes (Boucher et al. 1995; Scragg et al. 1995b; Isaia et al. 2001; Lucas et al. 2005; Mattila et al. 2007), whilst a correlation between hypovitaminosis D and insulin resistance has been identified in pregnant women and obese adolescents (Alemzadeha et al. 2008; Maghbooli et al. 2008). A 10-year prospective study identified an inverse relationship between baseline serum 25(OH)D concentrations and later risk of insulin resistance (Forouhi et al. 2008). Administration of supplemental vitamin D to subjects with elevated blood glucose levels has resulted in an improvement in insulin secretion (Kumar et al. 1994; Borissova et al. 2003) and similar improvements have been observed in vitamin D-deficient subjects following supplementation (Gedik et al. 1986; Boucher et al. 1995).

Tai et al (2008) found no improvement in glucose tolerance following the administration of 2 vitamin D doses (100,000 IU) with an interval of two weeks to 37 non-diabetic, vitamin D deficient adults. Nagpal et al (2009) reported a randomised controlled trial of vitamin D₃, 3 fortnightly doses of 120,000 IU or placebo, in centrally obese Indian men. The subjects were not necessarily insulin resistant, but there was some improvement in post-prandial insulin sensitivity following supplementation. A recent systematic review and meta-analysis on the role of vitamin D and calcium in type 2 diabetes concludes that "there appears to be a relationship" but due to the paucity of data, an understanding of the mechanisms is incomplete (Pittas et al. 2007).

To date there have been no randomised controlled trials with vitamin D supplementation of a dose sufficient to raise serum 25(OH)D to > 80 nmol/L in vitamin D deficient, non-diabetic, insulin resistant subjects. It has been shown that a dose of vitamin D > 2000 IU per day is required to raise and maintain serum concentration to 80 nmol/L (Vieth et al. 2007). However, the increase in serum levels is related to baseline concentration, and where a patient is severely deficient (<12.5 nmol/L), a higher dose may be required (Heaney et al. 2003). Earlier

concerns about the toxic effects of high doses of supplemental vitamin D have been allayed by safety and efficacy tests which have demonstrated that doses of 4000 IU and 10,000 IU per day for 5-6 months resulted in no toxic effects (Vieth et al. 2001; Heaney et al. 2003).

The population of interest in this study is women of South Asian origin living in Auckland, New Zealand. Self-reported diabetes is over three times higher for South Asians living in New Zealand compared to national incidence (Ministry of Health 2006) and we have previously reported a prevalence of hypovitaminosis D of 84% in South Asian women (Von Hurst et al. 2007).

Aim

The aim was to investigate the effect of improved vitamin D status on markers of metabolic syndrome, primarily insulin resistance, in South Asian women who were insulin resistant and vitamin D deficient.

Method

The study protocol is described in greater detail elsewhere (Von Hurst et al. 2008). In brief, the study design was a randomised, placebo-controlled, double-blind trial with 4000 IU vitamin D₃ (cholecalciferol) per day (4 capsules of 1000 IU each) or 4 capsules of placebo per day for six months. Women of South Asian origin were recruited and screened for hypovitaminosis D (serum 25(OH)D <50 nmol/L) plus insulin resistance (HOMA-IR ≥ 1.93) and/or triglyceride/HDL-C ratio ≥ 3.0 . There are no recognised HOMA-IR cut-offs for this population and the rather arbitrary selection of 1.93 was based on the findings of the Chennai Urban Population Study (CUPS) in India where the upper quartile was found to have a HOMA-IR score ≥ 1.93 (Deepa et al. 2002). An elevated TG/HDL ratio has been identified as a strong predictor of insulin resistance and metabolic syndrome (Reaven 2005). Exclusion criteria included fasting serum glucose ≥ 7.2 mmol/L, medication for diabetes and vitamin D supplementation ≥ 1000 IU per day.

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Massey University Human Ethics Committee (Southern A), Reference No. 06/67. Written informed consent was obtained from all subjects.

Subjects were matched into pairs by age and BMI. Randomisation of the vitamin D/placebo capsules and allocation of these to the pairs was performed by Blackmores Ltd using nQuery Advisor[®], version 6.0 (Statistical Solutions, Cork, Ireland). Randomisation and allocation were fully concealed from the researchers until after statistical analysis of the data.

Fasting blood samples and anthropometric measurements were obtained at baseline and the end of the study. The intervention in the original cohort commenced in July 2007 which is mid-winter in New Zealand, and a second small cohort (n=7) commenced in October 2007. Subjects were recalled for their final blood test 6 months later i.e. January 2008 (mid-summer) and April 2008. Subjects were also recalled for a blood test at 3 months. This was primarily to check for adverse effects in response to the high dose of vitamin D supplementation. Serum calcium results were immediately checked for abnormality by a colleague not associated with the study, and results were entered into the data base at the end of the study. Subjects were advised to contact research staff immediately if they suspected a reaction to the supplements.

The original HOMA1 model for insulin resistance was used for subject selection as explained above (Matthews et al. 1985). This model utilises a simple linear equation based on pairing fasting serum glucose (FSG) and fasting serum insulin (FSI) to establish a measure for insulin resistance: $HOMA1-IR = (FSI \times FSG)/22.5$. The technique is simple and inexpensive with relatively low subject burden, and has been shown to correlate well with the glucose clamp in predicting insulin sensitivity (Bonora et al. 2000; Wallace et al. 2004).

The revised HOMA2 model was utilised to assess outcomes of the intervention. This is a computer model consisting of nonlinear empirical equations which, when solved, allow the determination of insulin sensitivity (HOMA2%S) from FSG and FSI, and beta cell function (HOMA2%B) from paired FSG and C-peptide. C-peptide is a reliable marker for insulin secretion as, unlike insulin, it is not taken up by the liver (Wallace et al. 2004). Both β -cell function and insulin sensitivity are reported as a percentage, where 100% is normal (Levy et al. 1998). Insulin resistance (IR) is the reciprocal of % sensitivity, and 1.0 is normal.

Methods for all measurements and laboratory analysis (FSG, FSI, lipid profile, hs-CRP, calcium, 25(OH)D₃) with the exception of C-peptide are reported in chapter 3 of this thesis and in von Hurst et al (2008). C-peptide was measured in EDTA plasma samples stored at -80°C, by Canterbury District Health Board Laboratory (Christchurch New Zealand), performed on the automated Roche Elecsys 2010 analyser, CV (within batch) of 2.4% @ 620pmol/L. Insulin and glucose were measured by LabPlus, Auckland. Insulin method: micro-particle enzyme immunoassay technology (MEIA - Abbott Diagnostics), intra-assay CV 4.0% at 8.3 mU/L, inter-assay CV 4.5% at 8.4 mU/L. Glucose method: standard enzymatic colourimetric assay (Roche), intra-assay CV 0.8% at 6.6 mMol/L, inter-assay CV 1.8% at 6.55 m mol/L.

Statistical methods

It was calculated that 42 subjects would be required for each arm of the trial to demonstrate a significant difference at 80% power and 5% significance. Power calculations were based on the results of a lifestyle intervention in obese women which achieved a reduction in HOMA-IR of 0.98 ± 0.77 (Sari et al. 2007). Serum 25(OH)D, CRP, insulin, glucose, plasma C-peptide and HOMA1 and 2, were not normally distributed and are reported as median (25th, 75th percentiles). Normally distributed data is reported as mean \pm standard deviation. Non-parametric tests were used to compare groups (Mann-Whitney U), and to compare baseline and endpoint measures within groups (Wilcoxon). The Kruskal-Wallis test plus Bonferroni adjustments were used to compare more than 2 independent groups or conditions, and the Friedman test plus adjustments to compare more than 2 related groups. A two-tailed *P*-value of <0.05 was considered statistically significant.

Results

Two hundred and thirty-five women were recruited and screened for insulin resistance and hypovitaminosis D. One hundred and fourteen qualified for selection, and from those 106 women volunteered to take part in the intervention trial. Twelve were lost to the study due to becoming pregnant (n=3), moving overseas (n=4), perceived side-effects (headaches and constipation) (n=2), and medical practitioner prescribing vitamin D (n=3). Following the early loss of the above-mentioned subjects, the study was re-opened for recruitment to ensure sufficient numbers. Seven women joined the RCT 4 months after the initial cohort – their numbers are included in the totals above. A further 13 could not be contacted/traced at the end of

the trial (fig. 1) The baseline characteristics of this group of 25 (11 from the vitamin D group, 14 from the placebo group) did not differ significantly from those participants who remained in the study.

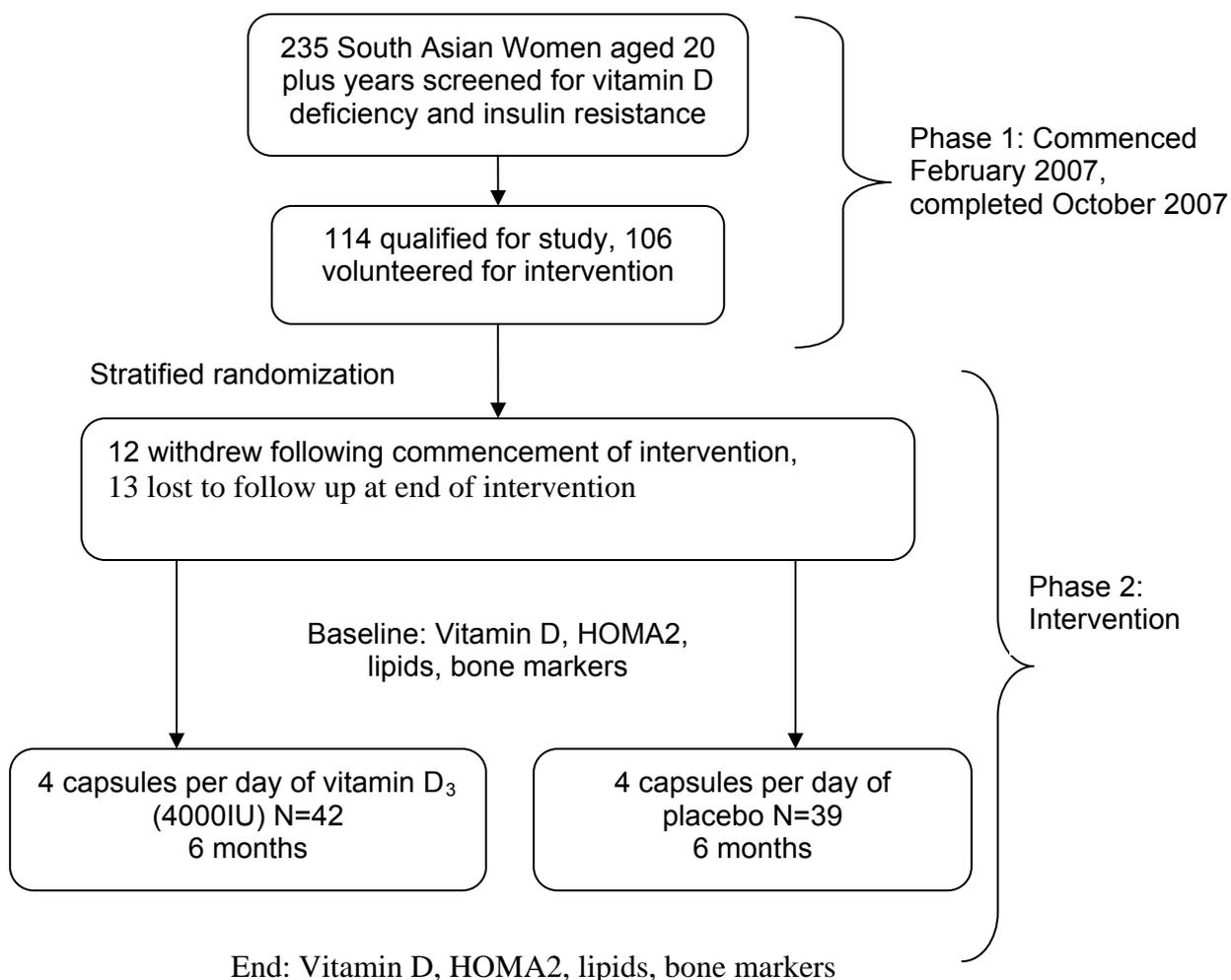


Figure 1. Participant status through duration of study

The majority of participants (91%) were Indian, with 6% from Sri Lanka and 3% from Pakistan. Seventy-nine percent had been in New Zealand for ≤ 10 years, and no relationship was seen between time in New Zealand and baseline 25(OH)D.

Baseline characteristics of the vitamin D and placebo groups are shown in table 1. There were no significant differences between the groups at baseline in any of the measures reported. BMI did not change significantly in either group during the study.

Whilst there was no relationship between HOMA-IR and age, a positive correlation was seen between HOMA-IR and BMI ($r = 0.316$, $P = 0.004$) No adverse effects were observed in the serum calcium results at 3 months.

Table 1. Baseline characteristics of trial participants

	Vitamin D group (n=42)	Placebo group (n=39)
Age (years)	41.8 ± 10.1	41.5 ± 9.1
BMI (kg/m²)	27.5 ± 5.0	27.4 ± 3.7
Waist-hip ratio	0.80 ± 0.07	0.80 ± 0.06
HOMA-IR (HOMA 1 model) *	2.70 (2.13, 3.61)	2.53 (2.11, 3.47)
Diastolic blood pressure (mmHg)	80.4 ± 8.9	80.9 ± 9.9
Systolic blood pressure (mmHg)	121.6 ± 17.6	124.0 ± 15.7
hs-CRP (mg/L)*	2.5 (1.0, 4.5)	2.4 (1.0, 4.6)
Total cholesterol (mmol/L)*	5.1 (4.5, 5.5)	4.7 (4.3, 5.5)
LDL-C (mmol/L)*	3.2 (2.9, 3.5)	3.0 (2.5, 3.3)
HDL-C (mmol/L)*	1.2 (1.0, 1.4)	1.2 (1.0, 1.4)
Triglyceride (mmol/L)*	1.4 (0.9, 1.7)	1.1 (0.7, 1.6)
Triglyceride/HDL-C ratio*	2.6 (1.9, 3.6)	2.0 (1.4, 3.3)

*Abbreviations: hs-CRP, high sensitivity C-reactive protein; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol. *Data are not normally distributed and expressed a median (25th, 75th percentile). Normally distributed data are expressed as mean ± standard deviation. There were no significant differences between the groups in any of the variables reported above. Baseline 25(OH)D, fasting serum glucose (FSG) and fasting serum insulin (FSI) are reported in table 2.*

Serum 25(OH)D concentrations increased significantly in the vitamin D supplemented group, from 21 (11, 40) nmol/L at baseline to 93 (69, 103) nmol/L at 3 months and then declined to 80 (67, 94) nmol/L at 6 months. There was also a significant increase in the placebo group between 3 months 21 (15, 36) nmol/L and 6 months 29(23, 46) nmol/L ($P = 0.014$). A significant inverse relationship was found between baseline concentrations of 25(OH)D and the change in serum 25(OH)D over 6 months in both the vitamin D ($r = -0.349$, $P = 0.023$) and placebo ($r = -0.456$, $P = 0.004$) groups (Spearman correlation). It is possible, as this relationship was seen in both groups, that this relationship was due to regression to the mean. There was no relationship between change in vitamin D concentration and BMI.

Significant improvements were seen in insulin sensitivity, insulin resistance and fasting insulin (table 2), with supplementation compared to placebo.

At 6 months, the interquartile range 25(OH)D concentrations in the vitamin D group was 67 – 94 nmol/L. Poor compliance was suspected, and subjects did report difficulties in taking 4 capsules per day for 6 months as required by the study protocol. Unfortunately, compliance was not measured objectively, only by subjects' verbal reports.

Table 2. Changes from baseline to endpoint measures of primary outcomes within vitamin D and placebo groups, and between groups.

	Vitamin D (n = 42)	<i>P</i> value (difference within group)	Placebo (n = 39)	<i>P</i> value (difference within group)	<i>P</i> value (difference between groups)
Serum 25(OH)D (nmol/L)					
Baseline	21 (11, 40)	<0.001	19(13,29)	<0.001	<0.001
End	80 (67, 94)		29 (23,36)		
Change: End - baseline	49 (21, 66)		8 (-1, 16)		
HOMA2%S					
Baseline	60.6(46.7,77.7)	0.01	65.9 (50.7,74.5)	0.69	0.003
End	68.0(52.6,102.1)		60.4 (44.8,74.0)		
Change: End - baseline	5.9(-4.1,29.8)		-5.9(-25.0,13.5)		
HOMA2%B					
Baseline	163 (129,181)	0.17	144 (120,182)	0.39	0.09
End	152 (126,180)		149 (122,181)		
Change: End - baseline	-11.2(18.0, -24.5)		0.6(-25.0,12.5)		
HOMA2%IR					
Baseline	1.7 (1.3,2.1)	0.03	1.5 (1.3,2.0)	0.27	0.02
End	1.5 (1.0,1.9)		1.7 (1.4,2.2)		
Change: End - baseline	-0.2(-0.4,0.1)		0.2(-0.3,0.6)		
Fasting insulin (mU/L)					
Baseline	13.2 (10.1,16.8)	0.02	11.9 (9.9,15.4)	0.27	0.02
End	11.2 (7.9,11.9)		13.1 (10.2,17.3)		
Change: End - baseline	-1.3(-3.6,1.0)		1.1(-2.5,4.2)		
Serum glucose (mmol/L)					
Baseline	4.7 (4.5, 5.1)	0.154	4.9 (4.5, 5.2)	0.07	0.82
End	4.8(4.6, 5.2)		5.0(4.7, 5.4)		
Change: End - baseline	0.1(0.4, -0.1)		0.1(0.4, -0.2)		
hs-CRP					
Baseline	2.5 (1.0, 4.5)	0.19	2.4 (1.0, 4.6)	0.38	0.05
End	2.15 (1.25, 3.4)		2.9 (1.5, 4.6)		
Change: End - baseline	0.00 (-1.05, 0.4)		0.2 (-0.1, 0.7)		
Fasting C-peptide (nmol/L)					
Baseline	0.81 (0.67, 1.1)	0.97	0.83 (0.64, 0.94)	0.11	0.15
End	0.81 (0.68, 1.0)		0.86 (0.69, 0.95)		
Change: End - baseline	-0.002(-0.09,0.07)		0.07 (-0.10,0.21)		

*All values are expressed as median (25th, 75th percentiles). Columns 3 and 5 are the significance (*P* value) for the change within each group from baseline to endpoint. Column 6 is the significance of the difference between groups in the change for each variable. With the exception of serum 25(OH)D, there was no significant change from baseline to endpoint in any variable in the Placebo group.*

There was a significant difference in the change in HOMA1-IR between groups ($P = 0.03$), with a decrease of -0.25 ($0.24, -0.81$) in the vitamin D supplemented group and an increase of 0.36 ($1.16, -0.41$) in the placebo group. Changes in hs-CRP, total cholesterol, triglyceride/HDL-C ratio, HDL-C or triglycerides were not significant within, and did not differ between, groups. In the vitamin D supplemented group (table 3)

Table 3. Changes from baseline to endpoint measures of secondary outcomes within and between vitamin D and placebo groups.

	Vitamin D (n = 42)	p value (difference within group)	Placebo (n = 39)	p value (difference within group)	p value (difference between groups)
hs-CRP					
Baseline	2.5 (1.0, 4.5)		2.4 (1.0, 4.6)		
End	2.15 (1.25, 3.4)	0.19	2.9 (1.5, 4.6)	0.38	
Change: End - baseline	0.00 (-1.05, 0.4)		0.2 (-0.1, 0.7)		0.05
Total cholesterol					
Baseline	5.1 (4.5, 5.5)		4.7 (4.3, 5.5)		
End	5.1 (4.4, 5.4)	0.47	4.6 (4.2, 5.2)	0.62	
Change: End - baseline	0.0 (-0.4, 0.5)		0.0 (-0.2, 0.3)		0.99
LDL-C					
Baseline	3.2 (2.9, 3.5)		3.0 (2.5, 3.3)		
End	3.2 (2.6, 3.6)	0.14	2.9 (2.4, 3.3)	0.37	
Change: End - baseline	0.0 (-0.2, 0.5)		0.0 (-0.2, 0.2)		0.76
Triglyceride/HDL-C					
Baseline	2.6 (1.9, 3.6)		2.0 (1.4, 3.3)		
End	2.3 (1.8, 4.3)	0.917	2.5 (1.5, 3.7)	0.008	
Change: End - baseline	-0.03 (-0.45, 0.47)		-0.25 (-0.58, 0.00)		0.08
Triglyceride					
Baseline	1.4 (0.9, 1.7)		1.1 (0.7, 1.6)		
End	1.3 (1.1, 1.9)		1.2 (0.9, 1.7)	0.005	
Change: End - baseline	0.05 (-0.23, 0.30)	0.37	0.1 (0.0, 0.3)		0.26
HDL-C					
Baseline	1.2 (1.0, 1.4)		1.2 (1.0, 1.4)		
End	1.2 (1.0, 1.5)	0.12	1.1 (1.0, 1.4)	0.17	
Change: End - baseline	0.0 (-0.1, 0.1)		0.0 (-0.1, 0.0)		0.06

Abbreviations: hs-CRP, high sensitivity C-reactive protein; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol

As shown in table 2, there was a significant difference in the change in HOMA2%S between the supplemented group and the placebo group. However, when the whole study group was divided into tertiles by endpoint 25(OH)D, only the upper tertile (> 80 nmol/L) showed a significant change in HOMA2%S (fig. 2). Sixteen of the 42 women in the vitamin D group achieved serum 25(OH)D concentrations of >80 nmol/L at both the 3 and 6 month tests. In these women HOMA2%S increased from 60.1 ($50.9, 70.5$) at baseline to 66.4 ($55.3, 84.5$) at 3 months ($P = 0.12$), but did not achieve significance until 6 months when HOMA2%S reached 85.8 ($47.3, 103.9$), ($P = 0.013$) (fig. 3).

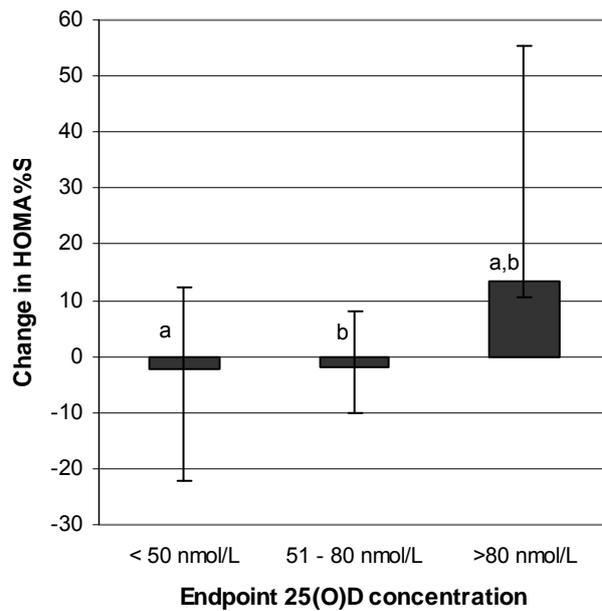


Figure 2. The change in HOMA2%S in the total study population grouped by tertiles of endpoint vitamin D concentration. Values with different superscript letters are significantly different (Kruskal Wallis test, Bonferroni adjusted; p value for multiple comparisons: $p < 0.02$)

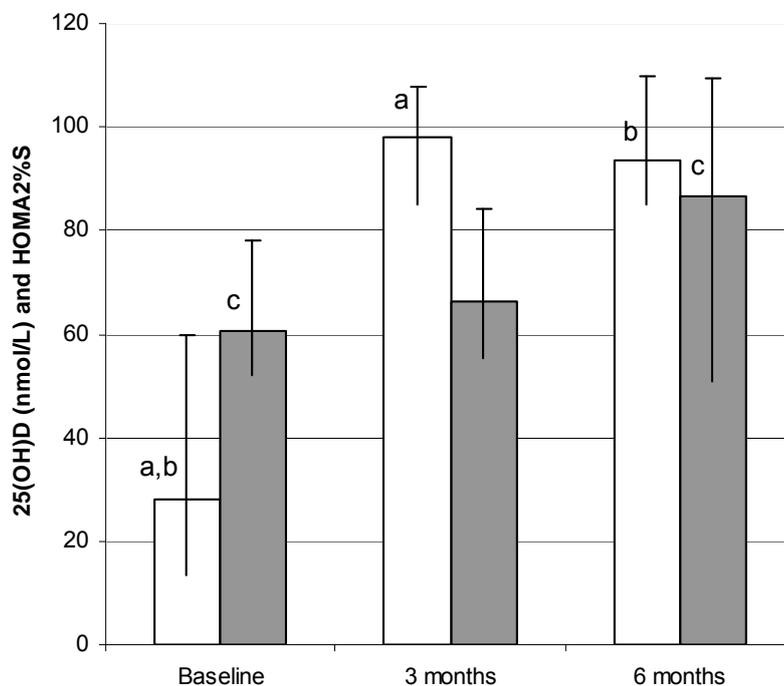


Figure 3. Changes in serum 25(OH)D (white bars) and HOMA2%S (grey bars) over time in subjects ($n = 16$) whose endpoint serum 25(OH)D was > 80 nmol/L. Values with same symbol are significantly different; a, b $P < 0.001$; c $P = 0.013$

Discussion

This study demonstrates that supplementation with vitamin D in women who are both vitamin D deficient and insulin resistant can enhance insulin sensitivity if the dose is large enough and continued over a sufficient length of time. Recent studies investigating the effect of high dose (100,000 and 120,000 IU per fortnight) vitamin D supplementation on glucose homeostasis have reported inconclusive results, (Tai et al. 2008; Nagpal et al. 2009) but have supplemented over a shorter time period (4 or 6 weeks), and subjects have not necessarily been insulin resistant. The women participating in this present study had elevated HOMA-IR, the intervention was for 6 months, and although there was a trend towards improvement in insulin sensitivity (HOMA2%S) after 3 months no significant change was seen until 6 months (Fig.1).

The wide range of endpoint serum 25(OH)D concentrations in the vitamin D group (10 -119 nmol/L) suggests variable compliance. Subjects reported finding it difficult to consistently consume 4 capsules per day for 6 months. The decline in median serum levels from the 3-month tests to endpoint in the vitamin D group probably reflects compliance dropping off in the latter half of the study. Meanwhile it is likely that the increase in serum 25(OH)D concentrations in the placebo group from mid-study is due to more incidental sun exposure with the approach of summer, to which the supplement group would also have been exposed. We have previously reported a small increase in the 25(OH)D concentrations in South Asian women who were tested during late summer, compared to those tested during winter and spring (Von Hurst et al. 2007).

The method of administration of the supplement in this study (daily dose) differed from the large fortnightly doses used in recent studies (Tai et al. 2008; Nagpal et al. 2009), and the approved prescription dose in New Zealand of 50,000 IU per month (Medsafe New Zealand 2006). Whilst the larger, less frequent doses may be advantageous from a compliance perspective, there is some evidence that daily doses are more effective than weekly or monthly doses as measured by serum 25(OH)D, PTH and bone markers (Chel et al. 1998), although this was not supported in a subsequent study (Ish-Shalom et al. 2008). It is possible that a single, large bolus could have negative outcomes; Taylor (1998) treated 3 vitamin D deficient individuals with type 2 diabetes with one intramuscular dose of 300,000 IU of vitamin D₂ and saw an increase in hyperglycaemia and lipidaemia (Taylor et al. 1998).

The significant inverse relationship between baseline concentration and increase in serum 25(OH)D suggests that baseline values influence the response to both cutaneous synthesis and supplementation. This is in agreement with other evidence that low baseline levels produce a steep dose-response slope in response to supplementation (Barger-Lux et al. 1998; Chel et al. 1998; Heaney et al. 2003), and is possibly due to feedback regulation, although synthesis of 25(OH)D in the liver is only loosely regulated (Jones et al. 1998).

Previous vitamin D supplementation studies, especially those in diabetic subjects, have concentrated on the role of vitamin D in increased insulin secretion (Borissova et al. 2003). There is also considerable evidence from animal studies that adequate 25(OH)D concentrations are required for normal insulin secretion (Chertow et al. 1983; Cade et al. 1986; Johnson et al. 1994).

Subjects in this present study were not diabetic, insulin secretion was not compromised and there was no change in C-peptide levels, suggesting that insulin secretion did not increase in response to supplementation. With the increase in serum 25(OH)D concentration in the vitamin D group, insulin resistance improved, driven by an upward shift in insulin sensitivity and a corresponding drop in fasting insulin as tissue extraction of insulin increased. Insulin sensitivity was significantly improved when the endpoint serum 25(OH)D concentrations exceeded 80 nmol/L. These findings support those of Chiu et al (2004) in a cross-sectional study which suggested enhanced insulin sensitivity might be seen if 25(OH)D concentration were to be increased from 25 to 75 nmol/L. The same study also found no independent effect of vitamin D status on β cell function in normo-glycaemic subjects, but did report a subtle variation in β -cell response with an oral glucose tolerance test with different vitamin D status (Chiu et al. 2004).

There have been at least two mechanisms postulated for an increase in insulin sensitivity in response to improved vitamin D status – suppression of chronic inflammation and increased expression of the insulin receptor and/or proteins of the insulin signalling cascade. A mild inflammatory state, marked by the presence of proinflammatory cytokines, is associated with obesity and insulin resistance. These cytokines, predominantly TNF- α and IL-6, are known to be released from adipose tissue (Weisberg et al. 2003) and increased serum concentrations are known to

induce insulin resistance in multiple tissues (Kershaw et al. 2004). Vitamin D has recognised anti-inflammatory actions: it has been shown to dose-dependently suppress the release of TNF- α and IL-6, (Muller et al. 1992; Schleithoff et al. 2006) whilst up-regulating synthesis of the anti-inflammatory cytokine IL-10, (Canning et al. 2001; Schleithoff et al. 2006) thus potentially partly counteracting the inflammatory consequences of increased adiposity. Plasma matrix metalloproteinases (MMPs) are also inflammatory markers and are associated with vascular damage and unstable angina. MMP2 and MMP9 have been shown to be inversely correlated with vitamin D status, and reduced with vitamin D supplementation (Timms et al. 2002)

CRP is considered to be a useful biomarker for the presence of TNF- α and IL-6. In this study we measured only hs-CRP; baseline levels were within the normal range in each group and the reduction following supplementation was not significant. Participants, although overweight, were not obese and mean waist/hip ratio did not exceed 0.80. These characteristics are similar to those found in women of other ethnicities, and in centrally obese Indian men (Kelley-Hedgpeeth et al. 2008; Nagpal et al. 2009). It is conceivable that the inflammatory effects of increased adiposity were not a major cause of their insulin resistance, thus the anti-inflammatory action of vitamin D is unlikely to explain the improved insulin resistance observed in this study. Future studies, especially in subjects susceptible to inflammation, should measure IL-6, TNF- α , IL-10 and possibly MMP2 and MMP9 to further explore this as a plausible mechanism.

A second potential mechanism for the influence of vitamin D on insulin sensitivity is in the regulation of the insulin signalling cascade. A vitamin D response element (VDRE) has been identified on the human insulin receptor (IR) gene promoter, (Maestro et al. 2003) and *in vitro* treatment with 1,25-dihydroxyvitamin D₃ resulted in increased transcription of the insulin receptor gene, together with improved insulin-dependent glucose transport (Maestro et al. 2000; Maestro et al. 2002). Additionally, 1,25(OH)₂D₃ appears to stimulate glucose oxidation either via the activation of IR transcription or by a direct regulation of phosphatidylinositol 3-kinase (PI3-kinase) activity (Maestro et al. 2002). Murine studies have shown PI3-kinase and the insulin receptor substrate proteins (IRS-proteins) to be important coordinators of insulin regulation (Withers et al. 2000). Insulin-stimulated activity of PI3-kinase and other proteins downstream of PI3-kinase such as protein kinase C (PKC) has been shown

to be impaired in obese and diabetic humans, and improved in obese subjects following weight loss (Kim et al. 2003).

Conclusions

Insulin sensitivity did improve in these insulin resistant women with supplementation, but the mechanism is unclear and such determination was beyond the scope of this study. The role of vitamin D in the expression of the insulin receptor gene, or in the insulin signalling pathway, requires further investigation. So too does the potential anti-inflammatory effect of vitamin D, although this is possibly more relevant if the subjects are obese and inflammatory markers are elevated.

Of particular importance is that in post-hoc analysis it was seen that no real change in insulin resistance occurred until serum 25(OH)D concentrations reached levels above 80 nmol/L. This finding provides further evidence for an increase in the recommended adequate levels of 25(OH)D from 50 nmol/L to 80 nmol/L (Vieth et al. 2007). The lack of a significant change in insulin sensitivity at 3 months, despite substantial increase in serum 25(OH)D concentration, suggests that duration of supplementation is also important. Differences in duration of study could explain the disparity between our results and those of Tai (2008) and Nagpal (2009), and the method of administration (daily vs 2-weekly) could also be a consideration.

This study highlights the importance of the long term maintenance of adequate vitamin D levels, especially in populations at higher risk of hypovitaminosis D and type 2 diabetes. The interpersonal variations in sun exposure requirements, together with the accepted risks of excessive UV radiation, make it very difficult to give recommendations about sun exposure. Supplementation may be the only answer for some populations, especially those with darker skin living in temperate climates. However, as sufficiency of dose is critical, we need to learn more about the long term safety and efficacy of high dose vitamin D supplements and the most effective way to deliver them.

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CHAPTER 6



Vitamin D supplementation and markers of bone turnover



Studies in South Asian immigrants in Europe, and from the Indian sub-continent, report a high incidence of low bone mineral density which correlates strongly with poor vitamin D status. The intervention trial with vitamin D provided the opportunity to obtain some preliminary data about the effect of vitamin D supplementation on bone metabolism. Changes in bone mineral density over the 6 month period of the trial would be difficult to detect, however biochemical markers of bone turnover are an ideal way to monitor changes in bone formation, demineralisation and total turnover over a short period of time.

Abstract

Background: There is a lack of evidence that improving vitamin D status, without changing calcium intake, has a positive effect on bone turnover as indicated by bone marker changes.

Aims: The objective of this study was to measure the effect of vitamin D₃ supplementation, in vitamin D deficient women, on markers of bone turnover - osteocalcin (OC) and C-telopeptide (CTX).

Method: The study design was a randomised controlled double-blind intervention administering 4000 IU vitamin D₃ (cholecalciferol) (n=42) or placebo (n=39) daily for 6 months to South Asian women, aged >20 years, living in Auckland, New Zealand. Subjects had serum 25(OH)D concentration < 50 nmol/L, and mean dietary calcium intake of 700±300mg/day. Exclusion criteria included vitamin D supplementation > 1000 IU/day. Subjects were stratified according to age and menopausal status.

Results: Median (25th, 75th percentile) serum 25(OH)D increased significantly from 21 (11, 40) to 75 (55,84) nmol/L with supplementation and also in the placebo group from 22 (15, 32) to 32 (24, 36) nmol/L probably due to seasonal variation. There were no changes in serum calcium or parathyroid hormone. There were 26 women who were older than 49 years or post menopausal; in those in that group who were not supplemented (n=13), CTX levels increased from 0.317±0.18 to 0.372±0.19 µg/L (P= 0.001), and OC increased from 20.00±6.56 to 23.38±8.03 µg/L indicating an increased rate of bone turnover. Supplementation appeared to abrogate the age-related increase in bone turnover; CTX decreased from 0.39±0.15 to 0.36±0.17 (P= 0.012) and there was no significant change in OC. In women who were under 49 years and premenopausal (n=55; 29 supplemented), there was no significant response to supplementation in either CTX or OC.

Conclusions: We conclude that increasing serum vitamin D levels in older women who are vitamin D deficient suppresses the age-induced increase in bone turnover and reduces bone resorption which would normally be exacerbated in conditions of low serum 25(OH)D.

Registered Trial No. ACTRN12607000642482

Introduction

The role of vitamin D in the control of calcium homeostasis and bone metabolism is well known. Calcitriol ($1\alpha,25(\text{OH})_2\text{D}_3$), the active metabolite of vitamin D has been shown to regulate the expression of the proteins involved in calcium absorption in the intestine and reabsorption in the kidney (Pike et al. 2007; Choi et al. 2008; Kim et al. 2009). Calcium absorption is reduced in a state of vitamin D deficiency, and is believed to plateau when circulating $25(\text{OH})\text{D}$ reaches a concentration of 80 nmol/L (Barger-Lux et al. 2002; Bischoff et al. 2003; Heaney et al. 2003; Holick 2007).

If there is insufficient dietary calcium to maintain homeostasis, calcitriol acts together with parathyroid hormone on the osteoclasts to stimulate bone resorption (Khosla 2001). However, if vitamin D levels are also low the primary response is an elevation of parathyroid hormone (PTH) level (secondary hyperthyroidism) and subsequent activation of the osteoclasts. This results in an increase in bone resorption and bone turnover, and loss of calcium in the bone (Lips et al. 2001; Jones et al. 2005). Although calcitriol acts in concert with PTH to activate osteoclastogenesis, it also appears to have a regulatory effect influencing the mature osteoblasts to inhibit bone resorption via a change in the ratio between osteoprotegerin (OPG) and receptor activator nuclear factor- κB ligand (RANKL) (Hofbauer et al. 1998; Baldock et al. 2006).

Biochemical markers of bone resorption and formation are measurable in the blood or urine, and provide an indication of the rate of bone turnover (Delmas et al. 2000). Increased bone turnover is associated with poor bone mineral density (BMD) and increased fragility (Eastell et al. 2008), and is associated with vitamin D levels < 50 nmol/L (Sahota et al. 1999; Lips et al. 2001; Need 2006; Cashman et al. 2008; Kuchuk et al. 2009). However despite this evidence of a relationship between vitamin D status and bone turnover, there has been to date no conclusive evidence that an improvement in vitamin D status brings about a measurable change in overall bone turnover, an increase in bone formation or decrease in bone resorption as indicated by changes in bone markers.

Gram et al (1996) observed a short term response in markers of bone formation following a 7-day supplementation with calcitriol, but both serum calcitriol and bone markers returned to baseline levels once the supplementation ceased. This result was not surprising as calcitriol concentration is very tightly regulated and catabolism

occurs as a result of a positive feedback loop as soon as serum levels are elevated (Sutton et al. 2003). Subsequent intervention studies have used vitamin D.

Of the limited number of intervention studies which measured bone markers, only one has supplemented with vitamin D₃ alone. This study, in adolescent girls (11.4 ± 0.4 years) with adequate calcium intake, showed a dose-dependent increase in bone mineral content (BMC) over 12 months, but no significant differences in bone markers between doses of 5µg (200 IU), 10µg (400 IU) vitamin D₃ and placebo (Viljakainen et al. 2006).

Two other reported studies which examined changes in bone markers supplemented with high doses of calcium (1200 and 1500mg) in addition to vitamin D₃. Hitz et al (2007) observed an increase in bone mineral density (BMD), concurrent with decreased PTH and bone turnover in older people under 70 years and reduced loss in those over 70 years. However there was no change in healthy students aged 18-27 years (Barnes et al. 2006).

The study reported here is a secondary outcome from the Surya Study, a trial investigating vitamin D supplementation and insulin resistance (Von Hurst et al. 2008). The primary aim of the Surya Study was to investigate the effect of improved vitamin D status on insulin resistance in women of South Asian origin who were insulin resistant and vitamin D insufficient or deficient (25(OH)D < 50 nmol/L). However, because the relationship between vitamin D and bone is so important, we also took the opportunity to measure the effect of supplementation on markers of bone turnover.

The selected subject group was women of South Asian origin living in New Zealand. South Asia encompasses the Indian sub-continent and Sri Lanka, and migrants from that area have been reported to have a high prevalence of vitamin D deficiency (Boucher et al. 1995; Nozza et al. 2001; Andersen et al. 2007 ; Meyer et al. 2008) as are urban-dwelling Indians in India (Harinarayan et al. 2007; Harinarayan et al. 2008; Puri et al. 2008). There is also evidence of South Asians living in New Zealand having a much higher prevalence of type 2 diabetes and cardiovascular disease than the general population (Ministry of Health 2006), and of Indian women having compromised bone health, possibly due to poor vitamin D status (Islam et al. 2008; Paul et al. 2008; Kadam et al. 2009).

Method

The study protocol is described in greater detail in chapter 3 of this thesis. The study design was a randomised, placebo-controlled, double-blind trial with 4000 IU vitamin D₃ (4 capsules) or 4 capsules of placebo per day for six months.

Volunteers were screened for hypovitaminosis D (< 50 nmol/L) plus insulin resistance (HOMA-IR \geq 1.93) and/or triglyceride/HDL-C ratio \geq 3.0. Exclusion criteria included fasting serum glucose \geq 7.2 mmol/L, medication for diabetes and vitamin D supplementation \geq 1000 IU per day.

Ethical approval was granted by the Massey University Human Ethics Committee (Southern A), Reference No. 06/67 and the subjects gave written informed consent for participation in the study.

Subjects were matched into pairs by age and BMI. Randomisation of the vitamin D/placebo capsules and allocation to pairs was performed by Blackmores Ltd using nQuery Advisor[®], version 6.0 (Statistical Solutions, Cork, Ireland). Randomisation and allocation were fully concealed from the researchers until after statistical analysis of the data.

Fasting blood samples and anthropometric measurements were obtained at baseline and the end of the study. The intervention in the original cohort commenced in July 2007 which is mid-winter in New Zealand, and a second small cohort (n=7) commenced in October 2007. Subjects were recalled for their final blood test 6 months later i.e. January 2008 (mid-summer) and April 2008. There is considerable circadian variability in bone markers; osteocalcin (OC) levels are increased by 20% at peak (very early morning) and C-terminal cross-linked telopeptide of type 1 collagen (CTX) levels at the nadir in the early afternoon may be half those at the nocturnal peak (Delmas et al. 2000). Accordingly, all blood samples were obtained within a consistent time period, between 8 am and 10 am.

Methods for the measurements and laboratory analysis of PTH, calcium, and 25(OH)D have been described previously (Von Hurst et al. 2008). Osteocalcin and CTX were measured in EDTA plasma samples stored at -80°C, by Canterbury

District Health Board Laboratory (Christchurch New Zealand), performed on the automated Roche Elecsys 2010 analyser.

Subjects were divided by age and menopausal status – Group 1 were pre-menopausal and < 49 years, Group 2 were postmenopausal and/or ≥ 49 years. Four day food diaries were completed by subjects at baseline and analysed using Foodworks 2007 (Xyris Software, New Zealand Foods Database).

Statistical methods

Power calculations were based on the requirements and outcomes of the primary objective of the study. Serum 25(OH)D was not normally distributed and is reported as median (25th, 75th percentiles). Normally distributed data is reported as mean ± standard deviation. Non-parametric tests were used to compare groups (Mann-Whitney U), and to compare baseline and endpoint measures within groups (Wilcoxon). A two-tailed *P* value of <0.05 was considered as statistically significant. Spearman's correlations were used for correlations involving 25(OH)D status, but where data was normally distributed, Pearson's correlations were used.

Results

Two hundred and thirty-five women were recruited and screened for insulin resistance and hypovitaminosis D. One hundred and fourteen qualified for selection, and from those, 106 women volunteered to take part in the intervention trial. Twelve were lost to the study due to becoming pregnant (n=3), moving overseas (n=4) perceived side-effects (n=2) and medical practitioner prescribing vitamin D (n=3). A further 13 could not be contacted/traced at the end of the trial. The baseline characteristics of this group of 25 did not differ significantly from those participants who remained in the study. Blood samples were taken at 6 months from 81 women, 42 from the vitamin D group and 39 from placebo group.

The majority of participants (91%) were Indian, with 6% from Sri Lanka and 3% from Pakistan. Seventy-nine percent had been in New Zealand for ≤10 years. Mean dietary calcium intake was 700 ± 300mg/day

Despite the low baseline serum 25(OH)D concentrations present in all subjects, there was still a significant inverse relationship at baseline between PTH and 25(OH)D in both groups and between PTH and serum calcium in Group 1 but not

Group 2. There was also a significant correlation between baseline 25(OH)D and serum calcium but this relationship was lost at 6 months. Although there was no significant change in serum PTH in response to the supplementation, the relationship between PTH and 25(OH)D was stronger at 6 months in both groups (Table 1).

Table 1. Correlations between serum 25(OH)D, parathyroid hormone (PTH) and calcium at baseline and endpoint, stratified by groups

		Correlation at baseline (r)	P value	Correlation at 6 months (r)	P value
Serum 25(OH)D and PTH	Group 1	-0.399	0.003	-0.465	<0.001
	Group 2	-0.502	0.009	-0.511	0.008
Serum 25(OH)D and calcium	Group 1	0.298	0.027	-0.100	0.468
	Group 2	0.455	0.022	-0.240	0.238
Serum PTH and calcium	Group 1	-0.352	0.008	0.013	0.923
	Group 2	-0.235	0.259	0.211	0.301

Group 1: Premenopausal and < 49 years. Group 2: ≥ 49 years and/or postmenopausal.

Serum 25(OH)D concentrations increased significantly in response to supplementation, from 21 (11, 40) nmol/L at baseline to 75 (55, 85) nmol/L at 6 months in the vitamin D group. There was a much smaller, but significant increase in serum 25(OH)D in the placebo group overall, from 22 (15, 32) nmol/L to 32 (24, 46) nmol/L. Baseline serum 25(OH)D concentration in placebo group 1 was significantly lower than in group 2 ($P = 0.001$), and the increase from baseline to end in the placebo group was only significant in group 1 (Table 2).

A significant inverse relationship was found between baseline concentrations of 25(OH)D and the change in 25(OH)D over 6 months in both the vitamin D ($r = -0.349$, $P = 0.023$) and placebo ($r = -0.456$, $P = 0.004$) groups.

Within the two groups, baseline values between the vitamin D and placebo arms were not significantly different in any of the variables reported. There were no significant differences in baseline values between group 1 and group 2 in PTH, OC or CTX, however the premenopausal group (group 1) had significantly lower baseline 25(OH)D concentrations than group 2: 19 (11, 29) vs 32 (22, 53) nmol/L ($P < 0.001$).

Table 2. Change in serum 25(OH)D, PTH, bone markers from baseline to endpoint.

	Serum 25(OH)D nmol/L		P value between groups	Parathyroid hormone pmol/L		P value between groups
	Vitamin D	Placebo		Vitamin D	Placebo	
Group 1 baseline end P-value within group Change: end - baseline	n=29 20 (11, 39) 75 (55, 83) <0.001	n=26 18 (12, 27) 30 (23,42) 0.001	<0.001	n=29 5.44±2.84 4.77±1.74 0.09	n=26 5.65±1.90 5.40±1.69 0.46	0.41
	48 (21, 69)	11 (0, 19)		-0.672±2.072	-0.246±1.673	
Group 2 baseline end P-value within group Change: end - baseline	n=13 31 (17, 57) 74 (56, 99) 0.002	n=13 32 (23, 40) 40 (27, 53) 0.17	<0.001	n=13 6.05±2.91 6.23±4.47 0.8	n=13 4.52±1.40 5.54±1.53 0.008	0.23
	49 (23, 57)	7 (-4, 9)		0.18±2.21	1.02±1.15	
	Osteocalcin µg/L		P value between groups	C-telopeptide µg/L		P value between groups
	Vitamin D	Placebo		Vitamin D	Placebo	
Group 1 baseline end P-value within group Change: end - baseline	n=29 19.88±7.45 19.55±7.12 0.60	n=26 20.04±5.44 22.01±7.27 0.022	0.026	n=29 0.30±0.15 0.29±0.14 0.574	n=26 0.31±0.15 0.32±0.16 0.906	0.636
	-0.036±3.35	1.96±4.03		-0.011±0.108	0.002±0.103	
Group 2 baseline end P-value within group Change: end - baseline	n=13 21.38±4.99 21.15±4.04 0.8	n=13 20.0±6.56 23.38±8.03 0.004	0.02	n=13 0.39±0.15 0.36±0.17 0.012	n=13 0.317±0.18 0.372±0.19 0.001	<0.001
	-0.231±3.876	3.385±3.500		-0.030±0.037	0.055±0.046	

Group 1 is women who are pre-menopausal and < 49 years; Group 2 is women who are postmenopausal and/or ≥ 49 years. Serum 25(OH)D is not normally distributed and is expressed as mean (25th, 75th percentiles). All other values expressed as mean ± standard deviation. P value between groups is the difference in change from baseline to end between Vitamin D and Placebo groups.

No significant response to supplementation was observed in the premenopausal group in any of the variables (other than 25(OH)D), although there was a decrease of borderline significance in PTH ($P = 0.09$). In group 2, there was a significant decrease in CTX ($P = 0.012$) in response to supplementation, but no change in OC. There were, however, significant increases in both OC ($P = 0.004$) and CTX ($P = 0.001$) in the group 2 women taking the placebo over the course of the study.

Discussion

In women who were under 49 years and premenopausal (Group 1), there was no significant response to supplementation in either CTX or OC, although OC did increase significantly in the placebo arm ($P = 0.02$). There are no established reference intervals for OC or CTX in South Asian women. However, Glover et al (2008) have published reference intervals for CTX in premenopausal women based

on an assessment of Caucasian women from Belgium, France, UK and USA. Their median of 0.299 $\mu\text{g/L}$ (geometric mean 0.317 $\mu\text{g/L}$) compares with the premenopausal women in this study at $0.310 \pm 0.150 \mu\text{g/L}$.

During the course of the trial, bone turnover rate continued to increase in the placebo arm of the postmenopausal women, with both OC and CTX increasing significantly. However, supplementation appeared to abrogate this menopause-related increase in bone turnover; CTX decreased ($P= 0.012$) and there was no significant change in OC.

OC is primarily a marker of bone formation, but because bone resorption and formation are closely linked, OC is also a reliable marker for bone turnover, and correlates with increased risk of fracture in postmenopausal women (Hoshino et al. 2000). Increased rate of bone turnover is associated with low bone mass (Ravn et al. 1996) and increased risk of fragility and fracture (Seeman et al. 2006). Higher levels of circulating CTX indicate increased bone resorption and a corresponding elevation in bone fragility (Delmas et al. 2000).

Osteoprotegerin (OPG) is a powerful protector of bone. Secreted by mature osteoblasts, it competes with the receptor activator of nuclear factor- κB (RANK) for binding with RANKL, and in this way it interrupts the RANK-RANKL signalling system and suppresses osteoclast formation and maturation (Simonet et al. 1997; Baldock et al. 2006). Both calcitriol and oestrogen appear to stimulate mature osteoblasts to produce more OPG and less RANKL, thus slowing bone resorption in preparation for the osteoblasts to commence rebuilding the bone (Hofbauer et al. 1999; Baldock et al. 2006), and consequently protecting the bone from excessive resorption.

In the perimenopausal period, when oestrogen concentrations are declining, the protective effects of oestrogen on bone are compromised (Hoshino et al. 2000), possibly by the reduced secretion of OPG (Hofbauer et al. 1999). Markers of bone resorption have been shown to decrease with hormone replacement therapy in postmenopausal women (Bjarnason et al. 2000). If, during this period, serum 25(OH)D concentrations are also less than adequate, bone is threatened by increased rates of both resorption and overall turnover. In the absence of sufficient 25(OH)D, absorption of dietary calcium from the intestine will be reduced and PTH

levels will increase to stimulate the reabsorption of calcium from the kidney, as well as activating osteoclasts to commence bone resorption and release calcium into circulation (Yamamoto et al. 1984; Holick 2004). At the same time, the regulatory effect of calcitriol on the osteoblasts (via increased OPG secretion) will be impaired due to low 25(OH)D levels and compounded by declining oestrogen concentrations (Hoshino et al. 2000; Baldock et al. 2006).

It is possible that this threat to bone is at least partially ameliorated if dietary calcium intake is sufficiently high. In the absence of adequate levels of serum 25(OH)D, calcium transport via the intestinal epithelia is reduced from approximately 30 – 40% to 10 – 15% of intake (Holick 2007). When Barnes et al (2006) supplemented young, healthy males and females with 600 IU vitamin D3 per day and compared bone marker activity with that of a control group receiving no vitamin D, no difference was observed between groups in markers of bone turnover or PTH. However, both groups were also receiving 1500mg supplemental calcium/day in addition to ~800mg calcium from their diet. These 18 – 27 year-olds would also have had optimum circulating levels of oestrogen or testosterone and their baseline 25(OH)D levels were ~50 nmol/L.

Secretion of PTH is regulated primarily by a drop in serum calcium (Suda et al. 2002; Pepe et al. 2005) and does not appear to be affected by vitamin D deficiency until serum 25(OH)D concentrations drop below 30 - 40 nmol/L (Mezquita-Raya et al. 2001; Pepe et al. 2005; Barnes et al. 2006). This may explain the lack of PTH response to supplementation in this study. There were small, not significant decreases in PTH levels in both the vitamin D and placebo arms of Group 1 at the end of the study. The median baseline 25(OH)D concentration in this group was 19 (11, 31) nmol/L and this had increased significantly in the placebo arm as well as the vitamin D arm after 6 months. A strong inverse correlation between 25(OH)D and PTH which was present at baseline was retained through the study and was actually stronger in both groups at 6 months (table 1). Meanwhile mean dietary calcium intake across the entire subject group was 700±300mg/day, which although less than the New Zealand RDI of 1000mg (Commonwealth Department of Health and Aging et al. 2006) is similar to the mean for all New Zealand adult females of 735mg/day (Russell et al. 1999), and possibly sufficient to suppress secondary hyperparathyroidism.

Conclusion

We conclude that increasing serum vitamin D levels in older women who are vitamin D deficient suppresses the increase in bone turnover induced by age and decline in oestrogen, and reduces bone resorption which would normally be exacerbated in conditions of low serum 25(OH)D. It is also possible that the dietary calcium intake in this group of South Asian women was at a level which also offered some protection against the stimulation of osteoclast activity by PTH. We suggest that a more effective test of the efficacy of vitamin D supplementation to reduce bone turnover rate would be to conduct a trials in more homogeneous groups. For example pre-menopausal women with low dietary calcium intake, removing the potentially protective effect of calcium and the additional variable of oestrogen deficiency. Longer term studies would also provide the opportunity for measuring potential changes in BMD.

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CHAPTER 7



Bone density, calcium intake and vitamin D status in South Asian women living in Auckland



As mentioned previously, the screening phase of the study provided the opportunity to build a “snapshot” of the health and lifestyle of South Asian women living in Auckland. The relationship between poor bone health and low vitamin D status is well documented in people of South Asian origin. Low vitamin D status was suspected, and subsequently confirmed in the women who volunteered for the study, so we took the opportunity to offer all participants a bone scan. Ninety-one women chose to accept the offer, and the results are discussed in this chapter.

Abstract

Purpose: To investigate the bone health and associated risk factors of a group of South Asian women living in New Zealand. Studies on the Indian sub-continent suggest a high incidence of low bone mineral density (BMD) in women with poor vitamin D status and low dietary calcium.

Design: Subjects were women of South Asian origin (n=91) living in Auckland, New Zealand. They completed a 4-day food diary, provided a blood sample and BMD was measured using dual X-ray densitometry.

Results Mean age of premenopausal (n=71) and postmenopausal (n=20) women was 39.8 ± 7.8 and 55.3 ± 5.4 years respectively. Osteoporosis (T-score ≤ -2.5) was present in 32% of postmenopausal and 3% of premenopausal subjects, but only in the lumbar spine. Adequate 25(OH)D levels (> 50 nmol/L) were found in only 22% of premenopausal, and 26% of postmenopausal women. Women < 30 years appeared at increased risk of osteoporosis, with 30% incidence of osteopenia and median serum 25(OH)D3 of 20(18,42) nmol/L.

Conclusion: The high incidence of osteoporosis in the postmenopausal group could be associated with the early age of oophorectomy or menopause together with low vitamin D status. There is an urgent need for further research to establish the level of osteoporosis risk in young South Asian women.

Introduction

Although many aspects of diet and lifestyle impact on bone, the three key environmental factors which determine the fate of the skeleton are dietary calcium, vitamin D and physical activity (Layne et al. 1999; Nieves 2005; Vieth 2005; Hind et al. 2007; Sakuma et al. 2007).

We have previously reported low vitamin D status in South Asian women living in New Zealand. Of 235 women tested for serum 25(OH)D during 2007, 84% had concentrations less than the currently accepted adequate level of 50 nmol/L (Working Group of the Australian and New Zealand Bone and Mineral Society et al. 2005), and 43% had concentrations less than 25 nmol/L (Von Hurst et al. 2007). The implications of this low vitamin D status for osteoporosis in this population group are unknown. However, serum concentrations of 25(OH)D \leq 25 nmol/L are associated with reduced bone mineral density and increased risk of fracture (Working Group of the Australian and New Zealand Bone and Mineral Society et al. 2005), and calcium absorption is reduced when serum 25(OH)D is less than 80 nmol/L (Heaney et al. 2003). We therefore hypothesised that South Asian women living in New Zealand are at risk of osteoporosis and osteoporotic fracture as they age.

More studies are emerging describing the bone health of people of South Asian origin, both from within the Indian sub-continent and in migrant populations around the world, including New Zealand. Cundy et al (1995) compared premenopausal Indian women (31.3 ± 8.2 years) and Caucasian women (32.9 ± 8.5 years) in New Zealand and found that the Indian women had slightly lower BMD. Pakistani men and women living in Denmark were found to have a high incidence of osteoporosis for age, very low vitamin D levels and less than adequate calcium intake (Andersen et al. 2007). Similarly, the BMD of a group of men and women in Lucknow, India was found to be strongly correlated with vitamin D status, and their calcium intake was also lower than recommended at 438 ± 125 mg/day (Arya et al. 2004). Recommendations for daily intake of calcium vary by country, but the current New Zealand and Australia nutrient reference values suggest 1000mg/day for adults and 1300mg/day for adolescents and postmenopausal women (Commonwealth Department of Health and Ageing et al. 2006). In Chicago, USA, 47 Indian and Pakistani women had lower vitamin D levels and higher risk of hip fracture than Caucasian women of similar age (Alekel et al. 1999). However, a study of young, Indian police men and women in India found adequate levels of vitamin D and

dietary calcium. In the women BMD did not differ significantly from Caucasian normative means at either lumbar or femoral neck, but it was significantly lower in the men at the lumbar spine (Tandon et al. 2003).

In New Zealand, the South Asian population is expanding rapidly due, mainly, to immigration from India (Statistics New Zealand 2006). As in most other developed countries, the population in general is aging and osteoporosis, a disease of older age, will increase in incidence as will the associated health costs.

Aims of study

- To describe the bone health of a group of women of South Asian origin living in Auckland, New Zealand, stratified for menopausal status.
- To describe the prevalence of dietary and lifestyle risk factors for osteoporosis, specifically vitamin D status and dietary calcium intake.
- To investigate the relationships (if any) between BMD, vitamin D status and calcium intake.

Method

This bone mineral density study was a sub-study of the Surya Study (Von Hurst et al. 2008). The Surya Study aimed to investigate the effect of vitamin D supplementation on insulin resistance in women 20 years and over, who were vitamin D deficient and insulin resistant. To find participants for the trial, 249 women of South Asian origin were screened. Invitations were extended to all subjects who participated in the screening phase of the Surya Study, and 91 women volunteered to participate in the bone mineral study.

Volunteers for the Surya Study were excluded if suffering from significant renal dysfunction, major systemic illness, or diabetes requiring medication. Use of vitamin D supplements exceeding 1000 IU/day (i.e. prescription dose), or any form of calcitriol (1, 25(OH)₂D₃) were also exclusion criteria.

As participants of the Surya Study, the women were weighed, height, waist and hip were measured and fasting blood samples taken. Ethnicity was confirmed with a questionnaire which established country of birth for subject, her parents and all grandparents. Demographic information, medical history, nutritional supplement and medication use was obtained by interviewer-based questionnaires. Methods,

including biochemical assays, are described in greater detail elsewhere (Von Hurst et al. 2008). Subjects were recruited and tested over a period of nine months, from February to November (summer to spring) 2007, and some seasonal variation in vitamin D status was expected. Participants were also requested to complete a 4 day food diary which was then followed up with an interview conducted by a dietitian to probe and confirm specific aspects of the diet including calcium-containing foods.

Women were defined as postmenopausal if 12 months or more had elapsed since they last menstruated.

Bone densitometry was measured at 2 sites, lumbar spine (L1 – L4) and right total hip (Hologic QDR 4000, Hologic, Waltham, Mass.); daily quality control scans were performed on the scanner and the co-efficient of variation ranged from 0.37 – 0.45% during the study. Individual results were compared with the manufacturer's normative data base for a Caucasian population, age and gender matched. World Health Organization standards were used for reporting osteopenia and osteoporosis. A T-score of ≤ -2.5 is diagnosed as osteoporotic, between -1.0 and -2.4 diagnosed as osteopenia, and > -1.0 regarded as normal (World Health Organization 1994).

Statistics

SPSS software (version 15) was used for all statistical analyses. Normally distributed data are reported as mean \pm standard deviation, independent t-tests were used to compare group means and Pearson's correlations to investigate relationships between variables. Data not normally distributed are reported as median (25th, 75th percentiles) and non-parametric tests were used to compare groups (Mann-Whitney). Serum 25(OH)D was not normally distributed, so Spearman's correlations were used when investigating the relationships between vitamin D and other variables.

Results

Of the 91 women who elected to participate in the bone mineral density study, 90% were of Indian ethnicity, 6% Sri Lankan and the remainder from Pakistan. The majority (74%) had been in New Zealand for 10 years or less, and 78% reported having the equivalent of an undergraduate degree, or three years tertiary education.

Physical characteristics, calcium intake, biochemical measurements and bone mineral density for both groups are shown in tables 1 and 2. There was no significant difference between the groups in height, BMI, serum vitamin D, parathyroid hormone (PTH) or dietary calcium intake. BMD at both sites was significantly lower in the post-menopausal women; lumbar spine ($P < 0.001$) and hip ($P = 0.005$).

Osteoporosis was present in 3% of the premenopausal, and 32% of the postmenopausal women, but only in the lumbar spine. A further 42% of the postmenopausal women had osteopenic T-scores in both lumbar spine and total hip. In the premenopausal group incidence of osteopenia was 40% in the lumbar spine and 32% in the total hip.

Table 1. Characteristics of participants, grouped by menopausal status. There was no significant difference between the groups in any variable except age and number of years in New Zealand.

	Pre-menopausal N = 71	Post-menopausal N = 20
Age (years)	39.8 ± 7.8	55.3 ± 5.4*
Height (cm)	158 ± 6.5	155 ± 5.8
BMI (kg/m ²)	25.6 ± 4.6	26.1 ± 3.9
Years in NZ	6 (4, 9)	17 (6, 37)**
Calcium intake (mg/day)	832 (638, 1029)	721 (528, 992)
25(OH)D (nmol/L)	32 (21, 49)	39 (29, 57)
Adequacy > 50 nmol/L	22%	26%
PTH (pm/L) <i>Ref. range 1.7 – 7.3</i>	4.7 ± 1.6	4.9 ± 2.0
Number of subjects reporting:		
HRT or estrogen-based birth control use	1	1
Family history of osteoporosis	8	1
Calcium supplement use	7	6
Vitamin D supplement use (< 1000 IU/day)	8	8

*Results are given as mean ± standard deviation or median (25th, 75th percentiles). **

*P < 0.001, ** p=0.001. Abbreviations: BMI – body mass index, NZ – New Zealand, PTH – parathyroid hormone, HRT – hormone replacement therapy*

The mean age in the postmenopausal group was 55.3 ± 5.4 years, and the ages ranged from 40 to 63 years. The median number of years since the last period was 5 (4, 18), and ranged from 1 to 28 years. The mean age at which menopause or hysterectomy occurred was 44.2 ± 6.0 years.

One woman, who reported having a hysterectomy 26 years ago, had been prescribed alendronate (a bisphosphonate drug which inhibits osteoclast-mediated bone resorption). Her lumbar and total hip T-scores were -0.30 and -0.60, respectively. She and 11 other women were also taking calcium supplements ranging from 500mg to 1000mg per day. Sixteen women also reported use of some form of vitamin D supplementation including cod liver oil and multivitamins. The dose available in the dietary supplements reported ranged from $1.2\mu\text{g}$ (48IU) in cod-liver oil capsules, to $10\mu\text{g}$ (400IU) in multi-vitamins.

Table 2: Bone scan results stratified by menopausal status

	Premenopausal	Postmenopausal	Significance of difference between groups
Total hip BMD (g/cm^2)	0.89 ± 0.11	0.81 ± 0.09	$t(89) = 2.873, P = 0.005$
T-score	-0.43 ± 0.95	-1.06 ± -0.74	$t(89) = 2.734, P = 0.008$
Z-score	-0.200 ± 0.97	-0.33 ± 0.64	
Lumbar 1- 4 BMD (g/cm^2)	0.99 ± 0.15	0.85 ± 0.13	$t(89) = 4.013, P < 0.001$
T-score	-0.49 ± 1.28	-1.78 ± 1.22	$t(89) = 4.171, P < 0.001$
Z-score	-0.18 ± 1.28	-0.66 ± 1.13	

Results are given as mean \pm standard deviation. Abbreviations: BMD – bone mineral density. Z-scores are age and gender based, so difference between groups is not meaningful.

Vitamin D levels increased slightly with age ($r = 0.238, P = 0.02$), and a moderate, inverse relationship was found between serum 25(OH)D and PTH ($r = -0.257, P = 0.02$). There was also a correlation between BMI and total hip BMD ($r=0.301, P = 0.004$) and T-score ($r=0.312, p = 0.003$), but no relationship could be found between BMI and lumbar BMD or T-score.

Dietary data were available from 68 women (51 pre- and 17 postmenopausal). There was no difference between groups in macro- or micronutrient intake. Mean energy intake was $6900 \pm 1325\text{kJ}$, protein contributed 15% of energy, fat 31% and carbohydrates 54%.

In the premenopausal group there was no difference between total hip and lumbar T-score ($P = 0.53$), however, in the postmenopausal women the lumbar T-score was significantly lower than the total hip T-score ($P = 0.005$).

The premenopausal group included 10 women between the ages of 20 and 29 years. When compared to the other premenopausal women, their mean BMD was not significantly different but prevalence of osteopenia was 30% in both sites, BMI was significantly lower $t(69)=-2.06$, $P = 0.04$, and there was a trend towards lower serum 25(OH)D and dietary calcium intake (table 3), but differences were not statistically significant.

Table 3. Characteristics of a subgroup of young women (n=10) 20 to 29 years of age.

Age (years)	24.9 ± 3.5	
Height (cm)	161.6 ± 5.3	
BMI (kg/m ²)	22.9 ± 4.0	
Years in NZ	5 (3, 10)	
Serum 25(OH)D (nmol/L)	20.0 (18, 42)	
Calcium (mg/day)	550 (512, 715)	
PTH (pmol/L)	4.4 ± 1.7	
Total hip	BMD (g/cm ²)	0.864 ± 0.009
Total hip	T-score	-0.75 ± 0.91
	T-score	Range
	Z-score	0.30 to -2.40
		-0.71 ± 0.87
Lumbar 1- 4	BMD (g/cm ²)	0.965 ± 0.014
Lumbar 1- 4	T-score	-0.60 ± 0.81
	T-score	Range
	Z-score	0.70 to -2.10
		-0.56 ± 0.80
Osteopenia prevalence	30%	

Results are given as mean ± standard deviation or median(25th, 75th percentiles)

Abbreviations: BMI – body mass index, PTH – parathyroid hormone, NZ – New Zealand

Discussion

The 32% prevalence of osteoporosis in the postmenopausal women is cause for concern given that the mean age of this group is 55.3 years. However, mean age at menopause/hysterectomy was 44 years, and some women were able to confirm that they had their ovaries removed at the same time as the hysterectomy. Others reported a hysterectomy, but did not know if they had retained their ovaries. Although little data is available regarding the prevalence of hysterectomies and associated oophorectomy in India, a report from Gujarat indicates that hysterectomies are common, often provided on demand without apparent disease present, and that the ovaries are routinely removed, especially if the woman is over 35 years (Ranson et al. 2002). The report also mentions that some surgeons neither informed their patients nor sought consent to remove the ovaries. The cessation of estrogen secretion from the ovaries results in an accelerated phase of (predominantly) trabecular bone loss. With natural menopause estrogen secretion begins to decline before cessation of menses and continues for approximately 3 years after. Following oophorectomy, however, the abrupt fall in circulating estrogen leads to a more rapid loss of bone (Riggs et al. 2002).

Reports of osteoporosis prevalence in India are also sparse, the available data are of poor quality, and based on hospitalisation for hip fracture. These data (reviewed by Malhotra and Mithal, 2008) seem to indicate that osteoporosis occurs at a younger age than in Caucasians, and with a higher incidence in men than in women (Malhotra et al. 2008). However, the reports do not differentiate between traumatic and fragility fractures, and where ages have been recorded, the average fracture age for men is lower than for women. This is in contrast to the USA (Burge et al. 2007) and Australia (Sanders et al. 1999) where osteoporotic fractures are more than twice as common in women as in men. The authors of the above review suggest as an explanation, that Indian men may be more likely to be taken to hospital than women, but recent bone health studies report lower BMD and higher incidence of osteopenia in South Asian men compared to South Asian women (Tandon et al. 2003).

A recently published study of bone health in Indian males (n = 683) and females (n = 858) aged 5 – 70 years from Pune, India, reported osteoporotic lumbar spine T-scores (< -2.5) in 16.9% of males and 22.4% of females in the 50 – 59 age group. These proportions increased in the next decade (60 to 69 years) to 23.9% (males)

and 31.7% (females) (Kadam et al. 2009). The post-menopausal group in the Surya Study had a mean age of 55.3 ± 5.4 years, and 32% were osteoporotic in the lumbar spine, suggesting that they are at higher risk than their contemporaries in India. The Pune study did not report vitamin D status.

Socio-economic status appears to be an important determinant of bone health in India, and is closely associated with nutritional status. Urban slum-dwelling women were shown to have a high prevalence of osteoporosis and osteopenia, together with inadequate intakes of both macro- and micronutrients (Shatrugna et al. 2005). The women in the present study were mostly well-educated and were relatively new migrants. Immigration regulations in New Zealand require new migrants have both professional qualifications and financial resources. Their macro- and micronutrient intake was similar to that of the general New Zealand adult female population as reported in the National Nutrition Survey 1997 (NNS97) (Russell et al. 1999), although mean total energy intake was approximately 1000kJ less.

Dietary calcium intake was below the current Australia/New Zealand recommended daily intake (RDI) (Commonwealth Department of Health and Aging et al. 2006), but higher than has been reported in Indian women in India (Alekel et al. 1999; Tandon et al. 2003; Arya et al. 2004; Shatrugna et al. 2005), and similar to the average intake of the New Zealand adult female population (Russell et al. 1999). Calcium supplementation, reported by 14% of participants, was often taken only sporadically and was not included in the 4-day food diaries. Food sources of dietary calcium were identified in a subset (n=102) of the Surya Study participants: milk contributed 29%, curry dishes (containing cream and yoghurt) 15%, and yoghurt 8% of calcium in the diets of these women (Tsai 2008). The high contribution of dairy products to calcium intake reflects that of the general New Zealand population (Russell et al. 1999), and is not dissimilar to that seen in upper socio-economic groups in India (Ganpule et al. 2006; Puri et al. 2008).

Dietary calcium absorption appears to increase proportionally with increasing vitamin D concentration up to a threshold around 80-90nmol/L serum 25(OH)D (Heaney et al. 2003). Similarly, an inverse relationship occurs between serum vitamin D and PTH, but the threshold for decreasing PTH may be as high as 100 – 110 nmol/L serum 25(OH)D (Dawson-Hughes et al. 1997). None of the women in this study achieved serum 25(OH)D concentrations of 80 nmol/L, in fact, the median

levels were well below the currently recommended minimum of 50 nmol/L (Working Group of the Australian and New Zealand Bone and Mineral Society et al. 2005). Therefore it is likely that bone health in these women is compromised more by their poor vitamin D status, than by dietary calcium intake.

BMD is also influenced by physical activity, and more specifically by weight-bearing or resistance exercise (Layne et al. 1999; Hind et al. 2007). We have not reported physical activity in these women. However, South Asian women have been identified as having the lowest level of physical activity of all New Zealanders (Scragg et al. 2005) so exercise is also an important factor when considering the skeletal health of these women.

The pattern of postmenopausal bone loss is probably explained by the young age and number of years since menopause of most of those women. Before menopause, the T-scores at both sites were not statistically different. However, in the postmenopausal women, the rate of bone loss appears to be much more rapid in the lumbar spine than in the hip, and osteoporosis was found only in the lumbar spine. Riggs et al (2003) classify the osteoporosis that occurs within the first 20 years after menopause as type 1 osteoporosis and describe it as being characterised by crush fractures which occur in the vertebrae (Riggs et al. 2003). There is some evidence that the risk of osteoporotic vertebral fracture is much greater (compared to hip fracture) in younger (50-54 years old) women than in older (>65 years) women (Kanis et al. 2004; Johnell et al. 2006). The risk of vertebral fracture (but not hip fracture) in American women with bilateral oophorectomy and no subsequent hormone replacement therapy (HRT) was almost double that expected in the general female population, whereas HRT in these women was protective for vertebral fracture, but not forearm or hip. It appears that the vertebrae, with a higher proportion of trabecular bone, respond sooner and more acutely to estrogen deficiency than the hip and other bones which also have substantial amounts of cortical bone. Studies of markers for bone resorption in postmenopausal women suggest that variability in the response to lowered serum estrogen may help explain why some women lose bone more rapidly, and are more susceptible to osteoporosis (Riggs et al. 2003).

The results for the small group of women (n=10) aged 20-29 years, highlight the need for further investigation of bone health in young South Asian women in New Zealand. This is the age that peak bone mass should be achieved, but T-scores were low, and 30% were osteopenic at both sites. One 26-year old had a total hip T-score of -2.40. Z-scores, which are age and gender matched, indicate that they are already falling behind the mean for the Caucasian reference group. It is also possible that these women do not attain peak bone mass until late into their 3rd decade – Kadam et al. (2009) found that bone mineral content in the lumbar spine of Indian women increased significantly till the age of 30 years.

Median serum 25(OH)D was very low in this group at 20 (18, 42) nmol/L, although not significantly different to the rest of the women, possibly due to the small numbers. The little information available about the bone health of other South Asian women in this age group suggests that BMD is strongly linked to vitamin D status (Alekel et al. 1999; Tandon et al. 2003).

The women who volunteered for this bone mineral study were a self-selected, and were mostly well-educated and recently arrived in New Zealand. The majority of New Zealand immigrants enter under the skilled migrant category, and education or professional qualifications are important criteria. The South Asian population in New Zealand almost doubled through migration between 2001 and 2006, increasing by 66,000 (Statistics New Zealand 2006). Therefore, although not representative of the local South Asian population, the women in this study are most likely very typical.

New Zealand is not the only country experiencing this influx of immigrants; Australia experienced the same increase as New Zealand in the 2001 – 2006 period (Australian Bureau of Statistics 2006), whilst Asian Indian immigration into the USA in 2000 – 2006 numbered 421,000, 24% more than the whole previous decade (U.S. Census Bureau 2005). Little or no information exists about the bone health or vitamin D status of South Asian people in either of these countries. This study highlights some very important concerns which may also be applicable to the above countries and require further investigation.

Conclusions

The findings of this study are constrained by the small numbers, and the wide range of ages, of participants. They do, however, indicate that the bone health of South Asian women living in New Zealand is more likely to be adversely affected by low vitamin D status than inadequate dietary calcium intake, especially once the protective effect of estrogen is lost after menopause. There was a high proportion of osteoporosis in the postmenopausal group given their relatively young age, possibly related to estrogen loss at an early age. That osteoporosis was present only in the lumbar spine may be explained by the young age of the post-menopausal group, together with their young age at time of menopause or oophorectomy. Thus, the combination of early menopausal stage or oophorectomy, and very low vitamin D status is of major concern in this population group.

From the very small sample of younger women in this study, it appears that young South Asian women living in New Zealand could be at high risk of poor bone health and subsequent osteoporosis. Further investigation is needed in this sub-group to establish a clearer picture of their skeletal health, together with their behaviours and attitudes with regard to calcium consumption, sun exposure and physical activity. Such findings could be of value to other countries with temperate climates and burgeoning numbers of South Asian immigrants.

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CHAPTER 8



Discussion and conclusions
Recommendations for future research



Discussion

The primary objective of the Surya Study was to conduct a randomised placebo-controlled trial to investigate the effect of improved vitamin D status on insulin resistance. South Asian women were selected as the subject group because they were identified as being at high risk of both hypovitaminosis D and type 2 diabetes. Over the course of the study, however, the health and lifestyle of the women themselves became increasingly more important to the researcher as awareness of the health risks in this population increased.

Vitamin D status

The evidence of vitamin D deficiency prior to the commencement of the study was largely anecdotal. The results of the screening phase, as reported in Chapter 4, revealed an incidence of deficiency (43%) and insufficiency (41%) that was unexpectedly high. Even if one considers only the well-established risk of poor vitamin D status on bone health, there is reason to be concerned about these women, but there is sufficient evidence to suspect that they may also be at increased risk for diabetes and CVD.

A number of factors seem to contribute to this poor vitamin D status apart from the obviously higher levels of melanin and the reluctance to cause further darkening of their skin. The women who responded to the sun exposure questionnaire expressed a high level of concern about the strength of the New Zealand sun, and the danger of skin cancer. They also reported a busy, indoor lifestyle which allowed them little time outside, and sensitivity to cooler temperatures meaning that bare skin was well covered at times when the sun is not so strong. Consequently, there seems to be scant opportunity for these women to achieve high levels of circulating 25(OH)D by endogenous synthesis even during summer, and they are at particular risk of deficiency during winter and spring.

Due to the lack of vitamin D fortified foods in New Zealand and the many reasons for sun avoidance mentioned above, vitamin D supplementation may be the most effective method of avoiding deficiency in populations such as this one. Before recommendations for ongoing supplementation can be made, it is important that

there is a clear understanding of the size of dose required and the efficacy of supplementation in the medium term.

Insulin Resistance and vitamin D

The HOMA-IR score of 1.93 set as inclusion criteria for the intervention study was an arbitrary figure based on an urban population study carried out in Chennai, India (Deepa et al. 2002). In that study, the upper quartile of the population scored over 1.93, but in the Surya Study half (49.6%) of the women screened were in that category. The HOMA-IR model is based on a simple equation using fasting serum glucose (FSG) and fasting serum insulin (FSI). As reported in Chapter 5, the women who qualified for the intervention study were mostly normoglycaemic, and their elevated HOMA-IR scores were due more to higher levels of circulating FSI than fasting hyperglycaemia. This suggests that they had reduced insulin sensitivity, but that insulin secretion was not impaired. This assumption is reinforced by the HOMA2 computer model results which present both insulin sensitivity and insulin secretion. Information provided by both HOMA models is limited to the basal fasting state; therefore it is impossible to deduce the dynamics of the insulin response, and to establish whether the insulin resistance is hepatic, peripheral or both. However, in light of the current knowledge about the progression from normoglycaemia to type 2 diabetes, it is possible that the majority of the Surya Study participants were in the early stages of impaired fasting glucose, when fasting hyperinsulinaemia compensates for hepatic insulin resistance and elevated hepatic glucose output.

It is important to consider the possible causes of insulin resistance in these women. Obesity, which sits in the primary position of the current definition of metabolic syndrome (International Diabetes Federation 2005), is a favourite candidate. The increase in levels of non-esterified fatty acids (NEFAs) as seen in obesity may be one of the most critical factors in the development of insulin resistance (Kahn et al. 2006). The inflammatory effects of adipokines such as interleukin-6, tumour-necrosis factor- α and plasminogen activator-inhibitor-1 are all associated with increased obesity and insulin resistance (Qatanani et al. 2008).

Whilst there was some evidence of central obesity (mean hip:waist = 0.80 ± 0.07 , BMI 27.5 ± 5.0), the women in the Surya Study were not universally obese. There was, however, a strong correlation between insulin resistance and BMI ($r = 0.56$) and waist/stature ($r = 0.53$) explaining 31% and 28% of the variance in insulin

resistance (Stonehouse et al. 2007). Although specific adipokines were not assayed, C-reactive protein (CRP) was measured, and was not elevated, suggesting that overall inflammatory markers were not increased. Lipid profiles were normal. This suggests that whilst obesity could partially explain the insulin resistance, other factors, such as vitamin D deficiency, could also contribute.

The big question, which is the crux of this study, is did vitamin D deficiency play a causative role in the reduced insulin sensitivity? Certainly, an improvement was seen in insulin sensitivity in the vitamin D group compared to the placebo group. The improvement was most significant in those subjects whose serum 25(OH)D reached (and was maintained at) 80 nmol/L or more, with the upper half of those subjects achieving a HOMA2%S (insulin sensitivity) of > 85% when 100% is normal. Insulin secretion, as measured by C-peptide levels did not change, but fasting insulin was lower after 6 months of supplementation, suggesting a more efficient uptake in the insulin sensitive tissues – either hepatic, peripheral or both.

There is some evidence for vitamin D influencing the insulin signalling cascade – either at the receptor level or in the downstream signalling proteins (Maestro et al. 2000; Maestro et al. 2002; Maestro et al. 2003). To date this evidence is limited to *in vitro* studies, but it presents a plausible explanation for insulin sensitivity being reduced when vitamin D status is low.

The progression from reduced insulin sensitivity, to type 2 diabetes also involves impaired β -cell function. That certain groups are predisposed to β -cell dysfunction, and consequently greater risk of developing type 2 diabetes has been demonstrated in first-degree relatives of individuals with type 2 diabetes (Knowles et al. 2002). This genetic association remains across different ethnicities, and when age and weight are controlled for (Jensen et al. 2002). South Asians, meanwhile, have been shown to have an increased susceptibility to type 2 diabetes which is more than doubled by family history (Chandalia et al. 1999; Ramachandran et al. 2001), possible genetic predisposition to β -cell dysfunction via a polymorphism in the VDR (McDermott et al. 1997), and a high frequency of another gene variant which appears to impair insulin sensitivity (Abate et al. 2003).

We were not able to detect impaired β -cell function in the participants in the Surya Study, due in part to the assessment methods used. However, whereas the subjects

in other studies who had either IGT or type 2 diabetes responded to vitamin D supplementation with increased insulin secretion (Gedik et al. 1986; Kumar et al. 1994; Boucher et al. 1995; Borissova et al. 2003), there was no such response in our subjects. This is an important differentiation, because it appears that the β -cell response to vitamin D supplementation might occur more quickly than the improvement in insulin sensitivity. Both Borissova (2003) and Boucher (1995) saw an increase in insulin secretion within 1 – 3 months of supplementation, but no improvement was detected in insulin sensitivity.

The recent publication of the results of two other vitamin D supplementation trials provides the opportunity to explore both this temporal difference in response, and the influence of baseline variables. Subjects in the Tai (2008) study were vitamin D insufficient (mean 25(OH)D 39.9 nmol/L), 6 of them had IFG and 6 had IGT, presumably the other 21 were normoglycaemic. They were treated at baseline and 2 weeks with large oral doses of vitamin D₃ (100,000 IU) and final blood tests were taken at 4 weeks from baseline. Despite a significant improvement in serum 25(OH)D, there was no change in insulin secretion or sensitivity. There was no control group in this study.

Nagpal et al (2009) conducted a randomised, placebo-controlled trial with 3 x 2-weekly doses of vitamin D₃ (120,000 IU). Subjects were Indian males with central obesity (waist circumference > 78 cm) which was used as a surrogate for potential insulin resistance. They were not selected for hypovitaminosis D, but mean \pm SD 25(OH)D concentrations were 36.5 \pm 14.55 nmol/L. Endpoint measures were taken 6 weeks after baseline and a small, but significant, improvement was seen in post-prandial insulin sensitivity (OGIS) but in no other measures including HOMA.

When comparing the above studies with the Surya Study there are some important differences in baseline conditions: Mean HOMA-IR in the Nagpal study was 1.47 \pm 1.16, in the Surya Study it was 3.0 \pm 1.42. Unfortunately, although Tai et al report HOMA, the results are nonsensical – they were possibly calculated using the wrong units of measurement. Baseline 25(OH)D was lower in the Surya Study subjects than both the other studies. Nagpal et al reported that high waist/hip ratio (WHR) and low 25(OH)D had a strong inverse correlation with OGIS at baseline, and were both significant predictors of the magnitude of improvement in OGIS following

supplementation. In the Surya Study baseline 25(OH)D was inversely correlated with improvement in both serum 25(OH)D and insulin sensitivity at 6 months.

When the Surya Study subjects were tested at 3 months (mid-study), 25(OH)D concentrations had increased significantly, and to a similar magnitude as seen in the two studies discussed above. Also, at that stage, there was no significant reduction in insulin resistance. By 6 months, however, although median 25(OH)D concentrations had dropped slightly in the supplemented group from the 3 month results, there was a significant improvement in insulin sensitivity and insulin resistance in the supplemented group compared to the placebo group, and a large, significant improvement in insulin sensitivity in those subjects whose endpoint serum 25(OH)D was ≥ 80 nmol/L.

The findings of the Surya Study make an important contribution to the current body of knowledge about the role of vitamin D in insulin resistance and the development of type 2 diabetes. This is especially true when the results are considered in contrast to the other recently published vitamin D supplementation trials discussed above. The inference from the findings of the 3 studies is that for vitamin D supplementation to improve insulin resistance, both insulin resistance and vitamin D deficiency need to be present. Also, whilst earlier studies have shown an almost immediate improvement in insulin secretion following vitamin D supplementation, the response in insulin sensitivity appears to take longer. Finally, both the magnitude of the increase and the endpoint serum 25(OH)D concentration achieved influence the improvement in insulin sensitivity.

Bone Health

The Surya Study provided an opportunity to investigate the status of the bone health of South Asian women living in New Zealand, and it was unfortunate that less than half of the women who volunteered for the screening phase of the study ultimately turned up for their bone scan. The changes in bone are so dynamic during a lifetime that the results from 91 women aged from 20 to 63 years do not present any conclusive evidence. However, there were some interesting and important findings which invite further investigation.

As discussed in Chapter 7, the small group of younger women (20 – 29 years) demonstrated some alarming risks for the later development of osteoporosis, with low calcium intake, very low serum 25(OH)D, and osteopenia present in 3 out of the 10. Their Z-scores, which are age and gender matched, were below those for the Caucasian reference group, suggesting that peak bone mass (PBM) acquisition would be less than ideal. However, it is also possible that PBM is reached later in South Asian women than in Caucasian women as Kadam et al. (2009) found BMD continued to increase in Indian women until after the age of 30 years.

When compared to older women in India (Kadam et al. 2009), the postmenopausal women in the Surya Study appeared to be losing BMD in the lumbar spine at an earlier age. This loss could be due to the premature decline in oestrogen levels following early menopause or oophorectomy. Alternatively, poor vitamin D status as observed in nearly all the women screened, could be to blame. Both oestrogen and vitamin D exert an influence over osteoblast maturation and OPG secretion, and potentially, a deficiency of both following menopause could result in higher bone turnover and greater BMD loss. Evidence for the protective role of both vitamin D and oestrogen in bone health was supported by the results of the RCT discussed below.

As described in Chapter 6, markers for bone turnover and resorption in the postmenopausal women did not continue to increase in the supplemented group, compared with the placebo arm. There was also a significant decrease in CTX which is a marker for bone resorption, suggesting that the improvement in vitamin D status was protective.

Oestrogen, vitamin D and dietary calcium singly and in combination have such a powerful effect on bone that distinguishing the influence of a single factor such as vitamin D requires that the others can be controlled for. This was not possible in the Surya Study due to small numbers of subjects in each group together with a wide range of age and hormonal status. The investigation of the bone marker response to supplementation was a secondary objective of the study, with the aim of obtaining some preliminary data as there have been so few studies conducted in this area. We were able to see that improving vitamin D status was protective of bone, reducing both turnover and resorption in the women who were postmenopausal.

Conclusions

Despite the rapidly growing interest in the role of vitamin D in health and disease, the Surya Study remains the first and only RCT in a population which was both insulin resistant and had low vitamin D status. It is also one of very few intervention studies investigating the influence of supplemental vitamin D on bone markers.

The primary objective of the study was to investigate the effect of vitamin D supplementation on insulin resistance. An improvement was seen in insulin resistance and insulin uptake increased, demonstrating the importance of adequate vitamin D status on insulin sensitivity in this population. It appears from the accumulating evidence that the actions of calcitriol in the control of glucose homeostasis are many and varied. Consequently, the response to vitamin D supplementation may depend on the degree of impairment and the stage of disease progression.

This study also demonstrated that an improvement in vitamin D status is protective of bone, especially in postmenopausal women. There was a high incidence of osteoporosis in the postmenopausal women who elected to have a bone scan, and it is possible that poor vitamin D status contributed to this.

The results of the Surya Study, together with evidence from around the world including India itself show a tendency for poor vitamin D status in people of South Asian origin due to a variety of causes. There is also sufficient evidence accumulating to support concerns for increased risk of osteoporosis in this population associated with low vitamin D status. A genetic predisposition for the development of type 2 diabetes has been well identified in South Asian people, and incidence of type 2 diabetes is triple that of the general population. If vitamin D deficiency also plays a role in the development of type 2 diabetes, it is important the low vitamin D status of South Asian people living in New Zealand be recognised as an important threat to the health of that community.

Recommendations for future research

1. Investigation of the long-term safety and efficacy of vitamin D supplementation versus fortification of the food supply, or amended messages about sun exposure, as methods to improve the vitamin D status of the population in the future.
2. Some population groups at risk of vitamin D deficiency have been identified. Further investigation of the vitamin D status of such groups is required to see if routine screening of vitamin D levels should be recommended for some populations.
3. The optimum level of serum 25(OH)D for protection against disease needs to be determined.
4. A randomised controlled trial with vitamin D supplementation in people with low vitamin D status, and with a range of baseline glycaemic conditions from IFG to type 2 diabetes, to investigate the effect of improving vitamin D status on insulin sensitivity and β -cell function. Such a study would be of longer duration than the Surya Study, ideally up to 3 years, to better monitor the ongoing response to improved vitamin D status. Study protocol would include more robust measures of insulin response.
5. An investigation of the BMD, vitamin D status and calcium intake of younger South Asian women living in New Zealand to try and get a clearer picture of the risk to their long term bone, and general, health.
6. The influence of improved vitamin D status on bone metabolism as measured by markers of bone turnover should be further investigated in more homogenous groups to control for some of the confounding variables such as hormonal status, number of years since menopause, calcium intake, gender and ethnicity.

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Appendices

1. Papers published or submitted

Von Hurst PR, W Stonehouse, MC Kruger, J Coad (2008). Vitamin D status of South Asian women living in New Zealand. *Proceedings of the Nutrition Society of New Zealand* **16** (Suppl 3): S50

Von Hurst PR, W Stonehouse, C Matthys, C Conlon, MC Kruger, J Coad (2008). Metabolic syndrome, vitamin D and bone status in South Asian women living in Auckland, New Zealand: a randomised, placebo-controlled, double-blind vitamin D intervention. *BMC Public Health* **8**: 267

Von Hurst PR, W Stonehouse, J Coad (2009). Vitamin D status and attitudes towards sun exposure in South Asian women living in Auckland, New Zealand. *Public Health Nutrition* Epub ahead of print, August 2009

Von Hurst PR, W Stonehouse, J Coad (2009). Vitamin D supplementation reduces insulin resistance in women who are insulin resistant and vitamin D deficient – a randomised, placebo-controlled trial. *British Journal of Nutrition* Epub ahead of print, 28 September 2009

Von Hurst PR, W Stonehouse, MC Kruger, J Coad (2009). Bone density, calcium intake and vitamin D status in South Asian women living in Auckland. *Proceedings of the Nutrition Society of New Zealand*

Von Hurst PR, W Stonehouse, MC Kruger, J Coad (2009). Vitamin D supplementation suppresses age-induced bone turnover in older women who are vitamin D deficient. *Journal of Steroid Biochemistry and Molecular Biology* (under review)

2. Conference presentations and abstracts

Von Hurst PR, W Stonehouse, MC Kruger, J Coad (2007). Vitamin D status of South Asian women living in New Zealand (abstract). *Asia Pacific Journal of Clinical Nutrition* **16** (Suppl 3): S50

Oral presentation at the Australia and New Zealand Nutrition Societies combined conference, Auckland 2007. Winner of "Best first time speaker" prize, Nutrition Society of New Zealand.

Von Hurst PR, W Stonehouse, C Matthys, J Coad (2008). Is waist circumference a useful indicator of markers of metabolic syndrome in South Asian women living in New Zealand? *Diabetes Research and Clinical Practice* **79** (S1): 112

Poster presentation at the Western Pacific International Diabetes Federation Congress. Wellington 2008.

Von Hurst PR, W Stonehouse, C Matthys, J Coad (2008). Observations on the health, diet and physical activity of South Asian women living in Auckland.

Oral presentation at the CAHRE (Centre for Asian Health Research and Evaluation) Conference, Auckland 2008.

Von Hurst PR, MC Kruger, W Stonehouse, J Coad (2008). Bone density calcium intake and vitamin D status in South Asian women living in Auckland. (abstract) *Proceedings of the Nutrition Society of New Zealand* (in press)

Oral presentation at the Nutrition Society of New Zealand Annual Scientific Meeting. Christchurch 2008.

Von Hurst PR, W Stonehouse, J Coad (2009). Vitamin D supplementation reduces insulin resistance in women who are insulin resistant and vitamin D deficient. *Journal of Diabetes* **1** (S1): A94

Poster presentation at the 3rd International Congress of Metabolic Syndrome and Pre-diabetes. Nice, France 2009.

Von Hurst PR, W Stonehouse, J Coad (2009). Vitamin D supplementation reduces insulin resistance in insulin resistant women with low vitamin D status: A randomised, placebo-controlled trial. Oral presentation at the New Zealand Dietetic Association Conference, Napier, New Zealand. 2009. Winner of the FSA NZ Research Award.

Von Hurst PR, W Stonehouse, J Coad (2009). Vitamin D supplementation reduces insulin resistance in women who are insulin resistant and vitamin D deficient. Oral presentation at the 14th International Vitamin D workshop, Bruges, Belgium 2009. Winner, Young Investigator Travel Award.

3. Participant information sheet



**Institute of Food, Nutrition and Human Health
Massey University
Albany, Auckland, New Zealand**

Surya Study

An investigation of the relationship between nutrition and risk factors for disease in South Asian women

Information for Participants

You are invited to take part in a university research project investigating the nutritional status of South Asian women, and its relationship with risk factors for diabetes, cardiovascular disease and bone health. The principal investigator (details below) is a PhD candidate in Nutritional Science at Massey University.

Principal Investigator: Pamela von Hurst Institute of Food, Nutrition and Human Health Massey University, Albany Tel: 414 0800 ext 41205 Email: P.R.vonHurst@massey.ac.nz	Supervisor: Dr Jane Coad Institute of Food, Nutrition and Human Health Massey University, Palmerston North Tel: 414 0800 ext 5962 Email: J.Coad@massey.ac.nz
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We are looking for 300-350 South Asian women to participate in this study.
To fit in to our study you should:

- Have been born in South Asia (or have parents or grandparents who were born there).
- Be female, over 20 years of age.
- Be willing to fast overnight for 10 to 12 hours and not drink any alcohol on the day before you come into the lab.
- Not be pregnant, breast-feeding or planning in the near future.
- Not be taking any medications which might interfere with our tests (we will ask you questions about your health).

If you decide you would like to take part in this study you will be first asked to complete a questionnaire which includes a medical history to ensure that you fit the needs of the study. If you do, we will then invite you to the Human Nutrition Unit at Massey University or another Auckland centre, where you will spend an hour with a researcher who will measure and weigh you, and take your blood pressure. You will be asked to provide a blood sample of about 25ml (about 5 tea-spoons) which will be taken at the same time. You will be asked to complete another questionnaire about your beliefs and knowledge about osteoporosis and a medical history form. Finally, you will be given a diary to complete with details about the foods you eat and what activities (such as walking and exercise) you do during the period of a week.

About the study

Phase 1 of the study seeks to recruit 300 women who were themselves born in the South Asian continent, or whose parents or grandparents were born there. The study is intended to find out about the nutritional status of South Asian women, how much vitamin D they get from their normal diet and daily activities, and how their diet affects other aspects of their health especially glucose tolerance and bone health.

Involvement in this part of the study would include the following:

- The researcher would make an appointment for you to visit the Massey University, Albany campus or another Auckland location, early in the morning before you have breakfast. We will reimburse you for your travel costs. You would first have blood samples taken (about 25 ml which is equivalent to about 5 tea-spoons). This “Fasting blood sample” needs to be taken early in the morning before you eat or drink (other than water). You also need to avoid alcohol in the 24-hour period before the blood test.
- Some of the blood taken will be analysed for calcium, vitamin D, glucose and insulin levels, B vitamins, lipid and cholesterol levels, fibrinogen and parathyroid hormone (which affects calcium handling by the body). Part of the sample will be stored at the laboratory at Massey University where a genetic analysis will be done to look for variations in the vitamin D receptor gene when the blood samples from all the subjects in the study have been collected.
- Next you would be offered a light breakfast meal (of fruit, cereal, milk, yoghurt, bread, tea and coffee). We will then measure your height, weight and waist and hip circumference. All measurements will be made by female researchers over light clothing so you do not need to get undressed. We will then also take your blood pressure measurement
- The researcher will give you a food and activity diary for you to complete over the following week or so and mail back.

We would also like to invite you to return to Massey at a later date to give you a bone density scan. DEXA bone densitometry is quick, accurate, non-invasive method of assessing bone health and risk for osteoporosis (fragile bones in older age) which does not involve anaesthetic. The amount of X-ray dose is very small (about the same as the average person receives from background radiation in one day or less than one tenth of the dose used in a standard chest X-ray).

It is possible that results from the blood tests in phase 1 of the study might show some potentially abnormal results (such as very high blood glucose levels or very low vitamin D levels) that should be investigated further. If any such problems are identified, we will invite you to come back to Massey to discuss these with the study doctor, Dr Shashikala Bhuthoji, who is a registered medical practitioner who works in the Massey University Health Centre. She may advise you to contact your General Practitioner or will contact your General Practitioner on your behalf if you prefer.

Phase 2

Phase 2 of the study seeks to recruit 100 women from the first part of the study who were found to have high blood glucose levels or other markers of metabolic syndrome in the blood sample taken at the start of the study but are not taking any medication for diabetes. This part of the study will investigate the relationship between vitamin D and glucose intolerance. It is an intervention study which means that participants will be given either 4 small capsules containing a vitamin D supplement, or 4 small capsules of identical appearance which do not

contain vitamin D, daily for a period of 6 months. During that period we will assess your vitamin status and blood glucose and insulin levels.

Participation in the study would also involve visiting a Diagnostic MedLab on 3 other occasions to have a fasting blood sample taken (in total about 19 mls which is equivalent to about 4 tea-spoons). These need to be taken early in the morning before you eat.

At some point during the 6 month period, you will need to come to Massey University Nutrition Laboratory where we will give you a bone density scan. DEXA bone densitometry is quick, accurate, non-invasive method of assessing bone health and risk for osteoporosis (fragile bones in older age) which does not involve anaesthetic. The amount of X-ray dose is very small (about the same as the average person receives from background radiation in one day or less than one tenth of the dose used in a standard chest X-ray).

Risks and benefits

There will be no charges made for any of the tests that you undertake. The principal benefit of taking part in this study is that you contribute to our better understanding of the health problems of South Asians in New Zealand. Over recent years the number people from these regions living in New Zealand has greatly increased but we know very little about the health of this growing segment of our population.

There are no personal risks to your health, but the blood tests and bone density scan could potentially identify undiagnosed health problems. If any such problems are identified, we will invite you to come back to Massey to discuss these with the study doctor, Dr Shashikala Bhuthoji, who is a registered medical practitioner who works in the Massey University Health Centre. She may advise you to contact your General Practitioner. At your request, we would be happy to contact your General Practitioner for you.

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 unless you give permission to the researcher;
- be given access to a summary of the project findings when it is concluded

General

If you want to discuss any aspect of this study you should contact the Principal Investigator, Pamela von Hurst at Massey University, 414 0800 ext. 41205.

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.

At the conclusion of the study we will provide a report of the outcome to those involved in the study, we will also hold a meeting 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.

Confidentiality

No material which could personally identify you would be used in any reports on this study. Information collected from you in the study will be stored securely in the Department of Nutrition and will only be available 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.

Genetic testing

Each person has a DNA make-up (their genes) which is different from that of everybody else - except in the case of identical twins. This genetic make-up is a mixture of the genes of our parents. The precise way they are mixed varies from child to child within the same family, so having the same parents does not mean that two children will have exactly the same genes. We already know that some health conditions and disorders are definitely inherited through the genes (hereditary conditions), but we do not know how many conditions are explained by genetic inheritance. Inherited genes may explain why some people are more resistant and some people more prone to disorders which have not yet been identified as hereditary. The research in which you are invited to participate will investigate genetic make-up to look for any link.

Because the research investigates genetic make-up, this identifies a participant and their particular genetic characteristics. This information is confidential and will not be disclosed, stored, or used in any way without the informed consent of the participant.

We have no intention of claiming the right, ownership or property in your individual genetic information or that of your kinship group. You consenting to participate in DNA sampling of the proposed study will not be construed as creating any right or claim on the part of the researcher to your genetic information.

Compensation for Injury

If physical injury results from your participation in this study, you should visit a treatment provider to make a claim to ACC as soon as possible. ACC cover and entitlements are not automatic and your claim will be assessed by ACC in accordance with the Injury Prevention, Rehabilitation and Compensation Act 2001. If your claim is accepted, ACC must inform you of your entitlements, and must help you access those entitlements. Entitlements may include, but not be limited to, treatment costs, travel costs for rehabilitation, loss of earnings, and/or lump sum for permanent impairment. Compensation for mental trauma may also be included, but only if this is incurred as a result of physical injury.

If your ACC claim is not accepted you should immediately contact the researcher. The researcher will initiate processes to ensure you receive compensation equivalent to that to which you would have been entitled had ACC accepted your claim.

Please feel free to contact the researcher if you have any questions about this study.

This project has been reviewed and approved by the Massey University Human Ethics Committee: Southern A, Application 06/67. If you have any concerns about the conduct of this research, please contact Professor John O'Neill, Chair, Massey University Human Ethics Committee: Southern A, telephone 06 350 5799 x 8635, email humanethicssoutha@massey.ac.nz

Please note: The formatting on this information sheet and the following medical history form and osteoporosis questionnaire has been altered in order to fit the formatting of the thesis, and allow for page layout requirements dictated by binding.

4. **Medical History Form**



Institute of Food, Nutrition and Human Health
Massey University
Albany, Auckland
New Zealand

Subject Identifier	
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Surya Study

**An investigation of the relationship between nutrition and
risk factors for disease in South Asian women**

DoB:

Participant details

First Name: _____ Family name _____

Name you would like to be called by: _____

Street address: _____

Suburb: _____

Phone (home): _____ Phone (mobile): _____

Email: _____

General Practitioner

Address

Phone No: _____

Medical History

Have you ever been diagnosed with any of the following:

	Tick for yes	Comments
High blood pressure	<input type="checkbox"/>
Heart disease	<input type="checkbox"/>
Angina	<input type="checkbox"/>
Stroke	<input type="checkbox"/>
Cancer	<input type="checkbox"/>
Osteoporosis	<input type="checkbox"/>
Lupus or ME	<input type="checkbox"/>
Rheumatoid arthritis	<input type="checkbox"/>
Psoriasis	<input type="checkbox"/>
Tuberculosis	<input type="checkbox"/>
Other

Does anyone in your immediate family (that is blood relatives) have any of the following conditions that you are aware of? If possible, please tell us the gender of the person, and the age at which they were diagnosed

Diabetes.....

Cardiovascular disease.....

Osteoporosis.....

Marital status.....

Years of education (from age 5).....

Country of birth: **Years in NZ**

Dental Health

Do you have, or have you had any treatment for, periodontal (gum) disease Yes

No

How many of your adult teeth have been extracted?.....

Medication:

Are you taking any form of medication, including traditional or homeopathic medicine? Please list:

.....

HRT/birth control.....

Supplements:

Are you taking any dietary supplements, minerals, vitamins etc.? Please list:

.....

Do you chew betel nut (paan) in any form? If so, please describe what form, and the frequency of use.....

<p>Do you smoke cigarettes?</p> <p>s <input type="checkbox"/> No <input type="checkbox"/></p> <p>If yes, how many per day</p> <p>If no, have you ever smoked? For how many years</p>	<p>Ye</p>
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<p>Do you drink alcohol?</p> <p>s <input type="checkbox"/> No <input type="checkbox"/></p> <p>If yes, approximately how many standard drinks per week</p>	<p>Ye</p>
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When was your last period?

Weight (kg)		Height (cm)	BMI
Waist		Hip:	Ratio
Blood pressure #1		#2	#3
Temperature			

Recall for DEXA bone density scan (optional) **Y** **N**

5. *Osteoporosis knowledge and sun exposure questionnaire*



Institute of Food, Nutrition and Human Health

Massey University

Albany, Auckland, New Zealand

Dear _____

Please complete the Osteoporosis Questionnaire enclosed and post it back to us in the self-addressed envelope provided as soon as possible. Once we have received your completed questionnaire, we will be able to send you the results of your blood tests, dietary analysis etc.

Select ONE answer for each question and check that ALL questions are answered before posting it back to us.

If you have any questions or problems, please email or phone for help:

Pamela von Hurst 414 0800 ext 41205

p.r.vonhurst@massey.ac.nz

Many thanks for your help with our study.

The Surya Study Team

Survey of your views about osteoporosis

Osteoporosis (os-te-o-po-ro-sis) is a condition in which the bones become very brittle and weak so that they break easily.

Please enter your name and subject id:

Family name:

First name:

ID:

Below is the list of things which may or may not affect a person's chance of getting osteoporosis. After you read each statement, think about if the person is:

More Likely to get osteoporosis, or

Less Likely to get osteoporosis ,or

It has nothing to do with (**neutral**) getting osteoporosis ,or

You **Don't Know**.

Please read each statement and select **ONE ANSWER** for each of the following questions.

1	Eating a diet LOW in milk products	<input type="radio"/> 1. More Likely
		<input type="radio"/> 2. Less Likely
		<input type="radio"/> 3. Neutral
		<input type="radio"/> 4. Don't Know
2	Being menopausal, "change of life"	<input type="radio"/> 1. More Likely
		<input type="radio"/> 2. Less Likely
		<input type="radio"/> 3. Neutral
		<input type="radio"/> 4. Don't Know

3	Having big bones	<input type="checkbox"/> 1. More Likely <input type="checkbox"/> 2. Less Likely <input type="checkbox"/> 3. Neutral <input type="checkbox"/> 4. Don't Know
4	Eating a diet high in dark green leafy vegetables	<input type="checkbox"/> 1. More Likely <input type="checkbox"/> 2. Less Likely <input type="checkbox"/> 3. Neutral <input type="checkbox"/> 4. Don't Know
5	Having a mother or grandmother who had osteoporosis	<input type="checkbox"/> 1. More Likely <input type="checkbox"/> 2. Less Likely <input type="checkbox"/> 3. Neutral <input type="checkbox"/> 4. Don't Know
6	Being a woman of South Asian origin	<input type="checkbox"/> 1. More Likely <input type="checkbox"/> 2. Less Likely <input type="checkbox"/> 3. Neutral <input type="checkbox"/> 4. Don't Know
7	Having ovaries surgically removed	<input type="checkbox"/> 1. More Likely <input type="checkbox"/> 2. Less Likely <input type="checkbox"/> 3. Neutral <input type="checkbox"/> 4. Don't Know
8	Taking cortisone (steroids e.g. Prednisone) for long time	<input type="checkbox"/> 1. More Likely <input type="checkbox"/> 2. Less Likely <input type="checkbox"/> 3. Neutral <input type="checkbox"/> 4. Don't Know
9	Exercising on a regular basis	<input type="checkbox"/> 1. More Likely <input type="checkbox"/> 2. Less Likely

- 3. Neutral
- 4. Don't Know

For the next group of questions, choose one answer from the 4 choices. If you think there is more than one answer, choose the best answer. If you are unsure, select Don't Know.

Please read each statement and select **ONE ANSWER** for each of the following questions.

10	Which of the following exercises is the best way to reduce a person's chance of getting osteoporosis	<input type="checkbox"/> 1. Swimming <input type="checkbox"/> 2. Walking briskly <input type="checkbox"/> 3. Doing kitchen chores, such as washing dishes or cooking <input type="checkbox"/> 4. Don't Know
11	Which of the following exercises is the best way to reduce a person's chance of getting osteoporosis?	<input type="checkbox"/> 1. Bicycling <input type="checkbox"/> 2. Yoga <input type="checkbox"/> 3. Housecleaning <input type="checkbox"/> 4. Don't Know
12	How many days a week do you think a person should exercise to strengthen the bones?	<input type="checkbox"/> 1. 1 day a week <input type="checkbox"/> 2. 2 days a week <input type="checkbox"/> 3. 3 or more days a week <input type="checkbox"/> 4. Don't Know
13	What is the LEAST AMOUNT OF TIME a person should exercise on each occasion to strengthen the bones?	<input type="checkbox"/> 1. Less than 15 minutes <input type="checkbox"/> 2. 20 to 30 minutes <input type="checkbox"/> 3. More than 45 minutes <input type="checkbox"/> 4. Don't Know

14	Exercise makes bones strong, but it must be hard enough to make breathing:	<input type="checkbox"/> 1. Just a little faster <input type="checkbox"/> 2. So fast that talking is not possible <input type="checkbox"/> 3. Much faster, but talking is possible <input type="checkbox"/> 4. Don't Know
15	Which of the following exercises is the best way to reduce a person's chance of getting osteoporosis?	<input type="checkbox"/> 1. Jogging or running for exercise <input type="checkbox"/> 2. Golfing using golf cart <input type="checkbox"/> 3. Gardening <input type="checkbox"/> 4. Don't Know
16	Which of the following exercises is the best way to reduce a person's chance of getting osteoporosis?	<input type="checkbox"/> 1. Bowling <input type="checkbox"/> 2. Doing laundry <input type="checkbox"/> 3. Aerobic dancing <input type="checkbox"/> 4. Don't Know

Calcium is one of the nutrients our body needs to keep bones strong.

Please read each statement and select **ONE ANSWER** for each of the following questions.

17	Which of these is a good source of calcium?	<input type="checkbox"/> 1. Apple <input type="checkbox"/> 2. Cheese <input type="checkbox"/> 3. Cucumber <input type="checkbox"/> 4. Don't Know
18	Which of these is a good source of calcium?	<input type="checkbox"/> 1. Watermelon <input type="checkbox"/> 2. Corn

		<input type="checkbox"/> 3. Canned Sardines <input type="checkbox"/> 4. Don't Know
19	Which of these is a good source of calcium?	<input type="checkbox"/> 1. Chicken <input type="checkbox"/> 2. Broccoli <input type="checkbox"/> 3. Grapes <input type="checkbox"/> 4. Don't Know
20	Which of these is a good source of calcium?	<input type="checkbox"/> 1. Yoghurt <input type="checkbox"/> 2. Strawberries <input type="checkbox"/> 3. Cabbage <input type="checkbox"/> 4. Don't Know
21	Which of these is a good source of calcium?	<input type="checkbox"/> 1. Ice cream <input type="checkbox"/> 2. Grapefruit <input type="checkbox"/> 3. Radishes <input type="checkbox"/> 4. Don't Know
22	Which of the following is the recommended amount of calcium intake for an adult?	<input type="checkbox"/> 1. 100 mg - 300 mg daily <input type="checkbox"/> 2. 400 mg - 600 mg daily <input type="checkbox"/> 3. 800 mg or more daily <input type="checkbox"/> 4. Don't Know
23	How much milk must an adult drink to meet the recommended amount of calcium?	<input type="checkbox"/> 1. 1/2 glass daily <input type="checkbox"/> 2. 1 glass daily <input type="checkbox"/> 3. 2 or more glasses daily <input type="checkbox"/> 4. Don't Know
24	Which of the following is the best reason for taking a calcium supplement?	<input type="checkbox"/> 1. If a person skips breakfast

		<input type="checkbox"/> 2. if a person does not get enough calcium from diet <input type="checkbox"/> 3. If a person is over 45 years old <input type="checkbox"/> 4. Don't Know
25	Which vitamin is required for the absorption of calcium?	<input type="checkbox"/> 1. A <input type="checkbox"/> 2. C <input type="checkbox"/> 3. D <input type="checkbox"/> 4. Don't Know
26	What is the best source of the vitamin required for calcium absorption?	<input type="checkbox"/> 1. Oranges <input type="checkbox"/> 2. Cheese <input type="checkbox"/> 3. Sunlight <input type="checkbox"/> 4. Don't Know

Below are some questions about your beliefs about osteoporosis. There are no right or wrong answers. We all have different experiences which will influence how we feel. After reading each statement, indicate if you **STRONGLY DISAGREE**, **DISAGREE**, are **NEUTRAL**, **AGREE** or **STRONGLY AGREE** with the statement.

It is important that you answer according to your actual beliefs and not according to how you feel you should believe or how you think we want you to believe. We need the answers that best explain how **you** feel.

Please read each statement and select **ONE BEST** option that explains what you believe.

27	Your chances of getting osteoporosis are high.	<input type="checkbox"/> 1. Strongly Disagree <input type="checkbox"/> 2. Disagree <input type="checkbox"/> 3. Neutral
----	--	--

		<input type="checkbox"/> 4. Agree <input type="checkbox"/> 5. Strongly Agree
28	Because of your body build, you are more likely to develop osteoporosis.	<input type="checkbox"/> 1. Strongly Disagree <input type="checkbox"/> 2. Disagree <input type="checkbox"/> 3. Neutral <input type="checkbox"/> 4. Agree <input type="checkbox"/> 5. Strongly Agree
29	It is extremely likely that you will get osteoporosis.	<input type="checkbox"/> 1. Strongly Disagree <input type="checkbox"/> 2. Disagree <input type="checkbox"/> 3. Neutral <input type="checkbox"/> 4. Agree <input type="checkbox"/> 5. Strongly Agree
30	There is a good chance that you will get osteoporosis.	<input type="checkbox"/> 1. Strongly Disagree <input type="checkbox"/> 2. Disagree <input type="checkbox"/> 3. Neutral <input type="checkbox"/> 4. Agree <input type="checkbox"/> 5. Strongly Agree
31	You are more likely than the average person to get osteoporosis.	<input type="checkbox"/> 1. Strongly Disagree <input type="checkbox"/> 2. Disagree <input type="checkbox"/> 3. Neutral <input type="checkbox"/> 4. Agree <input type="checkbox"/> 5. Strongly Agree
32	Your family history makes it more likely that you will get osteoporosis.	<input type="checkbox"/> 1. Strongly Disagree

		<input type="checkbox"/> 2. Disagree <input type="checkbox"/> 3. Neutral <input type="checkbox"/> 4. Agree <input type="checkbox"/> 5. Strongly Agree
33	The thought of having osteoporosis scares you.	<input type="checkbox"/> 1. Strongly Disagree <input type="checkbox"/> 2. Disagree <input type="checkbox"/> 3. Neutral <input type="checkbox"/> 4. Agree <input type="checkbox"/> 5. Strongly Agree
34	If you had osteoporosis you would be crippled.	<input type="checkbox"/> 1. Strongly Disagree <input type="checkbox"/> 2. Disagree <input type="checkbox"/> 3. Neutral <input type="checkbox"/> 4. Agree <input type="checkbox"/> 5. Strongly Agree
35	Your feelings about yourself would change if you got osteoporosis.	<input type="checkbox"/> 1. Strongly Disagree <input type="checkbox"/> 2. Disagree <input type="checkbox"/> 3. Neutral <input type="checkbox"/> 4. Agree <input type="checkbox"/> 5. Strongly Agree
36	It would be very costly if you got osteoporosis.	<input type="checkbox"/> 1. Strongly Disagree <input type="checkbox"/> 2. Disagree <input type="checkbox"/> 3. Neutral <input type="checkbox"/> 4. Agree

		<input type="checkbox"/> 5. Strongly Agree
37	When you think about osteoporosis you get depressed.	<input type="checkbox"/> 1. Strongly Disagree <input type="checkbox"/> 2. Disagree <input type="checkbox"/> 3. Neutral <input type="checkbox"/> 4. Agree <input type="checkbox"/> 5. Strongly Agree
38	It would be very serious if you got osteoporosis.	<input type="checkbox"/> 1. Strongly Disagree <input type="checkbox"/> 2. Disagree <input type="checkbox"/> 3. Neutral <input type="checkbox"/> 4. Agree <input type="checkbox"/> 5. Strongly Agree
39	Regular exercise prevents problems that would happen from osteoporosis.	<input type="checkbox"/> 1. Strongly Disagree <input type="checkbox"/> 2. Disagree <input type="checkbox"/> 3. Neutral <input type="checkbox"/> 4. Agree <input type="checkbox"/> 5. Strongly Agree
40	Regular exercise helps to build strong bones.	<input type="checkbox"/> 1. Strongly Disagree <input type="checkbox"/> 2. Disagree <input type="checkbox"/> 3. Neutral <input type="checkbox"/> 4. Agree <input type="checkbox"/> 5. Strongly Agree
41	Exercising to prevent osteoporosis also improves the way your body looks.	<input type="checkbox"/> 1. Strongly Disagree

		<input type="checkbox"/> 2. Disagree <input type="checkbox"/> 3. Neutral <input type="checkbox"/> 4. Agree <input type="checkbox"/> 5. Strongly Agree
42	Regular exercise cuts down the chances of broken bones.	<input type="checkbox"/> 1. Strongly Disagree <input type="checkbox"/> 2. Disagree <input type="checkbox"/> 3. Neutral <input type="checkbox"/> 4. Agree <input type="checkbox"/> 5. Strongly Agree
43	You feel good about yourself when you exercise to prevent osteoporosis.	<input type="checkbox"/> 1. Strongly Disagree <input type="checkbox"/> 2. Disagree <input type="checkbox"/> 3. Neutral <input type="checkbox"/> 4. Agree <input type="checkbox"/> 5. Strongly Agree

For the following 6 questions, "taking enough calcium" means taking enough calcium by eating calcium rich foods and/or taking calcium supplements.

Please read each statement and select **ONE BEST** option that explains what you believe.

44	Taking in enough calcium prevents problems from osteoporosis.	<input type="checkbox"/> 1. Strongly Disagree <input type="checkbox"/> 2. Disagree <input type="checkbox"/> 3. Neutral <input type="checkbox"/> 4. Agree <input type="checkbox"/> 5. Strongly Agree
45	You have lots to gain from taking in enough calcium to prevent osteoporosis.	<input type="checkbox"/> 1. Strongly Disagree

		<input type="checkbox"/> 2. Disagree <input type="checkbox"/> 3. Neutral <input type="checkbox"/> 4. Agree <input type="checkbox"/> 5. Strongly Agree
46	Taking in enough calcium prevents painful osteoporosis.	<input type="checkbox"/> 1. Strongly Disagree <input type="checkbox"/> 2. Disagree <input type="checkbox"/> 3. Neutral <input type="checkbox"/> 4. Agree <input type="checkbox"/> 5. Strongly Agree
47	You would not worry as much about osteoporosis if you took in enough calcium	<input type="checkbox"/> 1. Strongly Disagree <input type="checkbox"/> 2. Disagree <input type="checkbox"/> 3. Neutral <input type="checkbox"/> 4. Agree <input type="checkbox"/> 5. Strongly Agree
48	Taking in enough calcium cuts down on your chances of broken bones.	<input type="checkbox"/> 1. Strongly Disagree <input type="checkbox"/> 2. Disagree <input type="checkbox"/> 3. Neutral <input type="checkbox"/> 4. Agree <input type="checkbox"/> 5. Strongly Agree
49	You feel good about yourself when you take in enough calcium to prevent osteoporosis.	<input type="checkbox"/> 1. Strongly Disagree <input type="checkbox"/> 2. Disagree <input type="checkbox"/> 3. Neutral <input type="checkbox"/> 4. Agree <input type="checkbox"/> 5. Strongly Agree

50	You feel like you are not strong enough to exercise regularly.	<input type="radio"/> 1. Strongly Disagree <input type="radio"/> 2. Disagree <input type="radio"/> 3. Neutral <input type="radio"/> 4. Agree <input type="radio"/> 5. Strongly Agree
51	You have no place where you can exercise.	<input type="radio"/> 1. Strongly Disagree <input type="radio"/> 2. Disagree <input type="radio"/> 3. Neutral <input type="radio"/> 4. Agree <input type="radio"/> 5. Strongly Agree
52	Your spouse or family discourages you from exercising.	<input type="radio"/> 1. Strongly Disagree <input type="radio"/> 2. Disagree <input type="radio"/> 3. Neutral <input type="radio"/> 4. Agree <input type="radio"/> 5. Strongly Agree
53	Exercising regularly would mean starting a new habit which is hard for you to do.	<input type="radio"/> 1. Strongly Disagree <input type="radio"/> 2. Disagree <input type="radio"/> 3. Neutral <input type="radio"/> 4. Agree <input type="radio"/> 5. Strongly Agree
54	Exercising regularly makes you uncomfortable.	<input type="radio"/> 1. Strongly Disagree <input type="radio"/> 2. Disagree

		<input type="checkbox"/> 3. Neutral <input type="checkbox"/> 4. Agree <input type="checkbox"/> 5. Strongly Agree
55	Exercising regularly upsets your every day routine.	<input type="checkbox"/> 1. Strongly Disagree <input type="checkbox"/> 2. Disagree <input type="checkbox"/> 3. Neutral <input type="checkbox"/> 4. Agree <input type="checkbox"/> 5. Strongly Agree
56	Calcium rich foods cost too much.	<input type="checkbox"/> 1. Strongly Disagree <input type="checkbox"/> 2. Disagree <input type="checkbox"/> 3. Neutral <input type="checkbox"/> 4. Agree <input type="checkbox"/> 5. Strongly Agree
57	Calcium rich foods do not agree with you.	<input type="checkbox"/> 1. Strongly Disagree <input type="checkbox"/> 2. Disagree <input type="checkbox"/> 3. Neutral <input type="checkbox"/> 4. Agree <input type="checkbox"/> 5. Strongly Agree
58	You do not like calcium rich foods.	<input type="checkbox"/> 1. Strongly Disagree <input type="checkbox"/> 2. Disagree <input type="checkbox"/> 3. Neutral <input type="checkbox"/> 4. Agree <input type="checkbox"/> 5. Strongly Agree

59	Eating calcium rich foods means changing your diet which is hard to do.	<input type="checkbox"/> 1. Strongly Disagree <input type="checkbox"/> 2. Disagree <input type="checkbox"/> 3. Neutral <input type="checkbox"/> 4. Agree <input type="checkbox"/> 5. Strongly Agree
60	In order to eat more calcium rich foods you have to give up other foods that you like.	<input type="checkbox"/> 1. Strongly Disagree <input type="checkbox"/> 2. Disagree <input type="checkbox"/> 3. Neutral <input type="checkbox"/> 4. Agree <input type="checkbox"/> 5. Strongly Agree
61	Calcium rich foods have too much cholesterol	<input type="checkbox"/> 1. Strongly Disagree <input type="checkbox"/> 2. Disagree <input type="checkbox"/> 3. Neutral <input type="checkbox"/> 4. Agree <input type="checkbox"/> 5. Strongly Agree
62	You eat a well-balanced diet.	<input type="checkbox"/> 1. Strongly Disagree <input type="checkbox"/> 2. Disagree <input type="checkbox"/> 3. Neutral <input type="checkbox"/> 4. Agree <input type="checkbox"/> 5. Strongly Agree
63	You look for new information related to health.	<input type="checkbox"/> 1. Strongly Disagree <input type="checkbox"/> 2. Disagree

		<input type="checkbox"/> 3. Neutral <input type="checkbox"/> 4. Agree <input type="checkbox"/> 5. Strongly Agree
64	Keeping healthy is very important for you.	<input type="checkbox"/> 1. Strongly Disagree <input type="checkbox"/> 2. Disagree <input type="checkbox"/> 3. Neutral <input type="checkbox"/> 4. Agree <input type="checkbox"/> 5. Strongly Agree
65	You try to discover health problems early.	<input type="checkbox"/> 1. Strongly Disagree <input type="checkbox"/> 2. Disagree <input type="checkbox"/> 3. Neutral <input type="checkbox"/> 4. Agree <input type="checkbox"/> 5. Strongly Agree
66	You have a regular health check-up even when you are not sick.	<input type="checkbox"/> 1. Strongly Disagree <input type="checkbox"/> 2. Disagree <input type="checkbox"/> 3. Neutral <input type="checkbox"/> 4. Agree <input type="checkbox"/> 5. Strongly Agree
67	You follow recommendations to keep you healthy.	<input type="checkbox"/> 1. Strongly Disagree <input type="checkbox"/> 2. Disagree <input type="checkbox"/> 3. Neutral <input type="checkbox"/> 4. Agree <input type="checkbox"/> 5. Strongly Agree

Finally we would like to know about your attitudes to sun exposure and sun bathing. Please select the statement that best expresses your thoughts.

Please read each statement and select **ONE ANSWER** for each of the following questions.

68	I enjoy spending time outside in the sun	<input type="radio"/> 1. Often <input type="radio"/> 2. Rarely <input type="radio"/> 3. Never <input type="radio"/> 4. Don't Know
69	I believe that the sunlight can be good for your health	<input type="radio"/> 1. Always <input type="radio"/> 2. Sometimes <input type="radio"/> 3. Never <input type="radio"/> 4. Don't Know
70	I believe that the sunlight is bad for your health	<input type="radio"/> 1. Always <input type="radio"/> 2. Sometimes <input type="radio"/> 3. Never <input type="radio"/> 4. Don't Know
71	During summer I sunbathe	<input type="radio"/> 1. Often <input type="radio"/> 2. Rarely <input type="radio"/> 3. Never <input type="radio"/> 4. Don't Know
72	If I am going to be outside, I use sunscreen:	<input type="radio"/> 1. Always <input type="radio"/> 2. Sometimes <input type="radio"/> 3. Rarely <input type="radio"/> 4. Never
73	My main reason for avoiding sun is:	<input type="radio"/> 1. Custom or religious reason

74

I would spend more time in the sun if:

- 2. Public health messages say to avoid the sun
- 3. Specific health reasons
- 4. I don't want darker skin
- 5. I don't avoid the sun

75

Do you want to make any comments about sun exposure?

- 1. I had somewhere private to sunbathe
- 2. I had more time
- 3. I wasn't worried about skin cancer
- 4. I wouldn't spend more time in the sun