Associations between physical activity, body composition, nutrient intake, and bone mineral density in pre-menopausal Pacific Island women living in New Zealand.

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Maria Casale

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Abstract

Background/Aim: Anecdotally it is suggested that Pacific Island women have good bone mineral density (BMD); however little evidence for this or for associated factors exists. The aim of this study is to explore associations between several key predictors of bone health with bone mineral density, as measured by BMD (g/cm^2), in pre-menopausal Pacific Island women.

Methods: Healthy pre-menopausal Pacific Island women (*n*=91; age 16-45y) were recruited. Participants' body composition and total body BMD were assessed using DXA and air-displacement plethysmography (BodPod). A food frequency questionnaire (FFQ) and current bone-specific physical activity questionnaire (cBPAQ) were completed. Variables that significantly correlated with BMD were applied to a hierarchical multiple regression analysis.

Results: The mean BMD was 1.1 g/cm^2 ± 0.08. Bone-free, fat-free lean mass only (LMO, 52.4kg ± 6.9) and total mass (90.4kg ± 19) were the only factors to show a significant correlation with BMD. Body-fat (38.4% ± 7.6), cBPAQ score (1.7 (0.4,5.2)), and dietary calcium (1016mg ± 442), protein (18% ± 3.8) and vitamin C (125mg (94, 216)) showed no correlation with BMD. The regression analysis suggests that LMO is the most important predictor of BMD, explaining 13.4% of the variance, while total mass accounts for a further 2.5% of the variance. Together, these factors explain a total of 15.9% of the variability.

Conclusions: LMO is the strongest predictor of BMD, while many established contributors to bone health (calcium, physical activity, protein, and vitamin C) do not appear to be associated with BMD in this population. As just 15.9% of the variability can be explained, further research is needed in this area.

Key words: *Bone mineral density, Pacific Island, pre-menopausal, body composition, physical activity, dietary intake*

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List of Abbreviations

25-OH-D	25-hydroxycholecalciferol
AMDR	Acceptable Macronutrient Distribution Range
ASMM	Appendicular Skeletal Muscle Mass
BIA	Bioelectrical Impedance Analyser
BLHQ	Bone Loading History Questionnaire
BMAD	Bone Mineral Apparent Density
BMC	Bone Mineral Content
BMD	Bone Mineral Density
BMI	Body Mass Index
BMR	Basal Metabolic Rate
BPAQ	Bone-Specific Physical Activity Questionnaire
cBPAQ	current Bone-Specific Physical Activity Questionnaire
DXA	Dual-Energy X-ray Absorptiometry
EXPLORE	Examining Predictors Linking Obesity Related Elements
FFM	Fat Free Mass
FFQ	Food Frequency Questionnaire
IGF+1	Insulin-like Growth Factor
IL	Interleukin
LMO	Lean Mass Only
LRP	Lipoprotein Receptor Related Protein
MoH	Ministry of Health
MUHEC	Massey University Human Ethics Committee
NHANES	National Health and Nutrition Examination Survey
PAL	Physical Activity Level
PBM	Peak Bone Mass
pBPAQ	past Bone-Specific Physical Activity Questionnaire
PTH	Parathyroid Hormone
RANKL	Receptor Activator of Nuclear factor-Kappa B Ligand
RCT	Randomised Controlled Trial
RDI	Recommended Daily Intake
ROS	Reactive Oxygen Species
RPAQ	Regular Physical Activity Questionnaire
SD	Standard Deviation
SOP	Standard Operating Procedure
SPARC	Sport and Recreation New Zealand
tBPAQ	total Bone-Specific Physical Activity Questionnaire
UVB	Ultraviolet B
WHO	World Health Organisation

Contribution to Research

Table 1.1: *Contributions to this study*

Researchers	Contribution to this thesis
Maria Casale	Main researcher, participant recruitment, screening, and testing, data analysis, statistical analysis, interpretation and discussion of results.
Dr Pamela von Hurst	Main academic supervisor, DXA testing, and guidance with design of thesis, methods, statistical analysis, interpretation of results, and revision.
Dr Sarah Shultz	Academic supervisor and assistance with interpretation of physical activity measures, thesis design, interpretation of results, and revision.
Dr Marlena Kruger	Academic supervisor, assistance with design of thesis, methods, statistical analysis, interpretation of results, revision, and final approval.
Dr Rozanne Kruger	Principal Investigator of the Women's EXPLORE study, application for ethics, development of study design.
Wendy O'Brien and Shakeela Jayasinghe	Co-ordination of and participation in recruitment, screening, and testing of participants.
PC Tong	Analysis of DXA data and assistance with equipment for data collection.
Zara Houston, Richard Swift, Adrianna Hepburn, Jenna Schrijvers, Andrea Fenner, Sarah Philipsen, Owen Mugridge, Pamela von Hurst, Cath Conlon, Kathryn Beck, Rozanne Kruger	Participant screening, testing, and recruitment.
Jenna Schrijvers, Zara Houston, Chelsea Symons, Alex Lawn	Data entry of the FFQs

Chapter 1

Background of Research

There is little doubt that bone mineral density (BMD) is a cornerstone of healthy aging, and with a low BMD, quality of life is severely affected through loss of mobility and independence, and increased mortality. Peak bone mass (PBM) is reached in the third decade of life, and maintenance of bone density is crucial for avoiding osteoporosis and fragility fractures later in life. Anecdotally it is thought that Pacific Island women mostly have good BMD; however there is relatively scarce research into the factors determining this.

Although genetic factors account for approximately three quarters of the variation in BMD, individuals have the ability to determine their bone health through diet and lifestyle factors.

Physical activity is one lifestyle factor that is crucial for bone development, and reaching optimum PBM in early adulthood. In post-menopausal women with osteoporosis, physical activity reduces the likelihood of falls and subsequent fractures, thus improving quality of life (Caputo and Costa, 2014). Additionally, increasing daily activity can help prevent further decreases in bone mass of older adults (>75yrs) (Muir *et al.*, 2013). In New Zealand, there is mixed evidence for the level of physical activity amongst Pacific Island children, with indications that Pacific Island children are more likely to be active than European children, however there are conflicting reports that Pacific Island children have lower levels of physical activity than other groups (SPARC, 2003, Ministry of Health, 2003). However, it is likely that this is due to Pacific Island children having higher rates of incidental activity – such as walking or biking to school – but lower rates of participation in sport, which was the sole parameter measured when reporting lower physical activity rates.

Diet plays an important role in achieving and maintaining healthy bones. Specific nutrients involved in bone health are calcium, vitamin D, vitamin A, vitamin K, protein, phosphorous, omega-3 fatty acids, with lesser roles for iron, sodium, vitamin C, magnesium, and B vitamins (Anderson *et al.*, 2012). Intakes of these nutrients amongst Pacific Island women in New Zealand falls short of the recommended daily intake (RDI) set by the Ministry of Health for calcium (University of Otago and Ministry of Health, 2011). Data for folate, sodium, and

Vitamin D are not shown as the data was not reliable, and there is no data available for magnesium intake.

Body composition is also a key factor in bone health: it is well known that the greater the load on the body physical activity is, the more effect it has on bone density. As such, the positive association between higher body weight and greater BMD generally seen in Pacific Islanders is likely to be due in part to the greater mechanical force placed on the skeleton. Pacific Island women typically fall into the higher range of the international body mass index (BMI) cut-off points: 13.7% fall within the normal range (18.5 – 24.9 kg/m^2), 26.5% in the overweight range (25-30 kg/m^2), and 59.5% in the obese range (≥30 kg/m^2). Much of the contribution to BMI comes from lean muscle mass, and it is well established that Pacific Islanders have greater muscularity than European or Asian Indian groups. Considering that lean mass is a predictor for BMD in pre-menopausal women, it is likely that this is a primary determinant for the bone health of Pacific Island women.

Purpose of the study

The greatest focus on the health of Pacific Island people often tends to be around obesity and lifestyle-related diseases: it may be important to consider the potential cost to bone health when devising interventions around this. By identifying the determinants of bone health, whether it be genetic in terms of bone microstructure and architecture, or related to body composition, could allow for greater refinement of obesity interventions so the risk of any affect on bone health could be minimised. Additionally, identifying BMD determinants in Pacific Island women allows this study to contribute to an area that is currently somewhat lacking in specific research.

Hypothesis

Lean muscle mass is the primary determinant of bone mineral density in pre-menopausal Pacific Island women.

Aim

To explore the associations between nutrients, physical activity, body composition, and regional fat with bone quality in New Zealand-based Pacific Island women.

Objectives

1. To analyse total body BMD of participants.
2. To analyse body composition of participants.
3. To analyse physical activity of participants.
4. To analyse key bone-specific nutrient intake of participants.
5. To investigate the relationships between these factors and bone density.

Research Structure

This thesis has been set out in six chapters. The first chapter introduces the topic of BMD, and in particular with relation to Pacific Island women. The second chapter is the literature review, and looks at structure and function of bone, factors that affect BMD, and those factors in relation to Pacific Island women. Chapter three details the methods of the study, with an overview of the wider EXPLORE study methods, then specific detail on the methods required for the present study. The fourth chapter contains the results. Chapter five consists of the discussion of the study findings, and incorporates limitations within each section. Chapter six concludes the thesis with a brief summary, and recommendations for applicability and future research.

Chapter 2

Review of the Literature

Bone is a well-researched topic, and there is a wealth of knowledge around bone growth, development, maintenance, structure and function. However, there are areas of contention as well, with conflicting and controversial issues, such as the role of calcium supplementation, and protein intake.

2.1 Bone Structure and Function

Bones serve multiple important functions in the body: they are a support framework, they protect vital organs, they act as levers to help move the body, they are a repository for minerals, they house blood cell formation, and they produce the hormone osteocalcin which helps regulate bone formation (Marieb and Hoehn, 2013). To achieve its function, bone must be strong, flexible, and light in weight. These properties are due to the structural and material composition of bone.

Structural properties

The outer shell of bone is the smooth, compact, cortical layer, and coats every bone in the body, protecting the inner trabecular bone and resisting mechanical stress and pressure. Despite its solid appearance, cortical bone is made up of a labyrinth of tiny cylinders called osteons, the hollow centre of which (the central canal) houses nerves and blood vessels. They act as miniature weight-bearing shafts, and they owe this ability to the layout of the collagen fibres. Each osteon is made up of three progressively smaller tubes that sit inside each other. Each of these tubes is called a lamellar, and each particular lamellar has collagen fibres running in one direction. However, the collagen fibres of the three lamella run in alternating directions to each other, thus each lamella helps the adjacent one resist twisting: it is this that gives bone the ability to resist torsion stress (Marieb and Hoehn, 2013). Cortical bone is the predominant type of bone in the body, making up ~85% of the skeleton. In addition to the osteons, there are perforating canals (Volkmann's canals), which run parallel from the axis of the bone and feed the blood and nerve supply to where the spongy bone sits in the medullary cavity (Seeman and Delmas, 2006). These are not surrounded by lamellae as the osteons are. There

are small holes called lacunae at the junctions of the lamellae that surround osteons, and these are filled with osteocytes. The lacunae are connected to both each other and the central canal by canaliculi, which are tiny strands of canals. Surrounding the osteons are incomplete lamellae which are either remains of osteons that have been broken down during the bone remodelling process, or are filling gaps between osteons that are forming (Floyd, 2015). Adjacent to these, located on the outer layer, is circumferential lamellae, which are spread around the circumference of the diaphysis, and provide resistance to twisting stress on the long bones (Fyhrie and Christiansen, 2015). In the centre of the cortical layer of bone is the trabecular bone: a soft, spongy bone. Unlike the cortical bone, the layout of the bone appears like an intricate matrix of bone strands - however, the trabecular bone is carefully deposited: in fact, the mechanical stressed placed on bone as they develop determines how the trabeculae are laid out (Seeman and Delmas, 2006). This design helps the bone further resist stress.

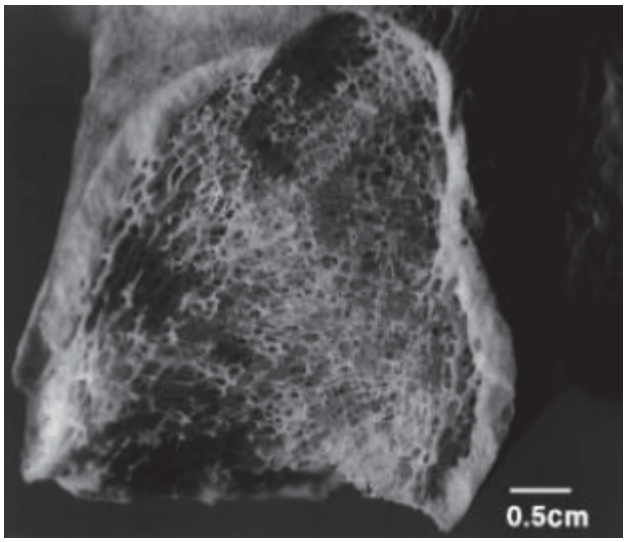


Figure 2.1: *Cortical and trabecular bone*
Weiner, S., & Wagner, H. D. (1998). The material bone: structure-mechanical function relations. *Annual Review of Materials Science, 28*(1), 271-298.

Material properties

Bone primarily consists of calcium carbonate and calcium phosphate (~60-70%), water (~25-30%), and collagen (Floyd, 2015). The collagen is primarily type I, which covalently cross-links to form a matrix, which is strengthened with hydroxyapatite that interlock with the matrix (Fyhrie and Christiansen, 2015, Seeman and Delmas, 2006). Hydroxyapatite is made up of calcium and phosphate: bone also contains small amounts of magnesium, sodium and

bicarbonate (Marieb and Hoehn, 2013). The more mineralised a bone is, the stronger it is and thus better able it is to withstand mechanical stressors: however, this is not a case of "more is better": bone that is 100% mineralised is fragile, and is not able to absorb impact by bending a little. Rather, its ability to withstand pressure will decline, and it will be more likely to fracture under pressure (Seeman and Delmas, 2006, Fyhrie and Christiansen, 2015). This effect occurs at a bone mineral content (BMC) of greater than 65% (Currey, 1969). If bone is under-mineralised, when remodelling increases after menopause, the older bone that may be well mineralised will be replaced with newer bone that is sparsely mineralised - this will cause the bone to become too flexible, and therefore more likely to fracture due to excessive bending with mechanical loading (Fyhrie and Christiansen, 2015, Seeman and Delmas, 2006).

Bone needs to be rebuilt due to either mechanical stresses endured by the bone from everyday activities, or because of bone breakdown in response to hormonal changes in response to increases or decreases of serum calcium. Osteoblasts, osteoclasts, and osteocytes are the three main types of cells that are involved in bone re-modelling, each with a different role, but all working in concert to maintain bone homeostasis (Huiskes *et al.*, 2000).

The function of osteoblasts is to build new bone matrix by producing collagen, into which bone salts can be deposited. As osteoblasts progress along the bone, they deposit densely cross-linked strands of type I collagen parallel then at right angles to the long axis of the bone, providing flexible strength (Reddi *et al.*, 1977). They then trap themselves in the very matrix they are secreting, and become osteocytes, resulting in stronger thicker bone (Franz-Odendaal *et al.*, 2006).

Osteocytes not only differentiate from osteoblasts, but also directly from osteoprogenitors. They reside in the lacunae and canaliculi, which they connect through to one another via elongated cytoplasmic extensions (Franz-Odendaal *et al.*, 2006). Osteocytes do not undergo mitosis, and have an average half-life of approximately 25 years. They respond strongly to mechanical loading (Burger and Klein-Nulend, 1999, Lanyon, 1996, Mullender and Huiskes, 1995, Noble *et al.*, 1997, Skerry *et al.*, 1989), which generates a complex cascade of signals that travel through the canaliculi and act upon osteoblasts which in turn lay down new bone (Burger and Klein-Nulend, 1999).

Osteoclasts on the other hand have the opposite effect on bone: they are multinucleated cells that break bone matrix down in response to various stimuli. Activated by cytokines produced

by the osteoblasts (Chambers and Fuller, 1985), potentially as a result of micro-fractures or mechanical disuse (Burger and Klein-Nulend, 1999, Noble *et al.*, 1997), osteoclasts secrete a lysosomal enzyme and collagenase which allows the disassembly and digestion of the bone, and releases calcium into the blood. This occurs by the osteoclast forming a tight seal around the area of the bone where resorption is to take place. Once the seal is formed, the portion of the osteoclast that is next to the bone changes to the distinctive ruffled shape, which allows increased secretory activity and the transcytosis of calcium and broken-down collagen into the extracellular matrix (Marieb and Hoehn, 2013).

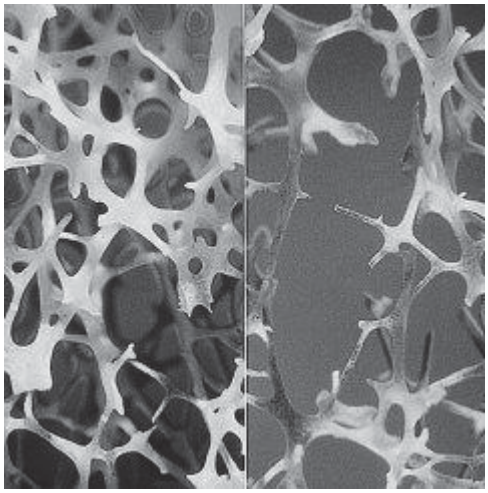
2.2 Osteoporosis

Pathogenesis

The literal translation of osteoporosis is “porous bone”, where the bone becomes brittle, weak, and is subsequently prone to fractures (figure 2.2). The development of osteoporosis is silent: the first symptom is typically a fracture, at which point osteoporosis is advanced, and more difficult to treat.

Osteoporosis results from abnormalities in the composition and structure of the bone. Additionally, as people grow older, less bone is laid down than is resorbed, which can be due to declining oestrogen that comes with menopause, calcium deficiency or malabsorption, or secondary hyperparathyroidism (Eriksen and Langdahl, 1997).

Bones reach their peak mass in the third decade (Mahan *et al.*, 2012). The quality of an individual’s PBM is dictated by many factors, with the predominant factor being genetics. Modifiable factors that play a major role are dietary (calcium & vitamin D), and lifestyle (physical activity, smoking, caffeine, and alcohol consumption). As such, the development of healthy bones earlier in life reduces the likelihood of developing osteoporosis later in life (Eriksen and Langdahl, 1997).



The bone on the left shows normal healthy bone, while the bone on the right shows the fragile porous bone that is characteristic of osteoporosis.

Figure 2.2: *Healthy versus osteoporotic bone*
U.S. Department of Health and Human Services. (2015). Menopause: Time for a Change. Retrieved 4/6/2015 2015 from https://www.nia.nih.gov/health/publication/menopause-time-change/postmenopausal-health-concerns

Definitions

Low bone density and osteoporosis are diagnosed by comparing a person's BMD to the established norm for a population, which produces a T-Score. (NIH Osteoporosis and Related Bone Diseases National Resource Centre, 2012). Osteoporosis is diagnosed when an individual's BMD T-Score is 2.5 standard deviations (SD) or more below the young adult mean BMD (table 1).

Table 2.1: *World Health Organisation definitions of osteoporosis based on T-scores*

Level	Definition
Normal	Bone density is within 1 SD (+/-) of the young adult mean
Low bone mass	Bone density is between 1 and 2.5 SD below the young adult mean
Osteoporosis	Bone density is 2.5 SD or more below the young adult mean
Severe (established) osteoporosis	Bone density is more than 2.5 SD below the young adult mean, and there have been one or more osteoporotic fractures

World Health Organisation. (2004). *WHO Scientific Group on the Assessment of Osteoporosis at Primary Health Care Level*. Geneva, Switzerland. Retrieved from http://www.who.int/chp/topics/Osteoporosis.pdf

There is potential for Z scores to help diagnose secondary causes of osteoporosis: if a Z-score is -2 or lower, something other than aging may be causing bone loss (Leib, 2004). Pre-menopausal women with a low BMD as determined by a Z-score of less than -2 would benefit from further medical evaluation to assess for secondary causes of osteoporosis.

Osteoporosis in pre-menopausal women is either idiopathic or secondary. One clinic reported 56% of patients with idiopathic variety, and 44% with secondary (Peris *et al.*, 2002). Patients with the idiopathic variety tended to have a lower BMI and higher femoral neck Z-Scores than those with secondary, as well as a family history of hypercalciuria and osteoporosis (Peris *et al.*, 2002). Micro-architectural defects of the bone is commonly seen in idiopathic osteoporosis, and such defects are similar to those seen in fractures, however it is not possible to predict future fracture risk due to a lack of longitudinal data (Cohen and Shane, 2013).

Osteoporosis from secondary causes can be diagnosed in pre-menopausal women using clinical diagnosis in conjunction with consideration of bone density measures: osteoporosis in pre-menopausal women who have a Z-score of -1 is likely due to secondary causes (Swaminathan *et al.*, 2009). Secondary causes of low bone mass may include excess glucocorticoids, early-onset menopause, anorexia nervosa, or pre-menopausal oestrogen deficiency (Peris *et al.*, 2002, Lewiecki, 2005). Women who are being treated with chemotherapy or endocrine therapy for breast cancer are subject to rapid bone loss (Hadji *et al.*, 2014). Gut conditions that affect nutrient absorption, such as Coeliac's disease, Crohn's disease, inflammatory bowel syndrome, or any other malabsoprtive disorders that prevent absorption and utilisation of key nutrients such as calcium, vitamin D, vitamin C, and protein may also increase the risk for osteoporosis, particularly in the case of juvenile onset during critical bone growth periods (Mauro and Armstrong, 2007, Katz and Weinerman, 2010, Bernstein *et al.*, 2003, Bertoldi Franco, 2014).

Measurements

BMD can be calculated by analysing either total body bone density, or specific sites of interest. The most common fragility fractures occur at the hip, spine, and wrist, with hip and spine fractures being the most devastating in terms of decreased mobility and independence, and increased mortality rates. As bone scans are typically conducted with the goal of helping diagnose possible osteoporosis or osteoporosis risk, regional scans of these areas of interest are most commonly carried out. The National Health and Nutrition Examination Survey

(NHANES) however changed to using total body bone density measurements in 1999 (Melton *et al.*, 2005). At this time, evidence for accuracy of total body scans to identify bone density at key sites of interest (spine and hip) was scarce, but consistently favourable (Franck and Munz, 2000, Hammami *et al.*, 2001, Hangartner *et al.*, 2000, Lu *et al.*, 1994, Nysom *et al.*, 1998). The rationale for the change was to obtain more complete data on body composition as well as bone density the NHANES. Since then, total body bone density has been shown to have strong correlations with dedicated site-specific scans of the hip, spine, and distal forearm, but with slightly different predictions of osteoporosis and fracture risk (Melton *et al.*, 2005).

Epidemiology

Approximately 50% of women will sustain an osteoporotic fracture, with the most common of these being hip, spine, or forearm fractures (Kanis *et al.*, 2001). Of these, hip fractures are the most devastating: they often require surgery, resulting in a substantial loss of mobility (Taylor *et al.*, 2004, Pasco *et al.*, 2005), and thus independence and quality of life. This is magnified by increased mortality, with 25% of people suffering an osteoporotic hip fracture dying within 12 months of sustaining the injury (Kanis *et al.*, 2003). Approximately 90% of hip fractures in both men and women occur as a result of a fall from standing height or less (Youm *et al.*, 1999). The pathophysiology of such falls can be attributed to several modifiable and non-modifiable factors, including the biomechanics of the fall: i.e., which part of the individual receives the greatest impact from the fall (Cummings and Melton, 2002).

People with vertebral fractures can experience loss of height, deformity (Dowager's Hump), and acute back pain, which again has a significant impact on mobility and independence (Ettinger *et al.*, 1992). Additionally, there is an increased risk of peripheral fractures subsequent to a vertebral fracture (Silverman, 1992), which further reduces quality of life. Vertebral fractures are less well quantified due to their low rate of diagnosis, however approximately a quarter are caused by a fall (Cooper *et al.*, 1992), while most result from load bearing during usual everyday activities (Myers and Wilson, 1997). Other risk factors identified were age, fracture history, physical activity, and osteoporosis history (Janott *et al.*, 1996).

2.3 Factors That Affect Bone Health

No one factor is solely responsible for the attainment and maintenance of good bone health: rather, it is a combination of many lifestyle and genetic influences, which weave together to create a tapestry that can be examined to assess likelihood of good bone health.

Dietary factors

Diet is a major modifiable factor in bone health, with many different nutrients playing a role in BMD, including protein, minerals, fat-soluble and water-soluble vitamins. Attainment of PBM is compromised in malnutrition and anorexia nervosa, with bone depending on dietary intake for its building blocks (Heaney, 2000, Rizzoli, 2014). The primary materials required for the synthesis of extracellular bone are calcium, phosphorous, and protein, with bone consisting of approximately 50% calcium phosphate, and 50% protein (Heaney, 2000).

Calcium

Calcium is an important micronutrient found in high quantities in dairy products, fish with soft bones such as sardines and mackerel, fortified non-dairy foods such as soy, nut, and rice milks, fortified cereals, and some nuts, and in lesser amounts in leafy green vegetables. Calcium is needed for several critical metabolic roles (Mahan *et al.*, 2012):

- Influence of transport functions in cell membranes
- Conduction of ions across cellular organelle membranes
- Neurotransmitter release across synaptic junctions
- Hormone function
- Activation and release of intra- and extra-cellular enzymes
- Nerve impulse transmission
- Regulation of cardiac muscle contractions
- Skeletal muscle tone and contraction
- Smooth muscle contractility
- Blood clot formation
- Enzyme co-factor

Calcium balance is therefore critical to life, and the body maintains calcium homeostasis within a narrow range of 2.2 to 2.7 mmol/L (Goldstein, 1990). Levels of calcium become low when daily losses such as in sweat, shedding of skin, hair, nails, urine, and digestive secretions (Heaney, 2000) are not replaced through dietary sources. The skeleton acts as a reservoir, containing approximately 99% of the body's calcium, and by providing calcium to the body through osteoclastic action when serum levels become low (Marieb and Hoehn, 2013). The skeleton is not merely a store for calcium however: it is also important for building and maintaining bones and teeth – in fact, the need of the skeleton for calcium is significant

compared with the small amount needed for these critical metabolic functions listed above (Mahan *et al.*, 2012).

It is well known that calcium is a key nutrient for good bone health : in a meta-analysis of 139 papers investigating the relationship between calcium and bone health, 52 of which were randomised controlled trials (RCTs), all but two of the RCTs showed that high intakes of calcium were positively associated with improved bone balance, higher attainment of bone mass during growth, reduced bone loss in the elderly, and lower fracture risk (Heaney, 2000). Of 86 observational studies, 64 reported a significant positive association between calcium intake and bone health (Heaney, 2000). Just two found a negative effect, one of which was explained by confounding factors not controlled for, and 19 observational studies found no effect (Heaney, 2000). A possible reason for the larger proportion of observational studies finding no effect of calcium is the difficulty of accurately quantifying calcium intake (Heaney, 1997). Overall, these findings emphasise the clear role of calcium in building and maintaining a healthy skeleton.

Calcium is primarily obtained through dietary intake of dairy products, except for those who have an allergy, intolerance, or aversion to dairy; as such the effects of calcium supplementation have been well researched. There is a clear role for calcium supplementation in the increase of bone mass: 17 RCTs showed calcium supplements significantly reduced fracture risk in post-menopausal women by 12%, with a further 23 RCTs showing a significant reduction in the rate of bone loss at the femoral hip and lumbar spine (Tang *et al.*, 2007). This is supported by another meta-analysis showing a small, non-significant effect of calcium supplementation on the BMD of post-menopausal women, and a trend towards a lowered risk of vertebral fractures (Shea *et al.*, 2002). However, another meta-analysis showed that calcium supplements had a negative effect on hip fracture risk (Bischoff-Ferrari *et al.*, 2007). This meta-analysis looked at 8 prospective cohort studies, and five RCTs that did not include consideration of Vitamin D status or supplementation. Because calcium absorption is dependent on Vitamin D, studies that do not consider Vitamin D may not reach accurate conclusions. It is also possible that people who already had adequate dietary calcium intake were being supplemented: this has not been defined, and it is unlikely that supplementation would improve bone health unless serum calcium concentrations in participants were low. In terms of children, a meta-analysis of 19 RCTs investigating calcium intake in children found only a small effect in the upper limb (Winzenberg *et al.*, 2006), which is unlikely to significantly

reduce fracture risk over a lifetime. Although overall calcium supplementation is shown to have a positive effect, it is important to consider the association found between supplementation and increased risk of myocardial infarction in people with coronary heart disease (Bolland *et al.*, 2008).

The RDI for calcium is 1300mg per day for adolescent girls, 1000mg per day for women aged 19-50, and 1300mg per day for women aged >51 (National Health and Medical Research Council, 2006). Calcium requirements are high for adolescent girls due to a greatly increased accrual of bone between the ages of 12 to 18 years, while an increase in women aged over 50 due to a decrease in intestinal absorption of calcium (Heaney and Recker, 1986, Heaney *et al.*, 1989, Morris *et al.*, 1991), and increased urinary calcium losses after menopause (Nordin *et al.*, 1999). In New Zealand, the median daily intake of calcium amongst women aged 15-71+ was 745mg, well short of the RDIs for all age groups.

Vitamin D

Vitamin D is a fat-soluble vitamin that is essential for good bone health. It is a hormonal complex that plays a key role in bone via calcium homeostasis, and stimulation of bone matrix growth and maturation (Lanham-New, 2008).

Vitamin D must undergo conversion into its biologically active form. It does this through a series of steps, starting from the skin, and involves the liver and kidneys. After the skin absorbs UVB rays, pro-vitamin D3 is converted to pre-vitamin D3 (cholecalciferol), which is taken into the liver. Here, it is hydroxylated by hepatic 25-hydroxylase, which converts it into 25-hydroxycholecalciferol (25-OH-D). This is transported to the kidneys, where it is hydroxylated again by renal 1α-hydroxylase, transforming it into 1,25-dihydroxyvitamin D3 (calcitriol), which is the active metabolite form. This binds to the vitamin D binding protein and is transported through the blood and stored in the fat deposits of various tissues and organs, and is released when needed (Holick, 2003).

Vitamin D acts upon the upper small intestine by increasing the efficiency of calcium absorption (Marieb and Hoehn, 2013). Indeed, calcium absorption is considered to be impaired when there is insufficient vitamin D present, with the rate of intestinal calcium absorption optimised at a 25-OH-D level of 86 nmol/L, a level notably higher than the minimum 25-OH-D

level of 50 nmol/L (Heaney *et al.*, 2003). This has interesting implications for the currently accepted reference range for vitamin D.

Vitamin D also works directly on osteoblasts to stimulate new bone growth and suppress bone degradation through the activation of key bone matrix proteins and other local factors (Marieb and Hoehn, 2013), as well as enhancing the activity of osteoclasts, and potentially playing a role in the differentiation of osteocyte pre-cursors (Underwood and DeLuca, 1984).

There is little doubt that vitamin D is one of the more important nutrients for bone health: several meta-analyses show that Vitamin D supplementation significantly reduces the incidence of fractures at the lumbar spine, and a trend has been shown for reduced fractures elsewhere in the body (Papadimitropoulos *et al.*, 2002, Bergman *et al.*, 2010, Richy *et al.*, 2005), affirming the critical role of vitamin D in good bone health. It is probable that a deficiency of vitamin D increases risk for fractures and osteoporosis in later life.

The primary source is sunlight skin exposure, and diet is a minor secondary source (Chen *et al.*, 2007). Precursors of vitamin D (pre-vitamin D3) are synthesised in the skin in response to exposure to ultraviolet B (UVB) rays, which varies in response to several factors:

1. Skin tone: melanin is the pigment in skin that is responsible for skin colour – the more melanin a person has, the darker their skin. Melanin acts as a natural sunscreen by absorbing broadband UV rays, which affects the skin's ability to produce pre-vitamin D3 (Holick *et al.*, 1981). The darker a person's skin tone is, the more UVB exposure they require to synthesise the same amount of vitamin D as a lighter skinned person (Chen *et al.*, 2007).
2. Latitude, season, time of day: in winter, although the sun is closest to the earth, the angle at which the sun's rays hit the earth is more slanting. This slanting angle results in the rays passing through the ozone layer for a longer distance, resulting in more UVB rays being absorbed by the ozone layer. Furthermore, the more slanted the sun's rays are, the fewer photons per area unit reach the earth (Holick, 2004). Other factors that affect how slanted the angles of the sun's rays are time of day, and latitude (Holick, 2003, Webb *et al.*, 1988). Latitudes above 37° have as much as 80-100% fewer photons reaching the earth from November to February, resulting in

little to no vitamin D synthesis during these months, with increasing amounts of vitamin D synthesised in the skin closer to the equator (Chen *et al.*, 2007). Time of day also affects the slant of the sun's rays, with the rays being at a much more oblique angle in the early morning and late afternoon (Holick, 2004).

3. Length of sun exposure: Typically 5-15 minutes of skin exposure to sunlight between 10am and 3pm in summer, autumn and spring is sufficient for individuals with type II or III skin. The time for darker skin tones will be longer due to increased melanin pigment.
4. Cloud cover and air pollution: when fossil fuels, wood and other materials are burnt, small particles in the air scatters and absorbs UVB rays (Kimlin, 2008). In some areas, such as over Canterbury in New Zealand, there are holes in the ozone layer. As the ozone layer absorbs UVB rays, this could lead to increased exposure and thus increased production of previtamin-D3 (Kimlin, 2008).
5. Sunscreen and clothing: Sunscreen reduces the amount of pre-vitamin D3 produced in the skin by over 95% (Holick, 2003, Holick, 2004, Matsuoka *et al.*, 1988), while clothing styles that cover much of the skin, such as traditional Middle Eastern, blocks the sun's rays and prevents previtamin-D3 production.
6. Age: there is reduced ability of the skin to synthesise pre-vitamin D3 in response to sun exposure as age increases, due to a decline in the amount of 7-dehydrocholesterol present in the epidermis. As a result, a person who is 70 years of age, and who receives the same sunlight exposure as a 20 year old, will only make approximately 25% of that of the 20 year old (Holick, 2004, Mazahery and von Hurst, 2015).

In addition to sun exposure, dietary intake can have a small impact on vitamin D status; however there are few foods that are good sources of vitamin D. Foods rich in vitamin D include egg yolk, oily fish such as salmon, tuna, and mackerel, with lesser amounts found in dairy sources such as milk and cheese (Chen *et al.*, 2007, Holick, 2003).

In New Zealand, 68.1% of adults have good levels of vitamin D (≥50 nmol/L), with 27.1% below the recommended level of 50 nmol/L, and 4.9% are deficient (<25nmol/L), with levels being similar across all age groups, and between men and women (Ministry of Health, 2012b). Vitamin D status is affected by body size, with people who are overweight or obese being at risk of having lower serum 25-OH-D levels (Institute of Medicine, 2011). In New Zealand, obese individuals have a significantly lower annual mean level of Vitamin D than those of a normal weight (Ministry of Health, 2012b).

Protein

While there is little doubt that protein has an important role in the health of the skeleton, the optimal balance of protein in order to have maximum positive effects on bone with no detrimental effects has been much debated (Ginty, 2003, Rizzoli and Bonjour, 2004, Roughead, 2003, Joint WHO/FAO/UNU Expert Consultation, 2007). This has been complicated by the potential differing effects of vegetable and animal proteins, but what has become clear is that protein in isolation is not solely responsible for bone health.

There are several mechanisms by which protein influences bone. Protein is needed for the maintenance of the bone structure (Darling *et al.*, 2009), and creates an anabolic drive which stimulates insulin-like growth factor (IGF-1) (Millward and Rivers, 1989). IGF-1 helps increase osteoblast activity and thus increase bone mass: it is also possible that IGF-1 helps with the mineralisation of bone (Rizzoli *et al.*, 2007). IGF-1's specific activities that contribute to bone health are to increase renal resorption of phosphate and calcitriol synthesis, and to indirectly control both intestinal calcium absorption and phosphate and calcium concentrations in the extracellular fluid (Caverzasio *et al.*, 1990).

A high protein intake is associated with an increased acid load as a result of sulphur-containing amino acid oxidation (Remer and Manz, 1995). Lower pH values can impact the ratio of osteoblastic to osteoclastic activity, and give an increase in urinary calcium excretion (Arnett and Dempster, 1986, Lemann *et al.*, 1967). This happens because the kidneys work to neutralise the acid load to restore pH homeostasis - however, kidney function declines with advancing age, and may not be able to neutralise the acid as efficiently, resulting in a need for buffering in the bone which may result in increased bone turnover (Kerstetter, 2009).

The above findings raise the question "is this endogenous acid production from a high protein diet of sufficient magnitude to affect bone?" (Kerstetter, 2009). The body has three major chemical buffering systems that maintain homeostasis (bicarbonate, phosphate, and protein buffer systems), and two physiological buffering systems (lungs and kidneys) (Marieb and Hoehn, 2013). The lungs and kidneys are the most powerful buffering systems, with the lungs removing CO_2 from the body and restoring O_2, and the kidneys which eliminate all other types of acid, and keep blood pH stable by regulating alkaline substances and restoring chemical buffers as they are depleted. The kidneys are extremely efficient at maintaining pH within a narrow range of 7.35-7.45, as evidenced by increased urinary acid excretion over a 24 hour period in response to ingesting more dietary protein (Kerstetter *et al.*, 2006). Post-prandial pH would need to be <7.2 for stimulation of osteoclastic action, which is not probable (Bonjour, 2005). The increased urinary calcium output that is observed with increased protein intake was originally thought to be due to increased bone turnover (Heaney and Recker, 1982, Kerstetter and Allen, 1990) is in fact likely due to the increased intestinal absorption of calcium that occurs with higher protein intakes: the amino acids arginine and lysine have a strong effect on absorption (Wasserman *et al.*, 1956). Furthermore, an intake of protein of 0.7 g/kg of body weight vs 2.1g/kg of body weight results in reduced intestinal absorption of calcium along with a rise in serum PTH (Kerstetter *et al.*, 1997, Kerstetter *et al.*, 1998).

Higher intakes of dietary protein is clearly linked with better bone health, however whether this translates to a lower long-term fracture risk is yet to be elucidated (Darling *et al.*, 2009, Genaro *et al.*, 2015, Sahni *et al.*, 2014, Zhu and Prince, 2015). What has been established is that higher dietary protein loads are not associated with an increased risk of fracture or decreased bone health (Beasley *et al.*, 2010, Hu *et al.*, 2014, Jia *et al.*, 2015, Kerstetter *et al.*, 2015).

The acceptable macronutrient distribution range (AMDR) for protein intake is 15-25% of an individual's total energy intake (National Health and Medical Research Council, 2006). The average protein intake of New Zealand adult females aged 15 years and over is 16.5%, which is adequate but at the lower end of the scale (University of Otago and Ministry of Health, 2011). Bread products are the most common dietary source of protein (11%), followed by milk and poultry (9% each), and beef and veal (8%) (University of Otago and Ministry of Health, 2011).

Vitamin C

Vitamin C is a potent antioxidant which is typically found in abundance in vegetables and citrus fruit. There is a well-established role for vitamin C in bone health: it is crucial for the development, function, and maintenance of healthy bone. Specifically, it modulates the production of collagen, a key component of bone matrix, and is involved with bone-related gene transcription. In addition, oxidative stress contributes to bone loss, with poor vitamin C intake positively associated with osteoporosis risk (Bai *et al.*, 2005, Ha *et al.*, 2004, Koh *et al.*, 2006, Lee *et al.*, 2005, Wittrant *et al.*, 2008, Garrett *et al.*, 1990).

There is a direct relationship between vitamin C and collagen formation. Vitamin C is a co-factor for prolyl and lysyl hydroxylases (Silverman, 1992, Bernstein *et al.*, 2003, Ettinger *et al.*, 1992, Bertoldi Franco, 2014), which is needed for the maturation, cross-linking, and maintenance of collagen. This role is demonstrated with *in vitro* experiments, in which collagen fibroblasts were grown in culture. When pure vitamin C was infused into the culture, production of the collagen fibres increased significantly. Furthermore, when vitamin C is added to osteoblast-like cells in culture, firstly deposition of collagen for the extracellular matrix is instigated (Heaney *et al.*, 2003, Silverman, 1992), then secondly, initiation of genes specifically associated with the osteoblast phenotype occurs (Heaney *et al.*, 2003, Underwood and DeLuca, 1984, McCarty, 2008, Lanham-New, 2008). Reinforcing this is the appearance of impaired collagen synthesis in animal cells that are deficient in vitamin C (Heaton, 1969, Tucker, 2009).

There has been some suggestion that vitamin C may also play a role in the mineralisation of bone (Felson *et al.*, 1993). It is likely that vitamin C has an indirect role in bone mineralisation: as osteoid trabeculae are in the process of being formed, there are already some deposits of bone salts (Morton *et al.*, 2001, Hall and Greendale, 1998), which suggests that matrix production, and mineralisation of that matrix, occur simultaneously. This suggestion is reinforced by the reduced mineralisation in the bones of animals that are fed a vitamin C-deficient diet as compared to those with vitamin C-replete diets (Floyd, 2015). The reduced calcification of deficient animals is likely due to a reduced amount of matrix available to be mineralised, and also would explain the reduced ability of individuals affected by scurvy to mineralise their bones.

Vitamin C not only regulates osteoblasts, but also osteoclast activity by both stimulating and inhibiting osteoclast action. Differentiation of osteoclast precursors by receptor activator of

nuclear factor-Kappa B ligand (RANKL) is inhibited by vitamin C, and the formation of bone resorption troughs *in vitro* is also depressed (Tucker *et al.*, 1995). Further to this, cell death of late-stage osteoclasts is brought on by the addition of vitamin C (Kalantar-Zadeh *et al.*, 2010). On the other hand, the addition of vitamin C to osteoclast precursors grown in culture significantly increased the occurrence and number of osteoclast precursors, particularly when added in the first four days of the growth stage (Rude *et al.*, 2009). Consistent with these findings, treatment of primary mouse bone marrow cultures with vitamin C resulted in a significant increase in the number, size, and nucleation of osteoclasts (Kalantar-Zadeh *et al.*, 2010). This seemingly conflicting role for vitamin C could perhaps be explained by the basic role it plays in the differentiation of mesenchymal cells, which in turn support osteoclast differentiation.

Reactive oxygen species (ROS) increase with age, placing the body under greater oxidative stress. ROS have a profound influence on the synthesis and survival of osteoblasts, osteoclasts, and osteocytes (Manolagas, 2010). Specifically, ROS products, especially the superoxide anion, activate osteoclast activity directly by replicating RANK signalling thereby stimulating differentiation of osteoclasts, or indirectly by initiating the coupling of osteoblasts and osteoclasts giving rise to osteoclast differentiation via RANKL expression, thus increasing bone turnover (Garrett *et al.*, 1990, Kim *et al.*, 2015, Manolagas, 2010, Wauquier *et al.*, 2009).

Markers of antioxidant defences in women with osteoporosis are notably low, and together with the above findings this suggests a pivotal role for vitamin C in the treatment and prevention of osteoporosis (Maggio *et al.*, 2003). Administration of vitamin C significantly attenuated bone loss and ROS activity, improved bone fragility, and increased osteoblast survival in vivo (Morikawa *et al.*, 2013, Nojiri *et al.*, 2011, Sanbe *et al.*, 2007). Furthermore, BMD in humans increases in response to vitamin C intake at clinically relevant sites (Chuin *et al.*, 2009, Maggio *et al.*, 2003, Sugiura *et al.*, 2011). In terms of doses, one case-controlled pilot study gave their control group 1000mg daily doses of vitamin C along with 600mg of vitamin E (Chuin *et al.*, 2009), which is significantly higher than the RDI set by the Ministry of Health of 45mg per day (National Health and Medical Research Council, 2006), and found positive effects of treatment on bone health. In addition to this, there are epidemiological studies that show a consistent relationship between vitamin C deficiency, and osteoporosis and fracture risk.

Vitamin C also has a direct role in the regulation of gene transcription in bone – specifically, the genes involved in osteoblast maturation and function (Katz and Weinerman, 2010).

In New Zealand, women aged 15 years and over have an average vitamin C intake of 106mg per day, well above the RDI set by the Ministry of Health (University of Otago and Ministry of Health, 2011). The most common source of vitamin C in the New Zealand diet is vegetables (28%), followed by fruit (22%), non-alcoholic beverages (15%), and potatoes, kumara, and taro (13%).

Vitamin K

There are two types of vitamin K: vitamin K1 (phylloquinone), and vitamin K2. Dietary vitamin K1 primarily comes from green leafy vegetables and some vegetable oils, while dietary vitamin K2 is predominantly found in soy (Mahan *et al.*, 2012).

Vitamin K functions in bone as a co-factor for several proteins, via the post-translational carboxylation of a number of proteins found in bone, including osteocalcin (Binkley and Suttie, 1995, Vermeer *et al.*, 1995, Weber, 1997). Other proteins dependent on Vitamin K are growth-arrest specific gene 6, transforming growth factor B-induced protein igh3, periostin, GLA-rich protein, and osteocalcin (Loeser *et al.*, 1997, Ohno *et al.*, 2005, Viegas *et al.*, 2009, Fortunati *et al.*, 2010), suggesting multiple roles for Vitamin K in bone health. Serum concentrations of under-carboxylated osteocalcin can be measured, along with serum vitamin K, and low levels of these are linked to lower measures of bone density and increased risk of fracture (Szulc *et al.*, 1993, Szulc *et al.*, 1994, Jie *et al.*, 1996, Kohlmeier *et al.*, 1997, Vergnaud *et al.*, 1997).

Another way in which vitamin K affects bone metabolism is through its influence on urinary calcium excretion: individuals on long-term anti-coagulant medication had a significant increase in urinary calcium loss, while increased intake of vitamin K reduced urinary calcium losses (Jie *et al.*, 1993). It is not currently clear as to what extent such losses may affect bone density however. Vitamin K also has a role in the inhibition of bone resorbing mediators prostaglandin E2 (Koshihara *et al.*, 1993) and interleukin 6 (Reddi *et al.*, 1995).

While these factors alone may not have a significant effect on bone, taken together it presents an active role for vitamin K in maintaining good bone health (Kanai *et al.*, 1997), and reduction of fractures (Hart *et al.*, 1985, Hodges *et al.*, 1993). Furthermore, vitamin K supplementation is positively associated with improved bone density (Akjba *et al.*, 1991).

Phosphate

Phosphate salts are required for the mineralisation of bone, and are needed in a ratio of approximately 1:1 with calcium for this to occur. Serum phosphate levels are carefully controlled by PTH, vitamin D, and fibroblast growth factor 23: if the ratio of phosphate to calcium increases, the concentration of calcium in the blood becomes diluted, which in turn stimulates PTH and thus osteoclast activity (Mahan *et al.*, 2012). It is likely that if this altered ratio becomes habitual, bone loss and increased risk of low bone mass may follow.

Phosphorous is in almost all foods, but particularly so in processed foods (Kalantar-Zadeh *et al.*, 2010). A particularly common source of dietary phosphorous is in soft drinks. On a background of adequate calcium intake, occasional soft drink consumption will not be likely to cause a problem – however, when milk is substituted for soft drinks, the phosphate to calcium ratio could become unbalanced, which would compound the primary effect of lower calcium intake from the replacement of milk.

Magnesium

Approximately half of the magnesium in the body is located in the bone, as part of the surface of the hydroxyapatite deposits (Heaton, 1969). It has many roles in the body, both bone-specific and non-bone specific. The bone-specific roles for magnesium are calcium metabolism through the transport of calcium, as the aforementioned hydroxyapatite constituent.

Magnesium deficiency is associated with osteoporosis (Mutlu *et al.*, 2007); however it is possible that a diet deficient in magnesium is also deficient in other key nutrients needed for good bone health (Mahan *et al.*, 2012). In rats, magnesium depletion from inadequate dietary intake leads to loss of bone mass through increased bone resorption and decreased bone growth: this is contributed to by changes in PTH and vitamin D, as well as increasing RANKL and decreasing osteoprotegerin (Rude *et al.*, 2009).

Supplementation with magnesium in post-menopausal women with osteoporosis increases bone density (Stendig-Lindberg *et al.*, 1993), and there is a positive relationship between BMD and magnesium status (Tucker, 2009, Tucker *et al.*, 1995).

The RDI for magnesium set by the MoH for women is 310 mg/day for 19-30 years, and 320 mg/day for 31 years and older (National Health and Medical Research Council, 2006). Dietary magnesium is relatively abundant, and primarily comes from leafy green vegetables, legumes, peas, nuts, and some shellfish (Mahan *et al.*, 2012).

Zinc

Zinc has an important role in bone health: in many conditions that are associated with low levels of zinc, decreased bone growth and mineralisation is evident (Kannus *et al.*, 1999, Michaëlsson *et al.*, 2005). The main role of zinc in bone growth is its effect on DNA, collagen synthesis, and alkaline phosphatase activity. These all show a dose-dependent increase in the femoral diaphysis of rats in response to zinc administration (R, 2005). Furthermore, zinc deficiency in rats results in not only impaired development of vertebrae and ribcage (Deng *et al.*, 2000), but atypical ossification in the offspring of these rats (Torgerson *et al.*, 1996). In rhesus monkeys who are zinc-deprived, bone mineralisation is impaired compared to that of zinc-replete controls (Cummings *et al.*, 1995).

The RDI for zinc for adult women is 8 mg/day (National Health and Medical Research Council, 2006). Zinc is found in many different foods, with meat, fish, and poultry being a primary source. There is also a substantial amount of zinc in dairy and cereals (National Health and Medical Research Council, 2006). Interestingly, the bioavailability of zinc is affected by protein, which itself is a significant contributor to bone health: zinc binds to protein, and diets that are rich in animal-based protein sources enable much greater absorption of zinc than those that primarily are high in plant-based protein (Willett, 2013).

Boron

Boron is a trace element that is found mainly in plant foods – in particular, in vegetables, nuts, legumes, and non-citrus fruit (Mahan *et al.*, 2012). Its main role in the body is to regulate the metabolic enzyme activity, as well as a positive influence on the metabolism of the bone-related nutrients calcium, vitamin D, phosphorous, and magnesium (Devirian and Volpe, 2003). Boron is also utilised by osteoblasts to play a role in bone formation (Hakki *et al.*, 2010). There have been animal studies conducted that show supplementation with boron results in increased plasma concentrations of magnesium and calcium, as well as increased growth (Hunt, 1989, Nielsen *et al.*, 1988). Boron supplementation has also been shown to have a positive effect on growth when vitamin D status is low in chicks, by normalising growth plate maturation through the increased utilisation of nutrients (Hunt *et al.*, 1994). In peri- and post-menopausal women who are supplemented with boron, urinary excretion of calcium and magnesium is reduced while serum oestradiol is increased (Nielsen *et al.*, 1987), through the conversion of oestrogen to the more active 17-beta-oestrodiol (Nielsen, 2008). Absorption of calcium is also increased with boron supplementation (Beattie and Peace, 1993). Boron

supplementation also has a positive effect on BMD in college age women (Meacham *et al.*, 1994). This indicates a role for boron in the attainment and maintenance of good bone health.

Copper

Copper is found in high amounts in lamb, pork, game meat, salmon, organ meat, and shellfish. Avocado, chocolate, soy milk, legumes, and dried fruit are also high in copper (Mahan *et al.*, 2012). Copper plays an important role in the formation and mineralisation of bone, as it facilitates the action of the enzyme lysyl oxidase which facilitates the cross-linking of collagen and elastin fibrils (Palacios, 2006). The effects of copper status on bone have been evaluated in several studies, and there are conflicting findings. Post-menopausal women with low serum copper also had decreased BMD (Lowe *et al.*, 2002, Mahdavi-Roshan *et al.*, 2015, Chaudhri *et al.*, 2009) –this was conflicted by other studies however (Murphy *et al.*, 1985, Mutlu *et al.*, 2007, Sadeghi *et al.*, 2014), who found no association between copper status and BMD. Heterogeneity is potentially an issue in these studies though, in terms of skeletal site of measurement and menopausal status of the participants. There is also scarce evidence for the role of copper supplements in bone health (Rondanelli *et al.*, 2013), and the small amount of literature available is contradictory (EatonEvans *et al.*, 1996, Nielsen *et al.*, 2011, Strause *et al.*, 1994). It isn't clear from the literature if copper is a nutrient that is worthy of further research specifically in terms of bone health, given the clear significance of other factors that regulate bone health.

Manganese

Manganese is a trace element that is found primarily in the bones, liver, kidneys, and pancreas. It has several roles in the body, which are to help form connective tissue, bone, sex hormones, and blood clotting factors, as well as helping to regulate calcium absorption, carbohydrate metabolism, and blood sugar homeostasis (Mahan *et al.*, 2012). There is a positive association between BMD and serum manganese, and also the number of fractures in post-menopausal women and serum manganese is negatively correlated (Palacios, 2006). The mechanism for this is likely to be due the regulatory effects of manganese on oestrogen. Additionally, patients receiving total parenteral nutrition suffered from bone loss that was thought to be due to manganese deficiency.

Physical activity

Physical activity is undertaken by the majority of people in many different ways, from incidental activity such as housework or walking to the bus stop, to organised sport,

recreational activities such as skiing, rowing, exercise such as running or aerobics classes, to work-related activity for those in active jobs such as blue collar workers, police force, or defence force. All forms of physical activity have a variety of beneficial effects, such as increased muscle mass, better cardiovascular health, and reduced risk of lifestyle diseases such as type 2 diabetes, hypertension, and cardiovascular disease to name just a few (Warburton *et al.*, 2006). Physical activity also has an important role in attaining and maintaining a good PBM, by promoting bone growth and reducing the risk of falls and subsequent fractures in older adults (Wolff *et al.*, 1999, Borer, 2005, Berard *et al.*, 1997).

When considering the possible effect of physical activity on BMD, it is critical to consider the type of activity: i.e. how much mechanical stress it places upon the skeleton. The reason for this is that when the bone is subjected to strain or force that is altered or greater than usual strain, an osteogenic signal is generated to the bone that gives a hypertrophic response (Lanyon, 1996, O'Connor *et al.*, 1982, Rubin and Lanyon, 1984, Rubin and Lanyon, 1985). Further to this, an altered pattern of mechanical loading has a strong osteogenic effect after just a few rounds of unusual physical activity (that is, physical activity that is different from what is normally done by a given person) (Rubin and Lanyon, 1984). The exact nature of the signal has been identified, and the primary mechano-sensory cell in the bone is mostly likely to be the osteocyte (Bonewald and Johnson, 2008, Burger *et al.*, 1995, Klein-Nulend *et al.*, 2013, Schaffler *et al.*, 2014). The signalling pathway involved is the low-density lipoprotein receptor-related protein 5 (LRP5)-mediated Wnt/B-catenin pathway, which is activated by loading both in vitro (Case *et al.*, 2008, Lau *et al.*, 2006, Santos *et al.*, 2010, Sunters *et al.*, 2010) and in vivo (Robinson *et al.*, 2006, Robling *et al.*, 2008, Sawakami *et al.*, 2006, Tu *et al.*, 2012), and has been shown to increase the sensitivity of osteoblasts and osteocytes to mechanical stress (Robinson *et al.*, 2006).The role of this pathway is further emphasised in an animal study that deleted a single b-catenin allele in osteocytes, resulting in the inability of the skeleton to increase bone mass in response to mechanical loading in vivo (Javaheri *et al.*, 2014). The importance of the LRP5 receptor in bone remodelling has been demonstrated in knock-out mice: these mice had a significantly lower BMD as well as decreased strength as compared to the non-knock-out mice (Sawakami *et al.*, 2006). Another role for LRP5 receptor signalling is its antagonistic effect on sclerostin. Sclerostin is a protein derivative of the SOST gene found primarily in osteocytes, and acts by strongly inhibiting bone formation. It is possible that by the osteocytes controlling how much sclerostin they contain, they can exert control over their mechanosensory action, which would affect Wnt/b-catenin signalling (Robling *et al.*, 2008). Increased mechanical loading reduces the SOST expression of sclerostin, which indicates a

possible local Wnt signal occurring in the osteocyte (Tu *et al.*, 2012). Additionally, the osteogenic effect of the Wnt/LRP5 pathway is site specific according to where the skeletal loading occurs (Lanyon, 1996, Moustafa *et al.*, 2012, Tommerup *et al.*, 1993).

Physical activity is critically important during childhood and adolescence, as this is the critical period in which PBM is attained. Longitudinal bone growth peaks one to two years before the pubertal growth spurt, while mineralisation peaks during the pubertal spurt (Fournier *et al.*, 1997). Thus, physical activity (and dietary measures) are crucially important during this time to achieve optimal bone growth (Behringer *et al.*, 2014).

Too much physical activity coupled with the aesthetic pressures of some sports such as gymnastics or ballet may be harmful and impair bone growth. Hypothalamic function can become disrupted when exercise is very prolonged and intense, which can result in a delay in the onset of menarche, and increases likelihood of amenorrhoea (Javed *et al.*, 2013, Gremion *et al.*, 2001). Both of these are strong risk factors for reduced bone mass (Warren and Perlroth, 2001, Chevalley *et al.*, 2009, Chevalley *et al.*, 2008): introduce to this the potential for insufficient energy intake if an individual is trying to reach a particular aesthetic ideal that is not realistic, then serious hormonal imbalances can ensue (Rizzoli, 2014).

The importance of physical activity beyond the age where PBM has been attained cannot be overlooked. There is a clear role for physical activity in the prevention of bone loss and osteoporosis in post-menopausal women (Tobias *et al.*, 2014, Talele *et al.*, 2014, Caputo and Costa, 2014). Load bearing physical activity not only stimulates an osteogenic signal as described earlier, but it will help strengthen skeletal muscle, which will in turn reduce the risk of falls and subsequent fractures. Additionally, given that lean muscle mass is a strong predictor for good bone mass in pre-menopausal women (Douchi *et al.*, 1997), it is clear that appropriate exercise is something that benefits bone throughout the lifecycle.

In terms of assessing physical activity in the context of bone health, there are many different methods available. Subjects can wear an accelerometer or pedometer, and there are a wide range of self-reporting tools. Many of these tools are designed with energy expenditure and metabolic equivalents in mind, with no emphasis on mechanical loading of bone. The bone-specific physical activity questionnaire (BPAQ) is one such tool that has been recently developed (Weeks and Beck, 2008). The BPAQ is a self-administered test that assesses current and past physical activity, and uses an algorithm which applies specific weightings to intensity, years of participation in, and frequency of both past, and current physical activity. Another

bone-specific tool is the bone-loading history questionnaire (BLHQ) which assesses the load of physical activity on the spine and hip of pre-menopausal women during certain stages of life (Dolan *et al.*, 2006). However, the BLHQ has limitations: unlike the BPAQ, the load measures of physical activity were not obtained from direct measures of force, and it is more time-consuming to use. Also, when compared to clinical densitometry measures, neither the BLHQ as well as other more traditional questionnaires that assess physical activity were able to predict bone strength (Weeks and Beck, 2008).

Body composition

Body composition is the ratio of fat mass to muscle mass, and is a major contributing factor to bone health. While there is little doubt that total mass has an effect on bone, whether it is the effect of fat mass or lean mass that predicts BMD is disputed.

Body mass plays an important role in bone turnover: women with low body mass have a greater rate of bone loss during menopause than those with a higher body mass (Finkelstein *et al.*, 2013). Greater mass will provide greater mechanical loading on the skeleton – the osteocytes respond by sending a signal which either increases the activity of osteoblasts, or decreases the activity of osteoclasts (Seeman and Delmas, 2006).

The role of fat mass in BMD is likely to be both hormonal and mechanical. From a hormone perspective, the effect of fat on bone is related at least in part to the production of oestrogen, however it has also been suggested that serum oestrone levels are independent of BMD (Lindsay *et al.*, 1992, Reid *et al.*, 1992a). There has been a suggested role for leptin also, with consistent positive associations established between circulating leptin and BMD in pre- and post-menopausal women (Pasco *et al.*, 2001, Thomas *et al.*, 2001, Yamauchi *et al.*, 2001, Blain *et al.*, 2002, Kaufman *et al.*, 2002). The proposed mechanism for this is two-fold: firstly, that osteoblast formation from the stromal cells of the bone marrow is activated by leptin with an accompanying inhibition of adipocyte maturation, and secondly through the inhibition of osteoclast activity (Thomas *et al.*, 1999, Ogueh *et al.*, 2000, Reseland *et al.*, 2001, Holloway *et al.*, 2002). Both fat and lean mass will also place a mechanical load on bone that will contribute to BMD (MacInnis *et al.*, 2003, Finkelstein *et al.*, 2013). It is probable that a combination of all of the above factors contribute to BMD, but to differing degrees depending on individual characteristics such as age and level of physical activity.

Whether fat mass or lean mass affects bone is not agreed on, however the effect appears to depend on several variables:

- Skeletal site of measurement (regional vs. total body)
- Indices used
- Menopausal status

The skeletal site of measurement varies between studies, and whether the site of measurement is a load bearing site or not, or if it is a total body measure, may affect the strength of body composition associations. Total body bone density appears to be affected by both lean mass and fat mass; however this is likely to be dependent on menopausal status, with the most powerful association being between total lean mass and total body BMD in pre-menopausal women (Douchi *et al.*, 1997) . Additionally, there are strong correlations between lean mass and BMD at load bearing skeletal sites such as the femoral neck and lumbar spine, but not at non-load bearing sites (Harris *et al.*, 1992), such as the distal radius, where fat mass appears to have a stronger effect, potentially through metabolic action (Glauber *et al.*, 1995). This finding has been contradicted by other studies however, showing that fat mass significantly affected lumbar spine and femoral neck BMD in Chinese and Turkish women (Liu *et al.*, 2011, Nur *et al.*, 2013), although it is possible that race could explain these different findings. Probable reasons for the role of lean mass is that the more load-bearing exercise an individual does, then the greater their lean mass will be: given the established effects of load-bearing exercise on bone as discussed previously, it is likely that there will be a positive relationship between the two. Added to that the reduced risk of falls and subsequent fractures in individuals – particularly older adults – with greater lean mass, and a clear picture for the role of muscle in bone health emerges.

Differing indices of bone mass are used in different studies, producing conflicting results. BMC is corrected to allow for body size differences by using BMD, or bone mineral apparent density (BMAD) (Kerr *et al.*, 2007, Makovey *et al.*, 2005, Taaffe *et al.*, 2000), and BMD/height (Reid *et al.*, 1992b). BMAD is obtained by dividing BMC by an estimated bone volume, while BMD/height is a simpler version where the BMD is adjusted for height. When height is adjusted for, the association between bone and lean mass is weakened, and in some cases eliminated completely (Gnudi *et al.*, 2007, Harris and DawsonHughes, 1996, Khosla *et al.*, 1996, Reid *et al.*, 1994, Reid *et al.*, 1992b). A possible reason for this is that height is typically strongly

correlated with lean mass, so adjusting for height may introduce a bias against the association between lean mass and bone (Khosla *et al.*, 1996). Adjusting for height may also result in a stronger positive effect of fat mass on bone, and reduce the effect of lean mass (Reid *et al.*, 1994, Reid *et al.*, 1992b, Khosla *et al.*, 1996). This illustrates how findings of body composition associations will depend upon which indices are used.

It is probable that menopausal status is a factor also: however, while there is a general consensus that fat mass is a significant predictor for BMD in post-menopausal women (Baumgartner *et al.*, 1996, Compston *et al.*, 1992, Douchi *et al.*, 1997, Kirchengast *et al.*, 2001, Liu *et al.*, 2011, Nur *et al.*, 2013, Reid *et al.*, 1994, Reid *et al.*, 1992a), and lean mass a significant predictor in pre-menopausal women (Aloia *et al.*, 1995, Bogl *et al.*, 2011, Douchi *et al.*, 1997, Ijuin *et al.*, 2002, Kerr *et al.*, 2007, Khosla *et al.*, 1996, Valdimarsson *et al.*, 1999), the effect of age is also disputed. There are claims that lean mass is the single best predictor for BMD in post-menopausal women (Chen *et al.*, 1997, Cho, 2005, Leena and Vidula, 2015, Makovey *et al.*, 2005), and reports that fat mass has a positive effect on BMD in women under 50 years, but not in those over 50 (Makovey *et al.*, 2005). It is likely that an individual's osteoporosis risk also determines the strength of association: lean mass may be more of a factor in post-menopausal women without osteoporosis, where both lean mass and fat mass are significant in those with osteoporosis (Gnudi *et al.*, 2007).

None of these factors can be considered in isolation when investigating the true effect of body composition on bone: they all inter-linked, and the one consistent factor is that the associations change depending on which combination of factors are analysed.

Hormonal factors

Bone remodelling is strongly affected by hormonal controls. The hormonal control of bone remodelling is primarily by parathyroid hormone (PTH), oestrogen, and vitamin D as discussed in the previous section. Calcitonin also contributes on a much lesser scale.

When blood calcium levels begin to drop, the parathyroid gland senses this, and secretes more PTH (Yamaguchi *et al.*, 2000). There are specific calcium-sensing receptors on the parathyroid cell surfaces, which bind calcium. When only enough calcium is bound to indicate 0.025mmol/L of calcium present, the parathyroid cells are stimulated to release PTH - this

causes calcium to be released, thus increasing the amount of calcium bound to the receptors, which lowers the stimulus for PTH production (Taniegra, 2004).

PTH drives the increase of serum calcium by helping to increase intestinal calcium absorption through the conversion of vitamin D to its active metabolite, increasing calcium reabsorption and phosphate excretion in the kidneys, and by stimulating the breakdown of bone, allowing calcium to be released into circulation (Meng and Wagar, 2015). There are specific PTH receptors expressed on osteoblasts, and through this PTH indirectly influence osteoclasts to break down bone, releasing calcium (Taniegra, 2004). The binding of PTH to osteoblasts creates a paracrine effect from the osteoblasts to the osteoclasts: PTH stimulates the osteoblasts to release interleukin (IL)-1, IL-6, tumour necrosis factor-α, and prostaglandin E, and other cytokines, which subsequently stimulate osteoclasts to resorb bone (Mahan *et al.*, 2012). As a result, when there are high amounts of PTH in response to low serum calcium, osteoclast activity is increased, however occasional low quantities of PTH has a favourable effect on bone anabolism (White, 2010). PTH also stimulates increased production of osteoclasts. PTH regulation relies on sufficient levels of 1,25-dihydroxyvitamin D: if an individual is deficient in vitamin D, PTH will not be as well supressed by normal blood calcium levels, which can result in increased bone loss (White, 2010, Carling *et al.*, 1998).

Oestrogen also has an important role in the maintenance of bone homeostasis, through the blocking of PTH-stimulated cytokines that stimulate osteoclastic action (Mahan *et al.*, 2012), which results in a reduction of the number of osteoclasts (Harada and Rodan, 2003a). Oestrogen also has a role in males, working through oestrogen receptor-α to close the epiphyses, and maintenance of post-pubertal bone mass (Harada and Rodan, 2003b). Because of the pivotal role that oestrogen plays, when women enter menopause, bone resorption rapidly increases due to the loss of oestrogen (Eriksen and Langdahl, 1997).

Calcitonin does not play such a significant role in bone turnover. Calcitonin is produced by the C cells of the parathyroid gland, and although the mechanism of its physiological role is yet to be elucidated, calcitonin has a role in regulating bone turnover as well as managing calcium homeostasis (Davey and Findlay, 2013). Calcitonin is used therapeutically to reduce bone resorption. The effectiveness is dose-dependent, and can reduce resorption by up to 50% (Akcay *et al.*, 2004, Gennari and Agnusdei, 1994, Karsdal *et al.*, 2008). There is a potential link between calcitonin treatment and cancer however, with rates of cancer varying between 0.7%

in studies with oral calcitonin, and 2.4% in those with nasal spray formulations (European Medicines Agency, 2012).

Genetics

Genetic differences have a substantial impact on bone: genes inherited from an individual's parents and how they are expressed accounts for approximately 70% of variance in BMD (Ralston and de Crombrugghe, 2006). The rate of an individual's bone growth is influenced by gene expression in much the same way as height is, and follows a path that is identifiable from pre-puberty. Other contributing factors for fracture risk are also highly heritable, such as bone turnover rate and skeletal geometry, with a family history of osteoporosis being a significant risk factor for fragility fractures and osteoporosis in later life (Ralston and de Crombrugghe, 2006).

In terms of fracture risk and heritability, there are conflicting findings about level of risk. In studies that found a high heritability of fracture (Torgerson *et al.*, 1996, Deng *et al.*, 2000, Cummings *et al.*, 1995, Andrew *et al.*, 2005), it appeared that the primary determinants were non-skeletal: i.e., muscle mass, cognitive function, neuro muscular control, and vision, which are all genetically determined to varying degrees (Ralston and de Crombrugghe, 2006, Andrew *et al.*, 2005). In contrast to these findings, a study looking at elderly twins did not find any genetic links to fracture risk (Kannus *et al.*, 1999). However, environmental factors such as dietary intake, muscle mass, vitamin D status play an increasingly important role in bone health as age progresses, while the role of genetics decreases, which explains these conflicting findings (Michaëlsson *et al.*, 2005).

Very severe osteoporosis, or unusually high bone density, can be products of rare genetic conditions that are inherited. This can commonly occur by a gene mutation resulting in the modification of the WNT signalling pathway. WNT signalling has a pivotal role in bone homeostasis: there are several WNT pathways, with the predominant pathway involved in bone homeostasis being the WNT/β-catenin pathway, or canonical pathway (Cadigan and Peifer, 2009). When the WNT ligands bind to a receptor complex which includes lipoprotein receptor-related protein (LRP)5 or LRP6, the β-catenin 'destruction complex' is inactivated, so β-catenin accumulates in the cytoplasm before trans-locating to the nucleus. Here, along with transcription factors, it regulates target gene transcription (Baron and Kneissel, 2013). In addition to this pathway, the WNT-PTH crosstalk pathway and WNT-PCP (non-canonical)

pathways are involved (Liu *et al.*, 2007). Over ten years ago, mutations in four groups of people resulting in low or high bone mass were identified, with all mutations altering the canonical WNT pathway with subsequent significant results on bone mass (Baron and Kneissel, 2013). In two of the groups, the mutation occurred in the LRP5 gene that encodes for the WNT co-receptor low density lipoprotein receptor-related protein 5. One had a loss-of-function effect, resulting in low bone mass (Gong *et al.*, 2001), and the other had a gain-of-function effect, resulting in high bone mass (Boyden *et al.*, 2002, Little *et al.*, 2002). In the other two groups, the mutation affected the SOST gene, which encodes for the protein sclerostin, which inhibits the WNT pathway by binding the LRP5 receptor (Poole *et al.*, 2005, van Bezooijen *et al.*, 2004). Both of these groups had unusually high bone mass due to a lack of production of sclerostin (Balemans *et al.*, 2002, Brunkow *et al.*, 2001). These mutations result in abnormally high or low bone mass, rather than bone mass that is lower or higher, but not abnormally so, from the norm.

2.4 Factors Affecting Bone Health of Pacific Island Women Living in New Zealand

There are three ethno-geographic subgroups that Pacific Islanders may belong to depending on what country they, or their ancestors are from (figure 2). Melanesian, Micronesian, or Polynesian. Melanesia (from the Greek word melas, “black”, and nesos, “island”) encompasses New Guinea, Solomon Islands, Vanuatu, New Caledonia, Fiji and the Bismarck Archipelago. Micronesia (“small islands”) contains Kiribati, the Marshall Islands, Nauru, and the Caroline Islands, and spans westward covering the Northern Mariana Islands, Guan, and Palau. Polynesia (“many islands”) encompasses countries in the eastern Pacific, starting with the Hawaiian Islands in the north, extending to New Zealand in the southwest, and Easter Island to the east. Other nations in Polynesia are Tuvalu, Wallis and Futuna, Tokelau, Samoa, Tonga, Niue, the Cook Islands, and French Polynesia.

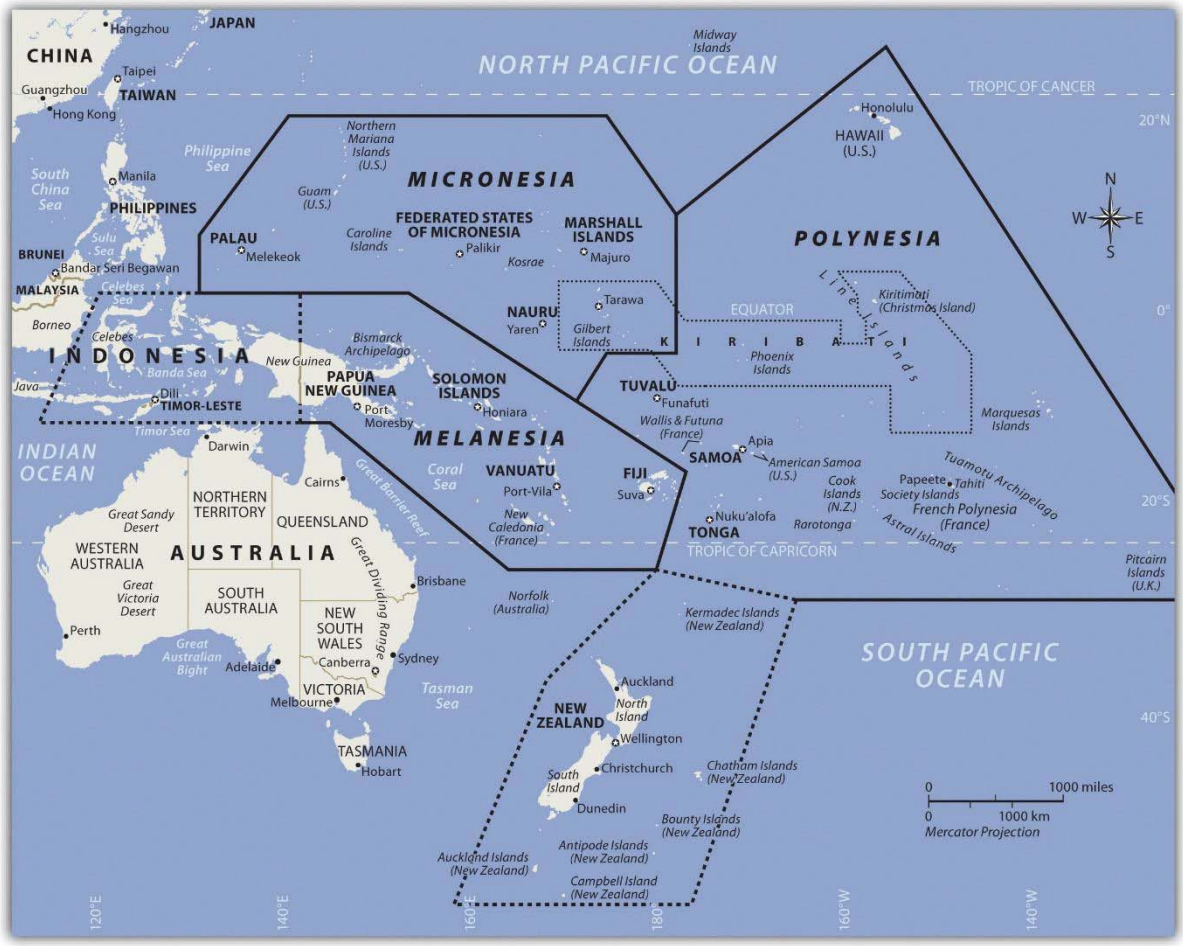


Figure 2.3: *Pacific Island geography*

Berglee, R. The Pacific and Antartica *Regional Geography of the World: Globalization, People, and Places* (Vol. 1): Saylor Academy Open Textbooks.

Intake of key nutrients affecting bone health

Calcium

A total of 92.3% of Pacific Island women living in New Zealand have an inadequate calcium intake (University of Otago and Ministry of Health, 2011). As stated earlier, the median intake of calcium by New Zealand women aged 15-71+ years is 745mg, well short of the RDI. Pacific Island women's calcium intake is lower still, with a median intake of 604mg (565-643) (University of Otago and Ministry of Health, 2011). The most common dietary sources of calcium for all women are milk (27%), bread and non-alcoholic drinks (both 10%), cheese (8%), and vegetables and dairy products (both 6%). There is relatively little data available on the typical dietary intake of Pacific Island women living in New Zealand; however there is data describing the typical intake of Pacific Island children at the age of four, which may reflect the

wider family's diet. The three most frequently consumed foods were bread (1.32 times per day), plain milk (0.86 time per day), then apples or pears (0.83 times per day) - yoghurt was the 14th most common food consumed, and other dairy products the 21st most common (Rush *et al.*, 2008). Milk intake was found to be associated with higher mass and BMI (Rush *et al.*, 2008), so any positive effects on bone health may come not only from the calcium in milk but the increased weight that is shown to result from a higher intake. Food security is an established issue for Pacific Island families, however 95% report they still buy milk when limited by money (Rush *et al.*, 2007).

Vitamin D

Pacific Island adults living in New Zealand are 2.3 times more likely to be deficient in vitamin D than non-Pacific Island adults, with 10% of Pacific Island adults having a vitamin D deficiency (Ministry of Health, 2012b). The National Adult Nutrition survey showed that there was a non-significant trend towards an increased risk of vitamin D deficiency in lower latitudes (central and southern regions) (Ministry of Health, 2012b). Pacific Island people most commonly live in urban areas, with 67% living in Auckland, 13% in Wellington, 4% in Waikato, and 4% in the South Island (Statistics New Zealand, 2007). This indicates that vitamin D deficiency is unlikely to be due to geographical reasons. Skin colour and weight are the two most probable factors for lower vitamin D status, with Pacific Island people tending to have darker skin tone, and a high prevalence of overweight and obesity, which will likely account for the increased risk of deficiency.

Protein

The average protein intake amongst adult Pacific Island women living in New Zealand is 16.9%, which is comparable to that of non-Pacific Island women (16.5%) (University of Otago and Ministry of Health, 2011). The Pacific Island Families study shows the most common types of protein consumed by Pacific Island children is milk, followed by chicken (0.57 times per day), eggs, and yoghurt or dairy food (0.49 times per day each). Other meats, poultry and fish are consumed less often (0.44 times per day) (Rush *et al.*, 2008). Although an intake of 16.9% of protein is at the lower end of the AMDR recommended by the Ministry of Health, it should be sufficient for maintaining good bone health.

Vitamin C

Vitamin C intake amongst Pacific Island women is on average 99mg per day (University of Otago and Ministry of Health, 2011), which is unsurprising given the wide range of fruit and

vegetables, especially traditional Pacific Island vegetables such as kumara and taro, that vitamin C is found in. Given that this is well above the RDI set by the Ministry of Health, vitamin C is not likely to account for any possible issues with bone health in Pacific Island women in New Zealand.

Table 2.2: *Key nutrient intake for bone health amongst Pacific Island women in New Zealand*

Nutrient	**Mean intake of Pacific Island women**	**Mean intake for all women**	**RDI**
Calcium	653 mg	784 mg	1000 mg
Vitamin A	671 µg RE	787 µg RE	700 µg RE
Protein	81 g	73 g	15 – 25% total energy
PUFA	10.3 g	10 g	
Iron	10.8 mg	9.9 mg	18 mg
Vitamin C	99 mg	106 mg	45 mg
Thiamin	1.3 mg	1.1 mg	1.1 mg
Riboflavin	1.6 mg	1.7 mg	1.1 mg
Niacin	33.4 mg	29.1 mg	14 mg
Vitamin B6	1.7 mg	1.6 mg	1.3 mg
Vitamin B12	4.1 µg	3.3 µg	2.4 µg

National Health and Medical Research Council. (2006). *Nutrient Reference Values for Australia and New Zealand Including Recommended Dietary Intakes*. Canberra: NHMRC

Physical activity prevalence of Pacific Island women living in New Zealand

It is important to consider the type of sport, given the importance of load bearing activity for bone health. Pacific Islanders are most likely to engage in sport for physical activity, specifically touch rugby, netball, volleyball, and rugby (Sport New Zealand, 2015). The most popular sport and recreation activities amongst Pacific Island people are listed in table 2.3

Table 2.3: *The 10 most popular sport and recreation activities for Pacific Islanders 2013-2014*

Activity	**%**
Walking	51.7
Jogging/Running	23.7
Equipment-based exercise	22.7
Swimming	20.4
Touch Rugby	17.7
Dance	17.4
Fishing	14.9
Netball	14
Volleyball	13.6
Rugby	13.5

Sport New Zealand. (2015). *Sport and Active Recreation in the Lives of New Zealand Adults. 2013/14 Active New Zealand Survey Results.* Wellington: Sport New Zealand.

There has been an increase in participation in sport amongst Pacific Islanders, with a 10% increase in sport and recreation over a seven day period between 2007/08 and 2013/14 (Sport New Zealand, 2015).

Body composition of Pacific Island women living in New Zealand

As previously discussed in this review, body composition is a complex but crucial predictor of BMD. The general consensus – although with some opposing views – is that lean muscle mass is the most significant predictor in pre-menopausal women, with fat mass the most significant in post-menopausal (Douchi *et al.*, 1997, Harris *et al.*, 1992, Glauber *et al.*, 1995, Liu *et al.*, 2011, Nur *et al.*, 2013). Total mass is also important, as greater mass results in a greater mechanical load on the skeleton.

In New Zealand, Pacific Island women are twice as likely to be obese compared to non-Pacific. When classified according to international BMI cut-off points, 13.7% fall within the normal range (18.5 – 24.9 kg/m^2), 26.5% in the overweight range (25-30 kg/m^2), and 59.5% in the obese range (≥30 kg/m^2) (Ministry of Health, 2014). However, while BMI may give a rough classification of size, it doesn't indicate actual body composition, as it uses total weight without considering to what extent that weight is contributed to by fat, or muscle. Due to muscle weighing considerably more than fat, a person with high muscle mass could easily fall into the overweight or obese categories – yet not have typical risk factors associated with

being overweight or obese. It is well known that Pacific Islanders typically have significantly greater muscle mass than European and Asian Indian groups (Rush *et al.*, 2009), and for this reason, BMI is not an appropriate predictor for bone density or mass.

BMI gives an indication of body fatness, by showing an indicator of weight adjusted for by height, and indeed BMI has been shown to be correlated with body fat. However, BMI doesn't distinguish between weight contributed to by muscle mass and weight from fat mass. Given the high proportion of muscle mass of Pacific Islanders, with a Pacific Islander with the same body fat% as a European typically having a higher BMI, the BMI is rendered less meaningful as a true measure of size (Rush *et al.*, 1997). BMI can be useful for providing an approximate estimation of general size of a population however. According to the international BMI cut-off points used by the Ministry of Health (table 2.4), almost two thirds of Pacific Island adults are considered to be obese. The Ministry of Health reports that Pacific Island adults have a higher BMI on average than European or Asian adults (Ministry of Health, 2015), however differences in muscle mass between ethnicities has not been considered. It is not helpful to compare different ethnic groups in this way, as there are many mitigating factors that makes different ethnicities unique, such as genetic influences, socio-economic status, and environmental factors. By comparing ethnicities, it suggests a need for one ethnic group, i.e. Pacific Islanders, to strive to be more like another, instead of simply looking within that group and examining how cultural, social, and environmental elements specific to that group can be approached.

Table 2.4: *International BMI Cut-Off Points*

Classification	BMI Score (kg/m^2)
Underweight	<18.5
Normal range	18.5-24.99
Overweight	25-29.99
Obese	≥30

World Health Organisation. (2015). BMI Classification. Retrieved 18/5/2015 from http://apps.who.int/bmi/index.jsp?introPage=intro_3.html

From this, it may be possible to extrapolate that muscle mass is a significant predictor of bone density and mass.

2.5 Bone Quality of Pacific Island Women Living in New Zealand and Australia

While anecdotally it is suggested that Pacific Island women have good bone density and mass, there is little data examining this. The general assumption of greater bone density could in part be attributed to the average body size of Pacific Island women: as discussed earlier in this

review total weight, as well as lean mass, has been shown to be significant determinants of bone density. Additionally, the fact that Pacific Island people have one of the lowest rates of non-traumatic hip fracture in the world, with the rate being approximately a quarter that of European women, also strongly indicates good bone density and mass (Cundy *et al.*, 1995). Further to this, pre- and post-menopausal Pacific Island women have a significantly higher BMC of the distal radius and ulna than European women (Reid *et al.*, 1986), however it is not clear if height and weight have been adjusted for, which may go some way towards explaining the difference. When BMI is adjusted for when examining the difference in bone mass in adults, significant differences still remain (Cundy *et al.*, 1995). Similarly to adults, Pacific Island children aged 3-7 years have a significantly greater bone density and mass compared to their age- and gender-matched European counterparts - however, this difference can be explained by greater height and weight, as when these factors are adjusted for, the differences in bone measures disappear (Grant *et al.*, 2005).

Studies involving Pacific Islanders were identified by searching the following databases: Web of Science, Scopus, PubMed, and Google Scholar. Keywords used were “BMD”, “bone mineral density”, “bone”, “Pacific Island”, “Pacific”. Six results were returned that related to bone mass and/or density in Pacific Islanders, and are summarised in table 2.5.

Table 2.5: *Summary of bone studies involving Pacific Islanders*

Author	Design	Purpose	Population	Methods	Main Findings
Reid et. al., 1986	Observational	To evaluate forearm BMC of Polynesian and European women in New Zealand.	Women aged 18-70 (50 Maori, 30 other Polynesian, 123 European).	The BMC of the distal radius and ulna in the non-dominant arm was measured using a Novo GT 35 osteodensitometer. Results of Maori and other Polynesian women were pooled due to no significant differences between the two. Exclusion criteria were major illness, glucocorticoid or hormone replacement therapy, and bilateral oophorectomy.	1. BMC was significantly higher in Polynesian women than European (p<0.0001). 2. The mean values were approximately 20% higher in Polynesians. 3. Bone density significantly declined with age in both races (p<0.0001).
Davis et. al., 1994	Observational	To compare the bone mass of Hawaiian, Filipino, Japanese, and white women living in Ohau, Hawaii.	Women aged 25-34 years (66 Hawaiian, 137 white, 144 Japanese, 74 Filipino).	Bone mass and density was measured at four sites: spine, calcaneus, and proximal and distal radius. Bone measures were compared between the four ethnicities, and body size was adjusted for.	1. The Hawaiian women had the greatest BMD, and Japanese the lowest at the spine and calcaneus. 2. Overall the Hawaiian women and the greatest BMC at all sites. 3. Most ethnic differences disappeared after adjusting for body size.
Cundy et. al., 1995	Observational	To assess the effect of adjusting for body size on interracial bone differences.	Pre-menopausal women (n=200) of Chinese, Indian, European, and Polynesian ethnicities (50 in each category).	The BMD of the lumbar spine and three femoral sites was measured using DXA, and compared across all four groups.	1. The Polynesian women had significantly greater BMD than the other three groups both before and after adjusting for body size (p<0.0001). 2. ANCOVA showed 10-40% of the difference in BMD may be explained

Author	Design	Purpose	Population	Methods	Main Findings
					by greater obesity in the Polynesian group, and the balance represents true interracial difference.
Norton et. al., 1995	Observational	To determine the incidence of hip fractures among those aged 60 years or older in the Auckland region.	All older people (≥60 years) hospitalised with a femoral neck fracture in Auckland between July 1991 and February 1994.	Participants were identified through ward registers and demographic data obtained. Fracture incidence was calculated using the number of people who had sustained femoral fractures as the numerator, and the 1991 census population estimate as the denominator. Individuals with fractures resulting from major trauma or who lived outside of the Auckland region were excluded.	1. Fracture incidence rates between Pacific Island men and women were very similar. 2. Pacific Island women had approximately a quarter of the fracture rates of European women. 3. Hip fracture rates amongst Maori and Pacific Islanders are some of the lowest internationally.
Grant et.al., 2000	Observational	To determine if there are differences in bone size and bone mineral in pre-pubertal Pacific Island and European children.	41 Pacific Island children aged 3-7, with 38 age- and gender-matched European children.	Body composition, bone size, and BMC were measured by DXA of the total body and non-dominant forearm.	1. Pacific Island children had significantly more BMC in the total body (12%), ultradistal radius (16%) and the 33% radius (8%). 2. BMD was higher at the ultradistal radius only (11%). 3. When adjusting for body weight, and in particular lean mass, there were no differences in bone measures, so it is suggested the differences in bone are due to greater weight and height of Pacific children.

Author	Design	Purpose	Population	Methods	Main Findings
Rush et. al., 2009	Cross-sectional	To investigate the relationships between body fatness and BMI, fat distribution, muscularity, bone mineral mass, leg length, and age-related changes in body composition between European, Pacific Island, Maori, and Asian Indian adults.	933 healthy adults: 454 male and 479 female, aged 17-80 years.	Body composition was measured using BMI, whole body composition was measured using DXA, and between-group differences were tested using a one-way ANOVA. ANCOVA was used to adjust body composition for comparison between ethnic groups.	1. There are marked ethnic differences in fat distribution, muscularity, bone mass, and leg length.

2.6 Summary

Bone health is contributed to by many different factors, with no one factor acting in isolation. Predominant factors are physical activity, body composition (total mass, lean mass, and fat mass all play roles), dietary factors (notably calcium, vitamin D, protein, and vitamin C), and genetics. The significance of these factors can vary according the menopausal status, ethnicity, and measures used (i.e. total body vs regional bone density). Although there is relatively little data examining the bone health of Pacific Island women, it is anecdotally accepted, and supported by the existing research, that Pacific Island women typically have excellent bone health. It is of scientific interest to investigate the possible reasons and main predictors of this, and find whether given the rise in many lifestyle-related diseases if this is one aspect of health that stands firm.

Chapter 3

Methods

3.1 Research Design

The Women's EXPLORE ("Examining Predictors Linking Obesity Related Elements" is a cross-sectional study investigating the implications for metabolic disease of different body composition profiles of post-menarcheal and pre-menopausal New Zealand European, Pacific Island, and Maori women. It also examines dietary and physical activity patterns as predictors for body composition profiles. The three different profiles explored are:

- Normal fat group: those with a BMI in the normal range (18.5-24.9 kg/m^2) and normal body fat (<30%)
- Hidden fat group: those with a BMI in the normal range, and a high body fat (≥30%)
- Apparent fat group: those with a high BMI (≥25 kg/m^2) and a high body fat (≥30%)

The current study is an observational investigation of predictors for BMD in Pacific Island women living in New Zealand. This analysis includes BMD data from DXA scans of the Pacific Island sub-group of the women's EXPLORE study, as well as physical activity data from a recent physical activity questionnaire, and anthropometric data from the BodPod. The BMD data, using standard BMD in grams per cm^2, is the dependent variable used in this research, with physical activity, body fat percentage, lean mass, total mass, and dietary calcium, protein, and vitamin C intake as the independent variables.

The EXPLORE study was not originally designed with bone analysis in mind, with DXA measurements to be used in the wider study to identify regional fat deposition. Nevertheless given that total body BMD and BMC data are available, the present study was born. Limitations resulting from this are detailed in the discussion section.

3.2 Ethical Approval

Ethical approval was gained for this study from the Massey University Human Ethics Committee (MUHEC): Southern A, Application 13/13. Additionally, written informed consent was gained from all participants prior to participating in the Women's EXPLORE Study.

3.3 EXPLORE Participants

The study participants are women aged 16-45 years, and were recruited in Auckland, New Zealand. Participants were recruited through local community and sports groups, universities, church groups, Kapa Haka groups, gala days such as PolyFest and Pasifika Festival, orientation days at educational institutes, sports games, local businesses, newspaper advertisements, magazine articles, and radio coverage. A total of 798 women were recruited for the EXPLORE study, 91 of whom were Pacific Islanders. Participants were included if they were aged between 16 and 45 years, were post-menarcheal or pre-menopausal (defined by continual regular menstruation for one complete past year), and identified as either Pacific Island ethnicity (with at least one parent identifying as Pacific Islander also). Participants were excluded in the presence of pregnancy & lactation, chronic illness (coronary heart disease, diabetes, cancer, gut disorders resulting in malabsorption, endocrine disorders, thyroid disease, kidney disease, liver disorders, and blood-borne illnesses such as hepatitis), low BMI (<18.5 kg/m^2), and dairy allergy.

3.4 Measurements

For the present study, the following measures were obtained: body composition (total mass, fat-free mass (FFM), body-fat %), physical activity, and dietary intake from a validated food frequency questionnaire (FFQ). Vitamin D was not included in this analysis.

3.5 Phases of Data Collection

Data collection was carried out in three phases: initial screening for eligibility, testing, and the at-home phase.

Phase 1: Screening

Participants were screened using a questionnaire and body composition analysis to assess for eligibility for inclusion into the study.

Phase 2: Testing

Participants visited Massey University's Human Nutrition Research Unit, where total body composition, BMD and content, and dietary intake were measured.

Phase 3: At home

Participants completed an RPAQ questionnaire one week after testing then either returned it to the Human Nutrition Research Unit by post, having been provided with a pre-paid addressed envelope, or completed the questionnaire online through Survey Monkey (www.surveymonkey.com).

3.6 Anthropometric Measurements

Phase 1: Screening

Participants were first provided with detailed information on the EXPLORE study, and were then given a consent form and screening questionnaire to assess suitability. If the inclusion criteria were met and the exclusion criteria not breached, height was recorded using a calibrated stadiometer, and weight, BMI and body fat percentage were obtained using a portable bioelectrical impedance analyser (BIA) (InBody 230, Biospace, Cerritos, CA). Participants were then divided into one of three body composition profile groups: NN (normal BMI, normal body fat %), NH (normal BMI, high body fat %), or HH (high BMI, high body fat %). Participants with a low BMI (<18.5 kg/m^2) were excluded. All eligible participants were invited to the second stage of testing.

Phase 2: Testing

Testing was carried out prior to 10am, and was timed to coincide with the first two weeks of each participant's menstrual cycle for the purposes of the wider EXPLORE study. Participants were asked to arrive fasted, with only having taken sips of water since 10pm the previous night. Upon arrival at the Human Nutrition Research Unit, the participants' name and date of birth was checked. The testing protocol was then explained, and a consent form was completed. A pre-testing questionnaire was filled out which gathered information on menstrual status, employment, smoking status, and alcohol consumption. Participants were asked if they had had anything to eat or drink since 10pm the previous night. If they answered yes, testing was re-scheduled for a different day.

Although height was recorded during the screening phase, it was recorded again as the first part of the testing phase, and it is this height that was used in the final data analysis. Participants were asked to remove their shoes and socks. Hair was checked to ensure it wasn't adding height, feet were positioned together with heels pressed against the stadiometer, and the participants jaw was lifted with both hands so the orbitale and ear canal were horizontally aligned. The participant was asked to inhale, and the measurement was recorded after

bringing the headboard down to the top of the skull. Three height measures were taken, then averaged and recorded to the nearest 0.1 cm

The waist and hip circumference was measured using a flexible steel tape measure (Lufkin W600PM, Apex Tool Group, NC, USA) using the cross-hand technique. For the waist circumference, the measure was obtained from the narrowest point between the iliac crest and lower costal border. The measure was recorded at the end of a normal exhalation. Hip circumference was measured at the widest point of the buttocks. Three measures were taken, with the average measurement recorded.

The participants' body composition was then measured using the BodPod system. This uses air-displacement plthysmography to measure body density by determining body volume and body weight, which are then used to calculate body fat percent.The BodPod was volume-calibrated using a 50 litre cylinder, and weight-calibrated using two 10 kg weights. Participants were asked to wear a tight fitting swimsuit or exercise clothing in which no air could be trapped. They also were asked to wear a swim cap, and empty their bladder. All jewellery was removed. Participants were then weighed using the BodPod scales, and the weight was entered into the BodPod computer. The participants' lung volume was then measured by expelling air from the lungs. The participant then needed to sit quietly for 50 seconds while the body volume procedure completed, with two measures being taken. Once this process was complete, the BodPod computer measured body density, and body fat percentage was then estimated using the Siri equation (Siri, 1961):

$$Body\ Fat = (495 / Body\ Density) - 450$$

Usually, only DXA measurements would be used in a study such as this, however a validation study shows that overall while the specific DXA and BodPod machines used in this study are in agreement with each other, individuals with very high or low body fat % are not as accurately measured by the DXA as the BodPod (von Hurst *et al.*, 2015). Given that the study population consisted of many participants with very high body weight, it was decided that BodPod measures would be used

The lean mass variable was calculated by subtracting fat mass (g) obtained from the BodPod and BMC (g) obtained from DXA from total mass obtained from BodPod:

Fat-free, bone-free lean mass = total mass from BodPod – (BMC from DXA + fat mass from BodPod)

This provided a measure of fat-free, bone-free lean mass, thus avoiding collinearity when looking for relationships between FFM, which includes bone, and BMD. For the purposes of this study, fat-free, bone-free lean mass is forthwith referred to as lean mass only (LMO).

3.7 Bone and Measurements

Total body BMD and content were measured using the DXA (Hologic Discovery A). The participants' date of birth, height, weight, gender and ethnicity were then entered into the DXA database, using the weight from the BodPod and height measured earlier. All participants were entered under the Caucasian database, regardless of whether they were Maori, Pacific Island or NZ European, as the only available database options were "white", "black", and "Hispanic". Participants were asked to change into a hospital gown to remove bias caused by artefacts in clothing, which may result in a falsely raised bone density. The participants then lay supine on the scan table, and they were checked to ensure that the centre line of the scan table aligned with the centre line of the participant to allow accurate cut locations when analysing the scan at a later date. Their head was positioned directly below the horizontal line that runs across the top of the scan table, and the entire body was checked to ensure the participant was within the scan lines on the table, with their arms slightly abducted from the trunk and palms resting downwards. In the case of participants who did not fit on the table, two scans were taken: one for the left side, and one for the right side. Participants were instructed to remain completely still throughout the scan, and if there was any body movement, the scan was aborted and re-started.

3.8 Physical Activity Measurements

In the third phase of testing, participants were asked to complete a regular physical activity questionnaire (RPAQ), shown in Appendix A. The data in this was then used by the research team to complete current bone-specific physical activity questionnaires (cBPAQ) (Appendix B) on behalf of each participant, which uses an algorithm to assess loading on the skeleton from physical activity (Appendix C).

3.9 Dietary Analysis

Participants completed an online validated 220-item FFQ (Appendix D) (Houston, 2014). Instructions were provided to the participants at the start of the FFQ, along with two example questions, which the participants worked through with a member of the EXPLORE research team, as per the standard operating procedure (SOP) (Appendix E). A research assistant checked and entered the participant ID number into the online form, and was available for questions throughout the process. When reporting their food intake, participants were asked to only consider the previous month. Dietary data was collected from July 2013 to March 2015, which minimised any possible discrepancy between seasonal food availability and dietary intake.

3.10 Data Handling

A total of 91 Pacific Island women were included in the final analysis. Of these, eight participants had no DXA data due to DXA machine malfunction, and so excluded from the analysis. Dietary data was analysed using FoodWorks 7 (NZ FOODfiles 2010), then the Goldberg equation using a physical activity level (PAL) of 1.55 was applied to identify under- and over-reporting. A total of 18 participants over-reported their dietary intake, and eight under-reported their intake. All 26 under- and over-reporters were excluded from analyses involving nutrients. The PAL was chosen due to overall very sedentary physical activity reported in the RPAQ. The over- and under-reporter were excluded from analyses looking at associations between dietary intake and BMD, but included in all other analyses.

3.11 Statistical Analyses

Statistical analyses were completed using SPSS (v.20, IBM Corporation, New York, USA). All variables were tested for normality using the Shapiro-Wilk and Kolmogorov-Smirnov tests. Non-normal data was tested for homogeneity using the Levene's test. If the data was shown to have a significant variance between groups using the Levene's test, it was log-transformed, and tested for normality again. Normally distributed data was reported as the mean ± standard deviation (SD), and non-normally distributed data was reported as the median [25^{th}, 75^{th} percentiles]. Significance was set at P=<0.05. Correlations were calculated using the Pearson's test for normal data and Spearman's Rho for non-normal data. A hierarchical block-wise multiple regression analysis was used to assess the ability of the independent variables to predict BMD (g/cm^2), with the independent variable with the strongest correlation entered

into the first block, and the variable with the weaker correlation in the second block. Only independent variables that showed a significant correlation with BMD were used in the regression analysis.

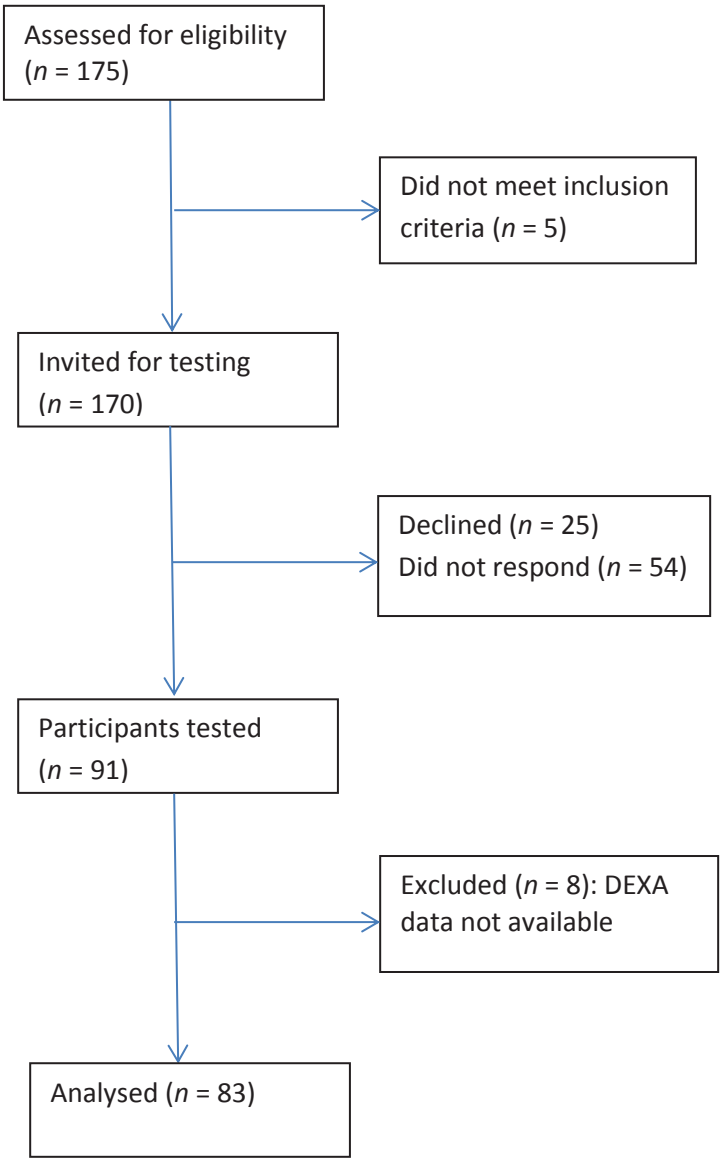
Chapter 4

Results

4.1 Study Participants

The present study is concerned with exploring total body mass, fat mass, muscle mass, physical activity, calcium, protein, and Vitamin C intake as predictors of BMD. A total of 175 Pacific Island women were recruited for the Women's EXPLORE Study, 5 did not meet screening criteria, 25 declined testing, and 54 did not respond to invitations for testing (figure 4.1). A total of 91 women took part in the testing phase. Of these, 8 were unable to undergo a DEXA scan due to equipment malfunction, 24 did not return a RPAQ for assessment of physical activity, and 28 either under or over-reported their dietary intake in the FFQ (11 under-reported, 16 over-reported as determined by the Goldberg equation, shown in Appendix F). A total of 43 participants accurately completed all steps of the testing phase that are investigated in the present study. Where data was missing that was an independent variable (i.e. dietary data or physical activity data), they were still included in any analyses that did not involve the missing variable.

Figure 4.1: *Participant recruitment*



4.2 Participant Demographics

For phase two of testing, 91 pre-menopausal Pacific Island women presented at the Massey University Nutrition Research Unit, where 83 women underwent a DEXA scan. The characteristics of the 83 participants who completed all testing procedures and included in the final analysis are shown in table 4.1.

Table 4.1: *Summary of **s**tudy population characteristics*

Parameters	***Participants (n=83)***
Age, years	28 (21, 37)*
Height (cm)	167.4 ± 5.8†
Weight (kg)	90.4 ± 19†
BMI, kg/m^2	32.4 ± 6.8†
Waist Circumference (cm)	92.3 ± 14.6†
Hip Circumference (cm)	115.9 ± 12.7†
Waist – Hip Ratio	.79 ± .07†
Body fat %	38.4 ± 7.6†
Fat-free, bone-free lean mass (kg)	52.4 ± 6.9†
Total Body BMD (g/cm^2)	1.1 ± 0.08†

† Mean ± SD, * Median (25th percentile, 75th percentile).

4.3 Bone Mineral Measures

In the present study, bone health is represented by a BMD (g/cm^2). All bone mineral measures are displayed in table 4.2

Table 4.2: *Bone mineral measurements for pre-menopausal Pacific Island women*

Bone Measurements	**Participants (*n*=83)**
TB-BMD (g/cm^2)	1.1 ± 0.08
TB-BMC (g)	2420 ± 300

Values are reported as mean ± standard deviation
Abbreviations: TB-BMD – total body bone mineral density; TB-BMC – total body bone mineral content

4.4 Body Composition Measures

As mentioned in the methods, the BodPod was used for body composition analysis due to a validation study showing that individuals with very high or low body fat % are not as accurately measured on the DXA as on the BodPod (von Hurst *et al.*, 2015). The participants had an average body weight of 90.4 ± 19kg, which indicated that the BodPod would be best used for body composition measures. A comparison of the DXA and BodPod body composition findings is shown in table 4.3.

Table 4.3: *Comparison between DXA and BodPod for body composition measures*

Parameter	Participants (*n*=83)			
	DXA	**BodPod**	**R***	**P value**
Total mass (kg)	91.5 ± 19.5	90.4 ± 19	.97	<.05
Body fat (kg)	33.1 ± 11.1	36.2 ± 14.5	.98	<.001
Body fat %	35.4 ± 5.2	38.4 ± 7.6	.85	<.001
Fat free mass (kg)	58.4 ± 9.6	54.8 ± 7.1	.92	<.001
Fat free bone free mass (kg)	55.9 ± 9.4	52.4 ± 6.9	.92	<.001

*Pearson's correlation coefficient

4.5 Physical Activity Measures

There were 67 RPAQs returned. These were transcribed into the BPAQ online interface (www.fithdysign.com/BPAQ/) to obtain the cBPAQ score for each participant. The cBPAQ scores were not normally distributed, with the curve skewed to the left (figure 4.2). The median score was 1.7 (0.4, 5.2). This score represents a scale, rather than a quantitative measure: the BPAQ focusses specifically on physical activity that is load bearing and has the capacity to affect load bearing, and as such this score has been shown to correlate with BMD (Weeks and Beck, 2008)).

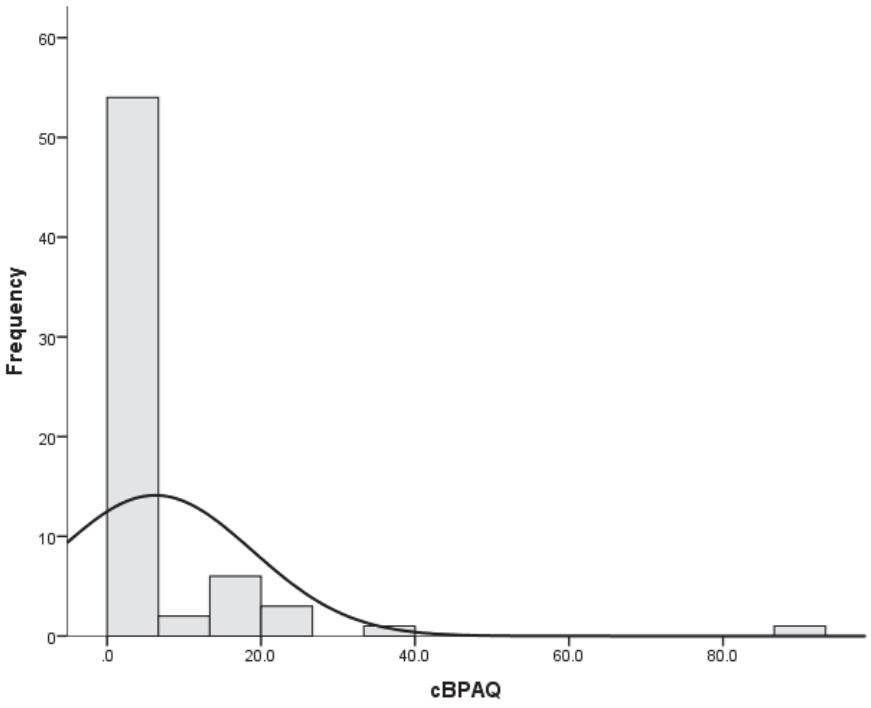


Figure 4.2: *cBPAQ score distribution with normality curve* (Kolmogorov-Smirnov normality test: *p*<.0001; Shapiro-Wilk normality test: *p*<.0001)

4.6 Dietary Analysis

A dietary analysis was obtained from the FFQs, and the Goldberg equation for under- and over-reporting was applied as described previously in the methods. Nutrient intake is shown in table 4.4, with an emphasis on bone-specific nutrients. A total of 90 participants returned FFQ data. Of these, 8 were identified as under-reporting energy intake based on their ratio of energy intake to basal metabolic rate being below the cut-off value of 1.49 (which is based on a lightly active PAL of 1.55), and 18 over-reported. This is shown in figure 4.3. The median daily energy intake was 2589 calories (1962, 3219). When determining the PAL cut off to be used in the equation, a conservative level of 1.55 was used based on the overall very low level of physical activity reported as shown in the previous section (figure 4.2). A complete list of energy intake along with calculations is shown in Appendix F.

Table 4.4: *Intake of key nutrients*

Nutrient	Study population intake (per day)	New Zealand-wide intake (adult Pacific Island women)[1]	Recommended intake[2]
Energy (kJ)	9334 (7210, 11821)*	8318	BMR x PAL
Carbohydrate (% of total energy)	43 ± 8.2[†]	47	45-65% of total energy
Fat (% of total energy)	35 ± 7[†]	35.2	20-35% of total energy
Protein (% of total energy)	18 ± 3.8 [†]	16.2	15-25% of total energy
Protein (total g)	108.5	81	0.8-1g per kg of mass
Calcium	1016 ± 442 mg[†]	653 mg	14-18 yrs: 1300 mg/day 19-50 yrs: 1000 mg/day
Vitamin C	125 (94, 216) mg*	99 mg	14-18 yrs: 40 mg/day 19-50 yrs: 45 mg/day
Phosphorous (mg)	1697 ± 602[†]	*No data available*	1000 mg/day (RDI)
Magnesium (mg)	405 ± 146[†]	*No data available*	320 mg/day (RDI)
Zinc (mg)	13.6 ± 4.7[†]	11.5	8 mg/day (RDI)
Copper (mg)	1.57 (1.1, 1.8)*	*No data available*	1.2 mg/day (EAR)
Potassium (mg)	3702 (3163, 4893)*	2764	2800 mg/day (AI)

[†] Mean ± SD, * Median (25th percentile, 75th percentile).

1. University of Otago, & Ministry of Health. (2011). *A focus on Nutrition: Key findings of the 2008/09 New Zealand Adult Nutrition Survey*. Wellington: Ministry of Health.

2. National Health and Medical Research Council. (2006). *Nutrient Reference Values for Australia and New Zealand Including Recommended Dietary Intakes*. Canberra: NHMRC

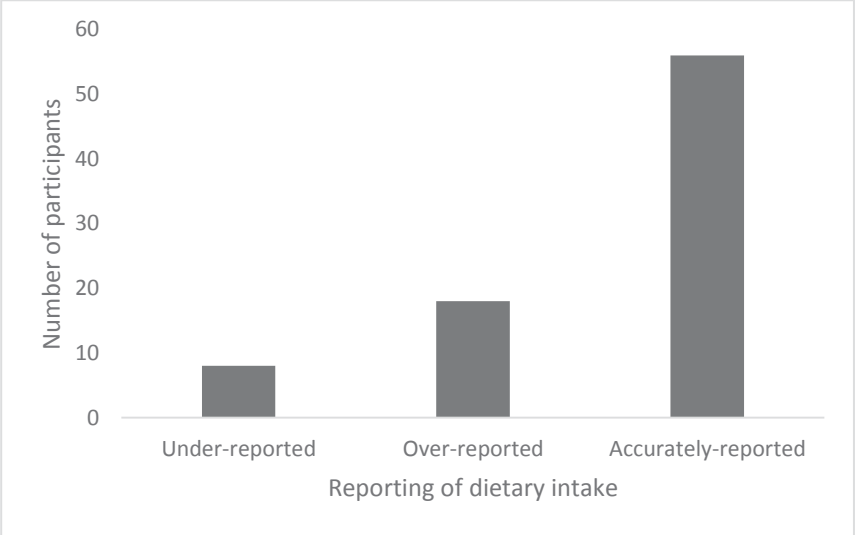


Figure 4.3. *Reporting of energy intake calculated using the Goldberg Equation*
Under reported=9.7%; over-reported=22%; correctly reported=68.2%.

Three key nutrients were chosen for use in the present study: calcium, protein, and vitamin C. The RDI of each of these nutrients was met, with participants reporting an average of 1075 ± 60 mg of calcium per day, a median daily intake of 131 (95, 222) mg of vitamin C, and a protein intake of 17.6% of their total energy intake.

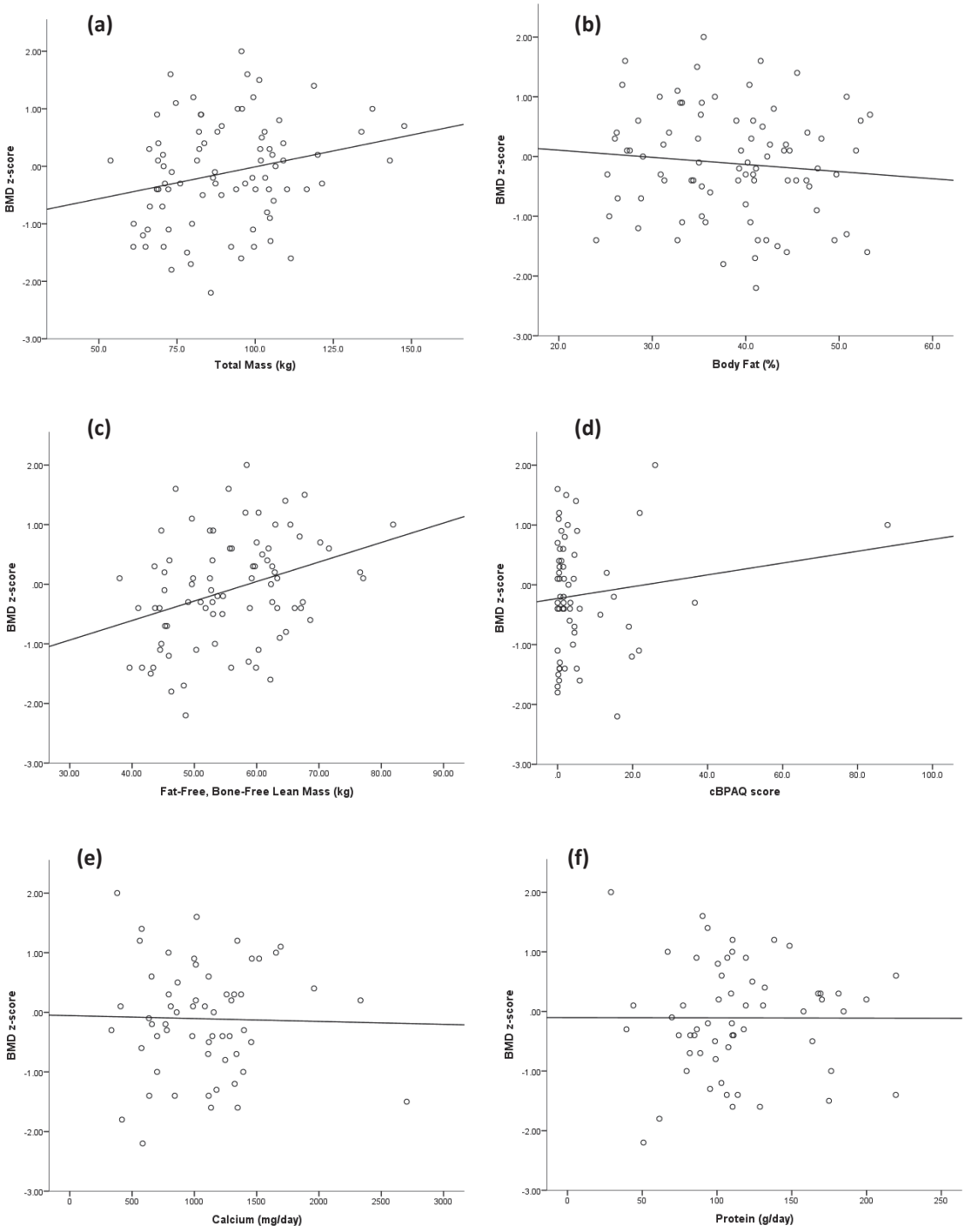
4.7 Associations between BMD and Predictor Variables

Correlation co-efficients were calculated to look for significant associations between the BMD and the factors measured in the present study that are well established as key predictors for BMD, which are:

1. Total mass
2. Lean mass
3. Body fat (kg)
4. Physical activity as measured by a cBPAQ score
5. Calcium intake
6. Protein intake
7. Vitamin C intake

Spearman's Rho was used for data that was not normally distributed (cBPAQ), and Pearson's correlation co-efficient was used for the normally distributed data (all other factors listed above). Total mass and lean mass were the only two factors to show a significant correlation

with BMD: r=.49, *n*=83, *p*<0.001 and r=.61, *n*=83, *p*<0.001 respectively (two-tailed). The scatter plots are shown in figure 4.4.



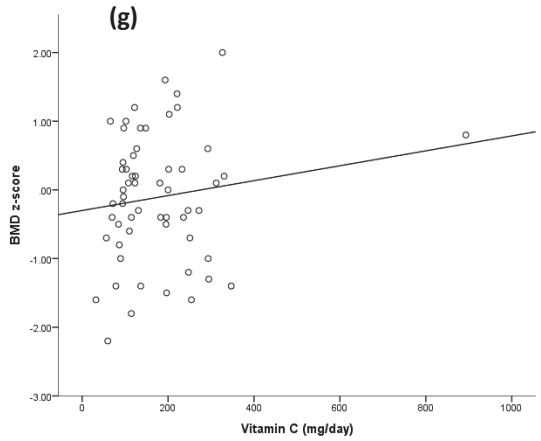


Figure 4.4: *Correlations between BMD (g/cm^2) and independent variables*. **(a)** BMD z-score versus total mass (R^2=0.06, n=83, *p*<.05). **(b)** BMD z-score versus body fat % (R^2=-0.1, n=83, *p*>.05). **(c)** BMD z-score versus fat-free, bone-free lean mass (R^2=0.12, n=83, *p*<.01). **(d)** BMD z-score versus cBAQ (R^2=0.02, n=60, *p*>.05). **(e)** BMD z-score versus calcium intake (R^2=0, n=58, *p*>.05). **(f)** BMD z-score versus protein intake (R^2=0, n=58, *p*>.05). **(g)** BMD z-score versus vitamin C intake (R^2=0.02, n=58, *p*>.05). Abbreviations: BMD=bone mineral density; cBPAQ=current bone-specific physical activity questionnaire.

There was one outlier that was identified in the vitamin C intake. The outlier for vitamin C may be explained by over reporting dietary intake (the participant didn't report a supplement). This was removed, and the correlation calculated again. There was no change in the result (figure 4.5). The results are tabulated in table 4.5.

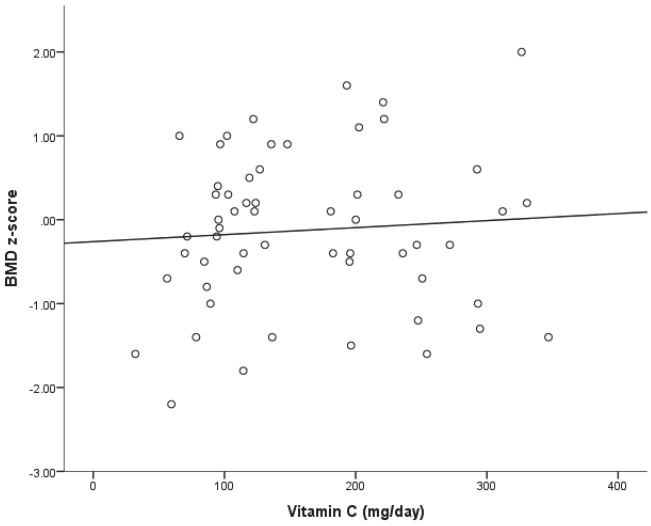


Figure 4.5: *Vitamin C intake with outlier removed* (R^2=0.02, n=58, *p*>.05)

Table 4.5: *Correlation of predictor variables with BMD*

Variables	*r*	*n*	*p*
Total mass*	.26	83	<.05**
Lean mass (kg)*	.37	83	<.01**
Body fat (kg)*	.12	83	>.05
cBPAQ†	.07	83	>.05
Calcium*	-.04	59	>.05
Protein*	.09	59	>.05
Vitamin C*	-.01	59	>.05

All tests were two-tailed. *Pearson's correlation; †Spearman's Rho correlation
**Significant

A hierarchical multiple linear regression model was used to determine how much of the variance in BMD was explained by lean mass and total mass. These predictors were selected as they showed a significant correlation with the dependent variable.

Prior to conducting the analysis, the relevant assumptions were tested. A sample size of 81 was considered sufficient given that three independent variables were included in the analysis (Tabachnick and Fidell, 2001). The assumptions for testing regression hypotheses have been met: the dependent variable (BMD) is normally distributed using the Kolmogorov-Smirnov and Shapiro-Wilk normality tests (p>0.05); the dependent variable is interval data; all residuals are independent (Durbin-Watson statistic = 1.6); the distribution of the residuals is normal when tested using the Kolmogorov-Smirnov and Shapiro-Wilk normality tests (p>0.05); the variance of the distribution of the BMD values shows homoscedasticity (figure 4.6); there is collinearity within the data: the tolerance value is greater than 0.1 (LMO = .14; total mass = .14), and the VIF value for each independent variable falls below 10 (LMO = 7.24; total mass = 7.26) (Field, 2009). It is suggested that a tolerance value below 0.2 indicates a potential problem with bias however (Menard, 1995), so this may indicate some bias given the above tolerance values: however, they are greater than the threshold of 0.1 or less that indicates a serious problem (Field, 2009). There was a correlation between LMO and total mass (R^2 = .68, n=83, p<.01), however as the collinearity statistics are within the accepted limits (Field, 2009), the assumption for no multicollinearity is deemed to have been met. This indicates that this model is generalisable to the population.

A two block hierarchical multiple regression analysis was conducted using BMD as the dependent variable. Lean mass was used in the first block, as it had the strongest correlation

with BMD, and total mass was entered in the second block. Table 4.6 summarises the regression analysis results.

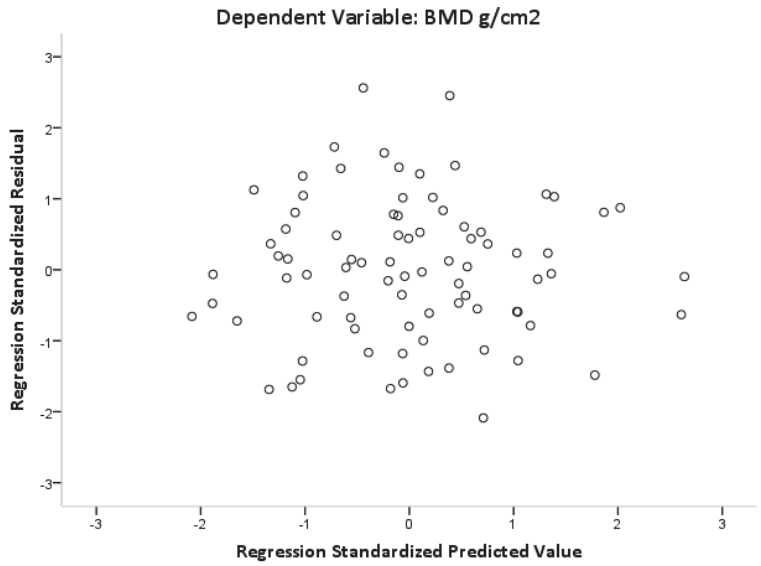


Figure 4.6: *Homoscedasticity of BMD distribution variance*

Table 4.6: *Hierarchical multiple regression (n=81)*

	B	***SE B***	**95% CI *B***	**Standardised β**	**R**	**R^2**	**ΔR^2**	***p***
Model 1					**.366**	**.134***	**12.5**	**.001**
Intercept	.922	.051	.821, 1.02					
LMO	.003	.001	.001, .005	.366				
Model 2					**.424**	**.159****	**4.4**	**.000**
Intercept	.872	.055	.764, 981					
LMO	.007	.002	.003, .012	.895				
Total mass	-.002	.001	-.004, .000	-.571				

Hierarchical block-wise enter technique. *F (1,82) = 12.5; **F (2,82) = 8.77
Abbreviations: LMO: lean mass only (fat-free mass g – BMC g)

The multiple regression analysis shows that lean mass significantly contributes to the model: *F* (1, 82) = 12.5, *p*< .01, and accounts for 13.4% of the variation in BMD. Introduction of the total mass variable explains a further 2.5% of the variation, with the change in R^2 being significant: *F* (2, 82) = 8.77, *p*<.001. Lean mass was the most important predictor for BMD, with the ability to uniquely explain 13.4% of the variance. Together, lean mass and total mass explain 15.9% of the variability in BMD.

Chapter 5

Discussion

The purpose of this study was to explore associations of key nutrients, physical activity, total mass, LMO, and body fat percentage with BMD in order to identify key predictors of bone health in Pacific Island women living in Auckland, New Zealand.

Maintaining good health is a vitally important factor in remaining independent in later years, and mobility is crucial for this. Bone loss leading to osteoporotic fractures severely impacts an older adult's ability to remain independent, and thus their quality of life: as such, osteoporosis is a serious health condition with potentially significant impact. It is estimated that 50% of women will incur an osteoporotic fracture during their lifetime, with the most common fractures being hip, spine, and forearm (Kanis *et al.*, 2001). The most catastrophic of these are hip fractures, which result in significant loss of mobility (Taylor *et al.*, 2004, Pasco *et al.*, 2005) with an estimated 50% of sufferers requiring long term care (Osteoporosis Australia, 2001). Compounding this is an increased likelihood of early death, with 25% of people who have sustained a hip fracture dying within 12 months of the injury occurring (Kanis *et al.*, 2003). The risk of osteoporosis can be managed with lifestyle choices from childhood, and while genetic factors play an important role, lifestyle choices can make the difference between healthy bones in old age and osteoporosis. Data looking at the contribution of different factors that affect bone health in Pacific Island women is relatively scarce. The aim of this study was to explore the associations between key predictors of bone health with bone density in New Zealand-based Pacific Island women. The objectives of this study were to firstly measure BMD, then analyse the body composition, physical activity, and key bone-specific nutrient intake of participants, then to investigate the relationships between these factors and BMD.

5.1 Summary of Outcomes

The main outcome of this study was that LMO and total mass were the only significant predictors of BMD of Pacific Island women. There was no association between dietary calcium, protein, and vitamin C, physical activity, or body fat %.

5.2 Participant Characteristics

Healthy, non-pregnant pre-menopausal women aged between 16 and 45 were recruited for this study. A total of 91 women were recruited, with a median age of 28 years (21, 37). They were of Pacific Island ethnicity.

5.3 Bone Mineral Measures

For this study, total-body BMD (g/cm^2) was used as a representation of bone health. Z-scores are often used in studies examining the bone health of pre-menopausal women. Z-scores are a measure that compares like with like: in this case, the bone density of age- and gender-matched individuals. It gives an indication of a particular score's relationship to the mean of that age-, race- and gender-matched group, with a score of 0 being the same as the mean group score. Z-scores can be positive or negative, with the value indicating the number of SDs the score falls either above or below the mean. When diagnosing osteoporosis in post-menopausal women, T-scores of the lumbar spine, femoral hip, or total hip, are used with reference to the World Health Organisation (WHO)-defined cut-off points (table 2.1). However, it is not recommend that T-scores be used in people who are under the age of 50: rather, Z-scores should be used (The Writing Group for the ISCD Position Development Conference, 2004). Additionally, the T-score classification is only applicable in measurements of the spine, hip, and forearm – not total body as is used in this study. Primary osteoporosis in post-menopausal women is defined according the T-score cut-off points set by the WHO (NIH Osteoporosis and Related Bone Diseases National Resource Centre, 2012). There is currently no agreed definition of osteoporosis in pre-menopausal women, however the International Society for Clinical Densitometry recommends that a Z-score of less than -2.0 defines having bone density that is "below the expected range for age" (Leib, 2004). A Z-score of -2 or less is likely to occur in 2.5% of pre-menopausal women (Lewiecki, 2005). A low PBM as defined by a Z-score of -2 or less is an established risk-factor for osteoporosis in later life (Matkovic *et al.*, 1990, Gordon *et al.*, 1991, Lloyd *et al.*, 1992, Matkovic *et al.*, 1994). For diagnosis of osteoporosis in pre-menopausal women, a non-traumatic fracture, and/or densitromic measures can be used, however routine screening of bone density in healthy pre-menopausal women is not recommended, as there is a scarcity of prospective studies examining fracture risk in relation to BMD by DXA (Cohen and Shane, 2013). However, due to the fact there is not a "Pacific Island" database available, using the Z-score as a variable in this study would not be a truly accurate representation of the bone health of the study group, thus it was decided that BMD would better serve the purposes of identifying the strongest predictors of bone health.

In the present study, subjects were age-matched and gender-matched. However, due to lack of data on the BMD of Pacific Islanders, the only ethnic groups available were Caucasian, Hispanic, and African. It was decided that the Caucasian category would be used as the reference population. This presented an opportunity to see how Pacific Island women fare compared to Caucasian women in lieu of other data being available at the present time. Regardless of what bone mineral indices are measured (i.e. BMC, BMD, or Z-score), the distribution of scores was approximately normal. In the Caucasian reference database, approximately 70% have a BMD Z-Score that falls within 1 SD of the age-matched, gender-matched, and ethnicity-matched mean, with 95% falling within 2 SDs. A further 15% have a BMD that is greater than 1 SD below the mean, and 2.5% more than 2 SD below the mean (Kanis *et al.*, 1997). This is summarised and compared to the study group in table 5.1. Although these reference points are established using T-Scores, Z-scores in pre-menopausal women are typically either identical or extremely similar (Lewiecki, 2005).

Table 5.1: *Percentage of European and Pacific Island Women in different Z-Score categories*

Reference Points	Reference Group (Caucasian)[1]	Study Group (Pacific Island)
Within 1 SDs of the mean	70%	59%
Within 2 SDs of the mean	95%	89%
Greater than 1 SDs below the mean	15%	17.6%
Greater than 2 SDs below the mean	2.5%	1.1%
Greater than 2.5 SDs below the mean	0.6%	0%

Abbreviations: SDs=standard deviations

1. Kanis, J., Delmas, P., Burckhardt, P., Cooper, C., & Torgerson, D. (1997). Guidelines for diagnosis and management of osteoporosis. *Osteoporosis International, 7*(4), 390-406.

This shows that compared to the standard Caucasian reference database, fewer Pacific Island women had low bone mas: however, this would benefit from a more in depth analysis of the raw data, which would provide insight into the statistical significance of this.

The participants in the present study can be compared to findings in an earlier study, in which the BMC of 96 Pacific Island women with a mean age of 42 ± 14 years was measured (table 5.2). This shows that the present study group has a lower BMC than the group from the other study.

The BMI of the other study group was normally distributed however, while the BMI of the present study wasn't, so there are some differences in body composition between the two groups which may explain the difference in BMC.

Table 5.2: *Comparison of findings between present study and study by Rush et. al.*

Variables	Present Study	Rush et. al[1]
Age (years)	28 (21, 37)*	42 ± 14†
BMI (kg/m^2)	31.4 (26.9, 36.9)*	33.1 ± 6.3†
BMC (kg)	2.424 ± 0.296	2.665 ± 0.403†

*Median (25th, 75th percentiles); †Mean ± SD

1. Rush, E. C., Freitas, I., & Plank, L. D. (2009). Body size, body composition and fat distribution: comparative analysis of European, Maori, Pacific Island and Asian Indian adults. *British Journal of Nutrition, 102*(04), 632-641.

For this study, total body BMD was calculated. Regional sites, most commonly lumbar spine and femoral hip, are often used - particularly when assessing the bone health of post-menopausal women. These are clinically relevant sites as the hip and spine are where the majority of fragility fractures are sustained, and additionally such fractures can have the greatest impact on mobility, independence, and mortality. Many methods of assessing bone density, including total body, are not volumetric (mass divided by a volume), but rather areal. There is potential for this missing volume value to cause different bone sizes to be a confounding factor, and it has been suggested that this could be reduced by dividing the areal BMD by the height (Reid *et al.*, 1992b). However, this adjustment was not applied to the present study.

In terms of site-specific versus total body scans, total body scans afford the opportunity to gain other important data relating to body composition. The aim of this study was to identify predictors of BMD in pre-menopausal Pacific Island women, which is not a group typically associated with risk of osteoporosis or fragility fractures. As such, is not necessary to conduct site-specific bone scans. Furthermore, given the strong correlations established between total body and site-specific scans (Franck and Munz, 2000, Hammami *et al.*, 2001, Hangartner *et al.*, 2000, Lu *et al.*, 1994, Nysom *et al.*, 1998), total body BMD is an appropriate approach for the present study.

5.4 Body Composition Measures

As discussed in the literature review, it is probable that body composition plays an important role in bone health, with evidence for lean mass and fat mass, as well as total mass.

The mean weight was 90.4kg ± 19, and median BMI 32.4 kg/m^2 ± 6.8. The overall range of BMI scores are comparable to New Zealand-wide BMI scores of Pacific Island women, and are shown in table 5.3.

Table 5.3: *Comparison of BMI scores between study group and NZ-wide Pacific Island women*

BMI Range (kg/m^2)	**Study group (%)**	**New Zealand-wide (%)[1]**
18.5-24.9	13.2	13.7
25-29.9	28.6	26.5
≥30	58.2	59.5

1. Ministry of Health. (2014). *Annual Update of Key Results 2013/14: New Zealand Health Survey*. Wellington: Ministry of Health.

As described in the results, the BodPod has a high level of agreement with the DXA except for when an individual has a very high or very low body fat %. The mean body fat % was 38.4% ± 7.6. While there is currently no consensus as to what constitutes an appropriate threshold for defining a high body fat % (Oliveros *et al.*, 2014), in 1995 the WHO published a report in which it defined obesity in women as ≥35% body fat (World Health Organization, 1995). However since then, the American Society of Bariatric Physicians (ASBP), who are an American Medical Association specialty board, published guidelines of ≥30% for women, which is used in most studies examining adiposity (Okorodudu *et al.*, 2010). Based on this, the average body fat % of 38.4% ± 7.6 found in the study population can reasonably considered to be high, therefore justifying the use of the BodPod for body composition measures.

There was a positive correlation between total weight and BMD, however the relationship was not as strong as expected. Other observational studies generally show a much stronger relationship between total weight and bone density (Finkelstein *et al.*, 2013, Felson *et al.*, 1993, Edelstein and Barrettconnor, 1993). There was a much stronger relationship between lean mass and BMD z-score however. This is consistent with other studies, in which there is a general consensus that lean mass is a significant predictor of total-body BMD in pre-menopausal women (Douchi *et al.*, 1997, Ijuin *et al.*, 2002).

It is well known that Pacific Island people are typically very muscular as compared to most other races. One study looking at the differences in body size and composition showed that Pacific Island women were significantly (*P*<.0001) more muscular than European, Maori, and Asian women, with a mean FFM of 46.4kg and appendicular skeletal muscle mass (ASMM) of 18.3kg (Rush *et al.*, 2009). The mean FFM of the current study group is 54.8 ± 7.1 kg, even

higher than that of the other study. This is interesting, as the reported physical activity was skewed to the left (see figure 4.2 in previous chapter), and high muscle mass that is implied from a high FFM is typically associated with greater physical activity. It is possible that this apparent discrepancy is due to a combination of genetic traits and the fact that everyday activity would have greater intensity for somebody who is carrying a high body weight. It could also be a limitation of how physical activity was assessed in this study: as mentioned earlier the cBPAQ focuses specifically on loading, and incidental activity, as well as history, could have a significant bearing on FFM.

A limitation of how the present study was analysed was that ASMM may have been a more appropriate variable to use than the LMO variable. ASMM is calculated by subtracting the total limb mass, minus the sum of wet bone mass (BMC divided by 0.55) and limb fat, a model which is thought appropriate as it is assumed that the weight of dermal tissue is negligible compared to the skeletal muscle (Heymsfield *et al.*, 1990). This is a practical method that could have been utilised for the present study with relative ease as the necessary data for the calculations are available on the DXA scans of each participant, and as such would have been a more appropriate choice.

5.5 Physical Activity Measures

Physical activity is an important factor when considering bone health, as it not only increases lean and total mass which has a beneficial effect on bone, it places the bones under mechanical force which has a direct osteogenic affect through the WNT/b-Catenin signalling pathway.

Participants were asked to complete an RPAQ, and a total of 67 RPAQs were returned. The RPAQ assesses physical activity in four domains over the previous month: leisure, work, commuting, and home. There has been a validity study amongst participants from 10 European countries, which show that the RPAQ can be confidently used in large-scale epidemiological studies (Golubic *et al.*, 2014). A systematic review shows the RPAQ has acceptable reliability with an intra-class correlation coefficient of 0.76, but just moderate validity at best (Helmerhorst *et al.*, 2012). Overall, the RPAQ is a valid way of assessing adult's self-reported physical activity (Besson *et al.*, 2010). However, for the purposes of this study, it was determined that the BPAQ would be more appropriate. This is a recently developed questionnaire that assesses physical activity that creates mechanical loading on bone that

results in an osteogenic effect. The RPAQ, while a valid and reliable way of assessing overall physical activity, doesn't specifically account for mechanical loading on bone.

Overall, the cBPAQ score for the majority of participants was extremely low, indicating little load-bearing physical activity. This is weakly reflected in the Sport and Recreation New Zealand (SPARC) Physical Activity and Sport survey from 2003 (SPARC, 2003), which shows that Pacific Island adults are less active than non-Pacific. However, it does show that 58% of Pacific Island women are considered to be active, which seems to be significantly more than the cohort in the present study. There are several possible reasons for the low physical activity reported by the women in this study: busyness due to family and/or church commitments; lack of culturally-specific resources and support; and lack of awareness of the importance of physical activity.

Being the primary caregiver of children in a family is arguably one of the most time-consuming roles a person can do. The participants in this study were all of child-bearing age: that is, post-menarcheal and pre-menopausal. Pacific Islanders typically have larger than average families: Pacific Island women have the highest birth rate of all ethnicities, with 11.4 completed pregnancies per 100 women of a reproductive age, as compared to the total for all ethnicities together of 7 completed pregnancies per 100 women (Ministry of Health, 2012a). Furthermore, there is an average of 4.4 people in a Pacific Island household, as compared to 2.7 for all of New Zealand (Tukuitonga, 2012b), which is due not just to higher birth rates but increased likelihood of extended family living in the same household (Commission, 2004). The median birth age for Pacific Island women is 27.7 years (Ministry of Health, 2012a, Tukuitonga, 2012a), which is almost identical to the median age of the study population of 28 years. In Pacific Island families, the traditional role of the mother is often to stay home to care for the children (Griffen, 2006). The amount of time required to care for large families can greatly impact the amount of free time the mother may have for sport and recreational activities, which in turn can result in low reporting of physical activity. However, the incidental activity that results from caring for children cannot be discounted. Such activity is not accounted for in the BPAQ, yet it likely contributes a significant amount of activity. Family size, completed pregnancies, and the number of people living in the household were not investigated as part of the present study, which is a limitation: this would have allowed better assessment of incidental physical activity related to family care activities.

Church involvement is another factor that can require a lot of time and commitment, which could further reduce time available for physical activity. In the Pacific Islands, church plays a central role, around which families and community life is built, with the church minister a powerful and respected person. In New Zealand, as much as 97% of Pacific Island groups have an association with a Christian religion (Statistics New Zealand, 2007), and 76% of Pacific Islanders regularly attending church (Dewes *et al.*, 2013). With church and family commitments, it is possible that there would be little time left for engaging in sport and recreational activity. However, it has been suggested that Pacific Island children who attend church are more physically active than Pacific Island non-attendees, as evidenced by rates of sedentary behaviours, such as watching TV or playing video games (Dewes *et al.*, 2013). Whether this translates to adults is unclear.

Awareness of the importance of physical activity may also be a factor, along with perceived associated cost. Physical activity is well promoted in New Zealand, but usually in conjunction with specific goals such as reducing diabetes risk and weight loss. It is possible that people may not think the exercise recommendations necessarily apply to them. Additionally, the potential perceived cost of exercise may be a barrier, such as the cost of gym memberships or team fees for sports.

An important limitation to consider of the use of the BPAQ in this study was that only the current bone-specific physical activity (cBPAQ) was able to be used, as historical data was not available from the RPAQ questionnaires. Bone mass attained in childhood and adolescence is one of the most crucial determinants of bone mass throughout the lifespan. Had historical data been obtained, the past bone-specific physical activity questionnaire (pBPAQ) score and total bone-specific physical activity questionnaire (tBPAQ) score could have been utilised in the present analysis, which would provide a much more robust picture of the determinants of bone health in Pacific Island women. This is further affirmed in a study of the correlations between pBPAQ and cBPAQ scores, and densitometry measures of bone, which showed that the cBPAQ predicts bone health at the hip, spine, and total body for healthy young men but not women, but the pBPAQ predicts bone strength at the heel for healthy young women (Weeks and Beck, 2008). Additionally, there was only a moderate association between the current and past scores of the BPAQ ($r=0.33$, $p=.04$), which suggests that low physical activity in adulthood does not necessarily mean low physical activity in childhood and adolescence – which as mentioned in the literature review is a key determinant of life-long bone health.

Indeed, it is feasible that this limitation could explain the left-skewed distribution of physical activity and subsequent lack of correlation with BMD Z-score: a pBPAQ may well have revealed greater physical activity in childhood and adolescence which would help explain the generally excellent BMD of Pacific Island women in this study. Backing this up is data from the SPARC Physical Activity and Sport Survey 2003, which shows a trend of decreasing physical activity with increasing age during childhood (table 5.4). This also shows that physical activity briefly increases between the ages of 18 and 20, before sharply dropping again between 25 and 34. This may reflect childbearing years, and more time committed to being spent at home caring for families.

Table 5.4: *Changing levels of physical activity with age in Pacific Islanders*

Age Groups	Inactive %	Active %
5-8 yrs	52	48
9-12 yrs	45	55
13-15 yrs	44	56
16-17 yrs	48	52
18-24 yrs	25	75
25-34 yrs	49	51
35-49 yrs	38	62

SPARC. (2003). *SPARC Facts: Results of the New Zealand Sport and Physical Activity Surveys (1997-2001).* Wellington: SPARC.

Another limitation is that by comparing the cBPAQ scores with a New Zealand-wide report on physical activity, like is not being compared with like. As mentioned previously, the BPAQ does focus specifically on loading activity, and doesn't take into consideration the duration of exercise – just the frequency. It also doesn't consider incidental exercise, which is a well-established way to reach the recommended 30 minutes a day of activity: indeed, it is entirely possible that this population may be reaching or exceeding 30 minutes a day of activity, but this is not quantified in the BPAQ.

5.6 Dietary Measures

Dietary intake makes up another piece of the puzzle of good bone health, with a wide range of nutrients having an effect on bone health. For this study, it was determined that calcium, protein, and vitamin C were the three nutrients of greatest importance to include in the analysis. Dietary calcium is typically thought to be one of the most important factors in the attainment of good bone health (Shea *et al.*, 2002, Tang *et al.*, 2007, Winzenberg *et al.*, 2006). Protein is another critical nutrient in bone health – although the nature of its role has been

contentious, is emerging as a positive factor (Bonjour, 2005, Rizzoli *et al.*, 2007, Sahni *et al.*, 2014). This is backed up by other research and sources: adequate dietary protein helps obtain and preserve lean muscle mass (Cooper *et al.*, 1996, Rizzoli and Bonjour, 2004), and is a significant determinant in the level of PBM achieved by pre-menopausal white women (Cooper *et al.*, 1996). Although the present study was conducted amongst Pacific Island women, the proposed mechanism by which protein promotes bone mass could likely still be applied (i.e. attainment and preservation of lean mass). Lastly, vitamin C was included due to its important role in collagen synthesis, which makes up a large proportion of the bone matrix. It is also likely that oxidative stress contributes to increasing fragility of the bone matrix, which dietary vitamin C may help combat (Wauquier *et al.*, 2009, Sugiura *et al.*, 2011).

Participants completed a validated FFQ to assess their intake of calcium, protein, and vitamin C, with a total of 91 returned. An FFQ is a cost-effective and efficient tool for assessing dietary intake in large populations. Previously, food records were commonly used to gather dietary data, with the participants recording their food and fluid intake for a set period of time: however both the participant burden and data handling overhead associated with this is significant (Willett, 2013). As such, a validated FFQ is a cost- and time-effective solution for a study such as this (Willett, 2013, R, 2005).

Validity of dietary data relies upon it meeting the basic premise that energy in should equate to energy out. One of the exclusion criteria of this study was that the participants were not losing weight, thus this premise can be applied to the present cohort. To check for over- and under-reporting prior to data analysis, the Goldberg equation was applied to the dietary data. The PAL used to calculate the cut-off points that determined under and over reporting was 1.55: this was decided upon due to the physical activity data showing overall low physical activity for the participants. This resulted in 25 of the 91 FFQs were eliminated due to either under- or over-reporting of data. Possible reasons for over- and under-reporting are social desirability bias, or lack of concentration/comparison with others. FFQs have been shown to be susceptible to social desirability bias (Smith *et al.*, 1991, Miller *et al.*, 2008), which may explain the nine under-reporters. However there were many more over-reporters (18). When a person is confronted with a long list of food items within a particular food category, actual intake tends to be over-estimated (Willett, 2013). However, while the raw FFQ data for the present study had an adjustment factor applied to it, it was only for the fruit and vegetable sections, as this has what has been shown to be most frequently over-reported in other

studies (Cade *et al.*, 2002). Another possible reason for the over-reporting in this particular group was that participants completed the FFQs on computers that were in the same room, and despite being instructed to complete the FFQ individually as set out in the standard operation procedure (SOP), due to many of the participants being recruited from Church groups, they knew each other well, and there was a lot of discussion and joking which was challenging to prevent. It is probable that the discussion and joking that was taking place provided sufficient distraction from the task at hand to result in the over-reporting of dietary intake. In future, this might be overcome by having computers in separate rooms, or having the participants complete the FFQ one at a time.

With the validated FFQ used for this study (Houston, 2014), raw FFQ data was multiplied by an adjustment factor for the fruit and vegetable sections, which then allowed the dietary data to be analysed as raw and adjusted data. When compared to a weighed four day food record, the FFQ had good relative validity, with minimal difference to the findings following fruit and vegetable adjustment (Houston, 2014).

Overall, participants reported adequate intakes of calcium, protein and vitamin C, which is discrepant with the most recent 2008/09 New Zealand Adult Nutrition Survey results (table 5.5). However, the reported intake meets the recommended intake set by the Ministry of Health (National Health and Medical Research Council, 2006).

Table 5.5: *Intake of key nutrients*

Nutrient	**Study population intake (per day)**	**New Zealand-wide intake (adult Pacific Island women)[1]**	**Recommended intake[2]**
Calcium	1075 ± 60 mg	653 mg	14-18 yrs: 1300 mg/day 19-50 yrs: 1000 mg/day
Protein	17.6% of energy	16.9% of energy	15-25% of energy
Protein (g/kg)	1.2 g/kg of mass	*Not available*	0.8-1 g/kg of mass
Vitamin C	131 (95, 222) mg	99 mg	14-18 yrs: 40 mg/day 19-50 yrs: 45 mg/day

1. University of Otago, & Ministry of Health. (2011). *A focus on Nutrition: Key findings of the 2008/09 New Zealand Adult Nutrition Survey*. Wellington: Ministry of Health.
2. National Health and Medical Research Council. (2006). *Nutrient Reference Values for Australia and New Zealand Including Recommended Dietary Intakes*. Canberra: NHMRC

A key nutrient that should ideally have been included in this study is vitamin D. Vitamin D enhances the absorption of calcium and another important nutrient in bone health,

phosphorous (National Health and Medical Research Council, 2006). Vitamin D is obtained from sunlight exposure and dietary sources. It is not known how much vitamin D is obtained from sunlight exposure; however it is undoubtedly the most important source (Glerup *et al.*, 2000, Holick, 1996, Rasmussen *et al.*, 2000). Indeed, it is probable that in certain situations, all of the required vitamin D can be provided by sun exposure alone. However, as noted in the literature review, there are many factors that determine the amount of cutaneous vitamin D synthesis that occurs in response to sunlight. Dark skin, latitude, time of year, dress, and time spent outdoors are all important. Where cutaneous production may be lower than usual, such as during winter, dietary sources become more important. Given these factors, assessing true exposure to vitamin D production in a study such as this (i.e. observational) is a challenging prospect. This is supported by a review of the literature that examines the validity of sunlight exposure questionnaires, which shows that such measures provide inexact estimates of vitamin D status (McCarty, 2008). A potential solution is to assess vitamin D status through a blood test; however this method is also susceptible to weaknesses. Serum 25-OH-D is a volatile measure that is prone to change: just two sunny days may make a significant difference. Participants would need to be all tested on the same day, and several tests over the space of a year – for example, one test each season, to obtain an average would also be needed for an accurate result. Unfortunately, such rigorous testing was not possible under the scope of this study. Although there is dietary vitamin D data available, it was determined that due to the negligible input this has into overall vitamin D status, it was not a suitable for use as part of the present study. With that said, there is evidence showing high rates of Vitamin D deficiency in dark-skinned Australian migrant groups (Grover and Morley, 2001), in which case dietary vitamin D may be considered an important aspect to consider. In New Zealand, rates of vitamin D deficiency among Pacific Island adults are 10%, and they are 2.3 times as likely to be vitamin D deficient as non-Pacific Island adults (Ministry of Health, 2012b). Not including vitamin D status or sunlight exposure is a weakness of this study, and in future research, vitamin D should be included in order to provide a more robust analysis of the contributing factors to bone health.

There were no associations between dietary calcium, protein, or vitamin C, and Z-score. This is at odds with other research, which consistently shows a strong relationship between these nutrients and BMD in both pre- and post-menopausal women of varying ethnicities, and in looking at both total body and site-specific BMD. Taking into consideration that there is such a large unexplained proportion in the variation of BMD, this reinforces the role of genetics in

determining BMD (Ralston and de Crombrugghe, 2006). This is further reinforced when considering that the 2008/09 New Zealand Adult Nutrition Survey indicates that Pacific Island women are having well below the daily recommended intake of calcium (Ministry of Health, 2014), yet all previous studies investigating bone in Pacific Islanders report general excellent bone health with the lowest rates of osteoporosis in the world (Rush *et al.*, 2009, Reid *et al.*, 1986, Norton *et al.*, 1995, Davis *et al.*, 1994, Cundy *et al.*, 1995).

Chapter 6

Conclusion

6.1 Summary

A wide-ranging review of the literature around factors contributing to bone health was conducted, along with a review of existing research on the bone health of Pacific Island women. It was found that diet, physical activity, and body composition all play complex and inter-linking roles in determining BMD. These factors must all be considered together as they have the ability to not only affect bone directly, but indirectly through effects on each other, such as the effect of diet and physical activity on fat mass. Additionally, how much one particular factor affects bone can also vary depending on the skeletal site of measurement (site-specific vs. total body), which indices are used (BMC, BMD, Z-score, T-score, height adjusted or not), menopausal status, and race.

To date, there has been six studies investigating bone content or density of Pacific Islanders, which consistently show high BMD or BMC compared to other races. For the present study, BMD was assessed using a total body scan, body composition was analysed in the DXA and BodPod, and dietary intake was taken from a validated FFQ. The participants in this study had high body mass, body fat, and LMO, with low rates of physical activity, and adequate intake of calcium, protein, and vitamin C. LMO and total mass has a significant positive associated with BMD, with LMO having the ability to uniquely explain 13.4% of the variance in BMD, and total mass explaining 2.5%. This leaves 84.1% of the variance unexplained, but it is likely due largely to genetic influences.

6.2 General Study Limitations

Although specific limitations relating to the gathering of body composition data, physical activity measures, and dietary intake have been detailed in the discussion, there was some general limitations around recruitment and participation.

This was a somewhat challenging group to recruit, and we found it was necessary to go through a person of authority in a church group. However due to social structures, there were times when it became apparent some participants were taking part out a sense of duty, which may have compromised the quality of self-reported data that was obtained. Due to the large

scope of the wider study, it was also necessary for participants to travel to the Nutrition Research Unit and Massey University's Albany campus, located on Auckland's North Shore. This is a considerable distance from South Auckland, where the majority of Pacific Island people live, and were thus recruited from. Many of the potential participants cited distance and travel time as barriers for taking part. In future, research utilising portable equipment may encourage greater participant recruitment and retention.

6.3 Future Applications and Research

There is currently a large focus around reduction of obesity rates. Awareness of the importance of LMO in determining BMD in pre-menopausal Pacific Island women could be useful when designing weight loss interventions or healthy lifestyle programs: a focus around good sources of protein, with a protein intake at the higher end of the AMDR, and increasing physical activity would help maintain muscle mass through any weight-loss efforts.

Accurate assessment of physical activity and dietary intake using more robust methods of assessment to establish if there truly is no association between these and BMD would be beneficial to undertake as a future research project. Future research could also examine patterns of physical activity and diet throughout childhood and adolescence leading to when PBM is achieved in order to assess their contribution to bone growth in Pacific Islanders, and to look at BMD in post-menopausal Pacific Island women to ascertain if high bone mass persists beyond the menopause.

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Appendices

Appendix A: *Recent Physical Activity Questionnaire (RPAQ)*

EXPLORE Recent Physical Activity Questionnaire (RPAQ)

1. Introduction

This questionnaire is designed to find out about your physical activity in your everyday life in the past 4 weeks.

The questionnaire is divided into 3 sections.

Please answer every question.

Section A asks about your physical activity patterns in and around the house.
Section B is about travel to work and your activity at work.
Section C asks about recreations that you may have engaged in during the last 4 weeks.

Your answers will be treated as strictly confidential and will be used only for the Massey University Women's EXPLORE study.

***1. Please enter your ID number (this is on the front of your Physical Acitivity Diary).**

***2. Please enter your name.**

***3. Please enter your date of birth.**

	DD	MM	YYYY
Date/Month/Year	/	/	

2. Section A - Home Activities

Getting about

***1. Which form of transport have you MOST OFTEN used in the last 4 weeks APART from your journey to and from work?**

- ◯ Car/motor vehicle
- ◯ Walk
- ◯ Public transport
- ◯ Cycle

TV, DVD or Video Viewing

***2. On average over the last 4 weeks, how many hours per day have you watched TV, DVD or video?**

	None	Less than 1 hour per day	1 to 2 hours per day	2 to 3 hours per day	3 to 4 hours per day	More than 4 hours per day
On a weekday before 6pm	◯	◯	◯	◯	◯	◯
On a weekday after 6pm	◯	◯	◯	◯	◯	◯
On a weekend day before 6pm	◯	◯	◯	◯	◯	◯
On a weekend day after 6pm	◯	◯	◯	◯	◯	◯

Computer use at HOME but NOT AT WORK (e.g. Internet, email, Playstation, Xbox, Gameboy, etc)

EXPLORE Recent Physical Activity Questionnaire (RPAQ)

***3. On average over the last 4 weeks, how many hours per day have you used a computer at home?**

	None	Less than 1 hour per day	1 to 2 hours per day	2 to 3 hours per day	3 to 4 hours per day	More than 4 hours per day
On a weekday before 6pm	◯	◯	◯	◯	◯	◯
On a weekday after 6pm	◯	◯	◯	◯	◯	◯
On a weekend day before 6pm	◯	◯	◯	◯	◯	◯
On a weekend day after 6pm	◯	◯	◯	◯	◯	◯

Stair Climbing at home

***4. On average over the last 4 weeks, how many times each day have you climbed up a flight of stairs (approx. 10 steps) at home?**

	None	1 to 5 times per day	6 to 10 times per day	11 to 15 times per day	16 to 20 times per day	More than 20 times per day
On a weekday	◯	◯	◯	◯	◯	◯
On a weekend day	◯	◯	◯	◯	◯	◯

3. Section B - Activity at Work

Please answer this section to describe if you have been in paid employment at any time during the last 4 weeks or you have done regular, organised voluntary work.
If you answer 'no' - you will move onto the recreation section.

***1. Have you been in employment during the past 4 weeks?**

◯ Yes

◯ No

4. Section B - Activity at Work (continued)

***1. During the last 4 weeks, how many TOTAL hours of work did you do per week (excluding travel)?**

4 weeks ago _____

3 weeks ago _____

2 weeks ago _____

1 week ago _____

Type of work

We would like to know the type and amount of physical activity involved in your work.

EXPLORE Recent Physical Activity Questionnaire (RPAQ)

***2. Please choose the option that BEST corresponds with your occupation(s) in the last 4 weeks from the following four possiblities:**

◯ Sedentary occupation: you spend most of your time sitting (such as in an office)

◯ Standing occupation: you spend most of your time standing or walking. However, your work does not require intense physical effort. (e.g. shop assistant, hairdresser, guard)

◯ Manual work: this involves some physical effort including handling heavy objects and use of tools (e.g. plumber, electrician, carpenter)

◯ Heavy manual work: this implies very vigorous physical activity including handling of very heavy objects (e.g. dock worker, miner, bricklayer, construction worker)

Travel to and from work in the last 4 weeks

***3. What is the approximate distance from your home to your work in kilometres? (e.g. 10.5)**

***4. How many times a week did you travel from home to your main work? (Count outward journeys only)**

***5. How did you normally travel to work?**

	Always	Usually	Occassionally	Never or rarely
By car/motor vehicle	◯	◯	◯	◯
By works or public transport	◯	◯	◯	◯
By bicycle	◯	◯	◯	◯
Walking	◯	◯	◯	◯

6. Please enter the postcode for your main place of work during the last 4 weeks. If you don't know your post code please go to question 7, otherwise skip to question 8.

7. If you didn't know your work post code, please enter your work address.

***8. What is the postcode of your home address?**

5. Section C - Recreation

The following questions ask about how you spent your leisure time.

Please indicate how often you did each activity on average over the last 4 weeks.

Please indicate the average length of time that you spent doing the activity on each occasion.

EXPLORE Recent Physical Activity Questionnaire (RPAQ)

1. Please indicate the NUMBER OF TIMES you did each activity in the past 4 weeks and the AVERAGE LENGTH OF TIME you spent doing that activity on each of those occasions.

Note: We want to know the AVERAGE TIME you spent doing the activity on EACH OCCASION, not the total time per week, e.g., if you did weeding for 1.5 hours twice a week, you would select '2 to 3 times per week' for the number of times, then '1' for hours and '30' for minutes.

Please answer for EVERY activity. If you DID NOT do an activity, choose 'None' for the number of times.
If you did an activity which doesn't fit into any of those listed below, please list them under the "Other" options and state what they were.

	Number of times in last 4 weeks	Average time per session (hours)	(minutes)
Swimming competitively			
Swimming leisurely			
Hiking or mountain climbing			
Walking for pleasure (not as a means of transport)			
Racing or rough terrain cycling			
Cycling for pleasure (not as a means of transport)			
Mowing the lawn			
Watering the lawn or garden			
Digging, shovelling or chopping wood			
Weeding or pruning			
DIY e.g. carpentry, home or car maintenance			
High impact aerobics or step aerobics			
Other types of aerobics			
Exercise with weights			
Conditioning exercises e.g. using an exercise bike or rowing machine			
Floor exercises e.g. stretching, bending, keep fit or yoga			
Dancing e.g. kapa haka, walata-a-ringa, lakalaka, hip hop			
Competitive running			
Jogging			
Bowling - indoor, lawn or 10 pin			
Tennis or badminton			
Squash			
Table tennis			
Golf			

EXPLORE Recent Physical Activity Questionnaire (RPAQ)

Football, rugby, hockey, touch			
Cricket or softball/baseball			
Rowing			
Netball, volleyball or basketball			
Fishing			
Horse-riding			
Snooker, billiards or darts			
Musical instrument playing or singing			
Ice skating			
Sailing, wind-surfing or boating			
Martial arts, boxing or wrestling			
Other 1			
Other 2			
Other 3			
Other 4			
Other 5			

2. If you selected "Other" activities above please state what each one was.

Other 1	
Other 2	
Other 3	
Other 4	
Other 5	

Appendix B: *Bone-Specific Physical Activity Questionnaire (BPAQ)*

Bone-Specific Physical Activity Questionnaire (BPAQ)

SUBJECT ID:	DATE:

1. **Please list <u>any</u> sports or other physical activities you have participated in regularly. Please <u>tick</u> the boxes to indicate how old you were for each sport/activity and how many years you participated for.**

Activities / Age:	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30

Activities / Age:	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60

Activities / Age:	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90

BONE-SPECIFIC PHYSICAL ACTIVITY QUESTIONNAIRE
Developed by B.K. Weeks and B.R. Beck
Griffith University, QLD, Australia

Bone-Specific Physical Activity Questionnaire (BPAQ)

SUBJECT ID:	DATE:

2. **Please list the sports or other physical activities (be as specific as possible) you participated in regularly during the <u>last 12 months</u> and indicate the average frequency (sessions per week)?**

Activity: _____ Frequency (per week): _____

Activity: _____ Frequency (per week): _____

Activity: _____ Frequency (per week): _____

Activity: _____ Frequency (per week): _____

Activity: _____ Frequency (per week): _____

Activity: _____ Frequency (per week): _____

Activity: _____ Frequency (per week): _____

Activity: _____ Frequency (per week): _____

BONE-SPECIFIC PHYSICAL ACTIVITY QUESTIONNAIRE
Developed by B.K. Weeks and B.R. Beck
Griffith University, QLD, Australia

Appendix C: *Current BPAQ (cBPAQ) algorithm*

cBPAQ algorithm (Weeks and Beck, 2008):

cBPAQ = [R + 0.2R(n-1)] x a

R = effective load stimulus (derived from ground force reaction testing)

n = frequency of participation (per week)

a = age weighting factor
(age weightings: <10 yrs = 1.2; 10-15 yrs = 1.5; 15-35 yrs = 1.1; >35 yrs = 1.0)

Appendix D: *Food Frequency Questionnaire*

EXPLORE Food Frequency Questionnaire

1. Please read carefully before you begin:

Please make sure when filling out this questionnaire that you:

- Tell us what YOU usually eat (not someone else in your household!).
- Fill in the form YOURSELF.
- Are correct, but don't spend too much time on each food.
- Answer EVERY question; the asterisk symbol (*) at the beginning of each question means that you must answer before moving onto the next question.

This will help us to get the most accurate information about your usual food intake.

Please answer by ticking the box which best describes HOW OFTEN you ate or drank a particular food or drink in the LAST MONTH and HOW MUCH you would usually have.

For example:

1. EXAMPLE: How often do you usually have sugar? (Please do not fill out)

	Never	<1x / month	1-3x / month	1x / week	2-3x / week	4-6x / week	Once / day	2-3x / day	4+ x / day
Sugar - 1 tsp	◯	◯	◯	◯	◯	◯	◯	◯	◯

If every day you have 2 cups of coffee with 1 tsp sugar, 4 cups of tea with 1 tsp sugar, one bowl of cereal with 1 tsp sugar and sugar on pancakes at dinner, you would choose four or more times per day = '4+ x / day'.

Adjust your portion size and frequency of intake to suit your eating habits.

2. EXAMPLE: How often do you usually eat bread? (Please do not fill out)

	Never	<1x / month	1-3x / month	1x / week	2-3x / week	4-6x / week	Once / day	2-3x / day	4+ x / day
Bread - 1 slice	◯	◯	◯	◯	◯	◯	◯	◯	◯

If every day you have two slices of toast for breakfast, and you have a sandwich for lunch three times per week, you would choose two - three times per day = '2-3x / day'.

Adjust your portion size and frequency of intake to suit your eating habits.

EXPLORE Food Frequency Questionnaire

2. EXPLORE Study Food Frequency Questionnaire

*1. Please enter your study ID (if you are unsure or don't know please ask the researcher)

EXPLORE Food Frequency Questionnaire

3. Eating Pattern

***1. How would you describe your eating pattern? (Please choose one only)**

- ◯ Eat a variety of all foods, including animal products
- ◯ Eat eggs, dairy products, fish and chicken but avoid other meats
- ◯ Eat eggs, dairy products and fish, but avoid chicken and other red meats
- ◯ Eat eggs and dairy products, but avoid all meats, chicken and fish
- ◯ Eat eggs, but avoid dairy products, all meats and fish
- ◯ Eat dairy products, but avoid eggs, all meats and fish
- ◯ Eat no animal products
- ◯ None of the above

Other (please state)

EXPLORE Food Frequency Questionnaire

4. Dairy

***1. Do you use milk? (e.g. fresh, UHT, powdered)**

- ◯ Yes
- ◯ No

2. What type(s) of milk do you have most often? (You can choose up to 3 options, but please only choose the ones you usually have)

- ☐ Not applicable
- ☐ Full cream milk (purple top)
- ☐ Standard milk (blue top)
- ☐ Skim milk (light blue top)
- ☐ Trim milk (green top)
- ☐ Super trim milk (light green top)
- ☐ Calcium enriched milk (yellow top) e.g. Xtra, Calci-Trim
- ☐ Calcium and vitamin enriched milk e.g. Mega, Anlene
- ☐ Calcium and protein enriched milk e.g. Sun Latte
- ☐ Standard soy milk (blue)
- ☐ Light soy milk (light blue)
- ☐ Calcium enriched soy milk (purple) e.g. Calci-Forte, Calci-Plus
- ☐ Calcium, vitamin and omega 3 enriched soy milk e.g. Essential
- ☐ Calcium and high fibre enriched soy milk e.g. Calci-Plus High Fibre
- ☐ Rice milk

Other (please state)

***3. On average, how many servings of milk do you have per day? (Please choose one only)**
(A 'serving' = 250 mL or 1 cup/glass)
e.g. 5 cups of coffee/tea using 50 mL of milk + ½ cup of milk on cereal = 1 ½ servings per day

- ◯ Not applicable
- ◯ Less than 1 serving
- ◯ 1-2 servings
- ◯ 3-4 servings
- ◯ 5 or more servings

EXPLORE Food Frequency Questionnaire

*4. How often do you usually have milk?

	Never	<1x / month	1-3x / month	1x / week	2-3x / week	4-6x / week	Once / day	2-3x / day	4+ x / day
Flavoured milk (milkshake, iced coffee, Primo, Nesquik) - 250 mL/ 1 cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
Milk as a drink - 250 mL / 1 cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
Milk on breakfast cereals or porridge - 125 mL/ 1/2 cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
Milk added to water-based hot drinks (coffee, tea) - 50 mL / 1/5 cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
Milk-based hot drinks (Latte, Milo) - 250 mL / 1 cup	◯	◯	◯	◯	◯	◯	◯	◯	◯

*5. How often do you usually eat cheese?

	Never	<1x / month	1-3x / month	1x / week	2-3x / week	4-6x / week	Once / day	2-3x / day	4+ x / day	4+ x / day
Cheddar (tasty, mild, colby) - 2 heaped Tbsp / matchbox cube	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯
Edam, Gouda, Swiss - 2 heaped Tbsp / matchbox cube	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯
Feta, Mozarella, Camembert - 1 heaped Tbsp / 1 med wedge	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯
Brie, blue and other specialty cheese - 1 heaped Tbsp / 1 med wedge	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯
Processed cheese slices - 1 slice	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯
Cream cheese - 2 heaped Tbsp	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯
Cottage or ricotta cheese - 2 heaped Tbsp	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯

*6. How often do you usually eat these dairy based foods?

	Never	<1x / month	1-3x / month	1x / week	2-3x / week	4-6x / week	Once / day	2-3x / day	4+ x / day
Ice cream - 2 scoops	◯	◯	◯	◯	◯	◯	◯	◯	◯
Custard or dairy food - 1 pottle / ½ cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
Yoghurt, plain or flavour - 1 pottle / ½ cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
Milk puddings (semolina, instant) - ½ cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
Fermented or evaporated milk (buttermilk) - ½ cup	◯	◯	◯	◯	◯	◯	◯	◯	◯

EXPLORE Food Frequency Questionnaire

5. Bread

***1. Do you eat bread?**

- ◯ No
- ◯ Yes

2. What type(s) of bread, rolls or toast do you eat most often? (You can choose up to 3 options, but please only choose the ones you usually have)

- ☐ Not applicable
- ☐ White
- ☐ White – high fibre
- ☐ Wholemeal or wheat meal
- ☐ Wholegrain

Other (please state)

***3. What type of bread slice do you usually have? (Please choose one only)**

- ◯ Not applicable
- ◯ Sandwich slice
- ◯ Toast slice
- ◯ Mixture of both sandwich and toast slices

***4. On average, how many servings of bread do eat per day? (Please choose one only)**
(A 'serving' = 1 slice of bread or 1 small roll)

- ◯ Not applicable
- ◯ Less than 1 serving
- ◯ 1–2 servings
- ◯ 3–4 servings
- ◯ 5–6 servings
- ◯ 7 or more servings

EXPLORE Food Frequency Questionnaire

*5. How often do you usually eat these bread based foods?

	Never	<1x / month	1-3x / month	1x / week	2-3x / week	4-6x / week	Once / day	2-3x / day	4+ x / day
Plain white bread - 1 slice	◯	◯	◯	◯	◯	◯	◯	◯	◯
High fibre white bread - 1 slice	◯	◯	◯	◯	◯	◯	◯	◯	◯
Wholemeal or wheat meal - 1 slice	◯	◯	◯	◯	◯	◯	◯	◯	◯
Wholegrain bread - 1 slice	◯	◯	◯	◯	◯	◯	◯	◯	◯
Fruit bread or fruit bun - 1 slice	◯	◯	◯	◯	◯	◯	◯	◯	◯
Wrap - 1 medium	◯	◯	◯	◯	◯	◯	◯	◯	◯
Focaccia, bagel, pita, panini or other speciality breads - 1 medium	◯	◯	◯	◯	◯	◯	◯	◯	◯
Paraoa Parai (fry bread) - 1 slice	◯	◯	◯	◯	◯	◯	◯	◯	◯
Rewena bread - 1 slice	◯	◯	◯	◯	◯	◯	◯	◯	◯
Doughboys or Maori bread - 1 slice	◯	◯	◯	◯	◯	◯	◯	◯	◯

*6. How often do you usually eat these other bread based foods?

	Never	<1x / month	1-3x / month	1x / week	2-3x / week	4-6x / week	Once / day	2-3x / day	4+ x / day
Crumpet or muffin split - 1 crumpet / 1 whole muffin split	◯	◯	◯	◯	◯	◯	◯	◯	◯
Scone - 1 medium	◯	◯	◯	◯	◯	◯	◯	◯	◯
Bran muffin or savoury muffin - 1 medium	◯	◯	◯	◯	◯	◯	◯	◯	◯
Croissant - 1 medium	◯	◯	◯	◯	◯	◯	◯	◯	◯
Waffle, pancakes or pikelets - 1 medium / 2 small	◯	◯	◯	◯	◯	◯	◯	◯	◯
Iced buns - 1 medium	◯	◯	◯	◯	◯	◯	◯	◯	◯
Crackers (cream crackers, cruskits, corn / rice crackers, vitawheat) - 2 medium	◯	◯	◯	◯	◯	◯	◯	◯	◯

*7. Do you have butter, margarine or spreads on bread or crackers?

◯ No

◯ Yes

EXPLORE Food Frequency Questionnaire

8. What type(s) do you have most often? (You can choose up to 3 options, but please only choose the ones you usually have)

- [ ] Not applicable
- [ ] Butter (all varieties)
- [ ] Monounsaturated fat margarine e.g. Olive, Rice Bran, Canola Oil Spreads
- [ ] Polyunsaturated fat margarine e.g. Sunflower Oil Spreads
- [ ] Light monounsaturated fat margarine e.g. Olivio Spread Light
- [ ] Light polyunsaturated fat margarine e.g. Flora Spread Light
- [ ] Plant sterol enriched margarine e.g. Pro Active, Logical Spreads
- [ ] Light plant sterol enriched margarine e.g. Pro Active Spread Light
- [ ] Butter and margarine blend e.g. Country Soft, Butter Lea

Other (please state) _____

***9. On average, how many servings of butter, margarine or spreads do you have per day? (Please choose one only)**
(A 'serving' = 1 level teaspoon or 5 mL)
e.g. 1 sandwich with butter thinly spread on two pieces of bread = 2 servings

- ◯ Not applicable
- ◯ Less than 1 serving
- ◯ 1–2 servings
- ◯ 3–4 servings
- ◯ 5–6 servings
- ◯ 7 or more servings

EXPLORE Food Frequency Questionnaire

6. Breakfast Cereals and Porridge

***1. Do you usually eat breakfast cereal and/or porridge?**

- ◯ No
- ◯ Yes

2. What breakfast cereal(s) do you eat most often? (You can choose up to 3 options, but please only choose the ones you usually have)

- ☐ Not applicable
- ☐ Weetbix
- ☐ Refined cereals e.g. Cornflakes or Rice Bubbles
- ☐ Bran based cereals including fruity varieties e.g. Special K, Muesli, All Bran
- ☐ Sweetened e.g. Nutrigrain, Cocoa Pops
- ☐ Porridge

Other (please state)

_____

***3. On average, how many servings of breakfast cereal or porridge do you have per week? (Please choose one only)**
(A 'serving' = ½ cup porridge, muesli, cornflakes or 2 weetbix)
e.g. ½ cup of porridge 3 times per week + 2 weetbix 4 times a week = 7 servings per week

- ◯ Not applicable
- ◯ Less than 4 servings
- ◯ 4–6 servings
- ◯ 7–9 servings
- ◯ 10–12 servings
- ◯ 13–15 servings
- ◯ 16 or more servings

EXPLORE Food Frequency Questionnaire

***4. How often do you usually eat porridge or these cereal foods?**

	Never	<1x / month	1-3x / month	1x / week	2-3x / week	4-6x / week	Once / day	2-3x / day	4+ x / day
Porridge, rolled oats, oat bran, oat meal - ½ cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
Muesli (all varieties) - ½ cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
Weetbix (all varieties) - 2 weetbix	◯	◯	◯	◯	◯	◯	◯	◯	◯
Cornflakes or rice bubbles - ½ cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
Bran cereals (All Bran, Bran Flakes) - ½ cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
Bran based cereals (Sultana Bran, Sultana Bran Extra) - ½ cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
Light and fruity cereals (Special K, Light and Tasty) - ½ cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
Chocolate based cereals (Milo cereal, Coco Pops) - ½ cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
Sweetened cereals (Nutrigrain, Fruit Loops, Honey Puffs, Frosties) - ½ cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
Breakfast drinks (Up and Go) - Small carton / 250 mL	◯	◯	◯	◯	◯	◯	◯	◯	◯

EXPLORE Food Frequency Questionnaire

7. Starchy Foods

***1. Do you eat any type of starchy foods such as rice, pasta, noodles and couscous?**

- ◯ No
- ◯ Yes

***2. On average, how many servings of starchy foods such as rice, pasta, noodles and couscous do you eat per week? (Please choose one only)**
(A 'serving' = 1 cup cooked rice / pasta)
e.g. 1 cup of rice + ½ cup of pasta included in a lasagne pasta dish + 1 cup of spaghetti = 2.5 servings

- ◯ Not applicable
- ◯ Less than 4 servings
- ◯ 4–6 servings
- ◯ 7–9 servings
- ◯ 10–12 servings
- ◯ 13–15 servings
- ◯ 16 or more servings

***3. How often do you usually eat these starchy foods?**

	Never	<1x / month	1-3x / month	1x / week	2-3x / week	4-6x / week	Once / day	2-3x / day	4+ x / day
Rice, white - 1 cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
Rice, brown or wild - 1 cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
Pasta, white or wholegrain (spaghetti, vermicelli) - 1 cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
Canned spaghetti (Watties) - 1 cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
Instant noodles (2 minute noodles) - 1 packet	◯	◯	◯	◯	◯	◯	◯	◯	◯
Egg and rice noodles (hokkien noodles, udon) - 1 cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
Other grain (quinoa, couscous, bulgar wheat) - 1 cup	◯	◯	◯	◯	◯	◯	◯	◯	◯

EXPLORE Food Frequency Questionnaire

8. Meat

***1. Do you eat beef, mutton, hogget, lamb, or pork**

- ◯ No
- ◯ Yes

***2. Do you trim any excess fat (fat you can see) off these meats? (Please choose one only)**

- ◯ Not applicable
- ◯ Always
- ◯ Often
- ◯ Occasionally
- ◯ Never cut the fat off meat

***3. On average, how many servings of meat e.g. beef, mutton, hogget, lamb or pork do you eat per week? (Please choose one only)**
(A 'serving' = palm size or ½ a cup of meat without bone)
e.g. ½ cup of savoury mince + 2 small lamb chops = 2 servings

- ◯ Not applicable
- ◯ Less than 1 serving
- ◯ 1-3 servings
- ◯ 4-6 servings
- ◯ 7 or more servings

***4. How often do you usually eat meat?**

	Never	<1x / month	1-3x / month	1x / week	2-3x / week	4-6x / week	Once / day	2-3x / day	4+ x / day
Beef mince dishes (rissoles, meatloaf, hamburger pattie) - 1 slice / patty / ½ cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
Beef or veal mixed dishes (casserole, stir-fry) - ½ cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
Beef or veal (roast, chop, steak, schnitzel, corned beef) - palm size / ½ cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
Lamb, hogget or mutton mixed dishes (stews, casserole, stir-fry) - ½ cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
Lamb, hogget or mutton (roast, chops, steak) - palm size / ½ cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
Pork (roast, chop, steak) - palm size / ½ cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
Canned corned beef - 1 medium slice	◯	◯	◯	◯	◯	◯	◯	◯	◯

EXPLORE Food Frequency Questionnaire

***5. How often do you usually eat these other meats?**

	Never	<1x / month	1-3x / month	1x / week	2-3x / week	4-6x / week	Once / day	2-3x / day	4+ x / day
Sausage, frankfurter or saveloy - 1 sausage / frankfurter/ 2 saveloys	◯	◯	◯	◯	◯	◯	◯	◯	◯
Bacon - 2 rashers	◯	◯	◯	◯	◯	◯	◯	◯	◯
Ham - 1 medium slice	◯	◯	◯	◯	◯	◯	◯	◯	◯
Luncheon meats or brawn - 1 slice	◯	◯	◯	◯	◯	◯	◯	◯	◯
Salami or chorizo - 1 slice / cube	◯	◯	◯	◯	◯	◯	◯	◯	◯
Offal (liver, kidneys, pate) - palm size / ½ cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
Venison/game - palm size / ½ cup	◯	◯	◯	◯	◯	◯	◯	◯	◯

EXPLORE Food Frequency Questionnaire

9. Poultry

***1. Do you eat poultry e.g. chicken, turkey or duck?**

- ◯ No
- ◯ Yes

***2. Do you remove the skin from chicken? (Please choose one only)**

- ◯ Not applicable
- ◯ Always
- ◯ Often
- ◯ Occasionally
- ◯ Never remove the skin from chicken

***3. On average, how many servings of chicken do you eat per week? (Please choose one only)**
(A 'serving' = palm size of chicken or ½ cup)
e.g. 1 chicken breast + 2 chicken drumsticks + 1 chicken thigh = 4 servings per week

- ◯ Not applicable
- ◯ Less than1 serving
- ◯ 1-3 servings
- ◯ 4-6 servings
- ◯ 7 or more servings

***4. How often do you usually eat poultry?**

	Never	<1x / month	1-3x / month	1x / week	2-3x / week	4-6x / week	Once / day	2-3x / day	4+ x / day
Chicken legs or wings - palm size / ½ cup / 1 unit (wing, drumstick)	◯	◯	◯	◯	◯	◯	◯	◯	◯
Chicken breast - palm size / ½ cup / ½ breast	◯	◯	◯	◯	◯	◯	◯	◯	◯
Chicken mixed dishes (casserole, stir-fry) - palm size / ½ cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
Crumbed chicken (nuggets, patties, schnitzel) - 1 medium / 4 nuggets	◯	◯	◯	◯	◯	◯	◯	◯	◯
Turkey or quail - palm size / ½ cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
Mutton bird or duck - palm size / ½ cup	◯	◯	◯	◯	◯	◯	◯	◯	◯

EXPLORE Food Frequency Questionnaire

10. Fish and Seafood

***1. Do you eat any type of fish or seafood?**

- ◯ No
- ◯ Yes

***2. On average, how many servings of fish and seafood (all types; fresh, frozen, tinned) do you eat per week? (Please choose one only)**
(A 'serving' = 80 - 120g or palm size or small tin (85g))
e.g. 1 fish fillet and 1 small tin of tuna = 2 servings per week.

- ◯ Not applicable
- ◯ Less than 1 serving
- ◯ 1-3 servings
- ◯ 4-6 servings
- ◯ 7 or more servings

3. How do you normally cook / eat fish? (You can choose up to 3 options, but please only choose the ones you usually have)

- ☐ Not applicable
- ☐ Raw / I don't cook it
- ☐ Oven baked / Grilled
- ☐ Deep fried
- ☐ Shallow fry
- ☐ Micro waved
- ☐ Steamed
- ☐ Poached
- ☐ Smoked

EXPLORE Food Frequency Questionnaire

***4. How often do you usually eat seafood?**

	Never	<1x / month	1-3x / month	1x / week	2-3x / week	4-6x / week	Once / day	2-3x / day	4+ x / day
Canned Salmon - 1 small can (85-95g)	◯	◯	◯	◯	◯	◯	◯	◯	◯
Canned Tuna - 1 small can (85-95g)	◯	◯	◯	◯	◯	◯	◯	◯	◯
Canned Mackerel, sardines, anchovies, herring - 1 small can (85-95g)	◯	◯	◯	◯	◯	◯	◯	◯	◯
Frozen crumbed fish (patties, fillets, cakes, fingers, nuggets) - 1 medium / 4 nuggets	◯	◯	◯	◯	◯	◯	◯	◯	◯
Snapper, Tarakihi, Hoki, Cod, Flounder - palm size / ½ cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
Gurnard, Kahawai or Trevally - palm size / ½ cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
Lemon fish or Shark - palm size / ½ cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
Tuna - palm size / ½ cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
Salmon, trout or eel - palm size / ½ cup	◯	◯	◯	◯	◯	◯	◯	◯	◯

***5. How often do you usually eat seafood?**

	Never	<1x / month	1-3x / month	1x / week	2-3x / week	4-6x / week	Once / day	2-3x / day	4+ x / day
Shrimp, prawn, lobster or crayfish - ½ cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
Crab or surumi - ½ cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
Scallops, mussels, oysters, paua or clams - ½ cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
Pipi or cockle - ½ cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
Kina - ½ cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
Whitebait - ¼ cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
Roe - ¼ cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
Squid, octopus, calamari, cuttlefish - ½ cup	◯	◯	◯	◯	◯	◯	◯	◯	◯

EXPLORE Food Frequency Questionnaire

11. Fats and Oils

***1. Do you cook meat, chicken, fish, eggs and/or vegetables with fat or oil?**

- ◯ No
- ◯ Yes

2. What type(s) do you use most often? (You can choose up to 3 options, but please only choose the ones you usually have)

- ☐ Not applicable
- ☐ Butter (all varieties)
- ☐ Margarines (all varieties)
- ☐ Cooking oils (all varieties)
- ☐ Lard, Dripping, Coconut oil, Ghee (clarified butter)
- ☐ Cooking spray

Other (please state) _____

***3. When you use fat or oil to cook, how many servings of fat or oil do you use per dish? (Please choose one only)**
(A 'serving' = 1 level teaspoon or 5 mL)

- ◯ Not applicable
- ◯ Less than 1 serving
- ◯ 1 serving
- ◯ 2 servings
- ◯ 3 servings
- ◯ 4 servings
- ◯ 5 or more servings

***4. On average, how many servings of fat or oil do you use to cook per week? (Please choose one only)**

- ◯ Not applicable
- ◯ Less than 1 serving
- ◯ 1-3 servings
- ◯ 4-7 servings
- ◯ 8-10 servings
- ◯ 11-14 servings
- ◯ 15 or more servings

EXPLORE Food Frequency Questionnaire

12. Eggs

***1. Do you eat eggs?**

- ◯ No
- ◯ Yes

***2. On average, not counting eggs used in baking / cooking, how many eggs do you usually eat per week? (Please choose one only)**

- ◯ Not applicable
- ◯ Less than 1 egg
- ◯ 1 egg
- ◯ 2 eggs
- ◯ 3 eggs
- ◯ 4 eggs
- ◯ 5 or more eggs

***3. How often do you usually eat eggs?**

	Never	<1x / month	1-3x / month	1x / week	2-3x / week	4-6x / week	Once / day	2-3x / day	4+ x / day
Whole eggs (hard-boiled, poached, fried, mashed, omelette, scrambled) - 1 egg	◯	◯	◯	◯	◯	◯	◯	◯	◯
Mixed egg dish (quiche, frittata, other baked egg) - 1 slice	◯	◯	◯	◯	◯	◯	◯	◯	◯

EXPLORE Food Frequency Questionnaire

13. Legumes

***1. Do you eat legumes e.g. chickpeas/dried peas, soybeans, dried/canned beans, baked beans, lentils or Dahl?**

- ◯ No
- ◯ Yes

***2. On average, how many servings of legumes (fresh, frozen, canned, dried) do you eat per week? (Please choose one only)**
(A 'serving' = ½ cup or 125g of cooked legumes)

- ◯ Not applicable
- ◯ Less than 1 serving
- ◯ 1 serving
- ◯ 2 servings
- ◯ 3 servings
- ◯ 4-5 servings
- ◯ 6-7 servings
- ◯ 8 or more servings

***3. How often do you usually eat these legumes?**

	Never	<1x / month	1-3x / month	1x / week	2-3x / week	4-6x / week	Once / day	2-3x / day	4+ x / day
Soybeans - ½ cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
Tofu - ½ cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
Dahl - ½ cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
Canned or dried legumes, beans (baked beans, chickpeas, lentils, peas, beans) - ½ cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
Hummus - 2 Tbsp	◯	◯	◯	◯	◯	◯	◯	◯	◯

EXPLORE Food Frequency Questionnaire

14. Vegetables

***1. Do you eat vegetables?**

- ◯ No
- ◯ Yes

***2. On average, how many servings of vegetables (fresh, frozen, canned) do you eat per day? Do NOT include vegetable juices. (Please choose one only)**
(A 'serving' = 1 medium potato / kumara or ½ cup cooked vegetables or 1/2 cup of lettuce)
e.g. 2 medium potatoes + ½ cup of peas = 3 servings

- ◯ Not applicable
- ◯ Less than 1 serving
- ◯ 1 serving
- ◯ 2 servings
- ◯ 3 servings
- ◯ 4 or more servings

***3. How often do you usually eat these vegetables?**

	Never	<1x / month	1-3x / month	1x / week	2-3x / week	4-6x / week	Once / day	2-3x / day	4+ x / day
Potato (boiled, mashed, baked, roasted) - 1 medium / ½ cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
Pumpkin (boiled, mashed, baked, roasted) - ½ cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
Kumara (boiled, mashed, baked, roasted) - 1 medium / ½ cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
Mixed frozen vegetables - ½ cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
Green beans - ½ cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
Silver beet, spinach - ½ cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
Carrots - 1 medium / ½ cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
Sweet corn - 1 medium cob / ½ cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
Mushrooms - ½ cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
Tomatoes - 1 medium / ½ cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
Beetroot - 1 medium / ½ cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
Taro, cassava or breadfruit - 1 medium / ½ cup	◯	◯	◯	◯	◯	◯	◯	◯	◯

EXPLORE Food Frequency Questionnaire

***4. How often do you usually eat these vegetables?**

	Never	<1x / month	1-3x / month	1x / week	2-3x / week	4-6x / week	Once / day	2-3x / day	4+ x / day
Green bananas (plantain) - 1 medium / ½ cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
Sprouts (alfalfa, mung) - ½ cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
Pacific Island yams - 1 medium / ½ cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
Turnips, swedes, parsnip or yams - ½ cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
Onions, celery or leeks - ¼ cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
Cauliflower, broccoli or broccoflower - ½ cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
Brussel sprouts, cabbage, red cabbage or kale - ½ cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
Courgette/zucchini, marrow, eggplant, squash, kamo kamo, asparagus, cucumber - ½ cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
Capsicum (peppers) - ½ medium / ¼ cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
Avocado - ¼ avocado	◯	◯	◯	◯	◯	◯	◯	◯	◯
Lettuce greens (mesculin, cos, iceberg) - ½ cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
Other green leafy vegetables (whitloof, watercress, taro leaves, puha) - ½ cup	◯	◯	◯	◯	◯	◯	◯	◯	◯

EXPLORE Food Frequency Questionnaire

15. Fruit

***1. Do you eat fruit?**

- ◯ No
- ◯ Yes

***2. On average, how many servings of fruit (fresh, frozen, canned or stewed) do you eat per day? Do NOT include fruit juice. (Please choose one only)**
(A 'serving' = 1 medium or 2 small pieces of fruit or 1/2 cup of chopped fruit)
e.g. 1 apple + 2 small apricots = 2 servings)

- ◯ Not applicable
- ◯ Less than one serving
- ◯ 1 serving
- ◯ 2 servings
- ◯ 3 or more servings

***3. How often do you usually eat these fruits?**

	Never	<1x / month	1-3x / month	1x / week	2-3x / week	4-6x / week	Once / day	2-3x / day	4+ x / day
Apple - 1 medium / ½ cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
Pear - 1 medium / ½ cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
Banana - 1 medium / ½ cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
Orange, mandarin, tangelo, grapefruit - 1 medium / 2 small	◯	◯	◯	◯	◯	◯	◯	◯	◯
Peach, nectarine, plum or apricot - 1 medium / ½ cup / 2 small	◯	◯	◯	◯	◯	◯	◯	◯	◯
Mango, paw-paw or persimmons / ½ cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
Pineapple - ½ cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
Grapes - ½ cup / 8-10 grapes	◯	◯	◯	◯	◯	◯	◯	◯	◯
Strawberries, other berries, cherries - ½ cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
Melon (watermelon, rockmelon) - ½ cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
Kiwifruit - 1 medium / 2 small	◯	◯	◯	◯	◯	◯	◯	◯	◯
Feijoas - 1 medium / 2 small	◯	◯	◯	◯	◯	◯	◯	◯	◯
Tamarillos - 1 medium / ½ cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
Sultanas, raisins or currants - 1 small box	◯	◯	◯	◯	◯	◯	◯	◯	◯
Other dried fruit (apricots, prunes, dates) - 4 pieces	◯	◯	◯	◯	◯	◯	◯	◯	◯

EXPLORE Food Frequency Questionnaire

16. Drinks

***1. On average, how many drinks do you have per day? (Please choose one only) (A 'serving' = 250 mL or one cup/glass)**

- ◯ Less than 1 serving
- ◯ 1-3 servings
- ◯ 4-5 servings
- ◯ 6-8 servings
- ◯ 9-10 servings
- ◯ 11 or more servings

***2. How often do you usually have these drinks?**

	Never	<1x / month	1-3x / month	1x / week	2-3x / week	4-6x / week	Once / day	2-3x / day	4+ x / day
Instant soup (Cup of soup) - 250 mL / 1 cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
Fruit juice (Just Juice, Fresh-up, Charlie's, Rio Gold) - 250 mL / 1 cup/glass	◯	◯	◯	◯	◯	◯	◯	◯	◯
Fruit drink (Choice, Rio Splice) - 250 mL / 1 cup/glass	◯	◯	◯	◯	◯	◯	◯	◯	◯
Vegetable juice (tomato juice, V8 juice) - 250 mL / 1 cup/glass	◯	◯	◯	◯	◯	◯	◯	◯	◯
Iced Tea (Lipton Ice tea) - 250 mL / 1 cup/glass	◯	◯	◯	◯	◯	◯	◯	◯	◯
Cordial or Powdered drinks (Thriftee, Raro, Vita-fresh) - 250 mL / 1 cup/glass	◯	◯	◯	◯	◯	◯	◯	◯	◯
Low-calorie cordial - 250 mL / 1 cup/glass	◯	◯	◯	◯	◯	◯	◯	◯	◯
Energy drinks small-medium can (V, Red Bull) - 250-350 mL	◯	◯	◯	◯	◯	◯	◯	◯	◯
Energy drinks large can (Monster, Mother, Demon, large V) - 450-550 mL	◯	◯	◯	◯	◯	◯	◯	◯	◯
Sugar-free Energy drinks (sugar-free V, Monster, Red Bull) - 1 small can	◯	◯	◯	◯	◯	◯	◯	◯	◯
Diet soft/fizzy/carbonated drink (diet sprite) - 250 mL / 1 cup/glass	◯	◯	◯	◯	◯	◯	◯	◯	◯
Soft/fizzy/carbonated drinks (Coke, Sprite) - 250 mL / 1 cup/glass	◯	◯	◯	◯	◯	◯	◯	◯	◯
Sport's drinks (Gatorade, Powerade) - 1 bottle	◯	◯	◯	◯	◯	◯	◯	◯	◯
Flavoured water (Mizone, H2Go flavoured) - 1 bottle	◯	◯	◯	◯	◯	◯	◯	◯	◯
Water (unflavoured mineral water, soda water, tap water) - 250 mL / 1 cup/glass	◯	◯	◯	◯	◯	◯	◯	◯	◯

EXPLORE Food Frequency Questionnaire

17. Dressings and Sauces

***1. How often do you usually have these dressings or sauces?**

	Never	<1x / month	1-3x / month	1x / week	2-3x / week	4-6x / week	Once / day	2-3x / day	4+ x / day
Butter (all varieties) - 1 tsp	◯	◯	◯	◯	◯	◯	◯	◯	◯
Margarine (all varieties) - 1 tsp	◯	◯	◯	◯	◯	◯	◯	◯	◯
Oil (all varieties) - 1 tsp	◯	◯	◯	◯	◯	◯	◯	◯	◯
Cream or sour cream - 1 Tbsp	◯	◯	◯	◯	◯	◯	◯	◯	◯
Mayonnaise or creamy dressings (aioli, tartae sauce) - 1 Tbsp	◯	◯	◯	◯	◯	◯	◯	◯	◯
Low fat/calorie dressing (reduced fat mayonnaise) - 1 Tbsp	◯	◯	◯	◯	◯	◯	◯	◯	◯
Salad dressing (french, italian) - 1 Tbsp	◯	◯	◯	◯	◯	◯	◯	◯	◯
Sauces (tomato, BBQ, sweet chilli, mint) - 1 Tbsp	◯	◯	◯	◯	◯	◯	◯	◯	◯
Mustard - 1 Tbsp	◯	◯	◯	◯	◯	◯	◯	◯	◯
Soy sauce - 1 Tbsp	◯	◯	◯	◯	◯	◯	◯	◯	◯
Chutney or relish - 1 Tbsp	◯	◯	◯	◯	◯	◯	◯	◯	◯
Gravy homemade - ¼ cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
Instant Gravy (e.g. Maggi) - ¼ cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
White sauce/cheese sauce - ¼ cup	◯	◯	◯	◯	◯	◯	◯	◯	◯

EXPLORE Food Frequency Questionnaire

18. Miscellaneous - Cakes, Biscuits and Puddings

***1. How often do you usually eat these baked products?**

	Never	<1x / month	1-3x / month	1x / week	2-3x / week	4-6x / week	Once / day	2-3x / day	4+ x / day
Cakes, loaves, sweet muffins - 1 slice / 1 muffin	◯	◯	◯	◯	◯	◯	◯	◯	◯
Sweet pies or pastries, tarts, doughnuts - 1 medium	◯	◯	◯	◯	◯	◯	◯	◯	◯
Other puddings or desserts - not including milk-based puddings (sticky date pudding, pavlova) - ½ cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
Plain biscuits, cookies (Round wine, Ginger nut) - 2 biscuits	◯	◯	◯	◯	◯	◯	◯	◯	◯
Fancy biscuits (chocolate, cream) - 2 biscuits	◯	◯	◯	◯	◯	◯	◯	◯	◯

EXPLORE Food Frequency Questionnaire

19. Miscellaneous

***1. How often do you usually eat these other foods?**

	Never	<1x / month	1-3x / month	1x / week	2-3x / week	4-6x / week	Once / day	2-3x / day	4+ x / day
Jelly - ½ cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
Ice blocks - 1 ice block	◯	◯	◯	◯	◯	◯	◯	◯	◯
Lollies - 2 lollies	◯	◯	◯	◯	◯	◯	◯	◯	◯
Chocolate - including chocolate bars (Moro bars) - 1 small bar	◯	◯	◯	◯	◯	◯	◯	◯	◯
Sugar added to food and drinks - 1 level tsp	◯	◯	◯	◯	◯	◯	◯	◯	◯
Jam, honey, marmalade or syrup - 1 level tsp	◯	◯	◯	◯	◯	◯	◯	◯	◯
Vegemite or marmite - 1 level tsp	◯	◯	◯	◯	◯	◯	◯	◯	◯
Peanut butter or other nut spreads - 1 level Tbsp	◯	◯	◯	◯	◯	◯	◯	◯	◯
Brazil nuts or walnuts - 2	◯	◯	◯	◯	◯	◯	◯	◯	◯
Peanuts - 10	◯	◯	◯	◯	◯	◯	◯	◯	◯
Other nuts (almonds, cashew, pistachio, macadamia) - 10	◯	◯	◯	◯	◯	◯	◯	◯	◯
Seeds (pumpkin, sunflower)	◯	◯	◯	◯	◯	◯	◯	◯	◯
Muesli bars - 1 bar	◯	◯	◯	◯	◯	◯	◯	◯	◯
Coconut cream - ¼ cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
Coconut milk - ¼ cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
Lite coconut milk - ¼ cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
Potato crisps, corn chips, Twisties - ½ cup / handful	◯	◯	◯	◯	◯	◯	◯	◯	◯

***2. Do you use salt in cooking?**

- ◯ Never
- ◯ Rarely
- ◯ Sometimes
- ◯ Usually
- ◯ Always

***3. Do you use salt at the table?**

- ◯ Never
- ◯ Rarely
- ◯ Sometimes
- ◯ Usually
- ◯ Always

EXPLORE Food Frequency Questionnaire

20. Miscellaneous - Takeaways

***1. On average, how often do you eat takeaways per week? (Please choose one only)**

- ◯ Never
- ◯ Less than 1 times
- ◯ 1-2 times
- ◯ 3-4 times
- ◯ 4-6 times
- ◯ More than 7 times

***2. How often do you usually eat these takeaway foods?**

	Never	<1x / month	1-3x / month	1x / week	2-3x / week	4-6x / week	Once / day	2-3x / day	4+ x / day
Meat pie, sausage roll, other savouries - 1 pie / 2 small sausage rolls or savouries	◯	◯	◯	◯	◯	◯	◯	◯	◯
Hot potato chips, kumara chips, french fries, wedges - ½ cup	◯	◯	◯	◯	◯	◯	◯	◯	◯
Chinese - 1 serve	◯	◯	◯	◯	◯	◯	◯	◯	◯
Indian - 1 serve	◯	◯	◯	◯	◯	◯	◯	◯	◯
Thai - 1 serve	◯	◯	◯	◯	◯	◯	◯	◯	◯
Pizza - 1 medium slice	◯	◯	◯	◯	◯	◯	◯	◯	◯
Burgers - 1 medium burger	◯	◯	◯	◯	◯	◯	◯	◯	◯
Battered fish - 1 piece	◯	◯	◯	◯	◯	◯	◯	◯	◯
Fried chicken (KFC, Country fried chicken) - 1 medium piece	◯	◯	◯	◯	◯	◯	◯	◯	◯
Bread based (Kebab, sandwiches, wraps, Pita Pit, Subway) - 1 medium	◯	◯	◯	◯	◯	◯	◯	◯	◯

EXPLORE Food Frequency Questionnaire

21. Other

***1. Are there any other foods or drinks that you can think of that you have on a regular basis that was not covered by this questionnaire?**

- ◯ No
- ◯ Yes

EXPLORE Food Frequency Questionnaire

22. Other

1. Please list these foods and drinks including; the serving size, and how many times per week you eat or drink these items (e.g. Pizza, 4 slices, one time per week)

Appendix E: *Standard Operating Procedure for Food Frequency Questionnaire*

Questionnaire Procedure

1. The questionnaires will be completed online using the computers in building 27. The two computers must first be turned on using the power button on the hard drive. You must then log in using the Explore network account, so that the computer is ready for the participants;

   Username: **xxxxxxx**

   Password: **xxxxxxx**

2. Once logged in open up each link to the FFQ, ensuring there is access. If there are any issues, please ask Zara, AJ, Sara, Kathryn Beck, or PC to help. If the issue cannot be resolved, there are hardcopies of the questionnaire which are kept in the pink Explore questionnaire folder which is will be beside the computers at the questionnaire station. However, the hardcopy version must be a last resort as this will have to be put into survey monkey manually by an Explore staff member at a later date, which is time consuming.

3. Participants will move to the questionnaire station after completing the blood pressure station, which is very quick (3-5minutes maximum) so ensure you are prepared by this stage. The participants are offered breakfast and a hot drink once blood pressure has been recorded, and if the participant declines food and drink please escort them straight to the questionnaire station. If the participant would like breakfast and/or a hot drink, please encourage them to consume this at the computer stations where they will begin the FFQ (as time may be short with multiple participants on the testing days). The questionnaire station is time consuming (25-40 minutes) so it is important to get this underway as soon as possible.

4. Read the first page of the FFQ to the participant, taking your time through the question examples, asking at the end if they have any questions.

5. Click ‘next’ at the bottom of the page and this will take you to the first questionnaire question which asks for the participant ID number, please insert this full number yourself i.e. 250018 and click ‘next’ at the bottom of the page.

6. The questionnaire is now ready for the participant to begin. Let the participant know that you will be there to answer any questions they have whilst completing it. Leave the participant and sit at the big round table behind the computer station, so that you are close enough for any possible questions, but so that you still give the participant some privacy.

7. It is important that conversation is kept to a minimum whilst participants are completing the questionnaires, they need full focus.

8. If any questions arise during this time from the participant that you are unsure of, please ask one of the team members stated above. It is important that we obtain the most accurate information possible from the FFQ.

9. If the FFQ stops working at any time, please see a team member stated above. You may need to provide a hardcopy for the participant to complete. If so, please ensure they complete this hardcopy version from the beginning, as their questionnaire data will not be saved unless they have 100% completed it.

Appendix F: *Energy intake and Goldberg Equation Cut-Offs*

ID	Weight (kg)	Height (m)	BMR (kcal)	Reported energy intake (kcal)	**Energy Intake:BMR
1	109	1.70	2061	940	0.456*
2	104.4	1.65	1692	833	0.492*
3	87.9	1.73	1558	783	0.502*
4	82	1.79	1719	1128	0.656*
5	100.1	1.61	1792	1236	0.690*
6	69	1.71	1519	1092	0.719*
7	61	1.62	1387	1007	0.726*
8	101.3	1.82	1989	1497	0.753*
9	87.3	1.67	1758	1425	0.810*
10	97.5	1.64	1636	1406	0.860*
11	72.3	1.65	1549	1333	0.860*
12	95.8	1.75	1622	1443	0.889
13	87.1	1.58	1729	1578	0.912
14	103	1.67	1972	1806	0.916
15	95.6	1.76	1621	1538	0.949
16	110.2	1.74	1739	1681	0.967
17	104.6	1.61	1694	1644	0.971
18	53.7	1.62	1281	1248	0.974
19	73.2	1.67	1567	1547	0.987
20	71.1	1.65	1532	1536	1.003
21	118.8	1.66	2183	2243	1.027
22	85.8	1.67	1541	1631	1.058
23	66.3	1.71	1483	1655	1.116
24	93.9	1.58	1823	2092	1.147
25	94.4	1.71	1611	1913	1.187
26	70.3	1.72	1416	1699	1.200
27	121.4	1.84	1830	2203	1.204
28	84.7	1.58	1532	1854	1.210
29	111.4	1.69	1749	2200	1.258
30	86.5	1.65	1547	1962	1.268
31	68.6	1.54	1467	1869	1.274
32	116.5	1.66	2151	2807	1.305
33	68.9	1.69	1513	1996	1.319
34	99.1	1.69	1924	2561	1.331
35	76	1.78	1635	2246	1.374
36	82.6	1.75	1516	2085	1.376
37	109	1.68	2056	2836	1.379
38	61.1	1.64	1392	1969	1.415
39	104.9	1.71	1696	2403	1.417
40	72.2	1.69	1431	2031	1.419
41	80.2	1.74	1496	2134	1.426
42	105.5	1.71	2016	2969	1.473
43	72.9	1.59	1554	2303	1.482
44	88.2	1.70	1779	2650	1.489

45	64.1	1.63	1365	2075	1.520
46	105.9	1.74	2030	3144	1.549
47	70.5	1.66	1417	2223	1.569
48	106.5	1.70	1709	2700	1.580
49	99.6	1.64	1653	2642	1.598
50	101.8	1.68	1671	2673	1.600
51	107.7	1.66	2032	3387	1.667
52	102.1	1.63	1673	2795	1.670
53	99.4	1.67	1921	3219	1.675
54	131.9	1.67	1915	3237	1.691
55	64.9	1.66	1517	2567	1.693
56	89.2	1.55	1569	2715	1.731
57	69	1.62	1534	2670	1.741
58	103.8	1.79	2017	3603	1.787
59	81.4	1.69	1506	2715	1.803
60	82.7	1.67	1695	3092	1.824
61	101.6	1.68	1669	3151	1.887
62	74.6	1.62	1451	2778	1.915
63	83.2	1.74	1723	3537	2.054
64	95.5	1.67	1868	3915	2.096
65	134	1.67	2393	5103	2.132
66	92.3	1.68	1830	3976	2.173
67	82.1	1.67	1687	3817	2.262
68	122.1	1.73	2249	5210	2.317
69	78.2	1.62	1619	3869	2.389
70	70.6	1.61	1418	3421	2.412
71	79.8	1.68	1493	3772	2.527
72	66.1	1.64	1462	3765	2.576
73	120	1.73	1818	4692	2.580
74	70.7	1.68	1536	4107	2.674
75	70.7	1.62	1419	3912	2.757†
76	137.5	1.73	2457	7246	2.949†
77	73.3	1.64	1559	4641	2.976†
78	143.1	1.73	2006	6322	3.152†
79	89.3	1.66	1783	5696	3.194†
80	103.2	1.62	1822	6105	3.350†
81	67.9	1.59	1396	5482	3.926†
82	79.4	1.62	1489	6656	4.469†
83	96.8	1.63	1877	8929	4.757†
84	83.7	1.67	1709	9026	5.280†
85	86.6	1.66	1548	9298	6.007†
86	65.6	1.59	1440	10082	6.999†
87	94.4	1.64	1847	13029	7.054†
88	68.5	1.61	1485	14974	10.086†
89	147.7	1.64	2043	20698	10.133†
90	104.7	1.65	1987	31042	15.619†

*=Under-reported energy intake; †=over-reported energy intake. **Energy Intake:BMR=reported energy (kcal)/BMR (kcal).Cut-off for under-reporting=0.873; cut-off for over-reporting=2.752.