Bone density, biomarkers and nutrient intake of postmenopausal women

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

Master of Science in Nutrition and Dietetics

at Massey University, Albany, New Zealand.

Amanda Smith

2024

Abstract

Background: Osteoporosis is associated with an increased risk of bone fractures and carry a significant burden on the individual and the health care system. Of these individuals, postmenopausal women have a high risk for developing osteoporosis due to decreased oestrogen production.

Objectives: This study explored the relationship between nutrient intake and bone health in postmenopausal women. Specifically, C-terminal of telopeptide of type 1 collagen (CTx-1), an indicator of bone turnover, and bone mineral density (BMD) were analysed in relation to the identified nutrient patterns. Correlations with additional markers associated with bone metabolism, parathyroid hormone (PTH), BMD, and vitamin D, were also examined.

Method: The present study was an analysis using baseline data from the COPES 4 Bones project. Eighty seven postmenopausal women participated (aged 48-77). Questionnaires were used to obtain participant demographics and physical activity levels. A three-day food record was self-reported by the participants. BMD was measured using dual x-ray absorptiometry. Weight, height and BMI were measured using standard techniques. Fasted venous blood samples were collected to measure CTx-1, 25-hydroxyvitamin D_3, and PTH. Nutrient patterns were derived from 3-day food records using principal component analysis. Linear regression was used to explore the association between BMD and the nutrient patterns. Age, weight, serum vitamin D, and physical activity were confounders in the model.

Results: Four nutrient patterns were identified from the data. Nutrient pattern 1 (NP1) was characterised by high amounts of phosphorus, protein, zinc, and niacin equivalents. Nutrient pattern 2 (NP2) was characterised by high amounts of dietary fibre, magnesium, potassium and a low amount of saturated fat. Nutrient pattern 3 (NP3) was characterised by high amounts of monounsaturated fat, vitamin E, polyunsaturated fat and a low amount of carbohydrates. Nutrient pattern 4 (NP4) was characterised by high amounts of alpha carotene and beta carotene. One significant negative correlation was identified between NP2 and hip BMD without consideration of confounders.

Conclusion: The current study identified NP2 as a potential factor contributing to bone health in a model exclusive of confounders such as weight and age. The research found weight to be positively associated with BMD. While dietary patterns were not explicitly studied, a diet consisting of high fruit and vegetable intake may be inferred from the characteristics of NP2. A negative relationship between a diet of this form and BMD challenges previously reported findings in postmenopausal women. Therefore, this work prompts future research to better characterise the role of nutrient intake in modulating bone health.

Acknowledgements

Completing my thesis has not been dissimilar to my other academic endeavours in regards to the fact that it would not have been possible without the extensive support from my family, friends and tutors.

I would like to offer my greatest thanks to my supervisor Professor Marlena Kruger. You have been the biggest support for me during this journey. Your extensive knowledge and experience provided me with the best guidance I could ask for. I am so grateful for amount of time and effort you committed to this project.

I would like to also thank my co-supervisor Dr. Lilian Ilesanmi-Oyelere. Thank you for your time and advice throughout this project. Your hard work in the primary study provided me with this academic opportunity.

I would like to express a big thanks to Dr Karen Mumme for your statistical knowledge. Your input and guidance for the statistical analysis and presentation allowed me to feel confident in the reliability of the data and findings.

To my parents, Catherine and Paul, thank you for emotionally and financially supporting me throughout this journey. To my partner, Peter, thank you for the constant support and love from home and across the globe.

My sincere thanks goes to the participants for their time they volunteered for this research. Without them sacrificing their time this study would not have been possible.

Table of contents

Abstract……...1
Acknowledgements……2
Table of contents……3
List of Tables and figures……5
Abbreviations definitions list……6
Chapter 1 Introduction……..7
1.1 Background……..…..7
1.2 Purpose of the study……..…..…9
1.3 Aims and objectives……..…..…9
1.4 Thesis structure……...……9
1.5 Researchers' contribution……..…..……..…..…...10
Chapter 2 Literature review……...……..11
2.0 Introduction…….……..……...11
2.1 Bone…….....……11
2.2 Bone cells…….…….…..12
2.2.1 Osteoclasts…….……..12
2.2.2 Osteoblasts……..…..…12
2.2.3 Osteocytes……....…12
2.2.4 Bone lining cells……...…13
2.3 Bone turnover…….....13
2.3.1 Bone turnover cycle……..…13
2.3.2 Mediators of bone turnover …….…….…...14
2.3.3 Bone growth throughout the lifecycle……..…14
2.4 Modifiable and non-modifiable factors affecting bone density…….……15
2.4.1 Physical activity……...……15
2.4.2 Hormonal control of bone homeostasis……15
2.5 Nutrients and bone health…….……...……...…..…18
2.5.1 Calcium……18
2.5.2 Magnesium……...…19
2.5.3 Phosphorus……...…19
2.5.4 Protein……..…19
2.5.5 Vitamin C……..…20
2.5.6 Vitamin K……20
2.5.7 Zinc…….……..…21
2.5.8 Copper……..21
2.5.9 Vitamin A……..…21
2.6 Measuring nutrient intake……..…22
2.7 Measuring physical activity……..23

2.8 Postmenopausal women and bone health……23
2.8.1 Hormonal effects of menopause on bone health……23
2.8.2 Dietary pattern's effects on bone health in postmenopausal women……24
2.8.3 Nutrient intake effects on bone health in postmenopausal women……25
2.9 Measuring bone health……26
2.9.1 Bone densitometry……26
2.9.2 Bone formation markers……26
2.9.3 Bone resorption markers……27
2.9.4 CTx-1 response to nutrients……28
2.9.5 Summary of findings……28
Chapter 3 Manuscript……29
3.0 Abstract……29
3.1 Introduction……30
3.2 Methodology……32
3.2.1 Study design……32
3.2.2 Ethics and trial registration……32
3.2.3 Sample size……32
3.2.4 Participants……32
3.2.5 Data collection……33
3.2.6 Data handling……34
3.2.7 Nutrient patterns……35
3.2.8 Statistical analyses……35
3.3 Results……37
3.3.1 Demographic data……37
3.3.2 Nutrient pattern data……40
3.3.3 Correlations……41
3.3.4 Linear regression……42
3.3.5 Sensitivity analysis……45
3.4 Discussion……47
3.5 Limitations & strengths……53
3.6 Conclusion……54
Chapter 4 Conclusion and recommendation……56
4.1 Overview……56
4.2 Main findings……56
4.3 Research impact……57
4.4 Strengths and limitations……58
4.5 Recommendations and future directions for research……60
References……61
Appendices……72

List of Tables and Figures

Table 1:……...……...38

Table 2:……...……...39

Table 3:……...……...40

Table 4:……...……...40

Table 5:…….....40

Table 6:……...…...41

Table 7:……...……...42

Table 8:……...…...42

Table 9:……...…...43

Table 10:…….... 44

Table 11:…….……...45

Table 12:…….……...45

Table 13:…….……...46

Table 14:…….……...46

Figure 1: ……..……..…...14

Abbreviations definitions list

Abbreviation	Definition
BMD	Bone mineral density
CTx-1	Carboxy-terminal crosslinked telopeptide of type 1 collagen
PTH	Parathyroid hormone
$25(OH)D_3$	25-hydroxyvitamin D_3
$1,25(OH)_2D_3$	1,25 di-hydroxyvitamin D_3
FFQ	Food frequency questionnaire
IGF-1	Insulin-like growth factor-1
TNF	Tumour necrosis family
NP	Nutrient pattern
FM	Femoral neck
MET	Metabolic equivalents
AEE	Activity energy expenditure
HRT	Hormone replacement therapy
DXA	Dual-energy X-ray absorptiometry
QUS	Quantitative ultrasound
CT	Computed tomography
PCA	Principal component analysis
EAR	Estimated average requirement
AI	Adequate intake
NRV	Nutrient reference value
MICE	Multivariate imputation by chained equations
BMI	Body mass index
P1NP	Procollagen type 1 N-terminal propeptide
PICP	Procollagen I carboxyterminal propeptide
ALP	Alkaline phosphatase
ALPL	Bone-specific alkaline phosphatase
ICTP	C-terminal cross-linked telopeptide of type I collagen
PYD	Pyridinoline
DPD	Deoxy-pyridinoline
NTX	N-terminal of telopeptide of type 1 collagen

Chapter 1 Introduction

1.1 Background

As the ageing population and life expectancy in the Western world increases, bone strength and maintenance is becoming a more important health marker. Osteoporosis is an irreversible disease where bones become brittle and weak. The World Health Organisation defines the disease as a bone mineral density T-score of less than 2.5 standard deviations below the average for a healthy young adult (Kanis, 2007). The skeletal fragility associated with osteoporosis is linked to increased risk of bone fractures. In New Zealand 1 in 3 females and 1 in 5 males will suffer an osteoporotic fracture in their lifetime (Osteoporosis New Zealand, 2017).

Not only does this result in poor health outcomes for individuals, but it also has a significant cost for the health care system. Ten years ago in New Zealand, a hip fracture which resulted in an average 3-week hospital stay equated to around $47,000. In 2007, the estimated total cost incurred by cases of osteoporosis in New Zealand equated to $330 million including the treatment of osteoporotic fractures, the aftercare of these fractures and the treatment of patients with osteoporosis (Brown et al., 2011).

There are multiple types of bone cells. Osteoclasts are the bone cells responsible for the breakdown of bone also known as bone resorption. Bone resorption plays an essential role in bone remodelling. Osteoblasts are the cells responsible for the bone formation and remodelling of broken-down bone (Marieb & Hoehn, 2015). At homeostasis, the rate of bone resorption is matched by the rate of bone formation.

Bone health can be measured by bone mineral density (BMD). Bone mass gained versus bone mass lost will determine an individual's bone mineral density. However with age, bone resorption rates surpass bone formation rates which results in a net loss of bone. Peak bone mass is the highest bone mineral density an individual will gain in their life. This occurs at the end of skeletal maturation between the age of 18-30 years. If peak bone mass is not maximised, then when the natural age-related decline in BMD occurs, the individual will have fewer bone mass reserves to lose before their BMD reaches the osteoporosis diagnostic criteria (Raymond & Morrow, 2022). Therefore, peak bone mass is a significant determinant of an individual's risk of developing osteoporosis. A 10% increase in peak bone mass has been predicted to delay osteoporosis by 13 years (Hernandez et al., 2003). Bone resorption can be measured through biomarker CTx-1 (Carboxy-terminal Crosslinked Telopeptide of type 1 collagen), a product of collagen breakdown.

Menopause is defined by the absence of a menstrual cycle for over 12 months (Cano, 2017). This life stage is physiologically characterised by the decline in the concentration of oestrogen. Females face an increased risk of osteoporosis due to the menopause associated reduction in oestrogen (Marieb & Hoehn, 2015).

Oestrogen is a hormone with many responsibilities in the body. One of these roles is the regulation of the osteoclasts involved with bone resorption. Research suggests that oestrogen induces apoptosis (cell death) of osteoclasts, which results in a reduction of bone resorption activity (Kameda et al., 1997). Therefore, after menopause, as oestrogen decreases the regulation of osteoclasts declines, and bone resorption activity increases. This exacerbates bone loss and reduces BMD.

Nutrition, physical activity, and other factors will determine the peak bone mass someone reaches (Marieb & Hoehn, 2015). Diet quality is significant during both periods of growth and development to reach peak bone mass as well as during ageing to minimise bone loss. Many nutrients such as calcium, vitamin D, vitamin K, vitamin B_6, vitamin B_{12} and vitamin C have significant impacts on BMD (Raymond & Morrow, 2022). Dairy foods are a rich source of calcium which makes them a prominent food group when considering bone health.

Research has found improved outcomes for osteoporotic fracture risk in postmenopausal Japanese women with a higher consumption of milk. However, the intake of cheese and yogurt, also calcium rich foods, was not associated with an improved fracture risk (Kojima et al., 2023). Another recent study investigated the efficiency of the calcium in milk to decrease CTx-1, a bone resorption biomarker, with a single 500ml dosage of high calcium cow's milk equating to 1000mg of calcium in postmenopausal women. The results reflected a significant decline in CTx-1 (Sharma et al., 2022). However, this research highlights only an acute result and is not reflective of a long-term dietary pattern. Other research in Swiss women found through the analysis of dietary patterns that those with osteoporosis had a lower dairy and calcium intake compared to those who did not have osteoporosis (Lanyan et al., 2020).

Despite the research promoting dairy foods for skeletal health a significant proportion of the New Zealand population is not meeting its calcium requirements. The 2008 New Zealand Nutrition survey highlighted that 72.7% of females did not meet their adequate intake of calcium. The post-menopausal women had the highest inadequacy rates, with 88.5% of 51-70 year olds, and 92.8% of 71+ years olds not having an adequate calcium intake (University of Otago & Ministry of Health, 2011). These demographic groups have the highest Recommended Daily Intake (RDI) for calcium of 1300mg per day. From the 2008 survey, across all demographics, milk was the largest contributor to calcium intake at 27%. The 2018-2020 New Zealand National Health Survey highlighted that 3.4% of the adult

population excluded dairy and 8% of the adult population reported drinking no milk (Ministry of Health, 2022).

1.2 Purpose of the study

Bone mineral density is a significant health parameter for postmenopausal women. The change that occurs in the bones between pre and postmenopausal life is the accelerated loss of bone. Therefore, the focus of this research was to investigate this bone loss in relation to nutrient intake. Calcium is an important nutrient for bone health and as the mentioned research (Sharma et al., 2022) suggests a modulator for bone loss. New Zealand postmenopausal women stand out as an at-risk population for osteoporosis due to their insufficient calcium intake. There is limited research focused on bone loss biomarker CTx-1 and nutrient intake. Therefore, this research investigated the relationship between bone markers CTx-1 and BMD, and nutrient intake through three-day diet records of postmenopausal women.

Research question

Do certain nutrients modulate bone turnover?

1.3 Aims and objectives

Aim

To explore the relationship between nutrient intake with bone resorption biomarker CTx-1 and bone mineral density in post-menopausal women using a 3-day diet record.

Hypothesis

A nutrient pattern including calcium will be positively associated with lower levels of bone resorption and higher bone mineral density in post-menopausal women.

Research objectives

1. To determine if there is a relationship between CTx-1 and BMD and/or PTH.
2. To determine if there is a relationship between PTH and vitamin D.
3. To create nutrient patterns and to determine if they have a relationship with the CTx-1 and BMD of postmenopausal women.

1.4 Thesis structure

The structure of this research is set out in 4 chapters. Chapter 1 introduces the topic of bone health in relation to postmenopausal women and diet. Chapter 2 reviews the current literature regarding bone structure, bone metabolism, nutrients and bone health, postmenopausal changes in relation to bone health and measuring bone density. Chapter 3 is the manuscript inclusive of the abstract, introduction,

methods, results, discussion, and conclusion. Chapter 4 consists of the conclusion of the research including the recommendations, achievement of the aims and objectives and evaluation of the strengths and limitations of the research.

1.5 Researchers contribution

Amanda Smith	Main researcher, data analysis, statistical analysis, interpretation, and discussion of results
Prof Marlena Kruger	Main academic supervisor
Dr Bolaji Lilian Ilesanmi-Oyelere, PhD	Academic supervisor, study design, recruitment, data collection
Dr Karen Mumme	Statistical analysis support

Chapter 2 Literature review

2.0 Introduction

This chapter reviews the literature relating to bone and bone turnover, factors affecting bone, postmenopausal women and bone, and nutrition's effect on postmenopausal women's bone health.

Search Strategy

Date searched: November 2023 – September 2024

Search criteria:
Postmenopausal AND ("bone density" OR "bone mineral density" OR "bone loss" OR "bone health") AND ("nutrient pattern*" or "dietary pattern*")

Filters: Peer reviewed.

Electronic databases: Massey Discover, PubMed, Google Scholar.

2.1 Bone

The skeleton is an essential part of the mammalian body that provides us with locomotive abilities, the protection of vital organs, the framework for muscle and other tissue, red blood cell production, mineral homeostasis and triglyceride storage (Tortora, 2019). The bone is the connective tissue that forms the skeleton. The two types of osseous tissue are compact and trabecular (spongy) bone. The membrane that surrounds the surface of the bone is the periosteum (Marieb & Hoehn, 2015). The extracellular matrix consists of collagen fibres, water and crystallised mineral salts such as calcium phosphate known as hydroxyapatite. The collagen fibres provide the structural framework for crystallised salts to be deposited for calcification (Tortora, 2019).

Compact bone has a smooth appearance and is the external layer of the bone. Compact bone is made up of units called osteons. Osteons are circular rings of lamella, mineralised extracellular matrix, that enclose around groups of blood vessels and nerves. Within the ring layers of lamella are spaces called lacunae where the bone cell osteocytes are found. There are channels called canaliculi within the lamella that allow the transport of nutrients to osteocytes and removal of waste (Tortora, 2019).

Trabecular bone is in the interior of the bone structure and has a layer of compact bone surrounding its exterior. It has a honeycomb appearance that is filled with bone marrow which produces red blood cells. The lamella of spongy bone is called trabeculae, which consists of randomly arranged cylinders. It is the unorganised arrangement that creates the honeycomb presentation (Tortora, 2019).

There are four different bone shapes including long bone, flat bone, irregular bone and short bone (Marieb & Hoehn, 2015). Within bone tissue, there are different types of cells and the careful orchestration of the different bone cells leads to the constant resorption and remodelling of bone tissue. (Tortora, 2019).

2.2 Bone cells

2.2.1 Osteoclasts

There are four types of bone cells. Osteoclasts are large multi-nucleate cells that come from hematopoietic stem cells like macrophages. These cells are responsible for the breakdown of bone. Osteoclasts break down the bone in resorption bay areas with enzyme degradation (Marieb & Hoehn, 2015). When osteoclasts come in contact with bone, a portion of the membrane joins to the bone. Integrins of the αv family are expressed in the osteoclasts' membranes and mediate the osteoclast to bone matrix recognition. An actin ring forms upon bone contact to anchor the cell to the bone and form a tight seal from the surrounding extracellular fluid called the sealing zone. Acidified intracellular vesicles containing proton pumps transit through the osteoclast towards the bone. This folds the shape of the bone interfacing membrane which is called the ruffled border (Teitelbaum, 2007). The osteoclast then releases hydrochloric acid and proteolytic enzymes such as cathepsin K that break down the collagen and minerals of the bone's extracellular matrix (Kenkre & Bassett, 2018).

2.2.2 Osteoblasts

Osteoblasts develop from divided osteoprogenitor cells. These are bone stem cells found in the periosteum, the endosteum and the blood vessel canals of bone. Osteoprogenitor cells come from the mesenchymal stem cells (Tortora, 2019). Osteoblasts are located along the surface of the bone and are responsible for forming new bone. The bone formation is completed in two steps. First, the components of organic bone matrix, collagen proteins and non-collagen proteins are released. This is followed by the mineralisation of the bone matrix (Florencio-Silva et al., 2015). The unmineralised bone is called osteoid. Osteoid requires 7 days to mature before it is mineralised to form hydroxyapatite (Marieb & Hoehn, 2015).

2.2.3 Osteocytes

Osteocytes are the mature bone cell that maintain the bone mineral complex. They are located in the lacuna within the bone mineral matrix. The osteocyte's role is the maintenance of bone tissue which includes exchanging nutrients and waste products with the blood. Osteocytes communicate with each other through gap junctions and canaliculi. Osteocytes have small projections into the canaliculi where extracellular fluid is transported (Tortora, 2019). These cells can live up to 25 years and make up most bone cells in the body (Florencio-Silva et al., 2015). Osteocytes are derived from osteoblast lineage and

the transformation from an osteoblast located on the bone surface to a mineral embedded osteocyte requires a significant decrease in size and changes in its cellular process (Franz-Odendaal et al., 2006).

Osteocytes act as loading sensors for the bone. Mechanical load triggers osteocytes to suppress osteoclast activity and promote osteoblast activity, resulting in bone formation. The absence of mechanical load and force causes the opposite response, leading to bone loss. Within the osteocyte cell, integrins, cilia, calcium channels and G-protein coupled receptors (GPCRs) have been identified as the sensors of force. Once the force has been sensed, the osteocytes transmit signals such as intracellular calcium, ATP, nitrogen oxide, prostaglandins and Wnt signalling to trigger its response (Uda et al., 2017).

2.2.4 Bone lining cells

Bone lining cells line the external surface of the bone and maintain the bone mineral matrix. Those lining the external surface of the bone are called periosteal cells, and those lining the internal surface are endosteal cells. Bone lining cells are also proliferated from osteogenic cells (osteoprogenitor cells) (Marieb & Hoehn, 2015).

2.3 Bone turnover

2.3.1 Bone turnover cycle

The bone turnover cycle is the replacement of old bone with new bone. The group of bone cells required is described as a basic multicellular unit. The bone remodelling compartment is a closed area to allow for the close coupling of resorption and formation. The bone remodelling occurs in 5 steps which are shown in Figure 1. The first step begins with the activation of precursor osteoclast cells and the separation of bone lining cells from the bone. This separation creates the boundary of the bone remodelling compartment. Multinucleated preosteoclasts bind to the bone matrix and form sealing zones to control the resorption pit as a microenvironment (Kenkre & Bassett, 2018).

The second step is resorption, where osteoclasts pump hydrogen ions via proton pumps and chloride ions into the resorption site, to remove the bone mineral. Then proteolytic enzymes cathepsin K and matrix metalloproteinases are realised to break down the bone matrix. This resorption process is approximately 14 days long and the cessation is determined by osteoclast cell death (Kenkre & Bassett, 2018).

The third step is the reversal phase, which is the interlude between resorption and formation. Unmineralised collagen matrix is removed and a boundary called a “cement line” of mineralised matrix is created to guide bone formation. The fourth step, bone formation, is the secretion of type 1 collagen

osteoid matrix by the osteoblasts, and the mineralisation of osteoid which is regulated by osteoblasts. Osteoblasts will then die or differentiate into bone lining cells or osteocytes (Kenkre & Bassett, 2018).

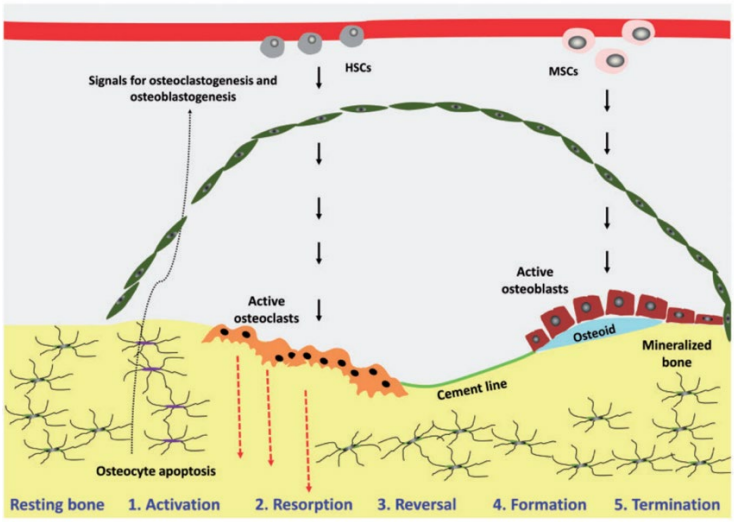


Fig 1, Bone Turnover Cycle (Kenkre & Bassett, 2018)

2.3.2 Mediators of bone turnover

RANKL is a protein from the tumour necrosis family (TNF) that has a role in bone metabolism. RANKL is made by osteoblasts, osteocytes and stromal cells. It is a modulator of osteoclast activity by triggering osteoclastogenesis when binding to receptor RANK on osteoclast precursors. Osteoprotegerin is an inhibitor of osteoclast activity. It is a decoy receptor for RANKL and upon RANKL binding to the decoy it is prevented from binding with RANK and therefore cannot trigger osteoclastogenesis (Florencio-Silva et al., 2015).

The Wnt-β-Catenin signalling pathway is a cascade of signal transductions that regulate important cellular processes such as proliferation, differentiation and apoptosis. It is essential in both the development of embryos and homeostasis in adults (Liu et al., 2022). The Wnt-β-Catenin signalling pathway induces bone formation by promoting the formation of osteoblasts and supressing mesenchymal stem cell differentiation to osteoclasts. Wnt signalling also causes the production of osteoprotegerin, further inhibiting bone resorption. The inhibition of this pathway leads to bone resorption domination and therefore bone loss (Baron & Kneissel, 2013).

2.3.3 Bone growth throughout the lifecycle

During adolescence, the skeleton grows in length and density. After adolescence, bone stops growing in length, but continues to increase in density by accumulating bone mass. This is called consolidation. In this period, bone formation exceeds bone resorption (Raymond & Morrow, 2022). During the third decade of life bone will cease growing and the bone density at that time is the individual's peak bone

mass (PBM). PBM is the greatest bone mass an individual reaches in their life before the age related bone mass decline begins. Therefore, the PBM reached in earlier adult life will largely determine how weak the bones will become with age. The PBM an individual reaches will be determined by their sex, genetics, diet and lifestyle (Raymond & Morrow, 2022).

2.4 Modifiable and non-modifiable factors affecting bone density

2.4.1 Physical activity

There are both modifiable and non-modifiable factors that contribute to BMD. Hormones are non-modifiable factors. Physical activity and nutrition are modifiable factors that can be optimised to improve BMD. Physical activity promotes bone formation as it is mechanical loading for the bones. As described earlier mechanical loading of bones is sensed by osteocytes and promotes osteoblast activity and supresses osteoclast activity. Multiple studies comparing adult monozygotic and dizygotic twins found that the twin with higher levels of physical activity had greater bone density in loaded bone areas than the other twin (Kujala et al., 2022, Nissen et al., 2023). Physical activity interventions of weight bearing resistance exercise appear to maintain and/or improve BMD greater than walking in older adults due to its increased loading. The increases in BMD are not reflected in the entire skeleton. Instead, they are localised to the weight bearing bones which receive the loading stimulus. Individuals with a lower BMD have greater changes from the exercise intervention (Gómez-Cabello et al., 2012).

2.4.2 Hormonal control of bone homeostasis

Growth hormone

Growth hormone is the key hormone responsible for bone growth during infancy and childhood. It is released by the anterior pituitary gland and stimulates the chondrocytes of the epiphyseal plate (Marieb & Hoehn, 2015). Growth hormone promotes bone growth indirectly too by increasing the expression of insulin-like growth factor-1 (IGF-1). Directly, growth hormone promotes the proliferation of pre-chondrocytes and stimulates osteoblasts (Ohlsson et al., 1998). Growth hormone's significance in bone growth is highlighted in growth hormone-deficient children having short stature (O'Neill et al., 2022).

Parathyroid hormone

Parathyroid hormone (PTH) is secreted by the parathyroid gland. This hormone is a significant regulator for serum calcium levels. It is responsible for triggering the bone resorption action of osteoclasts, breaking down bone and releasing calcium into the circulatory system. This cascade starts due to serum calcium levels reducing to below homeostatic levels (Marieb & Hoehn, 2015). This mechanism means that extended periods of low serum calcium will lead to prolonged bone breakdown. Though this process is at the expense of bone density, it is essential to maintain the tightly controlled serum calcium levels of 2.25-2.5mmol/L. The calcium ions are responsible for muscle contraction such as the heart and skeletal muscle tissue and function of the nervous system (Marieb & Hoehn, 2015).

Oestrogen

One of the main female sex hormones, oestrogen, acts as an inhibitor of bone resorption by inducing the apoptosis of osteoclasts. Oestrogen also promotes bone formation by suppressing the apoptosis of osteoblasts and osteocytes (Florencio-Silva et al., 2015). The secretion of this hormone is not linear through the life cycle. When females reach 40-50 years of age oestrogen levels begin to decline in association with menopause. Menopause is defined as the absence of a menstrual cycle for over 12 months due to the depletion of ovarian follicles. The low quantity of follicles leads to insufficient oestrogen to trigger a period (Cano, 2017). Therefore, postmenopausal women face increased bone resorption due to the reduced regulation of osteoclast cells.

Vitamin D

Vitamin D acts as a hormone inside the body for multiple essential processes including bone metabolism. Vitamin D's role in bone health is highlighted in the disease rickets, where a vitamin D deficiency leads to the development of weak and soft bones in children (Marieb & Hoehn, 2015). Vitamin D is taken into the body by ingesting food or is synthesised via an ultraviolet light-catalysed reaction in the skin. This reaction is the photochemical conversion of 7-dehydrocholestrol to pre-vitamin D_3. The conformational changes from the reaction result in the pre-vitamin D_3 being released from the plasma membrane. This allows vitamin D binding protein (DBP) to bind to and deliver the compound to the liver. The enzyme CYP2R1 then hydroxylates pre-vitamin D_3 to 25-hydroxyvitamin D_3 ($25(OH)D_3$) at the liver. $25(OH)D_3$ is the main serum form of vitamin D (Laird et al., 2010). DBP takes $25(OH)D_3$ from the liver to the kidneys where enzyme CYP27B1 hydroxylates the compound to 1,25 di-hydroxyvitamin D_3 ($1,25(OH)_2D_3$). This is the active form of vitamin D. The production of CYP27B1 is catalysed by PTH making PTH a stimulant for the active form of vitamin D (Laird et al., 2010).

One of vitamin D's roles within bone homeostasis involves calcium absorption. Calcium has two methods of intestinal absorption, passive paracellular absorption and active transcellular transport which is $1,25(OH)_2D_3$ dependent. In a state of insufficient dietary calcium or increased calcium requirements, active transcellular transport dominates (Christakos & Pike, 2020). Therefore, the elevated PTH levels in periods of hypocalcaemia will promote the production of $1,25(OH)_2D_3$ for calcium absorption. Insufficient $1,25(OH)_2D_3$ will lead to insufficient intestinal calcium absorption during periods of increased calcium needs as the active transcellular transport will not work. This will trigger bone resorption to release calcium from the bones to maintain serum calcium levels. $1,25(OH)_2D_3$ also increases intestinal phosphorus absorption (Christakos & Pike, 2020). Sufficient levels of absorbed calcium and phosphorus allow for the inclusion of these minerals in bone formation.

$1,25(OH)_2D_3$ promotes the production of osteopontin and osteocalcin. These are calcium-binding proteins involved in bone mineralisation. Equally, $1,25(OH)_2D_3$ has roles in bone resorption too. $1,25(OH)_2D_3$ promotes the expression of RANKL and decreases the expression of the antagonist of RANKL, osteoprotegerin, thereby increasing the function of RANKL (Christakos & Pike, 2020). Both $1,25(OH)_2D_3$ and PTH have minor roles in the regulation of calcium reabsorption within the distal convoluted tubule and connecting tubules of the kidneys. This accounts for approximately 10-15% of the filtered calcium (Christakos & Pike, 2020).

$25(OH)D_3$ is the form that is measured in serum tests to indicate vitamin D status. The New Zealand parameters of vitamin D deficiency in adults include mild deficiency being a range of 25-50nmol/L, moderate deficiency is between 12.5-25nmol/L and severe deficiency is <12.5nmol/L (Ministry of Health, 2017). For maximal muscle and bone benefits, the Endocrine Society recommends a serum $25(OH)D_3$ level of 72nmol/L (Holick et al., 2011).

Dietary sources of vitamin D include oily fish, dairy and eggs (Laird et al., 2010). The essential absorption of UV light from the sun for vitamin D synthesis can be influenced by many factors including skin tone, pollution, amount of skin exposed, duration of exposure, age and season. The darker an individual's skin is the greater the concentration of melanin there is in the skin. Melanin absorbs UV rays protecting other skin cells from its damage. Therefore, higher melanin concentrations in the skin lead to lower UV absorption by other skin cells (Webb, 2006). Age is a factor of vitamin D synthesis in multiple regards. Age related reduction in renal function reduces the conversion of $25(OH)D_3$ to $1,25(OH)_2D_3$ due to a decline in the enzyme activity of the reaction (Gallagher, 2013). The skin's ability to synthesise vitamin D decreases with age due to a reduction in the concentration of 7-dehydrocholesterol. With lower levels of 7-dehydrocholesterol less pre-vitamin D_3 can be produced (MacLaughlin & Holick, 1985). Considering this factor, and the decreased sunlight exposure older people have, they are an at-risk group for vitamin D deficiency. The New Zealand adequate intake of vitamin D for adults aged 19-50 years is 5µg/day. For adults aged 51-70 years it is 10µg/day and for 71+ years it is 15µg/day (Ministry of Health, 2017).

Glucocorticoids

Glucocorticoids are steroid hormones made in the adrenal gland with derivatives of cholesterol. Cortisol is a steroid hormone and at elevated levels, it interferes with osteoblast formation and decreases bone formation (Robey & Riminucci, 2020). Glucocorticoids also promote osteoclast proliferation and stimulate the synthesis of RANKL (Anderson et al., 2012). Cushing disease is the condition of over-endogenous production of cortisol by an adrenal or pituitary tumour. Cushing disease leads to osteoporosis, weakening of the bones and increased fracture risk (Anderson et al., 2012).

Glucocorticoids are also given exogenously as an anti-inflammatory medication to treat a wide range of inflammation related diseases, thereby increasing physiological concentrations. The use of this medication therefore does carry a cost to bone health. Inflammatory disorders exclusive of glucocorticoid treatment also cause bone loss (Briot & Roux, 2015). Glucocorticoids also indirectly influence bone by decreasing intestinal calcium absorption and increasing renal excretion of calcium (Briot & Roux, 2015).

2.5 Nutrients and bone health

2.5.1 Calcium

A range of nutrients hold important roles in bone formation and remodelling. These roles vary from minerals of the bone tissue to essential cofactors for reactions of bone metabolism. One of these bone minerals is calcium. Ninety-nine percent of calcium in the body is found within the skeleton (Raymond & Morrow, 2022). The skeleton acts as a reserve for calcium and when there is insufficient calcium provided in the diet to meet requirements, bone is broken down to provide the calcium (Marieb & Hoehn, 2015). During growth, calcium is required for the accumulation of bone mass and after growth, it is required for bone remodelling (Anderson et al., 2012). Low dietary calcium intake is associated with lower bone density in both adults and children (Pan et al., 2020, Kim et al., 2014). However, sufficient vitamin D levels may compensate for low dietary calcium's effect on BMD in adults (Kim et al., 2014). This is likely due to the previously mentioned role vitamin D has on the transcellular intestinal absorption of calcium. Intestinal calcium absorption increases from 10-15% during vitamin D deficiency to 30-40% during vitamin D sufficiency (Khazai et al., 2008).

Calcium is found in high concentrations in dairy foods and in lesser concentrations in green vegetables such as broccoli. Other food sources of calcium include fortified products such as plant milk and cereals. Calcium excretion in the urine can be influenced by other dietary factors such as sodium and animal protein intake (Ministry of Health, 2017). Animal protein is high in phosphate which enhances calcium excretion (Watson & Mahadevan, 2016). The recommended daily intake (RDI) of calcium in New Zealand is 1000mg/day for adult males and females. However, during adolescence (12-18 years of age), for elderly men (70 years +) and postmenopausal women (50 years +) the requirements are higher at 1300mg/day (Ministry of Health, 2017). The median intake of calcium from the 2008 New Zealand Adult Nutrition Survey was 919mg for males and 745mg for females (University of Otago & Ministry of Health, 2011). The Australian and American recommendations for calcium for adults and elderly are very similar to New Zealand's (National Institutes of Health, 2019; Ministry of Health, 2017). However England recommends a lower amount of 700mg for adults (NHS, 2020).

The bioavailability of calcium in food and supplements will vary with the calcium solubility and the presence of other food chemicals (Heaney et al., 1990). Oxalic acid and phytic acid are compounds

naturally occurring in food which inhibit the intestinal absorption of calcium. Oxalic acid is most commonly found in spinach and beans. Phytic acids are present in soy, beans, seeds and nuts (Ministry of Health, 2017). Additionally, with age, the intestinal absorption of calcium decreases by an average of 0.21% every year after the age of 40 (Heaney et al., 1989).

2.5.2 Magnesium

Magnesium has many responsibilities inside the body, including catalysing reactions involved in bone metabolism and contributing to the alkalinity of the pH of the body (Anderson et al., 2012). Greater than half the magnesium in the body is found in the bone where it is stored (Schuchardt & Hahn, 2017). Magnesium has significant roles in the regulation of vitamin D with it being an essential cofactor for enzymes within vitamin D metabolism (Uwitonze & Razzaque, 2018). Magnesium deficiency in rats leads to significant bone loss due to both increased bone resorption and decreased bone formation (Rude et al., 2003). In humans, positive associations have been found between serum magnesium and positive bone measures like greater BMD (Song et al., 2007, Wang et al., 1999). The New Zealand RDI of magnesium for adults (31-70 years old) is 420mg for males and 320mg for females. Good dietary sources of magnesium include green vegetables, nuts, legumes, and beans (Ministry of Health, 2017).

2.5.3 Phosphorus

Phosphate is a component of the bone mineral content. It is ingested through dietary sources as phosphorus and is excreted by the kidneys in the urine. Phosphorus is found in meat, seafood, nuts and legumes. Increasingly, phosphorus is found in processed foods in the form of additives or phosphorus salt (Anderson et al., 2012). The phosphorus RDI for adults is 1000mg a day (Ministry of Health, 2017). There are no general concerns about insufficient dietary phosphorus. Instead, there are concerns about excess phosphorus. Serum phosphate levels appear to be a modulator of the conversion of $25(OH)D_3$ to $1,25(OH)_2D_3$ (Portale et al., 1989). Therefore, high dietary phosphorus could indirectly influence bone loss via this mechanism. High serum phosphate, as seen in advanced chronic kidney disease (CKD), is known to cause CKD bone disease by leaching calcium from the bone. Additionally, excessive dietary phosphorus can hinder calcium absorption in the gut due to phosphate ions causing calcium to form an insoluble precipitate (Anderson et al., 2012).

2.5.4 Protein

Protein is one of the three macronutrients that provide the body with the energy it needs to survive. Protein also provides the body with the building blocks required for growth and development, amino acids. Protein is found in dairy, meat, fish, eggs, grains and cereals. Vegetables also have some protein, but animal-based sources have all essential amino acids while plant sources do not. The RDI of protein for a healthy adult is 0.84g/kg/day for males and 0.75g/kg/day for females. In regard to the acceptable macronutrient distribution (AMDR) protein is reccomended to contribute to 15-25% energy intake

(Ministry of Health, 2017). From the 2008 New Zealand Adult Nutrition Survey the median protein intake for males was 102g and for females was 72g and it contributed to 16.4% of males' and 16.3% of females' energy intake (University of Otago & Ministry of Health, 2011). A sufficient quantity of protein is required to maintain BMD. A longitudinal study found that older adults with lower protein intake had greater bone loss compared to those with higher protein intakes (Hannan, 2000). In other research, healthy individuals were put on a low protein diet and it induced hyperparathyroidism after only 4 days with elevated PTH and $1,25(OH)_2D_3$ levels as calcium absorption declined on a low protein intake (Kerstetter et al., 2003). High protein diets have been hypothesised to be harmful to BMD due to acidic disturbance in the acid-base balance causing bone resorption to restore balance, though the evidence is conflicting. In a study comparing a high protein diet (>2.2g/kg/day) to moderate protein for 6 months in exercise-trained women the high protein diet had no significant effect on BMD (Antonio et al., 2018). A higher protein and adequate calcium diet for women on bed rest led to a greater level of the bone resorption marker C-terminal of the type 1 collagen (CTx-1) than the control group on a moderate protein intake (Heer et al., 2017). However, this may be due to unloading activating osteoclasts and a high protein intake creating their preferred acidic environment.

2.5.5 Vitamin C

Vitamin C is a cofactor for collagen synthesis. It is required for the function of enzymes prolyl hydroxylase and lysyl hydroxylase which catalyse the hydroxylation of the proline and lysine residues in the conversion of procollagen to collagen (Anderson et al., 2012). Vitamin C as an antioxidant can also neutralise free radical oxygen species that would otherwise damage bone (Anderson et al., 2012). Additionally, it contributes to bone health by influencing the differentiation of osteoblasts (Thaler et al., 2022). Research has found positive associations between intake of vitamin C and bone health either through increased BMD or decreased bone loss (Sahni et al., 2008) Vitamin C is found in citrus fruits such as oranges and vegetables like tomatoes and capsicum. The RDI of vitamin C for adults is 45mg/day (Ministry of Health, 2017). The median intake of vitamin C from the 2008 New Zealand Adult Nutrition survey was 99mg for both females and males (University of Otago & Ministry of Health, 2011).

2.5.6 Vitamin K

Vitamin K is another important vitamin for bone health. There are two types of vitamin K. Vitamin K_1, phylloquinone which is found in green leafy vegetables and Vitamin K_2, menaquinones which are found in soy and cheese or fermented foods such as natto. Vitamin K_2 is also synthesised by intestinal bacteria, but vitamin K_1 is the major contributor to total vitamin K intake (Anderson et al., 2012). The adequate intake of Vitamin K for adults is 70μg/day for males and 60μg/day for females (Ministry of Health, 2017). Vitamin K serves as an essential cofactor for the posttranslational carboxylation reaction that is required for the formation of protein, gammacarboxyglutamate (Gla). Gla has a strong calcium binding

affinity and in the bone it forms the greater bone polypeptides osteocalcin, matrix Gla protein (MGP), growth-arrest-specific gene 6 protein, protein S and periostin. During deficiency Vitamin K is spared for essential processes only and therefore bone is compromised for processes regarding more immediate survival (Anderson et al., 2012).

2.5.7 Zinc

Zinc has a large range of roles to fulfil within the body including the cofactor for a range of reactions for bone mineralisation. Zinc promotes osteoblast proliferation and increases alkaline phosphatase activity, an essential enzyme for the mineralisation of bone. During zinc deficiency, the skeleton releases zinc to maintain the tightly controlled serum level (O'Connor et al., 2020). Deficiency of zinc in the diet has been linked to impaired skeletal growth, with rats being fed a low zinc diet having much lower bone mass and growth than the control rats (Eberle et al., 1999). Zinc is found in meat, legumes, shellfish, eggs and grains. The RDI of zinc for adults is 14mg/day for males and 8mg/day for females (Ministry of Health, 2017). The 2008 New Zealand Adult Nutrition Survey reflected a median intake of zinc of 12.9mg for males and 9mg for females (University of Otago & Ministry of Health, 2011).

2.5.8 Copper

Copper plays an important role in bone metabolism. Copper is a cofactor for many enzymatic reactions. One of these reactions is the cross-linking of collagen with the enzyme lysyl oxidase (Anderson et al., 2012). Organ meat, seafood, nuts and seeds are significant dietary sources of copper, but it is found in a large range of foods. The adequate intake of copper for adults is 1.7mg/day for males and 1.2mg/day for females (Ministry of Health, 2017). Both dietary copper intake and serum copper levels have been found to be positively associated with BMD (Fan et al., 2022, Chaudhri et al., 2009).

2.5.9 Vitamin A

Retinol and carotenoids are the two forms of dietary vitamin A. Retinol is the biologically functional form of vitamin A and carotenoids are a precursor the body can convert to retinol. Dietary sources of retinol are dairy products, meat, eggs, the liver of animals and fish and cod liver oil. Carotenoids such as beta and alpha carotenes are found in vegetables such as carrots, pumpkin, kumara and capsicum, and fruits such as oranges (Anderson et al., 2012). The RDI of vitamin A for adults is 900µg/day of retinol equivalents for males and 700µg/day of retinol equivalents for females (Ministry of Health, 2017). The 2008 New Zealand Adult Nutrition Survey reflected a median intake of retinol equivalent of 846µg for males and 727 µg for females (University of Otago & Ministry of Health, 2011).

In regards to bone health, the two forms of vitamin A are not equal. Early research investigating retinol toxicity in animals found effects of bone thinning, spontaneous fractures and skeletal lesions (Binkley

& Krueger, 2000). A more recent study of Spanish postmenopausal osteoporotic women found a negative association between serum retinol levels and BMD of the lumbar spine and femoral neck (Navarro-Valverde et al., 2018). Another study found that a higher vitamin A intake was positively associated with BMD for vitamin D-sufficient participants (Joo et al., 2015). Overall, the evidence for the relationship between retinol and bone health is mixed.

Carotenoids have been found to be protective of bone health by decreasing osteoclast differentiation through the suppression of RANKL (Hirata et al., 2019). Research investigating carotenoid intake and BMD changes over 4 years in an elderly population found carotenoids to be protective for bone loss (Sahni et al., 2009).

2.6 Measuring nutrient intake

There are multiple ways to measure food and nutrient intake. This includes food frequency questionnaires and diet records. Diet records typically last between 3-4 days and are a large commitment for the participants as it requires all food to be weighed and recorded. The statistical analysis of a sample of diet records also adds a significant cost. Food frequency questionnaires are a simpler and quicker method of measuring nutrient intake for both the participants and the researcher as it is one questionnaire that assesses the frequency of the consumption of different foods (Bailey, 2021). A limitation of food frequency questionnaires and diet records is that they are self-reported and therefore there is the risk of under or over-reporting that can reduce the accuracy of the results.

Research has been completed comparing the methods of diet record and food frequency questionnaire in a population of postmenopausal women to compare whether the two would produce similar results. When comparing the results of the two methods the nutrients that had the most similar results were fat, carbohydrates, alcohol, potassium, vitamin C and fibre. Calcium, energy and protein had the most different estimates when comparing the results of the two methods. The two methods produced the same significant positive associations between magnesium and iron and BMD (Farrell et al., 2009). Another study comparing a short food group questionnaire and a 7-day diet record found that the two methods produced matching results for carbohydrates, saturated fats, cholesterol, total sugar, fibre, calcium, vitamin C, riboflavin, and folate. However, the results of vitamin D, total fat, monosaturated fat, polyunsaturated fat, carotene and vitamin E had the most variation between the two methods. From the twenty-three nutrients investigated, nine had correlating results (Roddam et al., 2005).

Results from food records and food frequency questionnaires can be transformed into nutrient and dietary patterns. Dietary patterns are the quantity, variety and frequency of different combinations of food and beverages in a diet that is habitually consumed (Dietary Guidelines Advisory Committees, 2020). This method looks at the diet overall and at what foods are habitually eaten together. Nutrient

patterns are the quantity and variety of different combinations of nutrients that are habitually ingested from food together.

In research, investigating nutrient patterns is more useful than single-nutrient investigation because nutrients are not ingested individually, but rather come in varying combinations through food. The combinations of different nutrients may have a synergistic or dependent effect, or a single nutrient's effect may be too small to detect while the effect of a combination could be measurable (Hu, 2002). Nutrient patterns can be interpreted and referenced back to different food groups and diet patterns. As reviewed, there is a significant number of nutrients involved in bone metabolism and therefore nutrient patterns will be used for this study.

2.7 Measuring physical activity

There are multiple different ways to measure physical activity for research. There is technology-based measurement using devices such as pedometers which measure step count or accelerometers which measure the number of accelerations that is then converted into energy expenditure or a physical activity pattern. Additionally, there are heart rate monitors to measure energy expenditure and armbands that combine body temperature and motion sensors to measure physical activity (Sylvia et al., 2014). There are self-reported tools for measuring physical activity including written or verbal questionnaires or exercise diaries. Questionnaires can ask participants about transport, occupation and leisure, or average duration at different levels of excursion. Physical activity diaries require participants to record the duration and type of exercise they participate in over a set period and often require participants to judge and record the intensity of the physical activity (Sylvia et al., 2014). While devices bear the cost of the technology, self-reporting methods have the limitation of participants overreporting physical activity and an exercise diary increases the burden on the participant.

2.8 Postmenopausal women and bone health

2.8.1 Hormonal effects of menopause on bone health

As mentioned earlier, oestrogen induces the apoptosis of osteoclasts and promotes the activity of bone remodelling cells. Therefore, the decreased levels of oestrogen after menopause lead to less regulation of osteoclasts, greater bone resorption and greater bone loss. This puts postmenopausal women at a greater risk of osteoporosis. Osteoporosis is a disease of low BMD that causes weak and brittle bones. WHO defines osteoporosis as "A value for BMD that is 2.5 SD or more below the young female adult mean (T-score less than or equal to –2.5 SD)" (Kanis, 2007). Osteoporosis causes a greater bone fracture risk due to increased weakness of the bone. The age-related changes in the ratio of bone remodelling and bone resorption are inevitable but reaching a greater PBM in earlier age will reduce the likelihood of developing osteoporosis.

Postmenopausal women face the previously described age-related decrease in vitamin D synthesis secondary to decreases in the skin and renal reactions in the synthesis pathway. PTH is another hormone that changes with age. As previously stated, PTH concentrations can be influenced by other physiological conditions such as low vitamin D and serum calcium. However, PTH concentrations increase with age independent of vitamin D, serum calcium, phosphate and renal function (Carrivick et al., 2015). This is associated with the decline in BMD due to PTH's role in calcium release from bone. However, one study found that vitamin D status was a greater predictor of PTH levels in postmenopausal women than age or serum calcium (Need et al., 2004).

2.8.2 Dietary pattern's effects on bone health in postmenopausal women

Research has investigated different ways to reduce bone loss in postmenopausal women to reduce the likelihood of them developing osteoporosis. Diet has been a significant realm of research. Different dietary patterns have been investigated to see if they correlate with greater bone health outcomes for postmenopausal women. A dietary pattern is a combination of types and proportions of different foods that are often consumed together.

A study of Iranian postmenopausal women found the consumption of a lacto-vegetarian diet and in particular, the consumption of vegetables, fruits, legumes, nuts, dairy, soy protein and egg to be inversely associated with low bone density (Ansari et al., 2023). In a study of Korean postmenopausal women, the same positive association was seen between vegetable intake and BMD (Kim et al., 2016). Other research investigating dietary patterns of New Zealand postmenopausal women found positive associations between a dietary pattern high in dairy and moderate in fruit and spine BMD (Ilesanmi-Oyelere et al., 2020). Another western population of Scottish postmenopausal women found the dietary pattern of fruit, vegetables, white meat, oily fish and dairy to be positively associated with BMD and negatively associated with bone loss (Hardcastle et al., 2011).

The Mediterranean diet, known for its anti-inflammatory properties, consists of a high intake of fruit, vegetables, whole grains, and legumes, moderate amounts of fish, olive oil and poultry and dairy with limited amounts of red meat and processed foods (Raymond & Morrow, 2022). This dietary pattern has been identified to be positively associated with BMD. A study with a sample of premenopausal Spanish women found that participants having diets that scored higher for the Mediterranean diet was associated with having greater BMD (Perez-Rey et al., 2019). Another study of postmenopausal women found the same association with participants with a greater Mediterranean diet score having higher lumbar spine BMD and lean muscle mass (Silva et al., 2019).

Overall, the research investigating dietary patterns and what foods correlate with greater BMD for postmenopausal women reflects a general result of dairy, fruits and vegetables being positive for bone health.

2.8.3 Nutrient intake effects on bone health in postmenopausal women

Previous research investigating the nutrient intake of New Zealand postmenopausal women using a three-day diet record found positive associations between a nutrient pattern of calcium, protein, phosphorus, potassium, magnesium, riboflavin, and niacin with BMD. The results also showed a nutrient pattern of total fat, fatty acids and fat soluble vitamins such as vitamin A, E and beta-carotene to be negatively associated with BMD (Ilesanmi-Oyelere et al., 2019).

Other research in postmenopausal women from Brazil using a food frequency questionnaire identified two nutrient patterns one of which was characterised by an intake of vitamin B_{12}, pantothenic acid, phosphorus, riboflavin, protein, vitamin B_6, potassium, vitamin D, vitamin E, calcium cholesterol, β-carotene, omega 3, magnesium, zinc, niacin, and selenium (Grili et al., 2023). The other nutrient pattern was characterised by an intake of iron, vegetable protein, thiamine, folate, fibre, PUFA, vitamin A, vitamin K, alpha-tocopherol, copper, sodium, and retinol. Both these nutrient patterns were negatively associated with osteopenia, but there was no association with osteoporosis (Grili et al., 2023).

In another study, a nutrient pattern characterised by folate, fibre, vitamin B_6, potassium, vitamin A, vitamin C, b-carotene, vitamin K, magnesium, manganese and copper was found to be positively correlated with spine BMD in postmenopausal Iranian women (Karamati et al., 2014). Other research on New Zealand postmenopausal women found no correlation between calcium intake and bone loss (Bristow et al., 2019).

Research with Swiss postmenopausal women highlighted that those with osteoporosis had lower calcium intakes than women without the disease (Lanyan et al., 2020). A study of Korean postmenopausal women found positive correlations between vitamin C, beta-carotene, zinc, sodium and BMD (Kim et al., 2016).

The results of nutrient intake and BMD correlation research vary across studies much more in comparison to the dietary patterns and BMD in postmenopausal women. The impact of nutrient patterns on bone health and individual nutrients' impact on bone health will differ as nutrient patterns reflect the synergistic effect of nutrients and potential dietary patterns, whereas an individual nutrient captures a smaller picture of a person's diet. Therefore, the relationship an individual nutrient has with bone health may not translate to having the same effect when within a nutrient pattern with other nutrients.

2.9 Measuring bone health

2.9.1 Bone densitometry

Bone densitometry refers to the measurement of bone mineralisation. The gold standard method to diagnose osteoporosis is dual-energy X-ray absorptiometry (DXA). The DXA sends x-ray beams through the bones and the bone mineral content will absorb the wave lengths. The more x-rays that are absorbed, the higher the bone mineral content. The x-rays that are not absorbed by bone will pass through the body and is measured (Berger, 2002). The results of the DXA are interpreted with the WHO's T-score.

An individual's T-score is calculated by subtracting the average young adult bone density from the measured one and dividing it by the young adult population standard deviation (Blake & Fogelman, 2007). The T-score is used for the diagnosis criteria of osteoporosis because this provides a comparison with a healthy person between the ages of 20-29. The reference range for healthy bone is a T-score greater than -1, a T-score between -1 and -2.5 is classified as osteopenia (low bone mass) and a T-score less than -2.5 is classified as osteoporosis (National Institute of Arthritis and Musculoskeletal and Skin Diseases, 2023). A z-score is also derived from a DXA. The z-score is the comparison of the individual's bone density to the bone density of people of their demographic. A z-score of -2.0 or less indicates concern for possible secondary osteoporosis (Sheu & Diamond, 2016).

Quantitative ultrasound (QUS) is another method to measure bone density by calculating bone stiffness. The QUS is radiation free compared to the DXA which uses ionizing radiation but QUS has poor reproducibility. A computed tomography (CT) scan is another method to measure bone density. CT measures true volumetric bone density, but has the limitation of higher radiation at 200-1200 micro-sievert, much higher than DXA's radiation exposure of 4 micro-sievert (Sheu & Diamond, 2016).

2.9.2 Bone formation markers

Bone activity can be measured with biomarkers indicating bone resorption and formation. Osteocalcin is a calcium-binding protein that is expressed by osteoblasts. It can be measured by a blood test to assess bone formation. The majority of osteocalcin will become a part of the bone mineral matrix while a small portion will enter the circulation. Though serum osteocalcin is a marker of formation, it is also released during bone resorption (Greenblatt et al., 2017).

Procollagen type 1 N-terminal propeptide (P1NP) is formed during the formation of bone collagen from procollagen via the cleavage of the N-terminal of procollagen. Procollagen I carboxyterminal propeptide (PICP) is also formed by cleavage during the formation of bone collagen. Therefore serum

levels of P1NP and PICP provide a measure of collagen formation and thereby bone formation in the body (Hlaing & Compston, 2014).

There are four different alkaline phosphatase (ALP) genes. Bone-specific alkaline phosphatase (ALPL) is synthesized by osteoblasts proportionally to their activity. This enzyme degrades pyrophosphate which is a strong inhibitor of bone mineralisation. ALPL levels can be measured by an immunoassay test to measure bone formation (Greenblatt et al., 2017).

2.9.3 Bone resorption markers

Bone resorption biomarkers are a way of measuring bone loss and resorption activity. C-terminal cross-linked telopeptide of type I collagen (ICTP) is a product of the digestion of bone from enzymes metalloproteinase or trypsin. However, this biomarker is less used in the clinical setting because it requires a radioimmunoassay. Pyridinoline (PYD) and deoxy-pyridinoline (DPD) are other bone resorption markers as they are cross-links in collagen that are cleaved off during bone resorption. DPD is more specific to bone making it the preferred biomarker (Greenblatt et al., 2017).

N-terminal of telopeptide of type 1 collagen (NTX) is the N-terminal telopeptide part of type 1 collagen that is cleaved off during the breakdown of bone. C-terminal of telopeptide of type 1 collagen (CTx-1) is of the same nature, but from the C-terminal of the type 1 collagen. These products enter the circulation and reflect the osteoclastic activity. Both of these resorption markers can be measured via a serum or urinary test (Greenblatt et al., 2017). One study measured CTx-1 levels in a wide age range of women. There were two pre-menopause groups aged 20-29 years and 30-39 years and three post-menopause groups 1-10 years, 11 to 20 years and 21+ years since menopause. The average CTx-1 levels were highest in 1-10 years post-menopause group with 11-20 years and 21+ years since menopause groups having the second and third highest average CTx-1 level respectively. Interestingly the 30-39-year group had a lower average CTx-1 level than then 20-29-year group (Gossiel et al., 2018). In a sample of postmenopausal Korean women, those who were 6-10 years post menopause had the highest levels of CTx-1, DPD and osteocalcin compared to the 0-5 years and >10 years post-menopause groups. These bone biomarkers were all higher in the osteoporotic participants compared to the non-osteoporotic participants. The results found CTx-1 to be a more reliable indicator of bone status for the first 5 years after menopause compared to DPD (Park et al., 2018).

Table 1

Bone Biomarkers

Bone resorption markers	Bone formation markers
C-Terminal-Crosslinked Telopeptide (CTx-1)	Osteocalcin
N-Terminal-Crosslinked Telopeptide (NTX)	Procollagen type 1 N-terminal propeptide (PINP)
Urinary N-telopeptide of type 1 collagen (NTx)	Bone-specific alkaline phosphatase
	Procollagen type 1 c-terminal propeptide (PICP)

2.9.4 CTx-1 response to nutrients

Evaluating CTx-1 levels in response to consuming different nutrients could highlight the nutrients effect on the bone turnover cycle. Fruit and vegetable intake has been shown to influence CTx-1 level. One study rotated participants between a low fruit and vegetable diet and a high fruit and vegetable diet. The results showed that CTx-1 levels were higher when participants were on the low fruit and vegetable diet in comparison to when they were on the high fruit and vegetable diet. (Cao et al., 2018). In regards to energy, research found there were no significant differences in CTx-1 levels following meals varying between 250 to 1000kcal (Elnenaei et al., 2010).

For calcium, 1000mg from cow's milk or calcium carbonate was effective in reducing CTx-1 in a sample of lean and overweight postmenopausal women (Sharma et al., 2022). A lowering effect for CTx-1 was also found in postmenopausal Chinese women who consumed a calcium and vitamin D fortified milk compared to the control who had regular milk over the 1 year study period (Kruger et al., 2018). Magnesium was also shown to affect CTx-1 long term; postmenopausal women with lower dietary magnesium had higher CTx-1 levels over two years (Wright et al., 2019).

2.9.5 Summary of findings

Bones are constantly undergoing remodelling throughout the lifecycle. This balance shifts as women enter menopause and the physiological levels of oestrogen decline. Bone resorption surpasses bone formation leading to overall bone loss for postmenopausal women. This mechanism puts postmenopausal women at a greater risk of developing osteoporosis.

However, research has shown there are modifiable factors such as nutrition and physical activity to help reduce this bone loss. Both BMD measurement through DXA and bone biomarkers such as CTx-1 serve as important measurement tools in the research to help find correlations and establish relationships.

Chapter 3 Manuscript

3.0 Abstract

Background: Osteoporosis is associated with an increased risk of bone fractures and carry a significant burden on the individual and the health care system. Of these individuals, postmenopausal women have a high risk for developing osteoporosis due to decreased oestrogen production.

Objectives: This study explored the relationship between nutrient intake and bone health in postmenopausal women. Specifically, C-terminal of telopeptide of type 1 collagen (CTx-1), an indicator of bone turnover, and bone mineral density (BMD) were analysed in relation to the identified nutrient patterns. Correlations with additional markers associated with bone metabolism, parathyroid hormone (PTH), BMD, and vitamin D, were also examined.

Method: The present study was an analysis using baseline data from the COPES 4 Bones project. Eighty seven postmenopausal women participated (aged 48-77). Questionnaires were used to obtain participant demographics and physical activity levels. A three-day food record was self-reported by the participants. BMD was measured using dual x-ray absorptiometry. Weight, height and BMI were measured using standard techniques. Fasted venous blood samples were collected to measure CTx-1, 25-hydroxyvitamin D_3, and PTH. Nutrient patterns were derived from 3-day food records using principal component analysis. Linear regression was used to explore the association between BMD and the nutrient patterns. Age, weight, serum vitamin D, and physical activity were confounders in the model.

Results: Four nutrient patterns were identified from the data. Nutrient pattern 1 (NP1) was characterised by high amounts of phosphorus, protein, zinc, and niacin equivalents. Nutrient pattern 2 (NP2) was characterised by high amounts of dietary fibre, magnesium, potassium and a low amount of saturated fat. Nutrient pattern 3 (NP3) was characterised by high amounts of monounsaturated fat, vitamin E, polyunsaturated fat and a low amount of carbohydrates. Nutrient pattern 4 (NP4) was characterised by high amounts of alpha carotene and beta carotene. One significant negative correlation was identified between NP2 and hip BMD without consideration of confounders.

Conclusion: The current study identified NP2 as a potential factor contributing to bone health in a model exclusive of confounders such as weight and age. The research found weight to be positively associated with BMD. While dietary patterns were not explicitly studied, a diet consisting of high fruit and vegetable intake may be inferred from the characteristics of NP2. A negative relationship between a diet of this form and BMD challenges previously reported findings in postmenopausal women. Therefore, this work prompts future research to better characterise the role of nutrient intake in modulating bone health.

3.1 Introduction

Osteoporosis is the disease of brittle bones which is associated with fracture risk. Osteoporotic fractures affect 1 in 3 females and 1 in 5 males in New Zealand (Osteoporosis New Zealand, 2017). The 3,800 New Zealand hip fracture patients in 2007 cost the healthcare system $105 million. The cost can continue to rise with long term care needed in 50% of hip fracture patients (Osteoporosis New Zealand, 2020). Bone loss is inevitable with ageing, but modifications can slow the process to ward off osteoporosis, to improve quality of life and reduce avoidable healthcare expenditure.

Postmenopausal women face an increased risk of osteoporosis due to a decline in their physiological oestrogen production. Oestrogen is a regulator for bone degradation and therefore a decrease in this hormone leads to increased bone resorption. The increased bone loss for postmenopausal women puts them at greater risk of osteoporosis (Marieb & Hoehn, 2015). Therefore, more effort is required to reach a higher bone density in younger age and minimise bone loss in adult life.

Bone tissue is a dynamic structure that is constantly undergoing remodelling. Bone mineral density (BMD) is maintained by the balance between multiple different types of bone cells in the body. Osteoblasts synthesize the bone mineral matrix. Osteoclasts break down and degrade bone minerals. Osteocytes exist within and maintain the bone mineral matrix by regulating the exchange of nutrients and waste (Marieb & Hoehn, 2015). During youth, the bone is growing and developing so bone accumulation exceeds bone loss and during aging bone loss exceeds bone accumulation.

Lifestyle changes will contribute to the incidence of osteoporosis. Dietary changes leading to decreased intake of vitamins and minerals, decreasing exposure to the sun and increased sedentary behaviour are examples of behaviour change putting the population at greater risk of osteoporosis. The 2022 New Zealand Health Survey reflected that only 46.8 % of the adults reached the recommended 150 minutes of physical activity per week and 14% of adults reported doing little to no exercise (Ministry of Health, 2023). Exercise is a modifiable behaviour that influences bone turnover by stimulating the loading sensors in osteocytes and promoting bone formation (Uda et al., 2017). The results from the survey also reflected that 10% of adults met their recommended intake of vegetables and 49% met the recommended intake of fruit. From the 2008 New Zealand Adult Nutrition Survey, 4.9% of the adult population were vitamin D deficient and 27.1% were below the recommended ranges (Ministry of Health, 2012).

Nutrient intake can be analysed through nutrient patterns where the effect of nutrient combinations rather than the individual effect can be investigated. This has been done previously in postmenopausal women in relation to their bone health. A study identified a nutrient pattern characterised by riboflavin, phosphorus and calcium to be positively associated with the BMD of postmenopausal women.

Additionally, a nutrient pattern of dietary fats and fatty acids was negatively associated with BMD (Ilesanmi-Oyelere et al., 2019). Further research found other nutrients patterns to be negatively associated with osteopenia in postmenopausal women. One of these nutrient patterns was characterised by an intake of vitamin B_{12}, pantothenic acid, phosphorus, riboflavin, protein, vitamin B_6, potassium, vitamin D, vitamin E, calcium cholesterol, β-carotene, omega 3, magnesium, zinc, niacin, and selenium. The other nutrient pattern was characterised by an intake of iron, vegetable protein, thiamine, folate, fibre, PUFA, vitamin A, vitamin K, alpha-tocopherol, copper, sodium, and retinol (Grili et al., 2023). Overall research has found significant relationships between nutrient patterns and BMD in postmenopausal women.

Dual energy X-ray absorptiometry (DXA) is the gold standard method for measuring BMD. It provides a T-score that can be compared by reference ranges for osteopenia and osteoporosis diagnoses established by the World Health Organisation (Blake & Fogelman, 2007). Bone biomarkers such as C-terminal of telopeptide of type 1 collagen (CTx-1) that are measured through blood samples can offer another insight into bone status. CTx-1 is a bone turnover biomarker that is a by-product of bone resorption and therefore elevated levels reflect bone breakdown (Greenblatt et al., 2017).

There is limited research assessing CTx-1 and nutrient intake in postmenopausal women. One study investigated the acute effect of a 1000mg dosage of calcium through cow's milk or calcium carbonate on CTx-1 in postmenopausal women. The cow's milk was found to have a stronger effect than calcium carbonate on decreasing serum CTx-1 levels (Sharma et al., 2022). A long-term effect was measured in a year-long trial investigating the consumption of calcium and vitamin D fortified cow's milk in postmenopausal women. The fortification group showed significantly lower CTx-1 levels than the control group having regular milk (Kruger et al., 2018). Dietary magnesium intake has also been associated with CTx-1 changes. Over two years postmenopausal women with a low magnesium intake were associated with higher CTx-1 levels (Wright et al., 2019).

Life span in New Zealand increased by 4.62% from 2000 to 2020 (World Health Organisation, 2024) and therefore people are spending a greater proportion of their life in older age. Prevention of osteoporosis is crucial to avoid increased rates of the disease and corresponding increases in the personal and financial burdens that the disease incurs. This study investigated the relationships between nutrient patterns and BMD and CTx-1 in postmenopausal women, and the relationships between CTx-1 and PTH, CTx-1 and BMD, Vitamin D and PTH.

3.2 Methodology

3.2.1 Study Design

This analytical cross-sectional study investigated the associations of nutrient patterns, BMD and CTx-1 in postmenopausal women. It is a secondary study that used the baseline data from a post-doctoral research project, COPES 4 Bones. COPES 4 Bones was a randomised human placebo-controlled trial investigating the effect of exercise and pre and probiotic supplementation on postmenopausal women's bone health (Ilesanmi-Oyelere et al., 2021). The data collection from participants began on the 11th of May 2022 and ended on the 18th of November 2022. This study was completed in the Human Nutrition Research Unit at Massey University, Palmerston North, New Zealand.

3.2.2 Ethics and Trial Registration

The study was registered at the Australian New Zealand Clinical Trials Registry (ANZCTR), Trial registration number: ACTRN12620000998943p. Health and Disability Ethics Committee (HDEC) of New Zealand approved the study with the ethics reference number 21/NTB/47 (Appendix 1).

3.2.3 Sample Size

The sample size calculation was calculated for the COPES 4 Bones study where BMD and CTx-I were the primary outcome variables. For these variables a sample size of one hundred was required to detect a significant difference between BMD and CTx-I and exercise and pre and probiotic supplementation, with 80% power, 5% statistical significance and a buffer dropout rate of 20%. However, the COPES 4 Bones clinical study was only able to recruit ninety-two participants with three drop-outs and two with missing data, resulting in eighty-seven women completing the study.

3.2.4 Participants

There were eighty-seven females that completed the study. Participants were recruited from the Manawatu-Whanganui region of New Zealand in March of 2022 by a recruitment agency named Trial Facts (https://trialfacts.com/). All study participants read the information sheet and signed written consent forms (Appendix 2) . The inclusion criterion of females was menopause confirmed by at least five years of no menstruation. Body Mass Index (BMI) of all participants was between 17 and 40 kg/m^2.
The exclusion criteria of participants:
Confirmed by medical history and/or measurements:

1. Use of hormone replacement therapy (HRT).
2. Bisphosphonates in last six months.
3. Currently on estrogen, tamoxifen, aromatase inhibitors or other antiresorptive or anabolic treatment of osteoporosis.
4. Liver function test or creatinine above the normal range, or any other history suggesting liver or kidney disease to be confirmed by the baseline screening.

5. Incidence of diabetes mellitus, established by using the questionnaire and baseline screening.
6. Subjects with an estimated BMD T-score lower than -2.5 or fragility fracture in the previous six months.
7. Smoking and intake of alcohol > 2 units per day.

To be confirmed by the baseline questionnaire:

1. Intake of multivitamins and mineral supplements (prescribed or over the counter), or use of any other medication known to affect the bone metabolism.
2. Presence of any systemic disease.
3. Use of any medications such as HRT, glucocorticoids, oestrogen, systemic cortisone, bisphosphonates, diuretics, or other steroid hormones.

3.2.5 Data Collection

Baseline Questionnaire

Participants completed a health screening and baseline questionnaire asking for information regarding their sociodemographic, medication in the last 6 months, smoking status and alcohol intake (Appendix 3). Where any concerns arose from these tests, participants were advised to discuss them with their doctor.

Body Composition

Anthropometry measurements included body weight using a scale to the nearest 0.1kg and height using a stadiometer to the nearest 0.1cm with no shoes and light clothing. This was required for the calculation of BMI using the equation weight (kg) / height^2 (m). Other measurements included waist and hip circumference to the nearest 0.1cm that was measured with a non-stretchable tape measure to determine the waist to hip ratio. Weight was used instead of BMI as a confounder in the linear regression model due to it having a greater correlation with BMD.

Dual-Energy X-ray Absorptiometry (DXA) measured participants' body composition including muscle mass and body fat percentage, bone mineral content (BMC), BMD and provided T-scores of the femoral neck, lumbar spine and hip (Horizon A bone densitometer, Hologic, Marlborough, Massachusetts, US). The scanner was operated by a trained technician. Quality control was performed daily by standardisation of the densitometer to a standard phantom before the first participant scan as well as after the last participant for the day.

Dietary Assessment

The dietary intake of participants was measured by using a 3-day diet diary (Appendix 4). The 3-day diet record covered non-consecutive days and included one weekend day. The amounts of food were

measured by participants using the household measuring instruments. No supplements were included in the nutrient analysis.

The diet records were then entered into FoodWorks 10 to complete the nutrient analysis. The nutrient analysis was used to identify the nutrient patterns described below. The measured macronutrients included protein, fat, cholesterol, saturated fat, monounsaturated fat, polyunsaturated fat, carbohydrates and dietary fibre. The measured micronutrients included niacin equivalents, vitamin C, vitamin D, vitamin E, vitamin K, folate, total vitamin A, retinol, beta carotene, alpha carotene, calcium, sodium, potassium, magnesium, phosphorus, iron and zinc.

Physical Activity

The New Zealand Physical Activity Questionnaire short form was used to measure participants' physical activity level to provide their daily metabolic equivalent of tasks (METs) (Appendix 5). This result allowed participants' active energy expenditure (AEE) to be calculated using the equation, METs x 3.5 x (body weight in kg) / 200 = kcal/min.

Blood sampling

Venous fasting blood samples were collected by a phlebotomist between 8-10am after 12 hours of fasting. Serum/plasma concentration of 25-hydroxyvitamin D_3 ($25(OH)D_3$), parathyroid hormone (PTH) and the bone metabolism biomarker cross-linked C-telopeptide of type 1 collagen (CTx-1) were measured.

3.2.6 Data handling

Within the results of the research there were outliers and missing data. Statistical outliers were identified by plotting the results graphically. To improve the validity of the data, outliers and missing data were addressed. Outliers were addressed for both the physical activity and nutrient statistics. Physical activity outliers were adjusted using the truncation of data (Craig et al., 2003). Therefore walking, moderate and vigorous exercise minutes could not exceed 180 minutes each. Nutrient intake outliers were identified using the accepted energy range established by Willett (2012) of between 2,100kJ and 14,700kJ. Results outside this range had the diet record re-entered into FoodWorks 10 and recalculated. There is a small amount of missing data in the COPES 4 Bones data. This data was imputed using Multivariate Imputation by Chained Equations (mice). Missing values for CTx-1, PTH, serum $25(OH)D_3$ and vitamin K were imputed using mice (the predicted means measure), with 5 imputations and 20 iterations. Predictors used were age, BMI, blood measures, physical activity measure and nutrient values. Missing values were not imputed for BMD (as an outcome). There was no dietary data for 2 participants and this data was not imputed nor used for the nutrient patterns.

3.2.7 Nutrient patterns

Not all of the measured nutrients were included in the nutrient patterns. Total vitamin A was removed as it represented the combination of retinol, alpha carotene and beta carotene. Vitamin K was removed due to it not being measured in the majority of the foods on the Food and Composition database. Total fat was removed as it represented the combination of monounsaturated fat, polyunsaturated fat and saturated fat. Sodium was excluded from the nutrient patterns as the number of nutrients to be included in the pattern was limited due to sample size and sodium does not hold a direct role in bone health. The nutrient intakes were standardised by energy intake before completing the principal component analysis (PCA).

The nutrient data was first checked for suitability for PCA using the Bartlett test of sphericity and the Kaiser, Olkin Measure of Sampling Adequacy. PCA and varimax rotation was used to identify the nutrient patterns. Four patterns were obtained based on the scree plot, eigenvalue (> 2) and interpretability of patterns. Factor loadings measure the relative contribution (correlation) of the nutrient to a nutrient pattern. Positive loadings contribute to nutrient patterns, whereas negative loadings have an inverse association to the nutrient patterns. For a sample size of 80, a critical loading would be 0.572 (Stevens, 2009). Therefore only loadings greater than 0.572 or less than -0.572 were considered critical elements of the nutrient pattern. A standardised dietary pattern score was calculated per participant per nutrient pattern using the regression method.

3.2.8 Statistical analyses

The statistical package used was IBM SPSS statistics package version 24 (IBM corporation, New York, USA).

Participants were described with demographic, bone densitometry and nutrient analysis data. Means and standard deviations were used as the data was assumed to be normally distributed based on Central Limit Theorem. Categorical data was described using frequency and percentages. Correlations between variables were tested using Pearsons's correlation coefficient.

A multiple linear regression model was used to determine if the nutrient patterns had a relationship with BMD or CTx-1. The first model contained the nutrient pattern scores and the outcomes of spine, hip and femoral neck BMD or CTx-1 and was unadjusted. The second model added the confounding variables. Confounder variables for BMD included in the model were weight, age, serum 25(OH)D_3 and MET. Age was the only confounder variable for CTx-1. These confounders were added as they can affect BMD and CTx-1 independently to the nutrient pattern scores. The models were checked for collinearity using the Durbin Watson test. The enter method was used for selecting predictor variables.

There were no log transformations to the data. Plots were used (residuals v fitted, histogram) to examine linearity and homogeneity of the models. A p-value of 0.05 was considered significant.

A sensitivity analysis was completed for the correlations between CTx-1 and PTH, CTx-1 and BMD and PTH and 25(OH)D_3. This was done by repeating the correlations with the original variables rather than the imputed variables and comparing the results to check the results matched.

3.3 Results

3.3.1 Demographic data

Eighty-seven participants completed the study. The data was imputed for the missing variables PTH, CTx-1 and 25(OH)D_3 for 11 participants and dietary vitamin K was imputed for 1 participant. Demographic statistics of participants are reported in Table 1. The age range of the women was 48-77 years. The mean BMI was 26.52 kg/m^2 which falls in the overweight range of 25-30 kg/m^2 for BMI (Nutrition Foundation, 2022). The ethnicity of the majority of the participants was New Zealand European at 83.9%. The serum 25(OH)D_3 mean was 79.75 nmol/L, which is within the recommended range for bone health of 50-150nmol/L (Canterbury Health Laboratories, 2024c). The mean CTx-1 value was 0.37 ug/L which is within the adult reference range of <0.75ug/L (Canterbury Health Laboratories, 2024a). The mean PTH value was 4.01pmol/L which is within the reference range of 1.6-7.0pmol/L (Canterbury Health Laboratories, 2024b).

Table 1: Demographic statistics of participants

Characteristics	N	Mean ± SD or n (%)	Reference range
Age (yr.)	87	64.84 ± 5.74	
Weight (kg)	87	70.05 ± 13.10	
Height (m)	87	1.63 ± 0.06	
BMI (kg/m^2)	87	26.52 ± 4.93	
Ethnicity	87	Australia European 1(1%) Irish 1 (1%) Māori/New Zealand European 6 (7%) New Zealand European 73 (84%) Other British 2 (2%) Other Dutch 1 (1%) Other French 1 (1%) South African European 2 (2%)	
Alcohol Intake	87	Yes 71 (82%) No 16 (18%)	
MET (min/day)	86	295.79 ± 300.51	
AEE (kJ/day)	86	354.18 ± 359.22	
CTx-1* (ug/L)	87	0.37 ± 0.17	<0.75
PTH* (pmol/L)	87	4.01 ± 1.36	1.6-7.0
Serum 25(OH)D_3* (nmol/L)	87	79.75 ± 31.31	50-150
Nutrient Pattern 1 Score [a]	85	0.0 ± 1	
Nutrient Pattern 2 Score [a]	85	0.0 ± 1	
Nutrient Pattern 3 Score [a]	85	0.0 ± 1	
Nutrient Pattern 4 Score [a]	85	0.0 ± 1	

MET = Metabolic Equivalents
AEE = Activity energy expenditure
CTx-1 = C-terminal of the type 1 collagen
PTH = Parathyroid hormone
25(OH)D_3 = 25-hydroxyvitamin D_3
*Imputed using multivariate imputation by chained equations (mice) (n=11)
[a] Mean value of 0 and SD of 1 shows the nutrient pattern scores have been standardised

Bone densitometry

Bone densitometry measurements including the BMD of hip, femoral neck and spine and the corresponding T-scores are reported in Table 2. The mean femoral neck, hip and spine BMD were 0.7 g/cm^3, 0.82 g/cm^3 and 0.94 g/cm^3 respectively. The mean hip T-score was -1.34 and the mean spine T-score was -1.09, which are both in the osteopenia T-score range of -2.5 to -1. From spine BMD, 47.1% of participants had normal classified T-score while 52.9% met either the osteopenia or osteoporosis diagnosis criteria.

Table 2: Bone densitometry measurements

Bone densitometry	N	Mean ± SD or n (%)	Reference range
FM BMD (g/cm^3)	82	0.70 ± 0.10	
Hip BMD (g/cm^3)	82	0.82 ± 0.12	
Hip T-score	82	-1.34 ± 0.91	
Spine BMD (g/cm^3)	87	0.94 ± 0.16	
Spine T-score	87	-1.09 ± 1.42	
Osteoporosis classification based on Spine T-score	87	Normal 41 (47.1%) Osteopenia 30 (34.5%) Osteoporosis 16 (18.4%)	Normal: >-1 Osteopenia: -2.5 to -1 Osteoporosis: <-2.5
BMD = Bone Mineral Density FM = Femoral neck Osteoporosis T-score reference range (*Kanis, 2007)*			

Nutrient analysis

The macronutrient results are reported as means in Table 3. The vitamin and mineral results are reported as means in Table 4 and Table 5 respectively. The New Zealand Nutrient Reference Values (NRVs) were used to compare the group's average intakes against recommendations. Estimated average requirement (EAR) and adequate intake (AI) values were used for vitamins and minerals. The reference value for the demographic of women aged 51-70 years was used for the comparison. The majority of the nutrients met their respective NRVs. Vitamin D, Vitamin K and calcium were below their NRV. However, it is important to note that the average serum $25(OH)D_3$ level of participants was within the recommended range and the NRV for dietary vitamin D is set with the assumption of no to little UV exposure (Ministry of Health, 2017). Therefore, the participants' low dietary vitamin D intake does not imply inadequate $25(OH)D_3$. Additionally, the average vitamin K intake of the participants is likely inaccurately low due to the vitamin K value being missing from the majority of foods in the FoodWorks database. Consequently, the sole significant micronutrient the sample was below the NRV for was calcium. There are no specific requirement recommendations for energy, protein, fat and carbohydrates. Instead there is the acceptable macronutrient distribution range (AMDR) which is 15-20% of energy from protein, 45-65% of energy from carbohydrates and 20-35% of energy from fat (Ministry of Health, 2017). From the means of the sample's energy and macronutrient intake, the distribution of protein, carbohydrates and fats were 16%, 38% and 37% respectively.

Table 3: Estimated macronutrient intake from FoodWorks analysis of three-day food records (n=85)

Macronutrients	Mean ± SD
Energy (kJ)	8536.36 ± 2374.33
Protein (g)	85.88 ± 26.60
Total Fat (g)	84.60 ± 30.03
Saturated Fat (g)	32.53 ± 14.63
Polyunsaturated Fat (g)	12.68 ± 7.30
Monounsaturated Fat (g)	30.10 ± 11.73
Cholesterol (mg)	280.07 ± 168.8
Carbohydrate available (g)	205.10 ± 77.85
Dietary Fibre (g)	27.69 ± 10.02

Table 4: Estimated vitamin intake from FoodWorks analysis of three-day food records (n=85)

Vitamins	Mean ± SD	NRV
Vitamin C (mg)	114.27 ± 89.09	30[a]
Vitamin D (µg)	6.64 ± 5.24	10[b]
Vitamin E (mg)	9.50 ± 4.48	7 [b]
Vitamin K* (µg)	14.43 ± 16.11	60 [b]
Niacin equivalents (mg)	38.93 ± 13.26	11[a]
Total Vitamin A equivalents (µg)	976.34 ± 500.02	500[a]
Retinol (µg)	346.02 ± 222.63	-
Beta carotene (µg)	2573.24 ±2455.52	-

NRV = Nutrient reference value
*Imputed using multivariate imputation by chained equations (mice) (n=1)
[a] EAR
[b] AI

Table 5: Estimated mineral intake from FoodWorks analysis of three-day food records (n=85)

Minerals	Mean ± SD	NRV
Potassium (mg)	3539.95 ± 1188.01	2800 [b]
Sodium (mg)	2625.48 ± 1793.70	460-920 [b]
Calcium (mg)	909.79 ± 353.12	1100 [a]
Phosphorus (mg)	1487.16 ± 446.75	580 [a]
Magnesium (mg)	372.63 ± 124.55	265 [a]
Zinc (mg)	10.35 ± 3.49	6.5 [a]
Iron (mg)	12.92 ± 6.00	5 [a]

NRV = Nutrient reference value
[a] EAR
[b] AI

3.3.2 Nutrient Pattern Data

The principal component analysis identified four nutrient patterns shown in table 6, which explained 58% of the variation in dietary intake. The Kaiser–Meyer–Olkin measure of sampling adequacy was 0.63, and Bartlett's test of sphericity was significant ($p < 0.0001$), indicating the dietary data set was

suitable for principal component analysis. Table 6 displays the nutrient pattern loadings, range of nutrient pattern scores, and the variance of the total diet explained by each nutrient pattern.

NP1 was characterised by high amounts of phosphorus, protein, zinc, and niacin equivalents. NP2 was characterised by high amounts of dietary fibre, magnesium, potassium and a low amount of saturated fat. NP3 was characterised by high amounts of monounsaturated fat, vitamin E, polyunsaturated fat and a low amount of carbohydrates. NP4 was characterised by high amounts of alpha carotene and beta carotene. The fourth pattern was not included in the analyses as it only had two loadings and therefore was not considered reliable (Stevens, 2009).

Table 6: Loadings for the nutrient patterns calculated using principal component analysis

Energy adjusted nutrients	NP1	NP2	NP3	NP4
Protein	**0.83**	-0.18	-0.02	0.09
Saturated Fat	-0.01	**-0.59**	0.06	0.03
Polyunsaturated fat	-0.13	0.16	**0.83**	-0.06
Monosaturated Fat	-0.12	-0.21	**0.78**	0.15
Cholesterol	0.44	-0.38	-0.01	0.49
Carbohydrate available	-0.30	0.44	**-0.70**	-0.20
Dietary fibre	0.22	**0.85**	0.17	0.12
Niacin Equivalents	**0.65**	0.17	0.01	0.23
Vitamin C	-0.13	0.41	-0.10	0.22
Vitamin D	0.12	-0.23	0.28	-0.29
Vitamin E	-0.05	0.17	**0.82**	0.06
Total Folate	0.43	0.30	0.03	0.14
Retinol	0.28	-0.52	0.13	0.17
Beta Carotene	0.12	0.37	0.25	**0.73**
Alpha Carotene	0.22	0.00	0.05	**0.76**
Potassium	0.49	**0.63**	0.17	0.21
Magnesium	0.42	**0.62**	0.54	-0.03
Calcium	0.46	0.11	-0.09	-0.44
Phosphorus	**0.86**	0.05	0.11	-0.28
Iron	0.52	0.04	-0.07	0.09
Zinc	**0.68**	-0.21	-0.03	0.0
Participant score range	-2.13 – 2.32	-2.65 – 2.56	-2.14 – 3.22	-3.11 – 2.80
Variance explained	19%	15%	14%	10%
Loadings ≥ 0.57 or ≤ -0.57 A higher loading indicates a greater contribution to the dietary pattern. Positive loadings are positively associated, and negative loadings are negatively associated with the nutrient pattern.				

3.3.3 Correlations

Correlations between CTx-1 and PTH, and CTx-1 and BMD were tested. As shown in Table 7, there were no significant correlations found between CTx-1 and PTH, hip, spine or femoral neck BMD ($p>0.05$).

Table 7: Correlation of CTx-1 and PTH, hip, spine, and femoral neck BMD

	Statistic	PTH (pmol/L)*	Hip BMD (g/cm^3)	Spine BMD (g/cm^3)	Femoral Neck BMD (g/cm^3)
CTx-1 (ug/L)*	r	0.104	0.018	0.120	0.044
	n	87	82	87	82
	p	0.336	0.871	0.268	0.694
CTx-1 = C-terminal of the type 1 collagen PTH = Parathyroid hormone BMD = Bone mineral density *Imputed using multivariate imputation by chained equations (mice) (n=11)					

The relationship between PTH and serum 25(OH)D_3 was investigated. PTH was found to be significantly negatively correlated with 25(OH)D_3 with a medium effect (p=0.033), as shown in Table 8. After controlling for age, a confounder of PTH, the correlation between PTH and 25(OH)D_3 increased slightly.

Table 8: Correlation of 25(OH)D_3 and PTH

	Statistic	PTH (pmol/L)*
Serum 25(OH)D_3 (nmol/L)*	r n p	-0.229 87 0.033
Adjusted for Age		
Serum 25(OH)D_3 (nmol/L)*	r n p	-.249 84 0.021
25(OH)D_3 = 25-hydroxyvitamin D_3 PTH = Parathyroid hormone *Imputed using multivariate imputation by chained equations (mice) (n=11)		

3.3.4 Linear regression

Three of the four nutrient patterns (NP1, 2 & 3) were entered into the multiple linear regression. None of the models contained collinearity. This was checked using the Durbin Watson value. The Durbin Watson statistics were between 1.915 and 2.357 for all models. This is within the acceptable range of 1.5 and 2.5. Residual plots and histograms suggested the models were linear and variances were homogenous.

BMD Model 1

BMD Model 1 represents the regression analysis of the associations between hip, spine and femoral neck BMD and nutrient pattern scores with no adjustments, presented in Table 9. In BMD Model 1, only NP2 was associated with hip BMD. NP2 was negatively associated with hip BMD, standardised

beta = -0.232, p=0.04. No nutrient patterns were associated with spine BMD or femoral neck BMD. The nutrient patterns explained 6.0% of the variation in femoral neck BMD, 7.7% of the variation in hip BMD and 2.7% of the variation in spine BMD.

Table 9: Results of regression analysis for Model 1, showing associations between bone mineral density for hip, spine and femoral neck and the nutrient pattern scores.

	Unstandardised beta coefficient (standard error)	95% Confidence intervals for unstandardised beta	Standardised Beta coefficient	P-value
Femoral neck (n=81, R^2 = 0.060, df = 3, model p-value =0.19)				
NP1	-0.014 (0.11)	-0.036, 0.009	-0.0134	0.230
NP2	-0021 (0.11)	-0.044, 0.002	-0.205	0.067
NP3	-0.001 (0.11)	-0.023, 0.022	-0.007	0.949
Hip (n = 81, R^2 = 0.077, df = 3, model p-value = 0.10)				
NP1	-0.007 (0.012)	-0.032, 0.017	-0.064	0.56
NP2	-0.027 (0.013)	-0.051, -0.002	-0.232	0.04
NP3	-0.016 (0.012)	-0.041, 0.009	-0.0139	0.21
Spine (n = 85, R^2 = 0.027 , df = 3, model p-value = 0.533)				
NP1	-0.011 (0.018)	-0.046, 0.024	-0.069	0.529
NP2	-0.010 (0.018)	-0.045, 0.025	-0.063	0.570
NP3	-0.021 (0.018)	-0.056, 0.014	-0.134	0.226

BMD Model 2

Table 10 presents BMD Model 2, the regression analysis of the associations between hip, spine and femoral neck BMD and nutrient pattern scores with the confounders age, weight, MET and serum 25(OH)D_3. The association between NP2 and hip BMD, seen in BMD model 1 was no longer an association after controlling for confounders in BMD Model 2 (p=0.33). No other nutrient patterns were associated with BMD.

Positive associations were seen between weight and femoral neck, hip and spine BMD (p=<0.05). The results indicate an extra 10 kg of body weight increases femoral neck BMD between 0.01 and 0.05 g/cm^3, increases hip BMD between 0.03 and 0.07 g/cm^3 and increases spine BMD between 0.04 and 0.09 g/cm^3 (with 95% confidence).

The nutrient patterns and confounders, age, weight, MET and 25(OH)D_3 accounted for 17.4% of the variance in femoral neck BMD, 38.7% in hip BMD and 32.9% of the variance in spine BMD.

Table 10: Results of regression analysis for Model 2, showing associations between bone mineral density for hip, spine and femoral neck and the nutrient pattern scores with confounding variables age, weight, MET, 25(OH)D_3

	Unstandardised beta coefficient (standard error)	95% Confidence intervals for unstandardised beta	Beta Coefficient	P-value
Femoral Neck (n=81, R^2= 0.174, df = 7 , model p-value = 0.044)				
NP1	-0.012 (0.011)	-0.034, 0.009	-0.122	0.26.
NP2	-0.013 (0.011)	-0.035, 0.010	-0.123	0.273.
NP3	0.007 (0.011)	-0.016, 0.029	0.065	0.553.
Age (years)	-0.001 (0.002)	-0.005, 0.003	-0.034	0.755
Weight (kg)	0.003 (0.001)	0.001, 0.005	0.336	0.005
MET (min/day)	-1.212E-5 (0.0)	0.0, 0.0	-0.030	0.783
Serum 25(OH)D_3 (nmol/L)*	0.000 (0.0)	-0.001, 0.001	-0.044	0.698
Hip (n = 81, R^2 = 0.387, df = 7, model p-value = 0.10)				
NP1	-0.006 (0.011)	-0.027, 0.015	-0.056	0.550
NP2	-0.011 (0.011)	-0.033, 0.011	-0.095	0.325
NP3	-0.002 (0.011)	-0.024, 0.019	-0.019	0.840
Age (years)	0.001 (0.002)	-0.003, 0.005	0.058	0.54
Weight (kg)	0.005 (0.001)	0.003, 0.007	0.562	<0.001
MET (min/day)	-2.783E-5 (0.000)	0.0, 0.0	-0.062	0.511
Serum 25(OH)D_3 (nmol/L)*	0.000 (0.000)	-0.001, 0.001	-0.027	0.779
Spine (n = 85, R^2 = 0.329, df = 7, model p-value = <0.001)				
NP1	-0.011 (0.015)	-0.041, 0.019	-0.069	0.467
NP2	0.011 (0.016)	-0.020, 0.042	0.069	0.487
NP3	-0.001 (0.015)	-0.032, 0.029	-0.007	0.939
Age (years)	0.004 (0.003)	-0.001, 0.009	0.146	0.132
Weight (kg)	0.007 (0.001)	0.004, 0.009	0.532	<0.001
MET (min/day)	-3.450E-5 (0.0)	0.0, 0.0	-0.056	0.566
Serum 25(OH)D_3 (nmol/L)*	0.000 (0.0)	-0.001, 0.001	-0.039	0.695
MET = Metabolic Equivalents 25(OH)D_3 = 25-hydroxyvitamin D_3 *Imputed using multivariate imputation by chained equations (mice) (n=11)				

CTx-1 Model 1

CTx-1 Model 1 represents the regression analysis of the associations between CTx-1 and nutrient pattern scores reported in Table 11. In CTx-1 Model 1, no nutrient patterns were associated with CTx-1 ($p>0.05$).

Table 11: Results of regression analysis for Model 1, showing associations between serum CTx-1 and the nutrient pattern scores.

	Unstandardised beta coefficient (standard error)	95% Confidence intervals for unstandardised beta	Beta Coefficient	P-value
CTx-1 (n=85, R^2= 0.029 df = 3, model p-value = 0.496)				
NP1	-0.004 (0.019)	-0.043, 0.034	-0.026	0.816
NP2	-0.008 (0.019)	-0.046, 0.030	-0.046	0.674
NP3	-0.028 (0.019)	-0.066, 0.010	-0.161	0.144

CTx-1 Model 2

CTx-1 Model 2 represents the regression analysis of the associations between CTx-1 and nutrient pattern scores with the confounder of age shown in Table 12. In CTx-1 Model 2, no nutrient patterns were associated with CTx-1 ($p>0.05$).

Table 12: Results of regression analysis for Model 2, showing associations between serum CTx-1 and the nutrient pattern scores with confounding variable of age.

	Unstandardised beta coefficient (standard error)	95% Confidence intervals for unstandardised beta	Beta Coefficient	P-value
CTx-1 (n=85, R^2= 0.056, df = 4, model p-value = 0.327)				
NP1	-0.008 (0.019)	-0.046, 0.030	-0.044	0.689
NP2	-0.009 (0.019)	-0.47, 0.029	-0.050	0.650
NP3	-0.027 (0.019)	-0.065, 0.011	-0.152	0.166
Age (years)	0.005 (0.003)	-0.002, 0.012	0.165	0.136

3.3.5 Sensitivity analysis

A correlation analysis without imputed CTx-1, PTH and 25(OH)D_3 values was performed. There were no differences between the correlation results with and without imputed values. Table 13 shows the repeated correlations between CTx-1 and PTH and CTx-1 and BMD but with the original data for CTx-1 and PTH. There were no significant correlations. This is the same result seen with the imputed variables in Table 7. Table 14 shows the repeated correlations between 25(OH)D_3 and PTH with the original data. There was a significant negative correlation. This is the same result seen in Table 8 with the imputed variables.

Table 13. Correlation of original CTx-1 and original PTH, original hip, spine, and femoral neck BMD for sensitivity analysis

	Statistic	PTH (pmol/L)	Hip BMD (g/cm^3)	Spine BMD (g/cm^3)	Femoral Neck BMD (g/cm^3)
CTx-1 (ug/L)	r n p	0.100 76 0.391	-0.028 72 0.817	0.096 76 0.410	0.004 72 0.976
CTx-1 = C-terminal of the type 1 collagen PTH = Parathyroid hormone BMD = Bone mineral density					

Table 14: Correlation of original serum $25(OH)D_3$ and original PTH for sensitivity analysis

	Statistic	PTH (pmol/L)
Serum $25(OH)D_3$ (nmol/L)	r n p	-0.238 76 0.038
Adjusted for Age		
Serum $25(OH)D_3$ (nmol/L)	r n p	-.262 73 0.023
$25(OH)D_3$ = 25-hydroxyvitamin D_3 PTH = Parathyroid hormone		

3.4 Discussion

In this cross-sectional study, associations between nutrient patterns, BMD and CTx-1 of postmenopausal women were investigated. Correlations between CTx-1, PTH and BMD and correlations between 25(OH)D_3 and PTH were also investigated. Four nutrient patterns were identified from the analysis of the three-day food records. Only NP2 was found to be significantly associated with BMD. NP2 was characterised by high amounts of fibre, potassium and magnesium and a low amount of saturated fat. This nutrient pattern was negatively associated with hip BMD, but this association was attenuated when the confounders age, weight, MET and 25(OH)D_3 were included in the regression model. We observed no significant correlations between CTx-1, PTH and BMD. However, we did observe a negative correlation between 25(OH)D_3 and PTH.

PTH is produced by the parathyroid glands and maintains serum calcium balance by triggering bone degradation when serum calcium levels decrease (Marieb & Hoehn, 2015). The mean PTH in this study was 4.0pmol/L. This is within the acceptable reference range of 1.6-7.0pmol/L for adults (Canterbury Health Laboratories, 2024b).

CTx-1 is a byproduct of the bone degradation. CTx-1 is the c-terminal telopeptide from type 1 collagen that is released during bone resorption. The serum concentration of CTx-1 provides physiological insight into the balance of the bone turnover cycle (Greenblatt et al., 2017). CTx-1 concentrations increase as women become postmenopausal due to loss of bone turnover regulation and increased bone resorption that is associated with a decline in oestrogen.

The mean CTx-1 level of 0.37ug/L of the present study was within the acceptable CTx-1 reference range of <0.75ug/L for adults (Canterbury Health Laboratories, 2024a). Additionally, it was lower than the levels reported by Wright et al. (2019) who reported a mean CTx-1 of 0.47ug/L in a sample of postmenopausal women. In the present study, there was no significant correlation found between CTx-1 and PTH. A positive correlation between PTH and CTx-1 was expected. This is due to elevated levels of PTH indicating higher bone resorption and CTx-1 is a by-product of the bone resorption. Possible reasons for not seeing the expected results were the limited sample size of the study or because only participants with a PTH within the normal range were included in the study. The relationship between PTH and CTx-1 has been captured in the literature with treatment for hyperparathyroidism resulting in decreased serum CTx-1 levels (Rajeev et al., 2017).

Correlations between CTx-1 and BMD were also investigated. BMD is measured to provide insight into bone health and an individual's risk of osteoporosis. BMD is measured in postmenopausal women due to their increased risk of osteoporosis. When women enter menopause their physiological

concentrations of oestrogen decline. Oestrogen is a regulator for osteoclasts, the cells responsible for bone breakdown (Florencio-Silva et al., 2015). The loss of this regulator increases bone degradation and increases postmenopausal women's risk of osteoporosis.

The osteoporosis diagnosis criteria are defined by the WHO T-scores (Kanis, 2007). A T-score is calculated by measuring the individual's BMD and subtracting the average young adult BMD and dividing it by the young adult population standard deviation (Blake & Fogelman, 2007). Osteoporosis is defined by a T-score less than -2.5 and osteopenia is defined by a T-score between -2.5 and -1 (Kanis, 2007).

The prevalence of osteopenia and osteoporosis in the present study population, from classified T-scores of the spine BMD were 34.5% and 18.4% respectively. These prevalence rates are very similar to another cohort of New Zealand postmenopausal women who had a 52% prevalence of osteopenia and osteoporosis (Ilesanmi-Oyelere, 2020). Additionally, it is lower than a sample of Chinese postmenopausal women who had a 46.6% rate of osteopenia and a 25.0% rate of osteoporosis at the spine BMD (Gao et al., 2017).

An inverse correlation between CTx-1 and BMD was expected due to lower CTx-1 indicating less bone degradation (Greenblatt et al., 2017) and therefore higher BMD. In the present study, there was no significant correlation between the two variables. A negative correlation between these variables has been seen before in the literature. Gao et al. (2017) reported a negative relationship between CTx-1and BMD in postmenopausal women. Furthermore, Wei et al. (2021) found a negative correlation between CTx-1 and spine BMD in postmenopausal women with normal BMD. A potential reason this relationship was not observed in the present study was because CTx-1 is a marker of recent bone activity whereas BMD is a reflection of longer-term bone activity.

1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D)_3$ is the biologically functional form of vitamin D that has an important role in bone activity by facilitating the active transport of calcium across the intestinal wall (Christakos & Pike, 2020). Furthermore, serum $25(OH)D_3$ is the form of vitamin D that is measured to reflect sufficiency. The mean $25(OH)D_3$ of the women in the present study was 80nmol/L. This is within the reference range for optimum bone health of 50-150nmol/L (Canterbury Health Laboratories, 2024c). It is also greater than 72nmol/L which is recommended by the Endocrine Society for optimal bone and muscle health (Holick et al., 2011). Another study of New Zealand postmenopausal women found a similar average $25(OH)D_3$ levels of 78nmol/L (Ilesanmi-Oyelere, 2020). This was different in comparison to other studies with 55nmol/L being the average for a sample of postmenopausal Chinese women reported by Gao et al. (2017) and 89nmol/L being the average reported for a group of Black South African postmenopausal women reported by Wright et al. (2019).

It is important to consider the seasonal changes in serum $25(OH)D_3$ levels. The variation seen across the different study populations may be due to the differences in seasons when $25(OH)D_3$ was measured. Additionally, the present study commenced data collection in May which is the end of autumn and finished data collection in November which is the end of spring in the southern hemisphere. The changes in seasons during data collection may have influenced $25(OH)D_3$ levels in the present study. Another influence on the variation of vitamin D status across the study populations is ethnicity. Melanin in the skin reduces ultra violet light absorption. Darker skin tones have greater concentrations of melanin and therefore require more ultra violet light exposure for the synthesis of $25(OH)D_3$ (Laird et al., 2010).

The present study investigated the relationship between $25(OH)D_3$ and PTH. It found a negative correlation (with moderate effect) between $25(OH)D_3$ and PTH before and after adjusting for the confounder age. This was expected due to the physiological relationship the two hormones share. $1,25(OH)_2D_3$ facilitates intestinal calcium absorption, as previously mentioned and therefore helps regulate serum calcium levels. If serum $25(OH)D_3$ levels are sufficient, intestinal calcium absorption would be adequate and PTH would not be required to mobilise calcium stores from the bone (Laird et al., 2010).

Previous studies found this inverse correlation with PTH and vitamin D status in both men and women. Dawson-Hughes et al. (1997) found dietary calcium intake did not influence the negative correlation between PTH and serum $25(OH)D_3$. A recent study which found the same negative correlation also found a higher dietary calcium intake reduced the PTH levels in the vitamin D insufficient participants (<24.96nmol/L) (Steingrimsdottir et al., 2005). This negative correlation highlights the importance of maintaining sufficient levels of $25(OH)D_3$ through safe sun exposure, supplementation and/or dietary consumption of vitamin D. As sufficient levels can reduce elevated levels of PTH which will reduce the calcium that is extracted from the bone and minimise the loss in BMD.

The average nutrient intake of the postmenopausal women met the majority of the recommended values. Vitamin D, vitamin K and calcium were the only measured micronutrients that did not meet their nutrient reference values (NRV). The calcium NRV is higher for postmenopausal women compared to younger males and females due to their accelerated bone loss. It is the mineral of the highest concentration in the bone. Calcium is also an essential factor for the nervous system. If there is insufficient calcium supplied in the diet for these essential processes then it will be mobilised from the bone which will result in decreased BMD (Marieb & Hoehn, 2015).

Vitamin D, as previously mentioned, is important for bone health and while food is one way of getting vitamin D it is not the most important way. The body can synthesize it through an ultraviolet-light dependent reaction (Laird et al., 2010). Considering the average serum $25(OH)D_3$ of the participants fell within the recommended range for bone health and the NRV for dietary vitamin D is set under the condition of minimal sun exposure (Ministry of Health, 2017), it is not a significant factor that the average dietary intake of vitamin D for participants was below the NRV. Vitamin K is also an important micronutrient for bone health as it is required for the gammacarboxylation of osteocalcin (Anderson et al., 2012). Osteocalcin is a bone protein expressed by osteoblasts, it binds calcium ions to the bone with its calcium binding Gla residues (Zoch et al., 2016). The mean intake of dietary vitamin K of the participants is likely underestimated due to the FoodWorks database not accurately measuring vitamin K in a significant proportion of the foods. Therefore, it is difficult to determine whether the sample was meeting the NRV. Consequently, it is important to note the study sample of postmenopausal women on average did not meet the NRVs for at least one significant bone nutrient, calcium.

From the means of the sample's energy and macronutrient intake, the distribution of protein, carbohydrates and fats was 16%, 38% and 37% respectively. Therefore, the average macronutrient distribution of the participants was higher in fat and lower in carbohydrates than the AMDR (Acceptable Macronutrient Distribution Ranges) (Ministry of Health, 2017). Research in pre and perimenopausal women showed dietary fat was positively associated with calcium absorption (Wolf et al., 2000).

The average saturated fat intake in the present study contributed to 14% of the average energy intake. This exceeds the NRV of less than 10% of energy coming from saturated fat (Ministry of Health, 2017). Saturated fat was shown to negatively affect intestinal calcium absorption due to the formation of soaps (Gacs & Barltrop, 1977). Corwin et al. (2005) found saturated fat to have a negative effect on men's BMD but not on women's.

In regards to unsaturated fat, research showed rats fed a diet where omega 3 was the main source of fat increased calcium absorption and BMD in comparison to rats fed a diet where omega 6 was the main source of fat (Kruger & Schollum, 2005). Lavado-Garcia et al. (2018) found the dietary intake of polyunsaturated omega 3 to be positively associated with BMD in normal and osteopenic Spanish women.

Nutrient patterns allow for the effect of combinations of nutrients to be investigated. This is beneficial as nutrients are found in combinations within food. The analysis of the present study identified four nutrient patterns. NP1 was characterised by high amounts of phosphorus, protein, zinc, and niacin equivalents. NP2 was characterised by high amounts of dietary fibre, magnesium, and potassium and a low amount of saturated fat. NP3 was characterized by high amounts of monounsaturated fat, vitamin

E, polyunsaturated fat and a low amount of carbohydrates. NP4 was characterised by a high amount of alpha carotene and beta carotene.

The four nutrient patterns explained 58% of the variation in the diet. NP2 was negatively associated with hip BMD when not controlling for confounders. When confounders were included in the model the association was no longer significant. There were no other significant correlations between the other nutrient patterns and BMD.

NP2, a high amount of fibre, potassium and magnesium and low amount of saturated fat, was negatively associated with hip BMD in model 1 when there was no control for confounders. Fibre is found in fruits, vegetables, legumes and grains. Magnesium is found in green vegetables, nuts, legumes and unrefined grains. Magnesium's role in bone health is well established with it contributing to bone mineral, being a co-factor for chemical reactions and balancing pH levels. Potassium is found in fruit, vegetables, dairy and meat. Potassium does not have a direct role in bone health, but it does contribute to the alkalinity that supports bone (Anderson et al., 2012). Saturated fat is commonly found in animal products such as meat and dairy (Ministry of Health, 2017). This nutrient pattern may suggest a diet that is higher in fruits and vegetables and lower in animal fat sources or at least an intake of low-fat animal sources.

The present study's finding of the intake of fibre, potassium and magnesium and absence of saturated fat being negatively associated with BMD isn't seen across other research. A study in postmenopausal women found a nutrient pattern of fibre, potassium, magnesium, folate, vitamin B_6, vitamin A, vitamin K, copper, manganese and beta-carotene to be positively associated with spine BMD (Karamati et al., 2014). This pattern contains the three nutrients of NP2 of the present study. However, in contrast, NP2 had a negative correlation with hip BMD in model 1.

Fruit and vegetables, a source of these three nutrients, have been positively associated with BMD in postmenopausal women (Kim et al., 2016). Additionally, Zhang et al. (2024) found a negative correlation between fibre intake and osteoporosis risk in postmenopausal women. Soluble fibre increases calcium absorption; it is speculated that this is due to the effects the soluble fibre has on the gut microbiome (Coudray et al., 1997, Whisner et al., 2016). However, phytate and oxalate are compounds commonly found in high fibre foods (Ministry of Health, 2017), these compounds bind to calcium and prevent its absorption through the gastrointestinal wall (Raymond & Morrow, 2022).

NP1 of the present study was not seen in the other literature on postmenopausal women. However, Karamati et al. (2014) identified a nutrient pattern characterised by an intake of protein, calcium, zinc, phosphorus, vitamin B_2, vitamin B_{12}, and vitamin D and an absence of vitamin E. This pattern includes the nutrients of NP1 of the present study. Grili et al. (2023) found a nutrient pattern in postmenopausal

women containing the same nutrients as the present study's NP1 (phosphorus, protein, zinc, and niacin equivalents). Additionally, the pattern contained vitamin B_{12}, pantothenic acid, vitamin B_6, potassium, vitamin D, vitamin E, cholesterol, β-carotene, omega 3, magnesium, and selenium. Grili et al. (2023) found the nutrient pattern was inversely associated with osteopenia.

Ilesanmi-Oyelere et al. (2019)'s research on postmenopausal New Zealand women identified a nutrient pattern characterised by the intake of phosphorus, calcium and riboflavin. They found the nutrient pattern to be positively associated with spine and femoral neck BMD. However, this nutrient pattern is not similar to NP1 with phosphorus being the only nutrient in common.

NP3 of the present study was seen in other research. Ilesanmi-Oyelere et al. (2019)'s research on postmenopausal New Zealand women identified a nutrient pattern characterised by the intake of polyunsaturated fat and monounsaturated fat to be negatively correlated with hip BMD. The present study's NP3 is similar to this nutrient pattern. However, in the present study, the nutrient pattern did not have any significant association with BMD. Karamati et al. (2014) also found a nutrient pattern characterised by an intake of fat and the absence of carbohydrates similar to NP3 of the present study. They found no association between the nutrient pattern and BMD such as in the present study.

In model 1, hip BMD was negatively associated with NP2. However, when controlling for confounders, there were no significant associations between the nutrient patterns and BMD of the participants. This suggests that NP2 did have an effect on hip BMD, but the confounders had a greater effect. Weight was the only confounder that was significantly correlated to all three measured BMD sites. Therefore this would be the confounder that had the greater influence on BMD.

From the results, the relationship between body weight and BMD suggests an additional 10kg of body weight may be associated with increased BMD at the three sites between 0.01-0.09g/cm^3. The impact of weight on BMD has been thoroughly looked at in the research. Mechanical loading of the bones is a stimulus for bone formation (Uda et al., 2017). Therefore higher body weight is suggested to increase BMD. However, the results differ depending on the body weight composition.

Jain and Vokes (2022) reported an additional 1kg/m^2 of lean body mass was associated with a higher BMD T-score of 0.19 and an additional 1kg/m^2 of fat mass was associated with a lower BMD T-score of 0.10. However, when comparing the sexes individually the negative effect of fat mass was lesser for the females. For females, an additional 1kg/m^2 of fat mass was associated with a lower BMD T-score of 0.08. In the present study, body weight was not differentiated into lean mass and fat tissue. Ilesanmi-Oyelere et al. (2019) controlled for BMI rather than body weight when investigating the study's nutrient patterns and BMD associations. The model inclusive of confounder BMI had the same correlations as

the model without confounders dissimilarly to the present study, where the model inclusive of the confounder body weight had no association while the model without confounders did have an association.

No significant relationships were found between CTx-1 and the nutrient patterns. To our knowledge, no other studies have looked at nutrient patterns and CTx-1 in postmenopausal women. This was unexpected as nutrition has been shown to influence CTx-1 levels in the literature. Sharma et al. (2022) found a single dose of 1000mg of calcium through milk or supplement resulted in a decrease in CTx-1 levels in postmenopausal women. Additionally, Cao et al. (2018) found adults to have higher levels of CTx-1 when on a low fruit and vegetable diet and lower CTx-1 levels when on a high fruit and vegetable diet. Wright et al. (2019) found a dietary intake of magnesium to be negatively associated with CTx-1 levels over 2 years in postmenopausal women. CTx-1 is also influenced by age due to the increased bone degradation that is associated with increased age (Greenblatt et al., 2017). Therefore, age was a confounder in the CTx-1 model 2.

There are other nutrients in nutrient patterns from the previously mentioned studies that were not included in this study. The sample size of the study determined the number of nutrients that could be included in the principal component analysis for the nutrient patterns. The nutrients that were omitted were suggested by the literature to be of less significance to bone health. However, other nutrients may have been present in the patterns, but they had not been included in the analysis.

The inclusion criteria, described as the loading cut-off, for the measured nutrients to be included in a nutrient pattern was also determined by the sample size. In the present study, the loading cut-off for the nutrients to be included in the nutrient pattern was >0.57. The other mentioned studies had a lower loading cut-off allowing more nutrients to be including in the patterns. This is likely due to the larger sample sizes the studies had.

The nutrient data collection and statistical manipulation of the present study differed from the other referenced studies. Karamati et al. (2014) and Grili et al. (2023) used food frequency questionnaires to measure nutrient intake and statistically adjusted for energy intake in their nutrient pattern analyses. In contrast, Ilesanmi-Oyelere et al. (2019) used a three-day food record and did not statistically adjust for energy in the nutrient pattern analysis. The present study adjusted statistically for energy intake and used a three-day food record.

3.5 Limitations & Strengths

This research had both strengths and limitations that influenced its findings. A strength of the study was that it was one of the first studies to investigate the relationship between nutrient patterns and CTx-1 in

postmenopausal women. Furthermore, it measured and controlled for significant confounders that can influence bone density such as $25(OH)D_3$, physical activity, body weight and age. This ensured any possible relationships identified were not due to other factors. The use of a three-day diet record to measure nutrient intake was another strength of the study as this is considered the gold standard method. BMD was also measured using the gold standard method of DXA. Another strength was that missing data were imputed using multivariate imputation by chained equations and a sensitivity analysis was completed to ensure its validity.

There were limitations of the research which may have influenced the limited significant results that were found. The sample size of the research was small with participants dropping out and being excluded due to the exclusion criteria. The small sample size meant the number of nutrients included in the nutrient pattern analysis was reduced. The subjective decisions required for principal component analysis imposed a further limitation. This included the treatment of energy intake, the number of nutrients to include, the rotation method and the number of factors to retain. However, standard procedures were used overall.

An additional constraint was that the identified nutrient patterns are specific to this population and cannot be generalised. A further drawback was the recruitment of the sample. The sample was recruited through a recruitment website making it a convenience sample and possibly imposing another drawback by implying that the results cannot be generalised to the general population (Andrade, 2021).

3.6 Conclusion

The present study identified four nutrient patterns from the nutrient analysis of post-menopausal women. NP2 was negatively correlated with hip BMD when no confounders were in the model. The associations found between the nutrient patterns and BMD in the other research were not seen in this research. The present study also reviewed the complexity of nutrients and bone health.

This was the first study looking at nutrient patterns and CTx-1 in postmenopausal women but there were no significant associations found. The expected correlation between PTH and $25(OH)D_3$ was seen but other correlations between CTx-1, BMD and PTH were not. The study did not identify significant correlations between nutrient patterns and BMD when controlling for confounders. However, previous research has done so, therefore further research in this area is required to expand the understanding of nutrient patterns and BMD.

A larger sample size could provide more robust nutrient pattern statistics and possibly a greater variation in BMD among subjects. Future studies could also differentiate the confounder body weight into lean

and fat mass to investigate the differing effects. Additionally, future research is required to build the literature on nutrient patterns and CTx-1 with this being the first study to our knowledge of the relationship.

Chapter 4 Conclusion and Recommendation

4.1 Overview

Osteoporosis is the disease of brittle bones that increases individuals' fracture risk and subsequently reduces quality of life when bone fracture occurs (Kanis, 2007). Postmenopausal women face an increased risk to this disease due to the decline in oestrogen that occurs in the transition through menopause. Oestrogen is a regulator for osteoclasts, the cells responsible for bone degradation. The reduction in oestrogen concentrations results in greater bone loss (Cano, 2017). The prevention of osteoporosis through diet and lifestyle is possible before menopause. Therefore, research investigating nutrient's effects on bone in the vulnerable population is important to reduce the disease burden on the individual and the financial cost on healthcare. Bone mineral density (BMD) is the gold standard measurement of bone. However, biomarkers such as cross-linked C-telopeptide of type 1 collagen (CTx-1) provide insight into the short term responses different stimulus are causing in bone (Greenblatt et al., 2017). This research identified nutrient patterns in New Zealand postmenopausal women using 3-day food records and investigated the relationship between these nutrients patterns and BMD and CTx-1. Additionally, correlations between markers of bone metabolism, parathyroid hormone (PTH), 25-hydroxyvitamin D_3 ($25(OH)D_3$), CTx-1 and BMD were investigated.

4.2 Main findings

Objective 1: To determine if there is a relationship between CTx-1 and BMD and/or PTH.

Findings: The results found no significant relationship between CTx-1 and hip, spine or femoral neck BMD. A negative correlation was expected between CTx-1 and BMD, but this was not seen in this sample. Additionally, there was no significant relationship between CTx-1 and PTH. A positive correlation was expected between CTx-1 and PTH, but was not seen in this sample. However, these results do not mean that there is no relationship between CTx-1 and BMD or CTx-1 and PTH due to the limitations of the study.

Objective 2: To determine if there is a relationship between PTH and vitamin D.

Findings: The results found a significant negative correlation between PTH and $25(OH)D_3$ in the sample of postmenopausal women. These findings support the study's hypothesis of there being a negative relationship between PTH and $25(OH)D_3$. This correlation shows that maintaining optimal $25(OH)D_3$ levels can reduce PTH levels, which will consequently reduce the calcium that is mobilised

from the bone and reduce the bone mineral that would be lost. Minimising bone loss will assist in the prevention of osteoporosis.

Objective 3: To create nutrient patterns and to determine if they have a relationship to CTx-1 and BMD of postmenopausal women.

Findings: There were four nutrient patterns from the results. NP1 was characterised by a high amount of phosphorus, protein, zinc, and niacin equivalents. NP2 was characterised by high amounts of dietary fibre, magnesium, potassium and a low amount of saturated fat. NP3 was characterised by high amounts of monounsaturated fat, vitamin E, polyunsaturated fat and a low amount of carbohydrates. NP4 was characterised by a high amount of alpha carotene and beta carotene. NP4 was not included in the analysis between the nutrient patterns, BMD and CTx-1 due to it only comprising of 2 nutrients. NP2 was found to be negatively associated with the hip BMD of the post-menopausal women in model 1 where confounders were not controlled for. When confounders were controlled for in model 2 the correlation was no longer statistically significant. Weight was the only confounder that was significantly associated with BMD. From this result, weight is more of a significant predictor for BMD than NP2. No other nutrient patterns were significantly correlated with BMD in the postmenopausal women. No nutrient patterns had correlations with CTx-1 levels. The study's hypothesis that a nutrient pattern including calcium would be positively associated with lower levels of bone resorption and improved bone density in postmenopausal women were not proven in these results. A nutrient pattern that included calcium was expected to be associated with lower levels of CTx-1 and higher levels of BMD in the post-menopausal women. However, calcium was not in any of the study's nutrient patterns and therefore no relationships between calcium, BMD and CTx-1 were able to be analyzed.

4.3 Research impact

Osteoporosis burdens individuals lives and the healthcare budgets. It is important to identify lifestyle behaviours that can prevent the development of the disease. Nutrient patterns address the intake of nutrients together which is the way they are consumed through food. Finding nutrient patterns that do or do not support bone health in postmenopausal women can guide the dietary recommendations to help protect against osteoporosis in this vulnerable population.

Although the results of this study did not find the substantial significant relationships that were hypothesised between the bone variables and nutrient patterns, it was still another step in the direction for research in postmenopausal women's bone health. One nutrient pattern was found to be negatively associated with BMD when no confounders were controlled for. Additionally, this was the first study to investigate the relationship between nutrient patterns and CTx-1 levels in postmenopausal women.

4.4 Strengths and Limitations

The present study had both strengths and limitations within the recruitment, data collection and data analysis that impacted the final results of the research.

Limitations

One significant limitation of the study was the sample size. Due to the dropping out of participants there were eighty-seven subjects within the sample. Two of these participants had no nutrient analysis data as there was no submission of their three-day diet record. Having eighty-five participants data for nutrient analysis restricted the number of different nutrients that were evaluated in the nutrient patterns. Additionally, the sample size determined the loading cut-off for the nutrients to be included in the nutrient pattern. The smaller sample size increased the loading cut-off which excluded more nutrients from the patterns.

Another limitation of the study was the analysis of the three-day diet records. The majority of the data had already been analysed by a previous research assistant. However, the extreme outliers that were identified were recalculated by the author of the present study. Despite both using the FoodWorks software having the data evaluated by multiple people may lead to inconsistencies across the analysis results. Additionally, there was significant variation in the level of depth participants completed the food record. Some participants weighed all food while other participants omitted portions or estimated values.

Another limitation of the study was the missing data. Eleven participants did not have results for serum PTH, CTx-1 and $25(OH)D_3$ due to not completing the venous blood sample. This data was imputed using multivariate imputation by chained equations (mice) so the sample size was not further decreased.

Another limitation of the study was inaccuracy within the completion of the physical activity questionnaire. There were a significant number of outliers which signalled the questionnaire was not completed correctly. This suggested the instructions for the questionnaire were insufficient causing the participants to misunderstand the questions. This led to answers which were not possible. To account for these outliers the results were truncated as recommended by the IPAQ guidelines (Craig, 2005).

Another limitation of the study was the minimal cultural diversity represented in the participant sample. Only 6.9% of participants identified as Māori and there were no Pacifica women in the sample. This ethnic distribution does not represent the New Zealand population. Māori and Pacifica populations face the same risks in regard to bone health. Therefore, it is important they are represented in the research.

Another limitation of the study was that the time since menopause for the participants was not measured and reported. Time since menopause may have been a significant confounder of BMD to include in the regression models.

A limitation of the study was the practical application of nutrient patterns for dietary recommendations. Assumptions can be made of what foods and dietary patterns the nutrient patterns are coming from based on nutritional knowledge, but these are assumptions and cannot be confirmed. Relating the nutrients back to food is important to make the research applicable for behaviour and lifestyle change as people choose foods to eat not nutrients. Therefore, the recommendations need to be centred around foods.

Strengths

A strength of this study was that it investigated the relationship between CTx-1 levels and nutrient patterns in postmenopausal women. To our knowledge, there has been no other study in New Zealand of the relationship between nutrient patterns and CTx-1 in postmenopausal women. Though the present study did not find significant results we aim to encourage future research into the relationship.

Another strength of this study was that energy was controlled for in the nutrient patterns. Correlations between the individual nutrients and energy found the majority of them to be significantly correlated. Therefore, controlling for energy factored in this correlation. Despite this being the case in this sample it may not be sensible in other population groups where the consumption of ultra processed energy dense food is prevalent because these foods often have high caloric value but low micronutrient values. But for this sample, where majority of micronutrients were correlated with energy, the energy adjusted nutrient patterns were a strength in maximising accuracy of the nutrient patterns.

A strength of the study was the use of the gold standard techniques for measuring both BMD and nutrient intake. Dual-energy X-ray absorptiometry (DXA) is the gold standard method for measuring BMD. Three-day diet records are the gold standard method for measuring the food and nutrient intake of participants. The use of these methods improved to validity of the results compared to if less reliable methods were implemented.

The final strength of the study lay in the confounders of the study. Measuring and accounting for confounders that may have influenced the BMD of the participants eliminated the possibility that the identified relationships were due to other underlying factors.

4.5 Recommendations and future directions for research

Future research should continue to build the understanding between bone and nutrition in postmenopausal women. It should consider the findings, strengths and limitations of the present for a greater understanding.

Future study design recommendations

A future study should work to increase the ethnic diversity of the future study participants to accurately represent the New Zealand population. This may require changing the recruitment method to reach a greater range of people.

Future methodology recommendations

Investigate the relationship between muscle mass and BMD and fat mass and BMD. Then use muscle mass and fat mass individually rather than body weight as confounders if the correlations suggest this is statistically appropriate.

Expand the nutrients measured in the diet and included in the principal component analysis of the nutrient patterns. This would require increasing the sample size. The other research in this area had many nutrients that were not measured in the present study's analysis.

Future data collection recommendations

Collect the serum $25(OH)D_3$ of all participants within a smaller period. The present study completed participants venous blood samples over 6 months. Reducing this period to 6-8 weeks will decrease the seasonal influence that could have caused variation in the $25(OH)D_3$ data across the participants.

Improve the accuracy of the physical activity questionnaires and three-day diet records. Ensure sufficient instructions are provided and the answers are reviewed immediately after the completion to ensure they fit within the accepted range. Follow up with the participants if the results are in the outlier range. Provide kitchen scales to participants to encourage the weighing of food for the three-day diet records to increase accuracy of records.

References

Anderson, J., Garner, S. & Klemmer P. (2012). *Diet, nutrients, and bone health*. US: CRC Press.

Andrade, C. (2021). 'The inconvenient truth about convenience and purposive samples', *Indian J Psychol Med,* 43(1), pp.86-88. https://www.ncbi.nlm.nih.gov/pubmed/34349313

Ansari, S., Abbasi, B., Saneei, P., Heidari, Z. & Saraf-Bank, S. (2023). 'Higher lacto-vegetarian dietary score is associated with reduced risk of postmenopausal osteoporosis: A case-control study in a sample of iranian postmenopausal women', *Nutrition Research,* 120, pp.88-98. https://www.ncbi.nlm.nih.gov/pubmed/37950985

Antonio, J., Ellerbroek, A., Evans, C., Silver, T. & Peacock, C. A. (2018). 'High protein consumption in trained women: Bad to the bone?', *Journal of the International Society of Sports Nutrition,* 15, pp.6. https://www.ncbi.nlm.nih.gov/pubmed/29434529

Bailey, R. L. (2021). 'Overview of dietary assessment methods for measuring intakes of foods, beverages, and dietary supplements in research studies', *Current Opinion in Biotechnology,* 70, pp.91-96. https://www.ncbi.nlm.nih.gov/pubmed/33714006

Baron, R. & Kneissel, M. (2013). 'Wnt signaling in bone homeostasis and disease: From human mutations to treatments', *Nature Medicine,* 19(2), pp.179-192. https://www.ncbi.nlm.nih.gov/pubmed/23389618

Berger, A. (2002). 'Bone mineral desnity scans', *British Medical Journal,* 325, pp.484. https://www.bmj.com/content/325/7362/484

Binkley, N. & Krueger, D. (2000). 'Hypervitaminosis a and bone', *Nutrition Reviews,* 58(5), pp.138-144. https://doi.org/10.1111/j.1753-4887.2000.tb01848.x

Blake, G. M. & Fogelman, I. (2007). 'The role of dxa bone density scans in the diagnosis and treatment of osteoporosis', *Postgraduate Medical Journal,* 83(982), pp.509-517. https://www.ncbi.nlm.nih.gov/pubmed/17675543

Briot, K. & Roux, C. (2015). 'Glucocorticoid-induced osteoporosis', *RMD Open,* 1(1), pp.14. https://www.ncbi.nlm.nih.gov/pubmed/26509049

Bristow, S., Horne, A., Gamble, G., Mihov, B., Stewart, A. & Reid, I. (2019). 'Dietary calcium intake and bone loss over 6 years in osteopenic postmenopausal women', *Journal of Clinical Endocrinology & Metabolism,* 104(8), pp.3576-3584. https://www.ncbi.nlm.nih.gov/pubmed/30896743

Brown, P., McNeill, R., Leung, W., Radwan, E. & Willingale, J. (2011). 'Current and future economic burden of osteoporosis in New Zealand', *Applied Health Economics and Health Policy,* 9(2), pp.111-123. https://doi.org/10.2165/11531500-000000000-00000

Cano, A. (2017). *Menopause: A comprehensive approach,* Switzerland: Springer Nature.

Canterbury Health Laboratories (2024a). *C-telopeptide*. https://www.chl.co.nz/test/c-telopeptide/

Canterbury Health Laboratories (2024b). *Parathyroid hormone test*. https://www.chl.co.nz/test/parathyroid-hormone-test/

Canterbury Health Laboratories (2024c). *Vitamin D*. https://www.chl.co.nz/test/vitamin-d/

Cao, J. J., Whigham, L. D. & Jahns, L. (2018). 'Depletion and repletion of fruit and vegetable intake alters serum bone turnover markers: A 28-week single-arm experimental feeding intervention', *British Journal of Nutrition,* 120(5), pp.500-507. https://www.ncbi.nlm.nih.gov/pubmed/30022739

Carrivick, S. J., Walsh, J. P., Brown, S. J., Wardrop, R. & Hadlow, N. C. (2015). 'Brief report: Does pth increase with age, independent of 25-hydroxyvitamin d, phosphate, renal function, and ionized calcium?', *The Journal of Clinical Endocrinology and Metabolism,* 100(5), pp.2131-2134. https://www.ncbi.nlm.nih.gov/pubmed/25751107

Chaudhri, M. A., Kemmler, W., Harsch, I. & Watling, R. J. (2009). 'Plasma copper and bone mineral density in osteopenia: An indicator of bone mineral density in osteopenic females', *Biological Trace Element Research,* 129(3), pp.94-98. https://www.ncbi.nlm.nih.gov/pubmed/19139831

Christakos, S. & Pike, J. W. (2020). 'Vitamin d gene regulation', in Bilezikian, J. P (ed.) *Principles of bone biology.* US: Academic Press, pp. 739-756.

Corwin, R. L., Hartman, T. J., Maczuga, S. A. & Graubard, B. I. (2005). 'Dietary saturated fat intake is inversely associated with bone density in humans - analysis of nhanes III', *American Society for Nutrition,* 136. https://doi.org/10.1093/jn/136.1.159

Coudray, C., Bellanger, J., Castiglia-Delavaud, C., Remesy, C., Vermorel, M. & Rayssignuier, Y. (1997). 'Effect of soluble or partly soluble dietary ®bres supplementation on absorption and balance of calcium, magnesium, iron and zinc in healthy young men.', *European Journal of Clinical Nutrition,* 51, pp.375-380. https://doi.org/10.1038/sj.ejcn.1600417

Craig, C. (2005). Guidelines for data processing and analysis of the international physical activity questionnaire (IPAQ) - short and long forms.

Craig, C., Marshall, A., Sjöström, M., Bauman, A., Booth, M., Ainsworth, B., Pratt, M., Ekelund, U., Yngve, A., Sallis, J. & Oja, P. (2003). 'International physical activity questionnaire: 12-country reliability and validity.', *Medicine and Science in Sports and Exercise,* 35, pp.1381-1395. https://journals.lww.com/acsm-msse/fulltext/2003/08000/international_physical_activity_questionnaire_.20.aspx

Dawson-Hughes, D., Harris, S. S. & Dallal, G. E. (1997). 'Plasma calcidiol, season, and serum parathyroid hormone concentrations in healthy elderly men and women', *The American Journal of Clinical Nutrition,* 65(1), pp.67-71. https://doi.org/10.1093/ajcn/65.1.67

Dietary Guidelines Advisory Committees (2020). *Scientific report of the 2020 dietary guidelines advisory committee.* US: Dietary Guidelines for Americans.

https://www.dietaryguidelines.gov/sites/default/files/2020-07/ScientificReport_of_the_2020DietaryGuidelinesAdvisoryCommittee_first-print.pdf

Eberle, J., Schmidmayer, S., Erben, R. G., Stangassinger, M. & Roth, H. P. (1999). 'Skeletal effects of zinc deficiency in growing rats', *Journal of Trace Elements in Medicine and Biology,* 13. https://doi.org/10.1016/S0946-672X(99)80019-4

Elnenaei, M. O., Musto, R., Alaghband-Zadeh, J., Moniz, C. & Le Roux, C. W. (2010). 'Postprandial bone turnover is independent of calories above 250 kcal', *Annals of Clinical Biochemistry,* 47, pp.318-320. https://www.ncbi.nlm.nih.gov/pubmed/20400496

Fan, Y., Ni, S. & Zhang, H. (2022). 'Associations of copper intake with bone mineral density and osteoporosis in adults: Data from the national health and nutrition examination survey', *Biological Trace Element Research,* 200(5), pp.2062-2068. https://www.ncbi.nlm.nih.gov/pubmed/34283365

Farrell, V. A., Harris, M., Lohman, T. G., Going, S. B., Thomson, C. A., Weber, J. L. & Houtkooper, L. B. (2009). 'Comparison between dietary assessment methods for determining associations between nutrient intakes and bone mineral density in postmenopausal women', *Journal of the American Dietetic Association,* 109(5), pp.899-904. https://www.ncbi.nlm.nih.gov/pubmed/19394478

Florencio-Silva, R., Sasso, G. R., Sasso-Cerri, E., Simoes, M. J. & Cerri, P. S. (2015). 'Biology of bone tissue: Structure, function, and factors that influence bone cells', *BioMed Research International*, pp.421746. https://www.ncbi.nlm.nih.gov/pubmed/26247020

Franz-Odendaal, T. A., Hall, B. K. & Witten, P. E. (2006). 'Buried alive: How osteoblasts become osteocytes', *Developemental Dynamics,* 235, pp.176–190. https://doi.org/10.1002/dvdy.20603

Gacs, G. & Barltrop, D. (1977). 'Significance of ca-soap formation for calcium absorption in the rat', *Gut,* 18(1), pp.64-68. https://pmc.ncbi.nlm.nih.gov/articles/PMC1411254/

Gallagher, J. C. (2013). 'Vitamin d and aging', *Endocrinology and Metabolism Clinics of North America,* 42(2), pp.319-332. https://www.ncbi.nlm.nih.gov/pubmed/23702404

Gao, C., Qiao, J., Li, S. S., Yu, W. J., He, J. W., Fu, W. Z. & Zhang, Z. L. (2017). 'The levels of bone turnover markers 25(OH)D and PTH and their relationship with bone mineral density in postmenopausal women in a suburban district in china', *Osteoporos International,* 28(1), pp.211-218. https://www.ncbi.nlm.nih.gov/pubmed/27468899

Gómez-Cabello, A., Ara, I., González-Agüero, A., Casajús, J. A. & Vicente-Rodríguez, G. (2012). 'Effects of training on bone mass in older adults: A systematic review', *Sports Medicine,* 42(4), pp.301–325. https://doi.org/10.2165/11597670-000000000-00000

Gossiel, F., Altaher, H., Reid, D. M., Roux, C., Felsenberg, D., Gluer, C. C. & Eastell, R. (2018). 'Bone turnover markers after the menopause: T-score approach', *Bone,* 111, pp.44-48. https://www.ncbi.nlm.nih.gov/pubmed/29551751

Greenblatt, M. B., Tsai, J. N. & Wein, M. N. (2017). 'Bone turnover markers in the diagnosis and monitoring of metabolic bone disease', *Clinical Chemistry,* 63(2), pp.464-474. https://www.ncbi.nlm.nih.gov/pubmed/27940448

Grili, P. P. D., Vidigal, C. V., Cruz, G. F. D., Albergaria, B. H., Marques-Rocha, J. L., Pereira, T. S. S. & Guandalini, V. R. (2023). 'Nutrient patterns and risk of osteopenia in postmenopausal women', *Nutrients,* 15(7). https://www.ncbi.nlm.nih.gov/pubmed/37049510

Hannan, M. T. (2000). 'Effect of dietary protein on bone loss in elderly men and women: The framingham osteoporosis study', *Journal of Bone and Mineral Research,* 15(12), pp.2504-2512. https://doi.org/10.1359/jbmr.2000.15.12.2504

Hardcastle, A. C., Aucott, L., Fraser, W. D., Reid, D. M. & Macdonald, H. M. (2011). 'Dietary patterns, bone resorption and bone mineral density in early post-menopausal scottish women', *European Journal of Clinical Nutrition,* 65(3), pp.378-385. https://www.ncbi.nlm.nih.gov/pubmed/21179049

Heaney, R. P., Recker, R. R., Stegman, M. R. & Moy, A. J. (1989). 'Calcium absorption in women: Relationships to calcium intake, estrogen status, and age', *Journal of Bone and Mineral Research,* 4(4), pp.469-475. https://doi.org/10.1002/jbmr.5650040404

Heaney, R. P., Recker, R. R. & Weaver, C. M. (1990). 'Absorbability of calcium sources- the limited role of solubility', *Calcified Tissue International,* 46(5), pp.300–304. https://doi.org/10.1007/BF02563819

Heer, M., Baecker, N., Frings-Meuthen, P., Graf, S., Zwart, S. R., Biolo, G. & Smith, S. M. (2017). 'Effects of high-protein intake on bone turnover in long-term bed rest in women', *Appl Physiol Nutr Metab,* 42(5), pp.537-546. https://www.ncbi.nlm.nih.gov/pubmed/28177714

Hernandez, C. J., Beaupré, G. S. & Carter, D. R. (2003). 'A theoretical analysis of the relative influences of peak BMD, age-related bone loss and menopause on the development of osteoporosis', *Osteoporosis International: With other Metabolic Bone Diseases,* 14(10), pp.843-847. https://doi.org/10.1007/s00198-003-1454-8

Hirata, N., Ichimaru, R., Tominari, T., Matsumoto, C., Watanabe, K., Taniguchi, K., Hirata, M., Ma, S., Suzuki, K., Grundler, F. M. W., Miyaura, C. & Inada, M. (2019). 'Beta-cryptoxanthin inhibits lipopolysaccharide-induced osteoclast differentiation and bone resorption via the suppression of inhibitor of nf-kappab kinase activity', *Nutrients,* 11(2) pp.368. https://www.ncbi.nlm.nih.gov/pubmed/30744180

Hlaing, T. T. & Compston, J. E. (2014). 'Biochemical markers of bone turnover - uses and limitations', *Annals of Clinical Biochemistry,* 51, pp.189-202. https://www.ncbi.nlm.nih.gov/pubmed/24399365

Holick, M. F., Binkley, N. C., Bischoff-Ferrari, H. A., Gordon, C. M., Hanley, D. A., Heaney, R. P., Murad, M. H. & Weaver, C. M. (2011). 'Evaluation, treatment, and prevention of vitamin D deficiency: An endocrine society clinical practice guideline', *The Journal of Clinical Endocrinology & Metabolism,* 96(7), pp.1911–1930. https://doi.org/10.1210/jc.2011-0385

Hu, F. B. (2002). 'Dietary pattern analysis: A new direction in nutritional epidemiology', *Current Opinion in Lipidology,* 13(1), pp.3-9. https://doi.org/10.1097/00041433-200202000-00002

Ilesanmi-Oyelere, B. L. (2020). *The role of dietary patterns, inflammatory status and gut microbiome in bone health maintenance of postmenopausal women – a cross-sectional study.* Massey University. http://hdl.handle.net/10179/15775

Ilesanmi-Oyelere, B. L., Brough, L., Coad, J., Roy, N. & Kruger, M. C. (2019). 'The relationship between nutrient patterns and bone mineral density in postmenopausal women', *Nutrients,* 11(6) pp.1262. https://www.ncbi.nlm.nih.gov/pubmed/31163708

Ilesanmi-Oyelere, B. L., Coad, J., Roy, N. C. & Kruger, M. C. (2020). 'Dietary patterns, body composition, and bone health in New Zealand postmenopausal women', *Frontiers in Nutrition,* 7, pp.563689. https://www.ncbi.nlm.nih.gov/pubmed/33195365

Ilesanmi-Oyelere, B. L., Roy, N. C. & Kruger, M. C. (2021). 'Modulation of bone and joint biomarkers, gut microbiota, and inflammation status by synbiotic supplementation and weight-bearing exercise: Human study protocol for a randomized controlled trial', *JMIR Res Protoc,* 10(10), pp.30131. https://www.ncbi.nlm.nih.gov/pubmed/34698648

Jain, R. K. & Vokes, T. (2022). 'Fat mass has negative effects on bone, especially in men: A cross-sectional analysis of nhanes 2011-2018', *The Journal of Clinical Endocrinology and Metabolism,* 107(6), pp.2545-2552. https://www.ncbi.nlm.nih.gov/pubmed/35142799

Joo, N. S., Yang, S. W., Song, B. C. & Yeum, K. J. (2015). 'Vitamin A intake, serum vitamin D and bone mineral density: Analysis of the korea national health and nutrition examination survey (KNHANES, 2008-2011)', *Nutrients,* 7(3), pp.1716-1727. https://www.ncbi.nlm.nih.gov/pubmed/25763530

Kameda, T., Mano, H., Yuasa, T., Mori, Y., Miyazawa, K., Shiokawa, M., Nakamaru, Y., Hiroi, E., Hiura, K., Kameda, A., Yang, N. N., Hakeda, Y. & Kumegawa, M. (1997). 'Estrogen inhibits bone resorption by directly inducing apoptosis of the bone-resorbing osteoclasts', *The Journal of Experimental Medicine,* 186(4), pp.489-495. https://doi.org/10.1084/jem.186.4.489

Kanis, J. A. (2007). *Assessment of osteoporosis at the primary health care level*. UK: World Health Organization Collaborating Centre for Metabolic Bone Diseases, University of Sheffield. https://frax.shef.ac.uk/FRAX/pdfs/WHO_Technical_Report.pdf

Karamati, M., Yousefian-Sanni, M., Shariati-Bafghi, S. E. & Rashidkhani, B. (2014). 'Major nutrient patterns and bone mineral density among postmenopausal iranian women', *Calcif Tissue Int,* 94(6), pp.648-658. https://www.ncbi.nlm.nih.gov/pubmed/24687522

Kenkre, J. S. & Bassett, J. (2018). 'The bone remodelling cycle', *Annals of Clinical Biochemistry,* 55(3), pp.308-327. https://www.ncbi.nlm.nih.gov/pubmed/29368538

Kerstetter, J. E., O'Brien, K. O. & Insogna, K. L. (2003). 'Low protein intake: The impact on calcium and bone homeostasis in humans', *Journal of Nutrition,* 133(3), pp.855-861. https://www.ncbi.nlm.nih.gov/pubmed/12612169

Khazai, N., Judd, S.E. and Tangpricha, V. (2008). 'Calcium and vitamin D: Skeletal and extraskeletal health', *Current Rheumatology Reports*, 10(2), pp.110–117. https://doi.org/10.1007/s11926-008-0020-y

Kim, D. E., Cho, S. H., Park, H. M. & Chang, Y. K. (2016). 'Relationship between bone mineral density and dietary intake of beta-carotene, vitamin C, zinc and vegetables in postmenopausal korean women: A cross-sectional study', *Journal of International Medical Research,* 44(5), pp.1103-1114. https://www.ncbi.nlm.nih.gov/pubmed/27664069

Kim, K. M., Choi, S. H., Lim, S., Moon, J. H., Kim, J. H., Kim, S. W., Jang, H. C. & Shin, C. S. (2014). 'Interactions between dietary calcium intake and bone mineral density or bone geometry in a low calcium intake population (KNHANES IV 2008-2010)', *The Journal of Clinical Endocrinology and Metabolism,* 99(7), pp.2409-2417. https://www.ncbi.nlm.nih.gov/pubmed/24684465

Kojima, A., Kamiya, K., Kajita, E., Tachiki, T., Sato, Y., Kouda, K., Uenishi, K., Tamaki, J., Kagamimori, S. & Iki, M. (2023). 'Association between dairy product intake and risk of osteoporotic fractures in postmenopausal japanese women: Secondary analysis of 15-year follow-up data from the japanese population-based osteoporosis (jpos) cohort study', *The journal of nutrition, health & aging,* 27(3), pp.228-237. https://doi.org/10.1007/s12603-023-1898-1

Kruger, M. C., Chan, Y. M., Lau, L. T., Lau, C. C., Chin, Y. S., Kuhn-Sherlock, B., Todd, J. M. & Schollum, L. M. (2018). 'Calcium and vitamin d fortified milk reduces bone turnover and improves bone density in postmenopausal women over 1 year', *European Journal of Nutrition,* 57(8), pp.2785-2794. https://www.ncbi.nlm.nih.gov/pubmed/28975432

Kruger, M. C. & Schollum, L. M. (2005). 'Is docosahexaenoic acid more effective than eicosapentaenoic acid for increasing calcium bioavailability?', *Prostaglandins Leukot Essent Fatty Acids,* 73(5), pp.327-334. https://www.ncbi.nlm.nih.gov/pubmed/16154334

Kujala, U. M., Leskinen, T., Rottensteiner, M., Aaltonen, S., Ala-Korpela, M., Waller, K. & Kaprio, J. (2022). 'Physical activity and health: Findings from finnish monozygotic twin pairs discordant for physical activity', *Scandinavian Journal of Medicine & Science in Sports,* 32(9), pp.1316-1323. https://www.ncbi.nlm.nih.gov/pubmed/35770444

Laird, E., Ward, M., Mcsorley, E., Strain, J. J. & Wallace, J. (2010). 'Vitamin D and bone health: Potential mechanisms', *Nutrients,* 2(7), pp.693-724. https://www.ncbi.nlm.nih.gov/pubmed/22254049

Lanyan, A., Marques-Vidal, P., Gonzalez-Rodriguez, E., Hans, D. & Lamy, O. (2020). 'Postmenopausal women with osteoporosis consume high amounts of vegetables but insufficient dairy products and calcium to benefit from their virtues: The colaus/osteolaus

cohort', *Osteoporosis International,* 31(5), pp.875-886. https://doi.org/10.1007/s00198-019-05225-x

Lavado-Garcia, J., Roncero-Martin, R., Moran, J. M., Pedrera-Canal, M., Aliaga, I., Leal-Hernandez, O., Rico-Martin, S. & Canal-Macias, M. L. (2018). 'Long-chain omega-3 polyunsaturated fatty acid dietary intake is positively associated with bone mineral density in normal and osteopenic spanish women', *PLoS One,* 13(1), pp.0190539. https://www.ncbi.nlm.nih.gov/pubmed/29304057

Liu, J., Xiao, Q., Xiao, J., Niu, C., Li, Y., Zhang, X., Zhou, Z., Shu, G. & Yin, G. (2022). 'Wnt/beta-catenin signalling: Function, biological mechanisms, and therapeutic opportunities', *Signal Transduction and Targeted Therapy,* 7(1), pp.3. https://www.ncbi.nlm.nih.gov/pubmed/34980884

Maclaughlin, J. & Holick, M. F. (1985). 'Aging decreases the capacity of human skin to produce vitamin D3', *Journal of Clinical Investigation,* 76, pp.1536–1538. https://pmc.ncbi.nlm.nih.gov/articles/PMC424123/

Marieb, E. & Hoehn, K. (2015). *Human anatomy & physiology.* Essex: Pearson.

Ministry of Health (2012). *Vitamin D status of New Zealand adults: Findings from the 2008/09 New Zealand adult nutrition survey*. https://www.health.govt.nz/publications/vitamin-d-status-of-new-zealand-adults

Ministry of Health (2017). *Nutrient reference values for Australia and New Zealand*.

Ministry of Health (2022). *Adults' dietary habits – findings from the 2018/19 and 2019/20 New Zealand health survey*. Wellington: Ministry of Health. https://www.health.govt.nz/system/files/2022-10/adults-dietary-habits-oct22.pdf

Ministry of Health (2023). '*Annual update of key results 2022/23: New Zealand health survey'*. https://www.health.govt.nz/publications/annual-update-of-key-results-202223-new-zealand-health-survey

National Institute of Arthritis and Musculoskeletal and Skin Diseases (2023). '*Bone mineral density tests: What the numbers mean'*. https://www.niams.nih.gov/health-topics/bone-mineral-density-tests-what-numbers-mean

National Institutes of Health (2019). '*Calcium'*. NIH.gov. https://ods.od.nih.gov/factsheets/Calcium-Consumer/

Navarro-Valverde, C., Caballero-Villarraso, J., Mata-Granados, J. M., Casado-Diaz, A., Sosa-Henriquez, M., Malouf-Sierra, J., Nogues-Solan, X., Rodriguez-Manas, L., Cortes-Gil, X., Delgadillo-Duarte, J. & Quesada-Gomez, J. M. (2018). 'High serum retinol as a relevant contributor to low bone mineral density in postmenopausal osteoporotic women', *Calcified Tissue International,* 102(6), pp.651-656. https://www.ncbi.nlm.nih.gov/pubmed/29294148

Need, A. G., O'Loughlin, P. D., Morris, H. A., Horowitz, M. & Nordin, B. E. (2004). 'The effects of age and other variables on serum parathyroid hormone in postmenopausal women attending an osteoporosis center', *The Journal of Clinical Endocrinology and Metabolism,* 89(4), pp.1646-1649. https://www.ncbi.nlm.nih.gov/pubmed/15070925

NHS (2020). '*Calcium - Vitamins and minerals'*. NHS. https://www.nhs.uk/conditions/vitamins-and-minerals/calcium/

Nissen, F. I., Esser, V. F. C., Bui, M., Li, S., Hopper, J. L., Bjornerem, A. & Hansen, A. K. (2023). 'Is there a causal relationship between physical activity and bone microarchitecture? A study of adult female twin pairs', *Journal of Bone and Mineral Research,* 38(7), pp.951-957. https://www.ncbi.nlm.nih.gov/pubmed/37198881

Nutrition Foundation (2022). '*Body mass index (bmi): Is it a good measure of health?'*. https://nutritionfoundation.org.nz/body-mass-index-bmi/

O'Connor, J. P., Kanjilal, D., Teitelbaum, M., Lin, S. S. & Cottrell, J. A. (2020). 'Zinc as a therapeutic agent in bone regeneration', *Materials,* 13(10), pp.2211. https://www.ncbi.nlm.nih.gov/pubmed/32408474

O'Neill, C., Gangat, M. & Radovick, S. (2022). 'Growth hormone deficiency', *Endocrines,* 3(4), pp.736-744. https://doi.org/10.3390/endocrines3040060

Ohlsson, C., Bengtsson, B., Isaksson, O., Andreassen, T. & Slootweg, M. (1998). 'Growth hormone and bone', *Endocrine Reviews,* 19(1), pp.55-79. https://doi.org/10.1210/edrv.19.1.0324

Osteoporosis New Zealand (2017). *Osteoporosis New Zealand annual report.* New Zealand: Osteoporosis New Zealand. https://osteoporosis.org.nz/wp-content/uploads/ONZ-2017-Annual-Report-WEB.pdf

Osteoporosis New Zealand (2020). *Bone care 2020*. New Zealand: Osteoporosis New Zealand. https://osteoporosis.org.nz/wp-content/uploads/Bone-Care-2020-web.pdf

Pan, K., Zhang, C., Yao, X. & Zhu, Z. (2020). 'Association between dietary calcium intake and bmd in children and adolescents', *Endocrine Connections,* 9(3), pp.194-200. https://www.ncbi.nlm.nih.gov/pubmed/31990673

Park, S. G., Jeong, S. U., Lee, J. H., Ryu, S. H., Jeong, H. J., Sim, Y. J., Kim, D. K. & Kim, G. C. (2018). 'The changes of CTX, DPD, osteocalcin, and bone mineral density during the postmenopausal period', *Annals of Rehabilitation Medicine,* 42(3), pp.441-448. https://www.ncbi.nlm.nih.gov/pubmed/29961742

Perez-Rey, J., Roncero-Martin, R., Rico-Martin, S., Rey-Sanchez, P., Pedrera-Zamorano, J. D., Pedrera-Canal, M., Lopez-Espuela, F. & Lavado Garcia, J. M. (2019). 'Adherence to a mediterranean diet and bone mineral density in spanish premenopausal women', *Nutrients,* 11(3), pp.555. https://www.ncbi.nlm.nih.gov/pubmed/30841631

Portale, A., Halloran, B. & Morris, C. (1989). 'Physiologic regulation of the serum concentration of 1,25-dihydroxyvitamin D by phosphorus in normal men', *Journal of Clinical Investigation,* 83(5), pp.1494-1499. https://www.jci.org/articles/view/114043

Rajeev, P., Movseysan, A. & Baharani, A. (2017). 'Changes in bone turnover markers in primary hyperparathyroidism and response to surgery', *Ann R Coll Surg Engl,* 99(7), pp.559-562. https://www.ncbi.nlm.nih.gov/pubmed/28853593

Raymond, J. & Morrow, K. (2022). *Krause and Mahan's food and the nutrition care process,* Missouri: Elsevier.

Robey, P. G. & Riminucci, M. (2020). 'Skeletal stem cells: Tissue-specific stem/progenitor cells of cartilage, bone, stroma, and marrow adipocytes', in Bilezikian, J. P (ed.) *Principles of bone biology.* US: Academic Press, pp.45-71.

Roddam, A. W., Spencer, E., Banks, E., Beral, V., Reeves, G., Appleby, P., Barnes, I., Whiteman, D. C. & Key, T. J. (2005). 'Reproducibility of a short semi-quantitative food group questionnaire and its performance in estimating nutrient intake compared with a 7-day diet diary in the million women study', *Public Health Nutrition,* 8(2), pp.201-213. https://www.ncbi.nlm.nih.gov/pubmed/15877913

Rude, R. K., Gruber, H. E., Wei, L. Y., Frausto, A. & Mills, B. G. (2003). 'Magnesium deficiency: Effect on bone and mineral metabolism in the mouse', *Calcified Tissue International,* 72(1), pp.32-41. https://www.ncbi.nlm.nih.gov/pubmed/12370796

Sahni, S., Hannan, M., Gagnon, D., Blumberg, J., Cupples, L., Kiel, D. & Tucker, K. (2008). 'High vitamin C intake is associated with lower 4-year bone loss in elderly men', *Journal of Nutrition,* 138(10), pp.1931-1938. https://doi.org/10.1093/jn/138.10.1931

Sahni, S., Hannan, M. T., Blumberg, J., Cupples, L. A., Kiel, D. P. & Tucker, K. L. (2009). 'Inverse association of carotenoid intakes with 4-y change in bone mineral density in elderly men and women: The framingham osteoporosis study', *The American Journal of Clinical Nutrition,* 89(1), pp.416-424. https://www.ncbi.nlm.nih.gov/pubmed/19056581

Schuchardt, J. P. & Hahn, A. (2017). 'Intestinal absorption and factors influencing bioavailability of magnesium-an update', *Current Nutrition & Food Science,* 13(4), pp.260-278. https://www.ncbi.nlm.nih.gov/pubmed/29123461

Sharma, D. K., Anderson, P. H., Morris, H. A. & Clifton, P. M. (2022). 'Acute c-terminal crosslinking telopeptide of type I collagen (CTX-1) suppression with milk calcium or calcium carbonate is independent of visceral fat in a randomized crossover study in lean and overweight postmenopausal women', *The Journal of Nutrition,* 152(4), pp.1006-1014. https://doi.org/10.1093/jn/nxab384

Sheu, A. & Diamond, T. (2016). 'Bone mineral density: Testing for osteoporosis', *Australian Prescriber,* 39(2), pp.35-39. https://www.ncbi.nlm.nih.gov/pubmed/27340320

Silva, T. R. D., Martins, C. C., Ferreira, L. L. & Spritzer, P. M. (2019). 'Mediterranean diet is associated with bone mineral density and muscle mass in postmenopausal women', *Climacteric,* 22(2), pp.162-168. https://www.ncbi.nlm.nih.gov/pubmed/30661407

Song, C. H., Barrett-Connor, E., Chung, J. H., Kim, S. H. & Kim, K. S. (2007). 'Associations of calcium and magnesium in serum and hair with bone mineral density in premenopausal women', *Biological Trace Element Research,* 118(1), pp.1-9. https://www.ncbi.nlm.nih.gov/pubmed/17848724

Steingrimsdottir, L., Gunnarsson, O., Indridason, O. S., Franzson, L. & Sigurdsson, G. (2005). 'Relationship between serum parathyroid hormone levels, vitamin D sufficiency, and calcium intake', 294(18), pp.2336–2341. https://jamanetwork.com/journals/jama/fullarticle/201842

Stevens, J. P. (2009). *Exploratory and confirmatory factor analysis. Applied multivariate statistics for the social sciences.* New York: Routledge.

Sylvia, L.G., Bernstein, E.E., Hubbard, J.L., Keating, L. & Anderson, E.J. (2014). 'Practical guide to measuring physical activity', *Journal of the Academy of Nutrition and Dietetics*, 114(2), pp.199–208. https://doi.org/10.1016/j.jand.2013.09.018.

Teitelbaum, S. L. (2007). 'Osteoclasts: What do they do and how do they do it?', *The American Journal of Pathology,* 170(2), pp.427-435. https://www.ncbi.nlm.nih.gov/pubmed/17255310

Thaler, R., Khani, F., Sturmlechner, I., Dehghani, S. S., Denbeigh, J. M., Zhou, X., Pichurin, O., Dudakovic, A., Jerez, S. S., Zhong, J., Lee, J. H., Natarajan, R., Kalajzic, I., Jiang, Y. H., Deyle, D. R., Paschalis, E. P., Misof, B. M., Ordog, T. & Van Wijnen, A. J. (2022). 'Vitamin C epigenetically controls osteogenesis and bone mineralization', *Nature Communications,* 13(1), pp.5883. https://www.ncbi.nlm.nih.gov/pubmed/36202795

Tortora, G. J. (2019). *Principles of anatomy and physiology.* 2. US: Wiley.

Uda, Y., Azab, E., Sun, N., Shi, C. & Pajevic, P. D. (2017). 'Osteocyte mechanobiology', *Current Osteoporosis Reports,* 15(4), pp.318-325. https://www.ncbi.nlm.nih.gov/pubmed/28612339

University of Otago & Ministry of Health (2011). A focus on nutrition: Key findings of the 2008/09 New Zealand adult nutrition survey. Wellington.

Uwitonze, A. M. & Razzaque, M. S. (2018). 'Role of magnesium in vitamin D activation and function', *Journal of Osteopathic Medicine,* 118(3), pp.181-189. https://www.ncbi.nlm.nih.gov/pubmed/29480918

Wang, M., Moore, E., Crawford, P., Hudes, M., Sabry, Z., Marcus, R. & Bachrach, L. (1999). 'Influence of pre-adolescent diet on quantitative ultrasound measurements of the calcaneus in young adult women', *Osteoporos International,* 9, pp.532–535. https://doi.org/10.1007/s001980050181

Watson, R. & Mahadevan, D. (2016). *Handbook of nutrition and diet in therapy of bone*. Netherlands: Wageningen Academic Publishers.

Webb, A. R. (2006). 'Who, what, where and when-influences on cutaneous vitamin D synthesis', *Progress in Biophysics & Molecular Biology,* 92(1), pp.17-25. https://www.ncbi.nlm.nih.gov/pubmed/16766240

Wei, X., Zhang, Y., Xiang, X., Sun, M., Sun, K., Han, T., Qi, B., Xie, Y., Zhang, R. & Zhu, L. (2021). 'Exploring the relationship of bone turnover markers and bone mineral density in community-dwelling postmenopausal women', *Dis Markers*, pp.1-10. https://www.ncbi.nlm.nih.gov/pubmed/33968284

Whisner, C. M., Martin, B. R., Nakatsu, C. H., Story, J. A., Macdonald-Clarke, C. J., Mccabe, L. D., Mccabe, G. P. & Weaver, C. M. (2016). 'Soluble corn fiber increases calcium absorption associated with shifts in the gut microbiome: A randomized dose-response trial in free-living pubertal females', *J Nutr,* 146(7), pp.1298-1306. https://www.ncbi.nlm.nih.gov/pubmed/27281813

World Health Organisation (2024). *Health data overview for New Zealand*. https://data.who.int/countries/554

Willett, W. (2012). 'Issues in analysis and presentation of dietary data. ' in *Nutritional Epidemiology*, pp.306-333. https://doi.org/10.1093/acprof:oso/9780199754038.001.0001

Wolf, R. L., Cauley, J. A., Baker, C. E., Ferrell, R. E., Charron, M., Caggiula, A. W., Salamone, L. M., Heaney, R. P. & Kuller, L. H. (2000). 'Factors associated with calcium absorption efficiency in pre- and perimenopausal women', *The American Journal of Clinical Nutrition,* 72(2), pp.466-471. https://doi.org/10.1093/ajcn/72.2.466.

Wright, H. H., Kruger, M. C., Schutte, W. D., Wentzel-Viljoen, E., Kruger, I. M. & Kruger, H. S. (2019). 'Magnesium intake predicts bone turnover in postmenopausal black south african women', *Nutrients,* 11(10), pp.2519. https://doi.org/10.3390/nu11102519

Zhang, L., Zhao, L., Xiao, X., Zhang, X., He, L. & Zhang, Q. (2024). 'Association of dietary carbohydrate and fiber ratio with postmenopausal bone mineral density and prevalence of osteoporosis: A cross-sectional study', *PLoS One,* 19(2), pp.0297332. https://www.ncbi.nlm.nih.gov/pubmed/38354209

Zoch, M. L., Clemens, T. L. & Riddle, R. C. (2016). 'New insights into the biology of osteocalcin', *Bone,* 82, pp.42-49. https://www.ncbi.nlm.nih.gov/pubmed/26055108

Appendices

Appendix 1. Ethics approval letter

Health and Disability Ethics Committees
Ministry of Health
133 Molesworth Street
PO Box 5013
Wellington

6011
0800 4 ETHICS hdecs@health.govt.nz

23 March 2021

Dr. Bolaji Lilian Ilesanmi-Oyelere
Massey University
Private Bag 11222
Palmerston North 4442

Dear Dr. Ilesanmi-Oyelere,

Re:	**Ethics ref:**	**21/NTB/47**
	Study title:	Modulation of bone/joint biomarkers, gut microbiota and inflammation status by synbiotics (pre and probiotics) and weight-bearing exercise: A randomised controlled trial

I am pleased to advise that this application has been ***approved*** by the Northern B Health and Disability Ethics Committee. This decision was made through the HDEC-Expedited Review pathway.

Conditions of HDEC approval

HDEC approval for this study is subject to the following conditions being met prior to the commencement of the study in New Zealand. It is your responsibility, and that of the study's sponsor, to ensure that these conditions are met. No further review by the Northern B Health and Disability Ethics Committee is required.

Standard conditions:

1. Before the study commences at ***any*** locality in New Zealand, all relevant regulatory approvals must be obtained.

2. Before the study commences at ***any*** locality in New Zealand, it must be registered in a clinical trials registry. This should be a WHO-approved registry (such as the Australia New Zealand Clinical Trials Registry, www.anzctr.org.au) or https://clinicaltrials.gov/.

3. Before the study commences at ***each given*** locality in New Zealand, it must be authorised by that locality in Online Forms. Locality authorisation confirms that the locality is suitable for the safe and effective conduct of the study, and that local research governance issues have been addressed.

After HDEC review

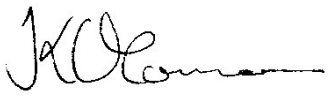
Please refer to the *Standard Operating Procedures for Health and Disability Ethics Committees* (available on www.ethics.health.govt.nz) for HDEC requirements relating to amendments and other post-approval processes.

Your next progress report is due by 23 March 2022

Participant access to ACC

This clinical trial is to be conducted principally for the benefit of the manufacturer or distributor of the medicine or item being trialled. Section 32 of the Accident Compensation Act 2001 provides that participants injured as a result of treatment received as part of this trial will **not** be eligible for publicly-funded compensation through the Accident Compensation Corporation (ACC).

Please don't hesitate to contact the HDEC secretariat for further information. We wish you all the best for your study.
Yours sincerely,



Mrs Kate O'Connor
Chairperson
Northern B Health and Disability Ethics Committee

Encl: appendix A: documents submitted
appendix B: statement of compliance and list of members

Appendix A
Documents submitted

Document	*Version*	*Date*
CV for CI: CV for CI	Version 1	23 January 2021
CVs for other Investigators: CV for other investigators	Version 1	23 January 2021
CVs for other Investigators: CV for other investigators	Version 1	23 January 2021
Protocol: Protocol chart	Version 1	23 January 2021
Survey/questionnaire: The physical activity questionnaire	Version 1	23 January 2021
Survey/questionnaire: Baseline participants' questionnare	Version 1	23 January 2021
Survey/questionnaire: Participants' screening questionnaire	Version 1	23 January 2021
Survey/questionnaire: 3-day diet diary	Version 1	23 January 2021
Covering Letter: Covering Letter for provisional approval	Version 2	12 March 2021
Evidence of scientific review: Evidence of scientific review	Version 1	26 January 2021
Evidence of scientific review: Evidence of scientific review	Version 1	26 January 2021
PIS/CF: Cleaned PIS/CF version	Version 3	12 March 2021
Protocol: Protocol	Version 1	27 January 2021
Evidence of scientific review: Evidence of scientific peer review	Version 2	15 February 2021
Protocol: Cleaned Version	Version 4	12 March 2021
Declined letter for previous application in respect of the same (or substantially similar) study: Previous invalid letter	Version 1	15 February 2021
Application		15 February 2021
Evidence of sponsor insurance	Version 1	12 March 2021
Evidence of CI indemnity	Version 1	12 March 2021
Protocol: COPES 4 Bones Research Proposal Clinical Study submitted for ethics_Mar 2021_TC.docx	version 4	01 March 2021
PIS/CF: COPES clinical study participant_information_sheetconsent-form_with update_Mar 2021_TC.doc	Version 3	01 March 2021
Response to Request for Further Information		

Appendix B
Statement of compliance and list of members

Statement of compliance

The Northern B Health and Disability Ethics Committee:

- is constituted in accordance with its Terms of Reference
- operates in accordance with the *Standard Operating Procedures for Health and Disability Ethics Committees*, and with the principles of international good clinical practice (GCP)
- is approved by the Health Research Council of New Zealand's Ethics Committee for the purposes of section 25(1)(c) of the Health Research Council Act 1990
- is registered (number 00008715) with the US Department of Health and Human Services' Office for Human Research Protection (OHRP).

List of members

Name	*Category*	*Appointed*	*Term Expires*
Mr John Hancock	Lay (the law)	14/12/2015	14/12/2018
Dr Nora Lynch	Non-lay (health/disability service provision)	19/03/2019	19/03/2026
Miss Tangihaere Macfarlane	Lay (consumer/community perspectives)	20/05/2017	20/05/2020
Mrs Kate O'Connor	Lay (ethical/moral reasoning)	14/12/2015	14/12/2018
Mrs Stephanie Pollard	Non-lay (intervention studies)	01/07/2015	01/07/2018
Mrs Leesa Russell	Non-lay (intervention studies), Nonlay (observational studies)	14/12/2015	14/12/2018
Ms Susan Sherrard	Lay (consumer/community perspectives)	19/03/2019	19/03/2022
Mrs Jane Wylie	Non-lay (intervention studies)	20/05/2017	20/05/2020

Unless members resign, vacate or are removed from their office, every member of HDEC shall continue in office until their successor comes into office (HDEC Terms of Reference)

http://www.ethics.health.govt.nz

Appendix 2. Participant information sheet & consent form

Participant Information Sheet

COPES (Combination of Physical Exercise and Synbiotics) 4 Bones

MASSEY UNIVERSITY
COLLEGE OF HEALTH
TE KURA HAUORA TANGATA

Formal Study title:

Modulation of bone/joint biomarkers, gut microbiota and inflammation status by synbiotics (pre- and probiotics) and weight-bearing exercise; a randomised controlled trial

Lead [Researcher / Study Doctor]: Dr. Bolaji Lilian Ilesanmi-Oyelere
Study Site: Human Nutrition Research Unit, Massey University, Palmerston North
Contact phone number: 021 0852 2308
Ethics committee ref.: 21/NTB/47

You are invited to take part in a study on **modulation of bone/joint biomarkers, gut microbiota and inflammation status by synbiotics (pre- and probiotics) and weight-bearing exercise (The COPES-4-Bones clinical study)**. Whether or not you take part is your choice. If you don't want to take part, you don't have to give a reason, and it won't affect the care you receive. If you do want to take part now, but change your mind later, you can pull out of the study at any time.

This Participant Information Sheet will help you decide if you'd like to take part. It sets out why we are doing the study, what your participation would involve, what the benefits and risks to you might be, and what would happen after the study ends. We will go through this information with you and answer any questions you may have. You do not have to decide today whether or not you will participate in this study. Before you decide you may want to talk about the study with other people, such as family, whānau, friends, or healthcare providers. Feel free to do this.

If you agree to take part in this study, you will be asked to sign the Consent Form on the last page of this document. You will be given a copy of both the Participant Information Sheet and the Consent Form to keep.

This document is 11 pages long, including the Consent Form. Please make sure you have read and understood all the pages.

VOLUNTARY PARTICIPATION AND WITHDRAWAL FROM THIS STUDY

Participation in this study is completely voluntary. You are under no obligation to accept this invitation. If you decide to participate, you have the right to:

- Decline to answer any particular question
- Withdraw from the study at any time
- Ask any questions about the study at any time during participation
- Provide information on the understanding that your name will not be used unless you give permission to the researcher
- Be given access to a summary of the project findings when it is concluded
- Withdrawing from the study, should you choose to, will not result in any disadvantage to you.

What is the purpose of the study?

Osteoporosis is a health problem in the elderly with hip and spine fractures occurring commonly after the age of 70 while osteoarthritis is also a major health concern in older age. Low bone mineral density (BMD) at an early age is strong risk factor for osteoporosis, with early detection allowing precautionary measures. There are a lot of factors that affect BMD leading to osteoporosis including genetics, age, diet, physical activity, hormones, alcohol intake, smoking and body weight.

Synbiotics are known to have beneficial effect on the gut microbiota (GM) and inflammation status which in turn could affect the BMD especially at postmenopausal years. Synbiotics are a mix of friendly beneficial bacteria and fiber. The gut microbiota is the microbial make-up/communities of the intestinal tract. Studying the effect of synbiotics and weight-bearing exercise on the gut, inflammation status and bone health might help in the development of therapies for osteoporosis and osteoarthritis during post-menopause.

The aim of this project is to investigate the role of weight-bearing physical exercise, probiotics and prebiotics on bone markers, gut health and inflammation status in postmenopausal women.

HOW IS THE STUDY DESIGNED?

The COPES clinical study will involve 120 eligible postmenopausal women 50 years and above. There will be 2 groups of 60 participants by stratified randomisation of exercise history. A method based on chance alone by which study participants are assigned to a treatment group. The chance of being in each group is 1/2. Participants will take part in online or telephone screening to check eligibility. If eligible they will visit the Human Nutrition Research Unit at Massey University twice at two time points; week 1 and week 12. Each visit will be for approximately 5 hrs for all the groups.

Description of groups

Control group: Placebo and exercise (7,000 steps required per day) required

Synbiotic + Exercise group: Both exercise (7,000 steps per day) and synbiotic intake required

The intervention capsule will consist of the probiotic supplement (6 billion colony forming units (CFU) of Lactobacillus rhamnosus) and 10 grams of prebiotic fiber), meanwhile, the placebo will be a maltodextrin.

All participants will be required to have body composition measurements, bone density measurement and fill out questionnaires regarding health, demographics, lifestyle, physical activity and dietary intake. In addition, participants will be asked to provide fasted blood, urine and faecal samples.

WHO CAN TAKE PART IN THE STUDY?

To be able to participate in this study you need to meet the following criteria:

- Aged 55 – 75 years
- 5 years past menopause
- Not taken antibiotics within the last 6 months
- Not taken laxatives, gastric motility medications, prebiotic or probiotic containing foods or supplements (i.e. Symbio probalance, Bio yoghurt, Yoplait Elivae, Activate, Bio farm organic, Yakult, Kefir, Psyllium, Sauerkraut, Kimchi, Kombucha) within the last month*
- BMI between 17 and 40 kg/m^2
- No significant weight loss or weight gain within the past year
- No food intolerances which cause gastrointestinal symptoms (i.e. lactose intolerance)
- Non-smoker
- Do not have a high intake of alcohol

*Please note: If you are taking prebiotic or probiotic containing foods or supplements and are willing to stop taking these foods or supplements for a month prior to starting the study and during the study then you are also eligible to take part in the study.

What will my participation in the study involve?

The following samples will be collected twice at two time points 12 weeks apart from all participants. And each appointment will be expected to take approximately 5 hours. Please kindly let Lilian or Marlena know if you will like the unused samples returned to you after the study.

Fasting blood samples

You will come into the lab around 9am having fasted since 9.00pm the night before. A light breakfast will be available. We will take a blood sample (25mL) by using a syringe for your fasting blood sample to analyse bone markers, C-Terminal telopeptide type I collagen (CTx), C-reactive protein (CRP), inflammation markers, 25-OH-D, Parathyroid hormone (PTH), COMP, a marker of cartilage degradation, osteocalcin etc. will be measured.

Faecal samples

Faecal samples will be collected and processed by the extraction total genomic DNA and 16s ribosomal DNA amplification. The total genomic DNA is the genetic material of the gut microbiota and not for you as the participant.

Questionnaires

You will be asked to complete questions about your dietary intake, demography and any physical activity or medications. You will be asked to fill in a screening questionnaire, 3-day food diary, Food Frequency Questionnaire (FFQ) and the New Zealand Physical Activity Questionnaire (NZPAQ).

Supplement

You will need to take the synbiotic (probiotic + prebiotic) food supplement daily as instructed.

Exercise

You will also need to make 7,000 steps daily.

Dual X-Ray Absorptiometry (DXA) measurement will be performed to measure body composition and bone density.

WHAT WILL HAPPEN TO MY *BLOOD AND FAECAL* SAMPLES?

All samples will be labelled with the participant's unique identity code/number and not by the participant's name.

The blood samples will be stored in a -80 degree freezer for up to 12 months during which time the biochemical analyses will be conducted. While waiting for analysis for bone/joint biomarkers, samples will be kept in the freezer at the Nutrition laboratory at Massey University, Riddet Building, Palmerston North campus. Some serum/plasma samples will also be sent to the accredited laboratories, for example the University of Otago, Canterbury Health Laboratories, LabPlus to assess DNA sequencing, inflammatory markers and vitamin D status.

Participants may ask to withdraw their samples at any time during the study.

Māori participants will be fully informed and have time to make their decision to be a part of the study. They will be given full information regarding the disposal of samples and the opportunity to observe appropriate tikanga Māori practice while taking part in the study. For example, the research team will offer participants the opportunity to karakia while blood samples are being taken and the option for disposal of whole blood samples if they wish. Bodily samples will be handled with integrity in the knowledge that the material is still considered living and therefore a treasure.

What are the possible risks of this study?

Some people may have a fear of having a blood sample taken or experience discomfort when blood samples are taken. Occasionally a slight bruising will result. The bruising usually disappears within a day or two. Blood samples will be taken by a certified phlebotomist. There may be social or cultural discomfort from having a blood sample, body composition measurements or performance-based measurements taken, however, you will be treated with respect and privacy will be ensured. We will explain all measurements being taken and ask for your permission prior to undertaking these measurements. You may also be accompanied by a support person if required. Every effort will be made to ensure your comfort and respect your participation.
We will use the Hologic DXA machine to estimate body fat percentage and bone density. The DXA has X-ray beams at 2 different energies. This dose is very low and unlikely to cause harm. The total effective dose of radiation to which you will be exposed is 10 microsieverts (μSv), this is much lower than the range normally used in medical diagnostics. To put it in perspective, the amount of radiation you are exposed to during a flight to the United Kingdom return is 100 μSv and from a dental X-ray 50 μSv. The room is private, and you can enter the DXA machine in complete privacy. We will provide you with a gown to wear during this measurement.

WHAT ARE THE POSSIBLE BENEFITS OF THIS STUDY?

A potential benefit of being involved in this study is that you will contribute to gaining a better understanding of the effect of synbiotics on the gut, inflammation status and bone health. If you are interested, you can also request to be sent information on your analyses and body composition measurements. You will also receive a summary of the main findings of the study which will either be posted or emailed to you.

Will any costs be reimbursed?

Participant will not incur any costs as part of being involved in the study and will receive reimbursement for time and travel ($75 in MTA vouchers).

What if something goes wrong?

If you were injured in this study, you would be eligible **to apply** for compensation from ACC just as you would be if you were injured in an accident at work or at home. This does not mean that your claim will automatically be accepted. You will have to lodge a claim with ACC, which may take some time to assess. If your claim is accepted, you will receive funding to assist in your recovery.
If you have private health or life insurance, you may wish to check with your insurer that taking part in this study won't affect your cover.

What will happen to my information?

During this study the researchers will record information about you and your study participation. This includes the results of any study assessments. You cannot take part in this study if you do not consent to the collection of this information.
Identifiable Information
Identifiable information is any data that could identify you (e.g. your name, date of birth, or address). The following groups may have access to your identifiable information:

- Research staff (to complete study assessments)

- Government agencies, like HDEC, ACC and its representatives, if you make a compensation claim for study-related injury. Identifiable information is required in order to assess your claim.
- Your usual doctor, if a study test gives an unexpected result that could be important for your health. This allows appropriate follow-up to be arranged.

De-identified (Coded) Information

To make sure your personal information is kept confidential, information that identifies you will not be included in any report generated by the researcher. Instead, you will be identified by a code. The researcher will keep a list linking your code with your name, so that you can be identified by your coded data if needed.

The results of the study may be published or presented, but not in a form that would reasonably be expected to identify you.

Security and Storage of Your Information.

Your identifiable information is held at Massey University during the study. After the study it is transferred to a secure archiving site and stored for at least 10 years, then destroyed. Your coded information will be entered into electronic case report forms. Coded study information will be kept in secure, cloud-based storage indefinitely. All storage will comply with local and/or international data security guidelines.
The linked data in this study will be destroyed at the end of the study.

Risks.

Although efforts will be made to protect your privacy, absolute confidentiality of your information cannot be guaranteed. Even with coded and anonymised information, there is no guarantee that you cannot be identified. The risk of people accessing and misusing your information (e.g. making it harder for you to get or keep a job or health insurance) is currently very small but may increase in the future as people find new ways of tracing information.

Rights to Access Your Information.

You have the right to request access to your information held by the research team. You also have the right to request that any information you disagree with is corrected.
Please ask if you would like to access the results of your screening and safety tests during the study. You may access other study-specific information before the study is over, but this could result in you being withdrawn from the study to protect the study's scientific integrity.
If you have any questions about the collection and use of information about you, you should ask researcher.

Rights to Withdraw Your Information.

You may withdraw your consent for the collection and use of your information at any time, by informing the study researchers.
If you withdraw your consent, your study participation will end, and the study team will stop collecting information from you.
Information collected up until your withdrawal from the study will continue to be used and included in the study. This is to protect the quality of the study.

WHAT HAPPENS AFTER THE STUDY OR IF I CHANGE MY MIND?

If you wish to withdraw from the study, please inform one of the research team. Information and data collected up until your withdrawal from the study will continue to be used and included in the study. This is to protect the quality of the study.
The data will be used for the purposes of this study, and fully anonymised, selected outcomes may be shared with other researchers on request for the purpose of accumulating data from individual studies. Only investigators and administrators of the study will have access to personal information, and this will be kept secure and strictly confidential. Participants will be identified only by a study identification number. Results of this project may be published or presented at conferences or seminars. No individuals will be able to be identified.
At the end of this study the list of participants and their study identification number will be disposed of. Any raw data on which the results of the project depend will be retained in secure storage for 10 years, after which it will be destroyed.
All participants will have access to a summary of the project findings and which treatment group they were in when the study is completed.

CAN I FIND OUT THE RESULTS OF THE STUDY?

All participants will have access to a summary of the project findings when it is completed.

The study is registered with the Australian New Zealand Clinical Trials Registry and can be accessed at www.ANZCTR.org.au.

WHO IS FUNDING THE STUDY?

This study is funded by Lottery Health Research, Fonterra and Massey University.

WHO HAS APPROVED THE STUDY?

This study has been approved by an independent group of people called a Health and Disability Ethics Committee (HDEC), who check that studies meet established ethical standards. The Massey University Human Ethics Committee has approved this study.

Who do I contact for more information or if I have concerns?

If you have any questions, concerns or complaints about the study at any stage, you can contact:

Dr. Bolaji Lilian Ilesanmi-Oyelere, Post-Doctoral Fellow
Phone: 021 08522308
Email: b.ilesanmi-oyelere@massey.ac.nz

Prof. Marlena Kruger
Phone: + 6469517571
Email: m.c.kruger@massey.ac.nz

If you want to talk to someone who isn't involved with the study, you can contact an independent health and disability advocate on:

Phone:	0800 555 050
Fax:	0800 2 SUPPORT (0800 2787 7678)
Email:	advocacy@advocacy.org.nz
Website:	https://www.advocacy.org.nz/

For Maori health support please contact:

Dr. Sharon Henare, Senior Lecturer
Phone: +6469517289
Email: s.j.henare@massey.ac.nz

You can also contact the health and disability ethics committee (HDEC) that approved this study on:

Phone:	0800 4 ETHIC
Email:	hdecs@health.govt.nz

SCOPE

The Covid-19 controls are over and above the existing Health and Safety policies within Massey University. They are intended to be read in addition to the University Operating Plan (appended) general guidelines for what the traffic light system means for Massey.

https://www.massey.ac.nz/massey/about-massey/news/advice-on-coronavirus-outbreak/alert-levels/alert-levels_home.cfm

Where possible and appropriate, these guidelines can also be followed at other levels of the protection framework allowing a smooth transition if levels change.

Cleaning

All cleaning of touch points will be undertaken with approved surface cleaning products, e.g. 70% ethanol wipes (e.g. Clinell hospital grade wipes) and sprays. (NB include any pens and clipboards used)

Extra cleaning will take place before and after the study visit including door handles, high use equipment and common areas. Note that cleaning is the responsibility of the research team.

After each participant visit (including visits to the toilet to provide a urine sample) researchers will clean the area and any equipment or surface they may have used or touched.

Basic Hygiene Protocols

- Hands must be washed/sanitised upon entrance to the HNRU and at the beginning and end of each appointment.
- Cough or sneeze into your elbow or by covering your mouth and nose with tissues
- Place used tissues in the biohazard bin or a bag immediately and wash your hands
- Avoid touching your eyes, nose or mouth
- Items that have come into contact with bodily fluids and discarded PPE should be placed in a yellow biohazard waste bag located in the clinical lab and examination room; full bags should be disposed of via the normal medical waste disposal system.
- Wear a government-approved mask.

Risk Identification

All participants will be asked to contact study staff if: they become aware they had contact with a confirmed or suspected case of Covid-19 within the 14 days prior to their visit to HNRU, or within two weeks following their study visit; they become unwell with symptoms consistent with Covid-19 (as per screening questions); if they are suspected of having Covid-19, or if they test positive for Covid-19.

The HNRU will stop operating if either a researcher or a participant is suspected or confirmed as having Covid-19 while an investigation is completed, and appropriate cleaning is carried out.

Social Distancing & Ventilation

Physical distancing of at least 1 m (ideally 2 m) should be practised whenever possible. Obviously, there are some procedures (e.g. blood draws, anthropometry, DXA scans etc) where physical distancing guidelines cannot be adhered to; in this case, note the Ministry of Health guidelines that procedures should be carried out in as short a time-frame as possible (less than 15 minutes). Where physical distancing of at least 1 m is not possible, use of masks. Use of the air conditioning units in the HNRU aids ventilation.

Consider the vulnerability of your participants but remain aware that use of mask is important. Reassure them.

Note that the requirement for distancing may mean extra rooms will need to be booked.

Protection

For non-contact activities, only social distancing and good hand hygiene is required.

When physical distancing of <1 m cannot be practised, an assessment must be made of the likelihood of contact with body fluids.

• Gloves must be worn for all contact procedures where contact with body fluids is possible) including blood-taking etc., and where possible for any contact with a participant e.g. positioning for a scan.

• Government-approved masks should be worn at all times by research staff.

- The number of participants in a single visit will be kept to a maximum of 2 unrelated participants. Participant arrival time will be staggered to reduce interaction between participants and contact between different groups

Consent Form

COPES (Combination of physical exercise and synbiotics) 4 Bones

Please tick to indicate you consent to the following

I have read, or have had read to me in my first language, and I understand the Participant Information Sheet.	☐	
I have been given sufficient time to consider whether or not to participate in this study.	☐	
I have had the opportunity to use a legal representative, whanau/ family support or a friend to help me ask questions and understand the study.	☐	
I am satisfied with the answers I have been given regarding the study and I have a copy of this consent form and information sheet.	☐	
I understand that taking part in this study is voluntary (my choice) and that I may withdraw from the study at any time without this affecting my medical care.	☐	
I consent to the research staff collecting and processing my information, including information about my health.	☐	
If I decide to withdraw from the study, I agree that the information collected about me up to the point when I withdraw may continue to be processed.	Yes ☐	No ☐
I agree to an approved auditor appointed by the New Zealand Health and Disability Ethics Committees, or any relevant regulatory authority or their approved representative reviewing my relevant medical records for the sole purpose of checking the accuracy of the information recorded for the study.	☐	
I understand that my participation in this study is confidential and that no material, which could identify me personally, will be used in any reports on this study.	☐	
I understand the compensation provisions in case of injury during the study.	☐	
I know who to contact if I have any questions about the study in general.	☐	
I understand my responsibilities as a study participant.	☐	
I wish to receive a summary of the results from the study.	Yes ☐	No ☐

Declaration by participant:
I hereby consent to take part in this study.

Participant's name: _____

Signature: _____ Date: _____

Declaration by member of research team:

I have given a verbal explanation of the research project to the participant, and have answered the participant's questions about it.

I believe that the participant understands the study and has given informed consent to participate.

Researcher's name: _____

Signature: _____ Date: _____

Appendix 3. Participant baseline questionnaire

MASSEY UNIVERSITY
COLLEGE OF HEALTH
TE KURA HAUORA TANGATA

ID:

COPES (combination of physical exercise and synbiotics) 4 Bones

Copes 4 BONES Study: Modulation of bone/joint biomarkers, gut microbiota and inflammation status by synbiotics (pre and probiotics) and weight-bearing exercise

Thank you for expressing an interest in participating in our research project. To ensure you are eligible to participate in the research project we would appreciate it if you can answer the following questions.

If you have any comments or questions relating to the research project or the questionnaire, please feel free to contact **Lilian Ilesanmi-Oyelere** during working hours on **021 085 22308** or email **b.ilesanmi-oyelere@massey.ac.nz**

OR

Professor Marlena Kruger during working hours on **(06) 951 7571**

Date of birth: _____

Ethnicity (please tick all that apply):
NZ European ☐ Maori ☐ Samoan ☐ Cook Island Maori ☐
Tongan ☐ Indian ☐ Chinese ☐ Other ☐ please specify: _____

How were you delivered Caesarean ☐ Normal ☐ Don't know ☐

Do you drink alcohol (please tick)? Yes ☐ No ☐
If yes, how many standard drinks do you consume per week? _____
(1 standard drink is 1 can/bottle of standard beer (330ml), 100ml wine or 30ml of spirits)
If yes, on how many occasions would you drink alcohol per week? _____

Were you breastfed as an infant? Yes ☐ No ☐ Don't know ☐

Have you been diagnosed with or experienced any of the following (tick for yes)?

Osteoporosis	
Heart disease	
Stroke	
High cholesterol	
High blood pressure	
Cancer	
Inflammatory bowel disease	
Irritable bowel syndrome	
Food intolerance or allergies causing diarrhoea, bloating, cramping or constipation	
Long term diarrhoea or constipation	
Fractures	
If yes, how many?	
Other (please specify):	

Are you taking any medications (traditional or homeopathic) **or nutritional supplements?**

Type of medication/supplement	**Taking?** (please tick)	**If you have answered <u>YES</u> for any of the medication or supplement options please provide the below information**	
		Medication/supplement name	***Dose and frequency***
Antibiotics	Yes☐ No☐		
Blood pressure lowering	Yes☐ No☐		
Cholesterol lowering	Yes☐ No☐		
Vitamins	Yes☐ No☐		
Minerals	Yes☐ No☐		
Laxatives	Yes☐ No☐		
Metamucil or Benefibre	Yes☐ No☐		
Phloe or Kiwicrush	Yes☐ No☐		
Probiotics	Yes☐ No☐		
Prebiotics	Yes☐ No☐		
Antacids or anti-reflux	Yes☐ No☐		
Anti-inflammatory	Yes☐ No☐		
Other	Yes☐ No☐		

Have you consumed prebiotic or probiotic yoghurt, or fermented drinks or foods within the <u>past month</u> (i.e. Symbio probalance, Bio yoghurt, Yoplait Elivae, Activate, Bio farm organic, Yakult, Kefir, Sauerkraut, Kimchi, Kombucha) (please tick)?

Yes ☐ No ☐

If yes, please specify the product name and frequency of consumption: _____

_____

Have you taken antibiotics within the <u>last 6 months</u> (please tick)?

Yes ☐ No ☐

If yes, please specify the name of the antibiotic and when you last took it:

_____

How often do you take antibiotics in <u>a year</u> (please tick)?

Once ☐ Twice ☐ 3 times ☐ 4 times or more ☐

Have you made any significant changes to your food intake over the <u>past year</u> (i.e. become vegetarian/vegan, stopped consuming gluten, dairy or sugar, increased your fruit and vegetable

☐ ☐

intake or increased/decrease the amount of food you are eating) (please tick)? Yes
No

If yes, what changes to your food intake have you made? _____
_____

Do you regularly experience any of the following (please tick all that apply)?

Abdominal pain ☐ Abdominal bloating ☐
Flatulence/wind ☐

If you experience abdominal pain, bloating or flatulence/wind is it mild (nagging/annoying)**, moderate** (strong negative influence on your daily living) **or severe** (disabling) (please tick the boxes that apply)?

	Absent	**Mild**	**Moderate**	**Severe**
Abdominal pain				
Abdominal bloating				
Flatulence/wind				

Thank you very much for taking the time to complete this questionnaire.

Appendix 4. Three-day food record

ID:

Copes 4 Bones clinical Study

3 Day Food Record

Thank you very much for taking part in the Copes 4 Bones clinical Study. We are extremely grateful for your time, effort and commitment!

(2 week days and one weekend)

If you have any questions, please contact Lilian Ilesanmi-Oyelere on 021-08522308 or email
b.ilesanmi-oyelere@massey.ac.nz

All information in this diary will be treated with the strictest confidence. No one outside the study will have access to this.

Please bring this diary with you for your second visit.

Reminders for your next appointments

- Bring this diary with you to your next appointment
- Wear comfortable, casual clothes including a top with either short-sleeves on no sleeves.
- Your appointment will last approximately 2 hours so bring something along to entertain yourself with – your laptop and personal DVDs, a book, magazines, iPod or study notes.

Second Appointment

Date
Time

If for any reason you are going to be unable to come for this appointment at the scheduled time and/or day, please let us know as soon as possible.

Email: **b.ilesanmi-oyelere@massey.ac.nz**
Phone: *Lilian Ilesanmi-Oyelere on 021-08522308*

3 day food diary - What to do?

- Record all that you eat and drink on the following dates (please include a weekend):

_____

_____

_____

- If possible record food at the time of eating or just after – try to avoid doing it from memory at the end of the day.
- Include all meals, snacks, and drinks, even tap water.
- Include anything you have added to foods such as sauces, gravies, spreads, dressings, etc.
- Write down any information that might indicate size or weight of the food to identify the portion size eaten.
- Use a new line for each food and drink. You can use more than one line for a food or drink. See the examples given.
- Use as many pages of the booklet as you need.

Describing Food and Drink

- Provide as much detail as possible about the type of food eaten. For example **brand names and varieties / types** of food.

General description	Food record description
Breakfast example – cereal, milk, sugar	1 cup Sanitarium Natural Muesli 1 cup Pam's whole milk 1 tsp Chelsea white sugar
Coffee	1 tsp Gregg's instant coffee 1 x 200ml cup of water 2 Tbsp Meadow fresh light green milk
Pasta	1 cup San Remo whole grain pasta spirals (boiled)
Pie	Big Ben Classic Mince and Cheese Pie (170g)

- Give details of all the **cooking methods** used. For example, fried (sort of oil/fat used), grilled, baked, poached, boiled…

General description	Food record description
2 eggs	2 size 7 eggs fried in 2tsp canola oil 2 size 6 eggs (soft boiled)
Fish	100g salmon (no skin) poached in 1 cup of water for 10 minutes

- When using foods that are cooked (eg. pasta, rice, meat, vegetables, etc), please record the **cooked portion** of food.

General description	Food record description
Rice	1 cup cooked Jasmine rice (cooked on stove top)
Meat	90g lean T-bone steak (fat and bone removed)
Vegetables	½ cup cooked mixed vegetables (Wattie's peas, corn, carrots)

- Please specify the **actual amount of food eaten** (eg. for leftovers, foods where there is waste)

General description	Food record description
Apple	1 x 120g Granny Smith Apple (peeled, core not eaten – core equated to ¼ of the apple)
Fried chicken drumstick	100g chicken drumstick (100g includes skin and bone); fried in 3 Tbsp Fern leaf semi-soft butter

- Because we are especially interested in your calcium intake, please take care to list **all** the milk you consume, and record what type of milk it was.

General description	Food record description

hot chocolate	l x cup hot chocolate made with Cadbury's powder and 150 mls Anchor Calcitrim milk, 100 ml hot water. No sugar

- **Record recipes** of home prepared dishes where possible: record how many the recipe fed and the proportion of the dish you ate. There are blank pages for you to add recipes or additional information.

Recording the amounts of food you eat

It is important to also record the quantity of each food and drink consumed. This can be done in several ways.

- By using household measures – for example, cups, teaspoons and tablespoons. eg. 1 cup frozen peas, 1 heaped teaspoon of sugar.

- By weight marked on the packages – eg. a 425g tin of baked beans, a 32g cereal bar, 600ml Coke

- Weighing the food – this is an ideal way to get an accurate idea of the quantity of food eaten, in particular for foods such as meat, fruits, vegetables and cheese.

- For bread – describe the size of the slices of bread (eg. sandwich, medium, toast) – also include brand and variety.

- Using comparisons – eg. Meat equal to the size of a pack of cards, a scoop of ice cream equal to the size of a hen's egg.

- Use the food record instructions provided to help describe portion sizes.

General description	Food record description
Cheese (Edam)	1 heaped tablespoon of grated cheese 1 slice cheese (8.5 x 2.5 x 2mm) 1 cube cheese, match box size Grated cheese, size 10B

- If you go out for meals, describe the food eaten in as much detail as possible.

- ***Please eat as normally as possible - don't adjust what you would normally eat just because you are keeping a diet record and be honest! Your food record will be identified with a number rather than your name.***

Example day

Time food was eaten	**Complete description of food (food and beverage name, brand, variety, preparation method)**	**Amount consumed (units, measures, weight)**
Example 7:55am	Sanitarium weetbix	2 Weetabix
" "	Anchor Blue Top milk	150ml
" "	Chelsea white sugar	2 heaped teaspoons
" "	Orange juice (Citrus Tree with added calcium – nutrition label attached)	1 glass (275 ml)
10.00am	Raw Apple (gala)	Ate all of apple except the core, whole apple was 125g (core was ¼ of whole apple)
12.00pm	Home made pizza (recipe attached)	1 slice (similar size to 1 slice of sandwich bread, 2 Tbsp tomato paste, 4 olives, 2 rashers bacon (fat removed), 1 Tbsp chopped spring onion, 3 Tbsp mozzarella cheese)
1.00pm	Water	500ml plain tap water
3.00pm	Biscuits	6 x chocolate covered Girl Guide biscuits (standard size)
6.00pm	Lasagne	½ cup cooked mince, 1 cup cooked Budget lasagne shaped pasta , ½ cup Wattie's creamy mushroom and herb pasta sauce, ½ cup mixed vegetables (Pam's carrots, peas and corn), 4 Tbsp grated Edam cheese
6.30pm	Banana cake with chocolate icing (homemade, recipe attached)	1/8 of a cake (22cm diameter, 8 cm high), 2 Tbsp chocolate icing
" "	Tip Top Cookies and Cream ice cream	1 cup (250g)
7.30pm	Coffee	1 tsp Gregg's instant coffee 1 x 300ml cup of water 2 Tbsp Meadow fresh blue top milk 2 tsp sugar

Date_____ DAY 1

Time food was eaten	Complete description of food (food and beverage name, brand, variety, preparation method)	Amount consumed

Date_____ DAY 1 continued

Time food was eaten	Complete description of food (food and beverage name, brand, variety, preparation method)	Amount consumed

Recipes (Day 1)

Date_____ DAY 2

Time food was eaten	Complete description of food (food and beverage name, brand, variety, preparation method)	Amount consumed

Date_____ DAY 2 continued

Time food was eaten	Complete description of food (food and beverage name, brand, variety, preparation method)	Amount consumed

Recipes (Day 2)

Date_____ DAY 3

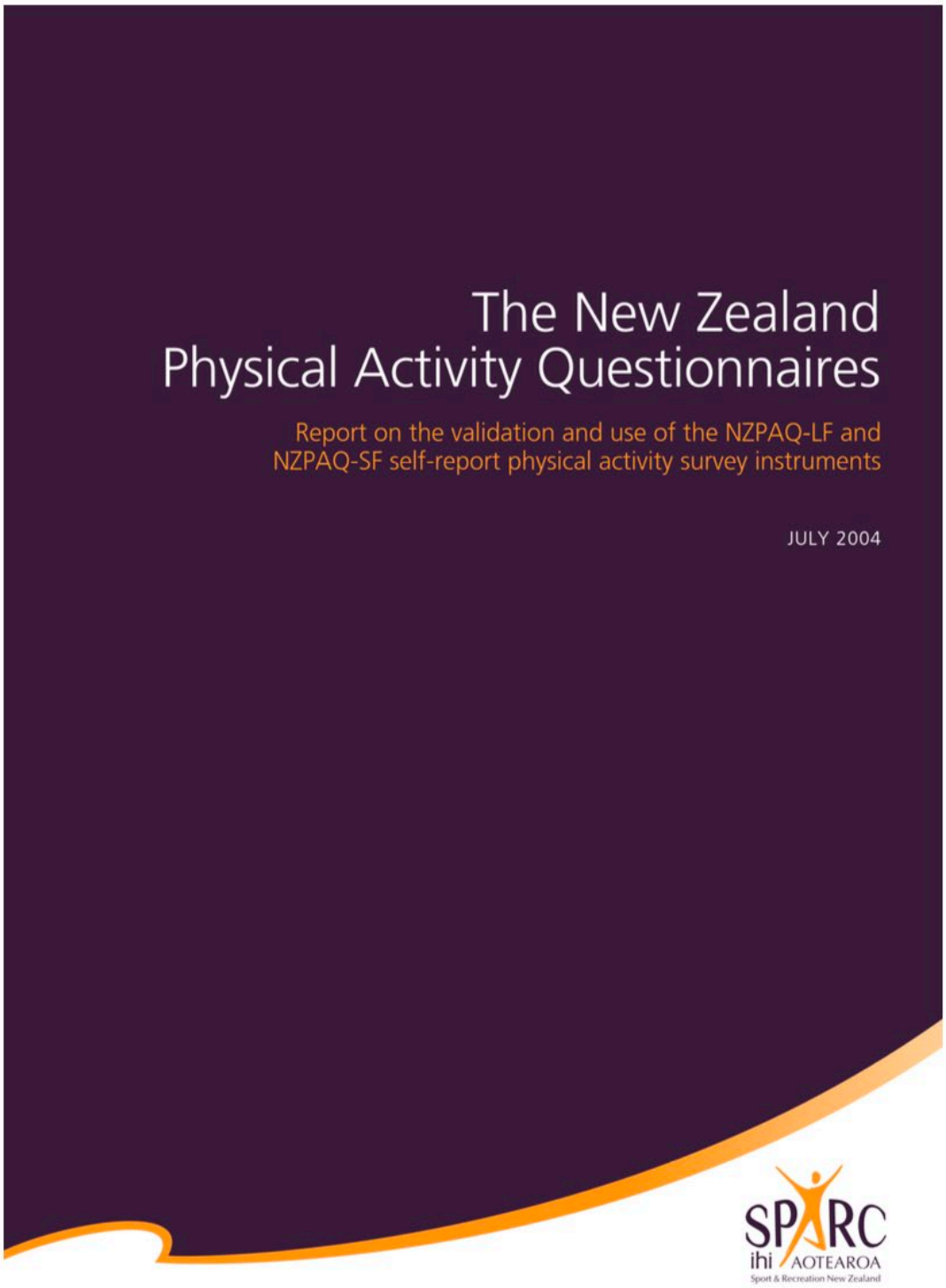
Time food was eaten	Complete description of food (food and beverage name, brand, variety, preparation method)	Amount consumed

Date_____ DAY 3 continued

Time food was eaten	Complete description of food (food and beverage name, brand, variety, preparation method)	Amount consumed

Recipes (Day 3)

Appendix 5. Physical activity questionnaire



New Zealand Physical Activity Questionnaire – Short Form (Version 1)

'I am going to ask you about the time you spent being physically active in the last 7 days, from last xxx to yesterday. Do not include activity undertaken today.

By 'active' I mean doing anything using your muscles.

'Think about activities at work, school or home, getting from place to place, and any activities you did for exercise, sport, recreation or leisure.

'I will ask you separately about brisk walking, moderate activities, and vigorous activities.'

Start Time:

Ask questions 1–7 (8 is optional)

Walking

1. During the last 7 days, on how many days did you ***walk at a brisk pace*** – a brisk pace is a pace at which you are breathing harder than normal? This includes walking at work or school, while getting from place to place, at home and at any activities that you did solely for recreation, sport, exercise or leisure.

   Think *only* about brisk walking done for at least 10 minutes at a time.

   ☐ _____ days per week (GO TO 2)

   ☐ None (GO TO 3)

2. How much time did you typically spend walking at a brisk pace on **each** of those days?

   ☐ _____ hours _____ minutes

Moderate physical activity

3. During the last 7 days, on how many days did you do **moderate** physical activities? 'Moderate' activities make you breathe harder than normal, **but only a little** – like carrying light loads, bicycling at a regular pace, or other activities like those on this card ***(Showcard 1 – Moderate Physical Activity).*** Do not include walking of any kind.

   Think *only* about those physical activities done for at least 10 minutes at a time.

   ☐ _____ days per week (GO TO 4)

   ☐ None (GO TO 5)

4. How much time did you typically spend on **each** of those days doing moderate physical activities?

   ☐ _____ hours _____ minutes

Vigorous physical activity

5. During the last 7 days, on how many days did you do **vigorous** physical activities? 'Vigorous' activities make you breathe **a lot** harder than normal ('huff and puff') – like heavy lifting, digging, aerobics, fast bicycling, or other activities like those shown on this card ***(Showcard 2 – Vigorous Physical Activity)***?

   Think only about those physical activities done for at least 10 minutes at a time.

   ☐ _____ days per week (GO TO 6)

   ☐ None (GO TO 7)

6. How much time did you typically spend on **each** of those days doing vigorous physical activities?

   ☐ _____ hours _____ minutes

Frequency of Activity

7. Thinking about all your activities over the last 7 days (including brisk walking), on how many days did you engage in:
   - At least 30 minutes of moderate activity (including brisk walking) that made you breathe a little harder than normal, OR
   - At least 15 minutes of vigorous activity that made you breathe a lot harder than normal ('huff and puff')?

   ☐ _____ days per week

   ☐ None

Stage of Change

Note: This question is optional

8. Describe your regular physical activity over the past six months. Regular physical activity means at least 15 minutes of vigorous activity (makes you 'huff and puff') or 30 minutes of moderate activity (makes you breathe slightly harder than normal) each day for 5 or more days each week. Include brisk walking.

   ☐ I am not regularly physically active and do not intend to be so in the next 6 months

   ☐ I am not regularly physically active but am thinking about starting in the next 6 months

   ☐ I do some physical activity but not enough to meet the description of regular physical activity

   ☐ I am regularly physically active but only began in the last 6 months

   ☐ I am regularly physically active and have been so for longer than 6 months

Finish Time:

Notes:

NZPAQ - Short Form Showcards

Showcard 1: Moderate Physical Activity

Carrying light loads	
Electrical work	Badminton (social)
Farming	Ballroom dancing
Heavy gardening (digging, weeding, raking, planting, pruning, clearing section)	Bowls (indoor, outdoor/lawn)
Heavy cleaning (sweeping, cleaning windows, moving furniture)	Cricket (outdoors – batting and bowling)
House renovation	Cycling (recreational – less than 15 km/hr – not mountain biking)
Machine tooling (operating lathe, punch press, drilling, welding)	Deer hunting
Lawn mowing (manual mower)	Doubles tennis
Plastering	Exercising at home (not gym)
Plumbing	Golf
	Horse riding/equestrian
Kapa haka practice	Kayaking – slow
Waiata-a-ringa	Skate boarding
	Surfing/body boarding
	Yachting/sailing/dingy sailing

Showcard 2: Vigorous Physical Activity

Carrying heavy loads	Boxing
Forestry	Aerobics
Heavy construction	Kayaking – fast
Digging ditches	Athletics (track and field)
Chopping or sawing wood	Aquarobics
	Skiing
Taiaha	Badminton (competitive)
Haka	Basketball
	Mountain biking
Soccer	Cricket – indoors (batting and bowling)
Rowing	Cycling – competitive
Rubgy League	Cycling – recreational (not mountain biking) – more than 15 km/hr
Rugby Union	Rock climbing
Hockey	Exercise classes / going to the gym (other than for aerobics) / weight training
Race walking	Netball
Running/jogging/cross country	Judo, karate, other martial arts
Table tennis (competitive)	Softball (running and pitching only)
Singles tennis	Squash
Touch rugby	Surf life saving
Tramping	Swimming – competitive
Triathlon	Waterpolo
Volleyball	

Interviewer Instructions: NZPAQ-SF (Version 1)

1. Administer the questionnaire only by face-to-face interview.
2. Do not use with children (<15 years).
3. Use translations when necessary (so far, Tongan and Samoan versions are available – please request these from Ministry of Health or SPARC if needed).
4. Note the start time for the interview in the box provided.
5. Begin with introductory statements, then proceed with the questionnaire, following the routing of questions as appropriate and showing the respondent the relevant showcard at the appropriate times.
6. Give the respondent the time they need to think about each question and formulate their response.
7. If the respondent requests clarification of a question, first read the question again. Then if clarification is still needed, try to use definitions or examples provided in the questionnaire and the showcards.
8. Once the interview has been completed, note the finishing time in the box provided (this is likely to be longer for older people, less educated people and those for whom English is a second language).
9. Also note (in the **Notes** box provided) if the information is possibly unreliable, together with reason for this, eg, lack of rapport, English is second language, cognitive defect, hearing/ speaking/communicating difficulty, lack of relevance (eg severe mobility disability).


MINISTRY OF HEALTH
MANATŪ HAUORA
SPARC
ihi AOTEAROA
Sport & Recreation New Zealand