Bone density and dietary calcium in New Zealand vegans

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Abstract

The restrictive aspect of a vegan dietary pattern warrants attention, as it may lead to individuals unknowingly obtaining low intakes of calcium. Moreover, several international studies have reported low calcium intakes in vegans. Furthermore, prolonged low calcium intakes can result in reduced bone mineral density (BMD) and increased risk of developing osteoporosis later in life. In older adults, a calcium deficiency will exacerbate bone loss as ageing is associated with a decline in BMD. Moreover, pregnant women are at increased risk of developing a calcium deficiency due to the increased demands of calcium that is required for the growth of foetal bone. The evidence of a vegan diet impacting BMD have been inconclusive in the literature, therefore further research is required to understand bone health of vegans. Especially, in the context of NZ vegan adults as the measurement of bone parameters and calcium intakes have not been investigated in this population.

Objectives: To describe calcium intake and bone health of NZ adults following a vegan diet.

Methods: This cross-sectional study included adults (N=212) (>18yrs), who followed a vegan diet for more than 2 years. Demographic and lifestyle information was obtained from questionnaires. A 4-day food record was completed for analysis of calcium, zinc, protein, magnesium, phosphorus and vitamin C intake and compared to the Estimated Average Requirement (EAR). Weight, height and BMI were obtained, BMD was measured at the hip and spine using dual x-ray absorptiometry (DXA) and reported as Z-scores. Participants were categorised based on BMD *Z* scores stratified as follows: low BMD (for age and sex) <-2.0 and normal BMD >-2.0. Blood samples were taken for PTH, 25(OH)D and plasma calcium concentrations were corrected for albumin. All values are presented as mean and standard deviation. Differences in bone parameters between BMD groups were analysed using multiple T-tests. A linear regression analysis examined the association between calcium intake, corrected calcium concentrations, serum PTH levels, BMI, and physical activity levels and BMD *Z* scores at the lumbar spine and femoral neck.

Results: Overall, Z scores at the lumbar spine and femoral neck were -0.29 ± 1.12 and -0.24 ± 0.89), respectively. Corrected calcium concentrations were 2.21 ± 0.33

mmol/L. Overall, calcium intake was 917 ± 347.23 (range 195 to 2,429 mg/day). The main source of calcium in the vegan diet was tofu and plant-based milks. The intake of protein (77 ± 27.80) g/day, magnesium (569 ± 181.05) mg/day, and vitamin C (145 ± 96.94) mg/day met the EAR, excluding vitamin and mineral supplements. However, the intake of phosphorus (1,472 ± 459.98) mg/day and zinc (10.6 ± 4.01) mg/day were below the EAR. Only BMI significantly predicted BMD Z-scores at the lumber spine (P = 0.004) and femoral neck (P = 0.003).

Conclusion: The study found that most vegans had normal BMD for their age and sex, adequate calcium intakes and bone homeostasis markers. Despite mean intake of calcium exceeding the EAR, very low intakes demonstrated significant variations between participants. Tofu was identified as the main plant-based source of calcium amongst participants. Moreover, longitudinal research is required to understand the long-term impact of a vegan diet on bone health.

Key words:

Vegan diet; calcium; bone; adults.

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List of abbreviations

%	Percentage
>	Greater than
≥	Greater than or equal to
≤	Less than or equal to
<	Less than
25(OH)D3	Circulating vitamin D
1,25(OH)2D3)	Biologically active vitamin D
µg	Micrograms
µg/day	Micrograms per day
4-DDD	4-day diet diary
BMD	None mineral density
BMI	Body mass index
COPD	Chronic obstructive pulmonary disease
DXA	Dual Energy- X-ray absorptiometry
EAR	Estimated average requirement
ECF	Extra Cellular Fluid
ECM	Extra cellular matrix
FN	Femoral neck
g/kg/day	Grams per kilogram per day
g/cm^2	Grams per square centimetre
HbA1c	Average 3 monthly blood glucose reading
KJ	Kilojoules
LS	Lumbar spine
MET	Metabolic equivalent of task
Mg	Milligrams
Mg/day	Milligrams per day
Mg/kg/day	Milligrams per kilogram per day
Mmol/L	Millimoles per litre
NCP	Non-collagenous proteins
Nmol/L	Nanomoles per litre

NRV	Nutrient reference values
NZ	New Zealand
NZPAQ-SF	New Zealand physical activity questionnaire form
PBDA	Plant-based dairy analogues
PBM	Peak bone mass
Pmol/L	Picomoles per litre
PTH	Parathyroid hormone
QUS	Quality ultrasound
RDI	Recommended daily intake
SD	Standard deviation
VIF	Variance Inflation Factor
VDR	Vitamin D receptor
WHO	World Health Organisation

Chapter One Introduction to the thesis

The constrictive nature of a vegan dietary pattern requires careful dietary planning to ensure essential nutrients important for bone health are adequately provided. Namely, the intake of calcium and protein which are required for the growth and mineralisation of bone (Galchenko et al., 2023).

To maintain an adequate supply of nutrients in the diet, the individual is required to consume mineral and vitamin fortified foods/and or dietary supplements if they suspect dietary sources are insufficient (Sobiecki et al., 2016). It is important to note that long-term inadequate intakes of these nutrients can result in the development of macro and micronutrient deficiencies (Bakaloudi et al., 2021; Craig, 2009). Deficiencies in calcium can significantly affect bone mineral density, leading to the increased susceptibility of developing osteoporosis later in life (Palacios, 2006). This is particularly important for vulnerable subgroups susceptible to the risks of calcium inadequacy, specifically in older adults, pregnant and lactating women (Petti et al., 2017), due to their increased requirements for calcium essential for rapid growth and development of the fetal skeleton and during infancy (Wawrzyniak & Suliburska, 2021).

Several international studies have reported that intakes of calcium and vitamin D amongst vegans were below the recommended dietary reference intake values (Hansen et al., 2018; Ho-Pham et al., 2012; Schüpbach et al., 2017; Sobiecki et al., 2016). However, the associations between the intake of calcium and low bone mineral density (BMD) in a vegan diet are inconclusive, highlighting the multifactorial influences on BMD amongst the participants. In addition to diet, these factors include hormonal characteristics, age, sex, physical activity and genetics (Iguacel et al., 2019; Justyna et al., 2021; Kranioti et al., 2019).

Ho-Pham et al. (2012), Karavasiloglou et al. (2020) and Iguacel et al. (2019) concluded that vegans had statistically significant lower BMD at the femoral neck and lumbar spine in comparison to omnivores. However, these findings were not supported by Knurick et al. (2015), as significant differences of BMD between vegans and omnivores were not detected. Although, the relationship between

veganism and reduced bone mineral density exists, it is unclear as to whether this is directly related to diet, participant demographics or a combination of both. Therefore, a nutritionally balanced vegan diet that includes adequate intakes of macro and micronutrients is essential for assisting in the maintenance of bone mineral density throughout life.

Several nutrients such as vitamin K, magnesium, zinc, phosphorus, and vitamin C are indirectly or directly involved in the biochemical processes relevant to bone metabolism (Palacios, 2006), and deficiencies in these nutrients are rare in well-planned vegan diets.

Purpose of study

The purpose of this study was to describe calcium intake, markers of bone homeostasis and bone mineral density (at the femoral neck and lumbar spine) in relation to *Z* scores of New Zealand individuals (n=212) who have adhered to a vegan diet for a minimum of 2 years.

To acquire nutrients necessary for the maintenance of bone from a vegan diet, the individual is required to engage in dietary planning to ensure their nutrient intakes are adequate. The exclusion of animal-derived foods in a vegan diet, which are recognised as rich sources of calcium can present challenges towards achieving the recommended daily nutrient requirements.

To date there has been no research evaluating the nutritional indicators of bone health amongst New Zealand vegan adults. Additionally, international research on adult vegans has been inconclusive. Research in New Zealand is required to assess bone density of individuals following a vegan diet and whether they are meeting their bone related nutrient/recommended calcium intakes. Identifying bone-related nutritional risk factors will help to inform future dietary recommendations for vegans to optimise their intake of nutrients that assist in the maintenance of bone mineral density in adults.

Aim

- To describe calcium intake and bone health of adults following a vegan diet.

1.1.1 Objectives

- To evaluate the sources of calcium in a vegan diet
- To describe bone mineral density of the femoral neck (FN) and lumbar spine using standard Z-scores
- To assess dietary intake of calcium, magnesium, phosphorus, protein, zinc, Vitamins C and D in relation to the estimated average requirements (EAR) and recommended daily requirements (RDI)
- To assess serum 25(OH)D3, PTH intact plasma and serum calcium concentrations in adult vegans
- To investigate the relationship between dietary intake of calcium, bone mineral density and calcium status in adult vegans

Thesis structure

The structure of this thesis consists of four chapters. Chapter one provides the background and introduction, followed by the purpose of the study, alongside the aims, hypothesis, objectives, and statement of the researcher's contributions. Chapter two is a literature review of the most recent and relevant research in the context of bone health in vegans. Chapter three includes the research manuscript, consisting of the abstract, introduction, methods, results and discussion of findings. Chapter four explains how the aims and objectives were achieved along with future research recommendations to improve bone health of vegans. The appendices consist of the recruitment poster, participant information sheet, consent form, dietary practice and supplement use questionnaires and a 4-day diet diary (4-DDD).

Contributors to the study

Table 1. Researcher's contributions

Author	Contribution
Abril Clark MSc Nutrition and Dietetic student	Primary author of the thesis, involved in participant recruitment, data entry of 4-day food diaries, completion of literature review, statistical analysis, interpretation and discussion of results.
Professor Pamela von Hurst Supervisor	Principal Investigator of the Vegan Research Program. Conception, Design, funding, study oversight.
Professor Marlena Kruger Co-supervisor	Advisor on bone section, co-supervision of thesis.
Dr Hajar Mazahery School of Health Sciences	Project manager. study design, ethical approval, recruitment and data collection.
Dr Karen Mumme	Assistance with statistical analysis
Rebecca Paul Research Assistant	Recruitment, data collection, DXA scans, study administration.

Chapter Two Literature Review

This review will examine the current literature on bone development throughout the lifecycle and its structural and mechanical properties, including the influences of ageing. Vegan diets and background information regarding calcium and vitamin D consumption will be explored in addition to bone mineral density (BMD) and other measures of bone health amongst vegans. The review will provide an overview of debates on whether veganism-related deficiencies in vitamins and minerals can negatively impact BMD. Scopus, Pubmed, Massey Discover and Google Scholar were used including the search terms as outlined below (Figure 1).

Figure 1. Research Methodology

Date searched: January 2023 –

Search criteria:
“vegan diet”, OR “micronutrient status vegans” OR bone remodeling OR bone health
OR peak bone mass“OR plant-based dairy analogues OR “veganism or vegan” OR “calcium fortified food” “vegans” OR “women” OR “bone mineral density” “dietary calcium intake” OR “dietary vitamin D intake” OR “bone health” OR “vegan food intake patterns” OR “serum calcium measurements” OR “protein intake in vegans” OR “zinc intake in vegans” OR “vitamin C intake in vegans” OR “magnesium intake in vegans”

Filters:
Past 10 years, Past 20 years,

Electronic Database:
Scopus, Pubmed, Massey Discover, Google Scholar

Overview of vegan diets

A vegan diet entails the adoption of dietary practices, which is based on the omission of animal-derived products from an individual’s diet (Petti et al., 2017). This includes

the exclusion of meat, eggs and dairy products in addition to foods that require the involvement of animal components during food production (Ostfeld, 2017; Richter et al., 2016). The motivations behind adopting a vegan diet can stem from ethical considerations related to animal exploitation, ecological, environmental, as well as the proposed health advantages associated with this particular lifestyle (Brunton, 2019).

According to a report from the Academy of Nutrition and Dietetics, appropriately structured vegan diets containing fruits, vegetables, whole grains, legumes, nuts and seeds with the regular inclusion of fortified foods and vitamin B12 supplementation can fulfil an individual's nutritional requirements (Melina et al., 2016; Richter et al., 2016). Nevertheless, conflicting evidence within the literature exists regarding careful dietary planning to ensure an adequate supply of nutrients from non-animal derived products are supplied in a vegan diet (Richter et al., 2016; Wegmüller et al., 2017).

Dietary components of a vegan diet

A traditional vegan diet typically includes plant-derived sources such as legumes, nuts, seeds, vegetables and wholegrain products (Alcorta et al., 2021; Richter et al., 2016; Sakkas et al., 2020). A study by Clarys et al. (2014) in Belgium found vegans (n= 104) obtained the highest scores for diet quality in comparison to vegetarians, semi-vegetarians, omnivores and pesco-vegetarians. The analysis indicated that the highest scores were strongly associated with increased adherence to dietary recommendations (Clarys et al., 2014). Indeed, these findings indicate the sources of food included in a vegan diet may represent eating patterns that are beneficial to health by facilitating the reduction of blood pressure, HbA1c (indicator of an average blood glucose level over a three-month period) and cholesterol (Richter et al., 2016; Tuso et al., 2013).

However, eating habits can differ from what is considered in a traditional vegan eating pattern i.e., usual intake of fruit, vegetables, wholegrains, legumes, nuts and soy products due to the increased availability of vegan processed foods. Gallagher et al. (2022) identified 38% of vegan participants (n=129) had an eating pattern associated with notable intake of vegan convenience snacks, meals, sweets and condiments. Overall, these results indicate heterogeneities in the composition of food across individuals adhering to a vegan diet. However, the participants were

predominantly female thus it is possible the findings regarding the composition of food in a vegan diet may not be applicable to the general vegan population.

Bone health

2.1.1 Bone composition and cells

The metabolism of bone is an intricate process and is affected by several factors including specific micronutrients, such as calcium and vitamin D (Mangels, 2014).

Bone is a composite material consisting of both inorganic and organic components within the extra cellular matrix (ECM) (Feng, 2009). The inorganic components constitute 60% of the matrix and are primarily made up of calcium and phosphate which are deposited as crystalline hydroxyapatite (Robey & Boskey, 2008). Additionally, mineral salts such as calcium carbonate and trace amounts of cations such as magnesium, sodium and fluoride, contribute to the composite material of bone (Wawrzyniak & Balawender, 2022). Moreover, mineral salts are to provide mechanical resistance and hardness to bone (Wawrzyniak & Balawender, 2022).

The organic components in the matrix make up 30% and the remaining 10% is water. Within the organic component, type 1 collagen proteins are the most abundant (90%) while the remaining are non-collagenous proteins (NCPs) (10%) (Feng, 2009). The tensile strength of bone is attributed to the structure of type 1 collagen, which is characterised by a triple helical molecule containing strong covalent crosslinks (Steiniche & Hauge, 2003). Furthermore, within the osteoid, non-mineralized bone matrix forms hydroxyapatite from calcium phosphate nanocrystals in-between the regions of the collagen fibrils to confer hardness to bone (Carvalho et al., 2021; Garnero, 2015). Although the physiological function of NCPs is not fully understood, they are claimed to have an important role in regulating the mineralisation of bone (Carvalho et al., 2021). This process requires the deposition of calcium phosphate into the matrix by osteoblast cells and mediated by NCPs (Carvalho et al., 2021) (Kuhn, 2001). In addition to these cells facilitating bone mineralisation, they are also involved with the coordinated process of bone metabolism. This occurs from the response of environmental signals to continuously regulate production of new bone and degradation of older bone (Wawrzyniak & Balawender, 2022). The three types of cells associated with bone metabolism are

osteoblasts (bone formation), osteoclasts (bone resorption) and osteocytes (regulation of osteoblast and osteoclast activity) (Wawrzyniak & Balawender, 2022).

2.1.2 Bone modeling and remodeling

Bone is a metabolically dynamic tissue that involves a highly regulated process between the interaction of osteoblast and osteoclast cells to balance bone formation and resorption (Siddiqui & Partridge, 2016). Osteoclasts play an essential role in remodeling by breaking down bone tissue and releasing minerals into the circulatory system (Sims & Martin, 2020). On the other hand, osteoblasts are responsible for the formation of the organic component of bone tissue. This is achieved by the synthesis of type 1 collagen to form the bone matrix in which minerals (calcium and phosphate ions) are deposited into (Noirrit-Esclassan et al., 2021; Wawrzyniak & Balawender, 2022). The transition from resorption to new bone formation is a complex process influenced by several different factors including adequate plasma vitamin D levels, hormonal status and physical activity (Florencio-Silva et al., 2015; Stagi et al., 2013). Consequently, this will significantly influence the repair and maintenance of bone tissue to maintain the structural integrity and strength of the adult skeleton (Wawrzyniak & Balawender, 2022).

Moreover, the metabolism of bone throughout life underpins two processes: bone modelling and remodeling (Kenkre & Bassett, 2018). Here, modelling determines the extent of the shape and size of bone to encourage growth, whilst remodeling involves the continuous renewal and repair of bone under the influence of hormones and mechanical loading for the duration of adulthood (Walsh, 2015). Both processes will occur at varying rates from skeletal development prior to birth and throughout adulthood (Katsimbri, 2017).

Childhood and adolescence are characterised by rapid growth in various skeletal sites. In particular, the period of longitudinal (length) and appositional (thickness) bone growth is mostly influenced by the modelling process (Stagi et al., 2013). Once the pubertal growth spurt is completed, longitudinal bone growth ceases, resulting in a slow decrease in modelling activity (Saggese et al., 2002). In early adulthood, bone continues to grow appositionally, in conjunction with the activity of osteoblast and osteoclast cells, which line the outer (periosteum) and inside of bone adjacent to the marrow (endosteum) as shown in Figure 2 (Biga et al., 2019).

Figure 2. shows the structure of bone cells within the rounded section at the base of long bone. The periosteum and endosteum comprise of cells involved with the repair, growth and maintenance of bone tissue.

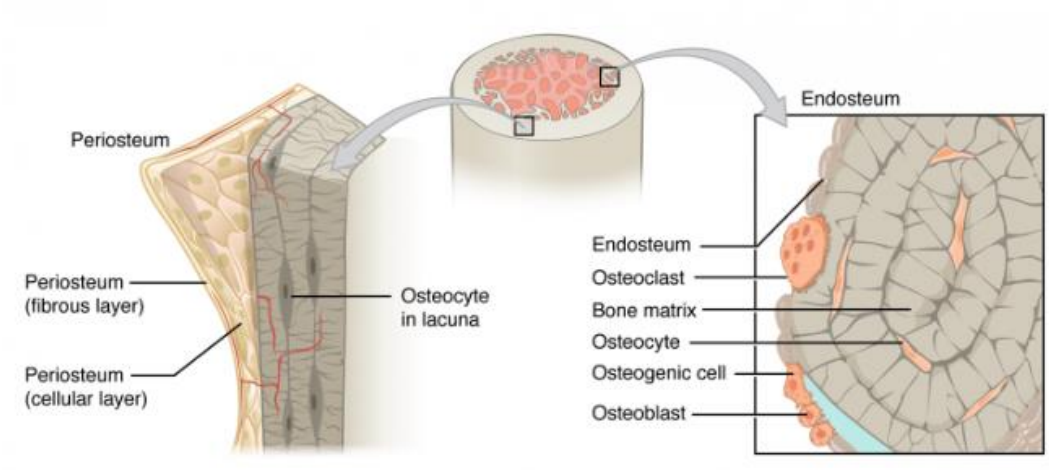

Figure 2. Structure of cells within the epiphysis region of long bone
Note. Anatomy and physiology (Chapter 6.3 Bone Structure), by Biga, LM, Dawson, S Harwell, 2019. Oregon State University. Copyright Creative Commons Attribution 4.0 International License 4.0 CCBY-SA4.0.

2.1.3 Peak bone mass (PBM)

Peak bone mass (PBM) is associated with the total accretion of bone tissue upon skeletal maturation. Although, debate within the literature exists, most specialists believe PBM is reached between the ages 20-25 in healthy men and women (Carey & Delaney, 2010; Chevalley & Rizzoli, 2022a).

The period between the start of puberty and early adulthood is associated with a 40-60% increase of adult bone mass and is considered a critical timeframe for skeletal development (Golden et al., 2014). Hence, the rapid accumulation of bone tissue during this period is considered a key determinant in influencing PBM. Importantly, the acquisition of high peak bone mass upon skeletal maturation will reduce the likelihood of developing osteoporosis later in life (Stagi et al., 2013).

2.1.4 Definition and diagnosis of Osteoporosis

Osteoporosis is defined as a type of bone disease indicated by low bone mass and microarchitectural deterioration of bone tissue with a subsequent increase in bone fragility (World Health Organization, 1998). The process underlying the physiological cause of osteoporosis is the imbalance between the ratio of bone resorption and

bone formation with increasing age (Walsh, 2015; Wawrzyniak & Balawender, 2022). As a consequence, this increases the activity of osteoclast cells which is widely recognised to influence the degradation of bone tissue (Noh et al., 2020). Moreover, individual variations in genetic and lifestyle factors in addition to inadequate calcium intake and vitamin D deficiency will determine the risk for development of osteoporosis with ageing (Wawrzyniak & Balawender, 2022).

Those who are most at risk of developing osteoporosis are post-menopausal women due to a loss of estrogen (Cauley, 2015), and it is estimated 40% will experience a fragility fracture during their lifetime (Runolfsdottir et al., 2015). Older men are also at risk of developing this condition, however this cohort only accounts for 20% of fragility fractures (Runolfsdottir et al., 2015).

The gold standard for measurement of BMD in clinical practice, is dual-energy X-ray absorptiometry (DXA) that uses two-dimensional imaging of the skeleton (Costa et al., 2022; Eastell et al., 2016; Kranioti et al., 2019; Punda & Grazio, 2014). The statistical values commonly used in the interpretation of BMD and DXA diagnostic criteria for osteoporosis are Z and T scores, which are derived by the comparison to a reference population (Carey & Delaney, 2010).

According to the World Health Organization (WHO), the clinically recognised diagnostic criteria for osteoporosis is defined as BMD in post-menopausal women and men (>50 years old) above or equivalent to 2.5 standard deviations below the mean value of BMD in the young-gender matched reference population (T-score ≤ -2.5 at the femoral neck, lumbar spine or total hip) (as cited in Eastell et al., 2016). Additionally, the diagnosis of osteoporosis can also be based on the occurrence of a fragility fracture (Carey & Delaney, 2010).

The remaining diagnostic criteria are as shown in Table 2 (World Health Organization, 1998)

Table 2. Diagnostic criteria for osteopenia and osteoporosis

Level of risk	Score
Normal	T-score ≤ - 1.0
Low bone mass (osteopenia)	T-score -1.0 to > -2.5
Osteoporosis	T-score ≤ -2.5
Severe osteoporosis	T-score < -2.5

Note. Adapted from World Health Organization (1998)

The classification of bone density is also in accordance with a Z-score for men and women under the age of 50, which is recommended by the International Society for Clinical Densitometry (Jeremiah et al., 2015). Review articles including Qaseem et al. (2017) and Dimai (2017) agreed to its use in clinical settings appropriate to identify if an individual's BMD is low for their age, excluding the diagnosis of osteoporosis. The BMD value of an individual is compared to an age-matched reference population, including sex and age by the number of SD above or below the expected BMD. A Z-score of -2.0 or lower is defined as low BMD for age and a number above -2.0 is predicted to be within the expected range (Qaseem et al., 2017).

To confirm the diagnosis of osteoporosis, conducting full patient history is required. Factors such as family history, diet, physical activity and fracture risk are considered in adjacency to low bone mass (Weaver et al., 2016).

Current research has indicated BMD using a bone densitometer as the primary diagnostic criteria for osteoporosis (Bagger et al., 2006; Boschitsch et al., 2017; Justyna et al., 2021), yet there is a lack of conclusive evidence concerning how vegan diets may impact BMD (Iguacel et al., 2019). This is significant, as vegans may be at a higher risk of lower BMD and fractures in comparison to omnivores due to deficiencies in nutrients essential for bone health which are commonly found in animal products (Iguacel et al., 2019). However, Ho-Pham et al. (2012), Iguacel et al. (2019), Karavasiloglou et al. (2020) and Li et al. (2021b) have reported conflicting findings, with results of lower BMD among vegans compared to other dietary groups such as vegetarians and omnivores.

Ho-Pham et al. (2012) concluded post-menopausal vegans aged >50 years old, (n=88) demonstrated a 4% decrease in BMD at the lumbar spine (LS) and femoral neck (FN) in comparison to omnivores. However, it is important to note that age and gender will influence BMD which may limit the generalisability of these findings to the adult vegan population. Contrasting findings indicate either no significant associations or modest differences that are not statistically significant in BMD of individuals following a vegan diet in comparison to other dietary groups (Knurick et al., 2015; Menzel et al., 2021). However, the clinical relevance of these small

differences in BMD have not been determined, given that the major nutrient of concern (calcium) can be insufficient in a vegan diet. A cross-sectional study conducted by Knurick et al. (2015) assessed the dietary quality of young adult vegans (19-50 years, n=28) and found no associations of low BMD in comparison to vegetarians and omnivores. However, it is important to note that the participants were required to follow a vegan diet for a minimum of 1 year to be eligible for the study. Furthermore, it takes several years for inadequate intakes of dietary calcium to result in the increased risk of developing osteoporosis (Cashman, 2002) Therefore, it may be challenging to detect changes in BMD during this relatively short timeframe with mean intakes of calcium 768mg/daily.

On the contrary, Menzel et al. (2021) concluded that vegans (n=36) exhibited lower levels of calcaneal ultrasound (QUS) parameters compared to omnivores. Additionally, this was accompanied by decreased serum levels of zinc and calcium. These findings suggest a potential relationship between nutritional markers and reduced bone mineral density. However, it is important to note that the small sample size may limit the generalisability and robustness of the results to a larger vegan population. Furthermore, Dane et al. (2008) and Dubois et al. (2001) concluded that QUS measurements correlated weakly with DXA which poses uncertainty with the validity and sensitivity of QUS as a sole measurement of BMD. This supports recent research by Rerkswattavorn (2023), who reported a 9% detection of osteoporosis by QUS calcaneal parameters in COPD patients (utilized a T score cut off value of -$\leq$ 2.5), which was significantly lower than a DXA diagnosis of osteoporosis at both the LS and FN (p <0.001).

The conflicting results among these studies may be attributed to potential confounding factors that affect BMD among participants, such as the various levels of physical activity, BMI, and nutrient intake leading to heterogeneity in the findings. Therefore, it remains unclear whether the associations of low BMD in vegans were solely based on dietary habits. Further analysis by Karavasiloglou et al. (2020) reported lower BMD in the hip and spine amongst vegans and vegetarians (n= 207) was associated with lower BMI and waist circumference. However, the lumbar spine BMD measurement remained significantly lower than non-vegetarians after BMI and waist circumference were controlled for. It should be emphasised that the distinction between vegetarians and vegans was not made in this study. Therefore, again these

results may not be generalisable to the vegan population due to the unidentified number of vegan participants with low BMD. Overall, these findings suggest that the observed differences in BMD are influenced by anthropometric measurements as an associated factor.

The contradictory results in differences of BMD among vegans highlight the need for further research to understand the relationship between a vegan diet and BMD. Overall, it appears that BMD is greatly influenced by anthropometric factors, genetics, age, physical activity and nutrient intake (Iguacel et al., 2019). The examination of potential confounding factors influencing BMD also highlights the requirement for larger and more diverse studies in the vegan population.

2.1.5 Factors influencing bone mineral density

The achievement of higher PBM in an individual's third decade of life will provide protection against the subsequent age-related loss of bone density. However, there remain several factors that can influence an individual's bone mass after PMB is achieved which will determine the progression of bone loss. Genetic factors (accounting for 60-80% variance) and age are responsible for the non-modifiable determinants of bone mass. Physical activity, nutrition and body weight are modifiable determinants considered to greatly influence the loss of BMD.

2.1.6 Gender, sex steroids and age

BMD begins to gradually trend towards a decline after PBM is achieved. Ageing results in the changes of circulating sex steroids responsible for the regulation of bone remodeling in men and women. Furthermore, the rate at which BMD decreases may differ at varying ages between sexes.

In men, testosterone is significantly associated with the regulation and maintenance of bone mass during adolescence and adulthood (Runolfsdottir et al., 2015). Indeed, total testosterone deficiency (hypogonadism) in older men has been linked to the development of secondary osteoporosis (Golds et al., 2017). This may be explained by the expression of androgen receptors in osteoblasts which are likely to influence bone metabolism (Golds et al., 2017). However, research by Adler (2014) and Golds et al. (2017) reported challenges determining if reduced testosterone is independently correlated to age-related bone loss. Particularly, as systematic reviews including Golds et al. (2017) and Cooke et al. (2017) indicated testosterone

is responsible for the synthesis of estrogen which is likely to result in indirect effects on bone metabolism. Additionally, a cross-sectional study by Ho-Pham et al. (2013) found a positive association between estradiol and BMD amongst men (n=200) and women (n=415). One reason for this may be due to the contribution of estrogen to the regulation of bone metabolism by inhibiting the effect of osteoclast activity via apoptosis to favor the maintenance of bone formation (Cauley, 2015). Male mice lacking the estrogen osteocyte receptor demonstrated a 20% reduction in trabecular bone mass in comparison to the control group (Windahl et al., 2013). These results indicate the importance of estrogen as the main sex steroid influencing bone mineral density in men.

In women, a decrease in estrogen production is associated with termination of the menstruation cycle which unfavorably affects BMD. In comparison to men, BMD will experience an accelerated loss during menopause in relation to significantly reduced estrogen levels (Runolfsdottir et al., 2015). Research by Daly et al. (2013), Kim et al. (2018) and Shanbhogue et al. (2016) reported women over the age of 60 years have reduced BMD and higher incidence of non-vertebral fractures in comparison to men within the same age range. These results suggest that the variations of BMD loss amongst sexes indicate an association of gender-specific changes with ageing in relation to declining estrogen levels.

Two phases of bone loss are apparent in women: the first phase involves 3-5 years of rapid bone loss located in the trabecular bone (menopause-related bone loss) and a second phase of 10-20 years progressive loss of both cortical and trabecular bone (age-related) (Eastell et al., 2016). The deterioration of the microstructure of bone begins to manifest via a decrease in volume resulting in bone becoming thin and porous. The second phase of bone loss in ageing affects men and women and can account for the explained delayed reduction of BMD and fracture incidence in men (Eastell et al., 2016).

According to a review by Chen et al. (2013) the differences in cortical BMD loss are more pronounced in women over the age of 50 years old in comparison to women younger than 50. These findings are consistent with the effects of increased resorption during the post-menopausal period (Kranioti et al., 2019; Noh et al., 2020). Consequently, this places post-menopausal women at an increased risk of

osteoporotic fractures as the cortical component of bone is recognised for its role in strength.

2.1.7 Physical activity

Bone is a metabolically living tissue that can respond to strain applied by muscular activity and mechanical load during exercise, resulting in the maintenance or net gain of bone mass (Chevalley & Rizzoli, 2022b). In response to mechanical load, osteocyte signaling is activated to influence bone remodeling (Giangregorio & Ponzano, 2022). Conversely, in the absence of mechanical loading to the skeleton, the demineralisation of bone occurs. This decline in BMD is evident amongst immobilised patients from a lack of weight-bearing activity resulting in muscle and bone atrophy, as reported in a cross-sectional study by Lala et al. (2014).

The general recommendations for physical activity resulting in the greatest effect on increasing bone density include exercises involving muscle contraction and gravitational loading forces on the skeleton, such as high-impact activities (e.g., running) or resistance training (Strope et al., 2015). Importantly, the type of exercise resulting from impact and strain on bones may determine the variation in changes of BMD. Costa et al. (2022) and Gheitasi et al. (2022) reported significantly higher BMD of the lumbar spine and lower extremities amongst footballers and long-distance runners in comparison to swimmers due to differences in gravitational loading forces.

The period of rapid skeletal growth in childhood and adolescence provides the advantage to maximise the accruement of bone mass from participating in high-impact physical activity. Strope et al. (2015) and Janz et al. (2014) reported positive correlations between moderate-intense high impact physical activity with maintenance and increased BMD of the hip and lumbar spine in children and young adults. Indeed, high-impact exercises resulting in mechanical stress applied to bone during childhood and adolescence is beneficial for the maintenance of bone mass. Furthermore, continuing to participate in exercise after the age of 60 is advised to prevent the loss of bone mass, resulting in the delayed onset of osteoporosis and related fractures (Troy et al., 2018). Strope et al. (2015), Allison et al. (2013), Allison et al. (2015), Hinton et al. (2015) and Watson et al. (2018) reported maintenance and improvement of hip and spine BMD resulting from adherence to weight-bearing (2.7-

3 times body weight/70-80% one-repetition maximum) and high-impact exercise (~50 jumps, 3-7 times per week) in older men and post-menopausal women.

2.1.8 Body Mass Index (BMI)

Low-body weight is an indicator for reduced BMD and increased risk of developing osteoporosis and fragility fractures (Cheng et al., 2022). Moreover, a thin body shape along with a body mass index (BMI) of under 18 is considered a well-established risk factor for osteoporotic fractures (Emaus et al., 2014b)

A study conducted by Karavasiloglou et al. (2020) found vegans and vegetarians (n=207) with lower BMI were associated with reduced lumbar spine BMD in comparison to omnivores. These findings indicate that BMI amongst vegans may have a relatively minor effect on BMD, considering factors such as body composition, age, gender, dietary and lifestyle factors as major determinants of BMD. Similarly, Tong et al. (2020) conducted a prospective cohort study and found vegans (n=1982) had attenuated but significant associations of increased risk of fractures in comparison to omnivores after adjustment for BMI.

2.1.9 Genetics

Genetic differences have been reported to determine an individual's BMD, accounting for an estimate 60-80% of osteoporosis risk and variability in BMD (Weaver et al., 2016). Heritable factors including individuals with a family history of a first-degree relative with osteoporosis will have a markedly high risk of a fragility fracture (Mullin et al., 2016). A genome wide association systematic review by Kemp et al. (2017) identified 153 loci via ultrasound of the heel involved in the development of osteoporosis. These results indicate the strong heritability of BMD as a predictor of osteoporosis.

2.1.10 Calcium homeostasis

Total serum calcium concentrations are maintained within a narrow physiological range between 2.2 – 2.6 mmol/L (Canterbury Health Laboratories, n.d.-a). This is controlled by the interaction of the biologically active form of vitamin D (1,25(OH)2D), parathyroid hormone (PTH) and calcitonin within a feedback system to regulate the transport of calcium between extracellular fluid (ECF) and in the gastrointestinal tract, kidneys and bone (Jeon, 2008; Mundy & Guise, 1999; Yu & Sharma, 2018).

In response to a decline in serum calcium concentrations, the parathyroid gland stimulates bone resorption by secretion of PTH into the bloodstream to release calcium and phosphate from bone tissue into the ECF (Mundy & Guise, 1999; Yu & Sharma, 2018).

Additionally, to restore serum calcium concentrations the production of the active metabolite of vitamin D (1,25 (OH)2D) in the kidneys is initiated by PTH to stimulate renal tubular reabsorption of calcium (Mundy & Guise, 1999; Yu & Sharma, 2018). Furthermore, the production of serum (1,25 (OH)2D) in response to circulating PTH levels promotes calcium absorption in the gastrointestinal tract (Mundy & Guise, 1999).

Conversely, an increase in serum calcium concentrations is regulated by the reduction of PTH and serum 1,25(OH)2D (Mundy & Guise, 1999; Peacock, 2010). This triggers the release of calcitonin from the parafollicular cells of the thyroid gland to promote deposition of calcium in bone from the activation of osteoblasts, therefore restoring normal serum calcium concentrations (Mundy & Guise, 1999; Yu & Sharma, 2018). The tight homeostatic control in plasma calcium is supported in research by Hansen et al. (2018), indicating vegans with normal mean serum corrected calcium concentrations (2.3 mmol/L), despite calcium intakes below the EAR in 40% of participants.

In the context of long-term calcium deficiencies, PTH secretion is enhanced resulting in increased 1,25(OH)2D formation to the detriment of 25(OH)D levels (Hansen et al., 2018; Lemoine et al., 2022; Nissenson & Jüppner, 2013). This is evidenced in research by Fillée et al. (2012), demonstrating high PTH concentrations associated with lower 25(OH)D levels in healthy adults.

Essential nutrients influencing bone mineral density

2.1.11 Calcium

Calcium is an essential mineral involved with several physiological functions in the body and 99% of it is stored in bone (Vannucci et al., 2018). The sources of calcium with the highest bioavailability are dairy foods, followed by certain fortified soy products, green-leafy vegetables and fortified plant-based dairy analogues (PBDA) (Theobald, 2005). Despite the availability of calcium-fortified food for vegans,

research by Hansen et al. (2018), Schüpbach et al. (2017) and Ho-Pham et al. (2012) reported adult vegans with low calcium intakes below the RDI.

A study by Warensjö et al. (2011) reported that intakes of calcium <800mg/day among British women aged >50 years led to increased risk of hip fractures compared to intakes of 1200mg/day. Overall, these findings suggest that prolonged insufficient intakes of dietary calcium can have detrimental effects on bone density. Therefore, sufficient intakes of calcium are essential for maintaining bone health throughout all stages in life. This will vary, with higher intakes of calcium required for bone formation during childhood, pregnancy, lactation, and for maintenance of bone during ageing (Vannucci et al., 2018).

Low calcium intake during periods of high bone resorption at the age of 70 years and over can have significant implications on bone health (Galchenko et al., 2023). Ho-Pham et al. (2012) found that calcium intakes in post-menopausal vegan women were substantially lower than omnivores without adverse effects on BMD. It was indicated that vegans were consuming 38% of the RDI. However, inadequate intakes of calcium were also reported in omnivorous women, with 68% of the RDI achieved. Similarly, Knurick et al. (2015) found low intakes of calcium amongst vegan adults in comparison to omnivores. However, it is important to account for the influence of modifiable and non-modifiable risk factors in addition to prolonged low calcium intakes which will contribute to BMD. Therefore, it is impossible to determine the duration of low calcium intake that would appear clinically significant regarding the impact on BMD without including lifestyle, nutrition and genetic risk factors.

Additionally, previous data regarding vegans' calcium intake is difficult to generalise to the New Zealand vegan population. This may be partially explained by differences in the availability of calcium fortified products, supplement use and the nutrient databases utilised for analysis of intake. Schüpbach et al. (2017) and Elorinne et al. (2016) reported adequate intakes of calcium in reference to the RDI amongst adult vegans in Europe. However, calcium supplementation was prevalent amongst the participants, resulting in high daily intakes of calcium that would be difficult to achieve exclusively from plant-based sources. Indeed, the use of single-nutrient supplementation is common amongst vegans (>50%) in comparison to non-vegans (Elorinne et al., 2016; Kristensen et al., 2015; Schüpbach et al., 2017). However, the

individual must obtain nutritional knowledge about the potential inadequacies of calcium arising from a poorly planned diet. Hence, the interpretation of results regarding intakes of calcium is to be evaluated with consideration of supplementation.

PBDAs are a common alternative to dairy milk amongst individuals adopting a vegan lifestyle (Smith et al., 2022). Moreover, the demand for PBDAs has increased exponentially and is estimated to have an annual growth rate of 10.18% between 2020 and 2024 (Fructuoso et al., 2021). PBDAs require the fortification of minerals and vitamins, namely calcium, vitamin D and B12, to closely resemble the nutrient profile of bovine milk (Aksoylu Özbek et al., 2023; Pua et al., 2022) and meet the nutritional requirements of consumers (Aksoylu Özbek et al., 2023). However, the type of inorganic calcium salt commonly used for fortification can reduce the bioavailability of calcium due to its interaction with plant proteins (Aksoylu Özbek et al., 2023).

According to a recent study conducted by Smith et al. (2022) in New Zealand, variations were observed in the calcium content of plant-based milks in relation to the calcium levels stated on the nutritional information label of shaken versus unshaken products. These variations were mainly attributed to the sedimentation of calcium in unshaken products, with 59% of calcium content equal to the label stated value of shaken products. Moreover, the type of plant-based milk analogue will affect the calcium content. Therefore, it is important for consumers to be well informed to select the product with higher calcium content to ensure adequate intake.

The market for plant-based cheese alternative products is also experiencing rapid growth and is now considered as the fastest growing category of plant-based foods (Craig et al., 2022). However, their nutritional composition can vary, particularly regarding the extent of calcium fortification required by the food manufacturers. To date, no research has analysed fortified calcium levels in plant-based cheese alternatives within the context of the New Zealand food industry. However, a study conducted in North America by Craig et al. (2022) analysed the calcium content of 245 plant-based cheese alternatives in supermarkets and reported 19% to meet the guidelines of calcium per serve. Notably, the criteria for calcium fortification were set at a minimum of 10% of the daily value per serving. This criterion demonstrated that

several plant-based cheese alternatives had low levels of calcium fortification. Similarly, a European study by Boukid et al. (2021) analysed 114 new plant-based cheese alternatives. These findings indicated 10.5% of plant-based cheeses were fortified with calcium. However, for this study a criterion to determine the extent of fortification appropriate for the contribution to the RDI of calcium was not used.

It should be noted that the main components of a vegan diet typically include wholegrains, legumes and plant-based milk alternatives which contain high levels of phytic acid (McClements & McClements, 2023). Phytic acid is characterised by its ability to bind to minerals and trace elements (calcium, zinc, magnesium and copper) to form insoluble complexes resulting in the reduced absorption of calcium in the small intestine (Chalupa-Krebzdak et al., 2018). Additionally, the presence of oxalate in cashews, almonds and soy will contribute to the inhibition of calcium absorption (Chalupa-Krebzdak et al., 2018). Consequently, the presence of calcium inhibitors in a vegan diet combined with the reduced bioavailability of calcium in some PBDAs may result in difficulty for accurately quantifying the extent of calcium that is bioavailable.

2.1.12 Vitamin D formation and metabolism

The two main forms of vitamin D are ergocalciferol (vitamin D2) found in plant-based sources and cholecalciferol (Vitamin D3) which is mainly synthesised from 7 dehydrocholestrol (pre-vitamin D3) in the skin via the exposure to sunlight (Charoenngam et al., 2023; Haussler et al., 2013). Vitamin D3 is also found in small quantities from animal-derived foods and fortification in food products (Charoenngam et al., 2023).

Once vitamin D2 and D3 enter the blood stream they are both transported to the liver and undergo hydroxylation to the circulating form of vitamin D (25(OH)D) via the enzyme 25-hydroxylase (Charoenngam et al., 2023). Moreover, the conversion from 25(OH)D to the biologically active form of vitamin D (1,25(OH)2D) occurs in the kidney from 1 α-hydroxylase (Charoenngam et al., 2023). Vitamin D receptors (VDRs) expressed in several tissues (skin, skeletal muscle, blood vessels and intestine) bind to 1,25(OH)2D and regulate calcium and phosphorus metabolism (Charoenngam et al., 2023).

2.1.12.1 Effect of vitamin D (1,25(OH)2D) on calcium homeostasis

The active form of vitamin D (1,25 (OH)2D) plays a significant role in the homeostasis of serum calcium concentrations, thereby regulating the metabolism of bone (Mostafa & Hegazy, 2015). Notably, the presence of adequate concentrations of serum 1,25(OH)2D results in the activation of VDRs on the small intestine which facilitates the absorption of calcium (Haussler et al., 2013; Mostafa & Hegazy, 2015). However, when low serum 1,25(OH)2D concentrations are present this can lead to the impaired absorption of calcium which can result in the demineralisation of bone overtime if left uncorrected (Laird et al., 2010).

2.1.12.2 Evaluation of serum 25-hydroxyvitamin D (25(OH)D) levels

At present, controversy exists regarding the concentration of circulating serum 25(OH)D levels considered as sufficient. Nonetheless, in accordance with the NZ threshold for vitamin D deficiency, sufficient vitamin D levels are characterised by serum concentrations of 25(OH)D as equal or greater than 50 nmol/L, mild to moderate Vitamin D insufficiency as 25-50 nmol/L, and moderate to severe deficiency as <25 nmol/L (Ministry of Health, 2012). However, it has been proposed that higher serum 25(OH)D levels may be required to maintain normal musculoskeletal health (Kannan & Lim, 2014). In the Endocrine Society Practice Guidelines on vitamin D, Holick (2017) defined vitamin D sufficiency as at least 75 nmol/L. A prolonged vitamin D deficiency among adults can subsequently develop into osteomalacia due to the absence of calcium hydroxyapatite deposition into the bone matrix (Zimmerman & McKeon, 2022). This is characterised by softening of bone and the increased susceptibility to fractures from falls (Zimmerman & McKeon, 2022).

In New Zealand, geographical latitude plays a role in the seasonal variations of adult serum vitamin D concentrations in the southern and central regions (Ministry of Health, 2012). In accordance with the 2008/09 Adult Nutrition Survey findings, individuals living in the central and southern regions between August and October had significantly lower levels of serum 25(OH)D levels compared to those in the northern regions (Ministry of Health, 2012). Furthermore, based on the cut-off values for serum 25(OH)D levels ($\leq$50 nmol/L), the results identified 27.1% of New Zealand adults were vitamin D insufficient (Ministry of Health, 2012) However, it is difficult to determine the proportion of NZ vegan adults at risk of vitamin D insufficiency as the

survey did not stratify participants based on diet patterns. Consequently, these results may not accurately indicate those at increased risk of vitamin D insufficiency within the NZ vegan population.

2.1.12.3 Dietary sources of vitamin D

There is very limited food sources of vitamin D3, they contain very small quantities and include egg yolks, cheese, oily fish such as mackerel and tuna, cod liver and fortified food products such as plant-based dairy substitutes (Laird et al., 2010; Mendes et al., 2018; Mostafa & Hegazy, 2015). New Zealand dietary intake data reported the sources of vitamin D in the New Zealand diet were fish, milk, eggs and margarine (Ministry of Health, 2012). However, due to the absence of animal-based foods in a vegan diet, the availability of dietary sources of vitamin D are considerably lower. Moreover, the EPIC-Oxford cohort study conducted by Sobiecki et al. (2016) found vegans (n=803) had low daily mean dietary intakes of vitamin D among men (1.77µg) and women (1.57µg).

Similarities in the high rates of vitamin D supplementation amongst vegans were reported in research by Hansen et al. (2018) and Weikert et al. (2020). Though, with the exclusion of supplements in the study by Weikert et al. (2020), the daily mean intake and serum 25(OH)D levels were lower amongst vegans. It is also important to note that the majority of vitamin D3 (90%) is obtained from skin exposure to sunlight (Mendes et al., 2018), which has a greater impact on circulating serum 25(OH)D concentrations than dietary intakes of vitamin D (Crowe et al., 2011). These findings may suggest the importance of supplementation in a vegan diet to achieve adequate serum 25(OH)D concentrations if sunlight exposure and dietary sources are insufficient.

2.1.13 Protein

An adequate intake of protein is important for facilitating bone mineralisation by indirectly increasing intestinal calcium absorption, given that dietary intake of calcium is adequate (>1000 mg/day) for this process to occur (Galchenko et al., 2023; Knurick et al., 2015; Mangano et al., 2014). Such findings suggest an adequate protein intake of 1.0 – 1.5 g/kg per day is presumably beneficial to bone health (Cuenca-Sánchez et al., 2015; Iguacel et al., 2019). Nevertheless, an effect of 1-2% on bone mineral density has been solely attributed to the intake of dietary protein (Lousuebsakul-Matthews et al., 2014).

Three studies assessing intake of protein in vegans reported intakes below the dietary reference intake values adjusted for age and gender (Dawczynski et al., 2022; Kristensen et al., 2015; Weikert et al., 2020). However, the effects of protein intake below the dietary reference intake values on bone mineral density were not investigated. Due to the lack of evidence regarding the adequacy of specific macronutrient intake in New Zealand vegans, these findings may only be able to provide insight into mean intakes of protein among this population.

In addition to the existing perception that a vegan diet may result in reduced intake of specific micronutrients, the dietary intake of protein is also represented in this category (Clarys et al., 2014; Kristensen et al., 2015; Ogilvie et al., 2022). Knurick et al. (2015) reported the intake of protein was reduced by 30% in vegans (n=28) with a mean intake of 69 g/day in comparison to omnivores. Furthermore, dietary protein remained significantly associated with BMD among vegans which indicates a potential relationship between the two factors.

Additionally, Tong et al. (2020) reported a statistically significant higher risk of fractures (in the hip, leg, and spine) among vegans (n=1982) in comparison to omnivores after controlling for protein intake (>0.75 g/kg per day) alongside BMI. However, protein intake could not be investigated as a sole factor influencing fracture risk as additional confounding factors including BMI and calcium intake were considered, which potentially affected the risk of fractures in vegans.

Similarly, Lousuebsakul-Matthews et al. (2014) reported vegans and vegetarians (n=3776) had increased risk for hip fractures in comparison to omnivores. However, 64% of the risk was reduced from the intake of protein derived from plant-based sources (legumes and meat analogues) in vegans and vegetarians (n=17,300). Vegans and vegetarians were included within the same analysis resulting in the inability to differentiate intake of plant-based protein. Furthermore, median intakes of total daily protein were not analysed, therefore the study was unable to determine if the minimum dietary reference intake values for protein were achieved.

2.1.14 Zinc

Zinc is a cofactor involved in the metabolism of bone tissue, including osteoblast activity and collagen synthesis (Molenda & Kolmas, 2023; Palacios et al., 2021).

Furthermore, bone tissues store 30% of the total body zinc (Huang et al., 2020; Molenda & Kolmas, 2023).

The bioavailability of zinc from plant sources may be reduced in a vegan diet due to the high presence of phytate found in several plant-based foods including legumes, unrefined cereals, nuts and seeds (Saunders et al., 2013; Wegmüller et al., 2017). The inhibitory effects of phytate arise from the formation of insoluble complexes by chelating with zinc, resulting in reduced intestinal absorption (Saunders et al., 2013).

Therefore, the requirement for dietary zinc may be 50% greater in an individual with a strict vegan diet with high intakes of grains and legumes (Capra, 2006; Tucker, 2014). Particularly, if a vegan diet has a phytate: zinc molar ratio of >15 (15% absorption) as it can potentially lead to suboptimal zinc status (Saunders et al., 2013). The WHO (1998) recommends 14 mg/day of zinc for vegan men (if a high consumption of phytates is indicated), however no recommendations have been set for vegan women (as cited in Saunders et al., 2013). The existing RDI for zinc among non-vegan women aged 19-30 is 6.5mg/day (Capra, 2006). Therefore, careful attention must be applied towards the planning of a vegan diet to ensure adequate intakes of zinc are obtained from plant-based sources (Saunders et al., 2013).

Schüpbach et al. (2017) and Elorinne et al. (2016) reported similarities in adequate mean intakes of zinc among vegans. However, a study by Kristensen et al. (2015) reported dietary intake of zinc in male vegans (n=33) was below the dietary reference intake values. Nevertheless, the greater requirement for zinc in a vegan diet was not adjusted into the dietary reference intake values. This may indicate unrecognised marginal intakes of zinc among the vegan population, especially due to the reduced bioavailability of zinc found in typically consumed plant-based foods. Moreover, it is unclear if suboptimal zinc status will have implications on bone mineral density given its structural and regulatory role in the metabolism of bone tissue.

2.1.15 Vitamin C

Vitamin C is required for the hydroxylation of lysine and proline in the cross-linking of collagen fibrils within bone (Malmir et al., 2018; Mangano et al., 2021; Palacios, 2006). A well-planned vegan diet has been associated with an adequate supply of

vitamin C due to a high consumption of fruit and vegetables (Craig, 2009; Kristensen et al., 2015).

This is supported in research conducted by Weikert et al. (2020), Schüpbach et al. (2017), Neufingerl and Eilander (2022) and Kristensen et al. (2015), which demonstrated the mean intakes of vitamin C among vegans exceeded the dietary reference intake values adjusted for age and gender. Given the intakes of vitamin C among vegans are considered adequate, this is unlikely to be a nutrient of concern for bone health in New Zealand adult vegans.

2.1.16 Vitamin K

Another vitamin essential for bone health is vitamin K, which exists in two forms: K1 (phylloquinone) primarily found in green leafy vegetables (Hu et al., 2021; Rodríguez-Olleros Rodríguez & Díaz Curiel, 2019), and K2 (menaquinone) in animal products and fermented soybean (natto) (Hu et al., 2021; Rodríguez-Olleros Rodríguez & Díaz Curiel, 2019). It is an essential co-enzyme involved in bone mineralization, enabling carboxylation of osteocalcin to ensure minerals such as calcium ions and hydroxyapatite crystals are integrated into the bone matrix (Rodríguez-Olleros Rodríguez & Díaz Curiel, 2019). The intake of vitamin K1 amongst vegans (243 μg/day) have been reported to be high in comparison to omnivores (154 μg/day) (*P* 0.01*)* in research by Weikert et al. (2020) and (Dawczynski et al., 2022).

2.1.17 Phosphorus

Phosphorus is a highly abundant mineral in the human body, with 85% of its stores bound to the skeleton (Galchenko et al., 2023; Ilich & Kerstetter, 2000). Research by Allès et al. (2017), Kristensen et al. (2015) and Schüpbach et al. (2017) found dietary intakes of phosphorus among vegans exceeded the dietary reference intake values adjusted for age and gender. These results suggest that vegans exhibit a high compliance to the recommended daily intakes for phosphorus, indicating a low prevalence of deficiency that may impact bone mineral density.

2.1.18 Magnesium

Magnesium has a significant effect on the formation of the structural properties of bone, particularly the mineralised inorganic composition known as hydroxyapatite (Castiglioni et al., 2013). Furthermore, a deficiency in magnesium indirectly reduces

the secretion of parathyroid hormone and 1,25(OH)D resulting in an imbalance of calcium homeostasis (Castiglioni et al., 2013; Palacios et al., 2021; Rude et al., 2009).

Vegan diets that are nutritionally adequate are associated with a sufficient intake of magnesium (Richter et al., 2016). This may be attributed to the low incidence of magnesium deficiencies among individuals following a vegan diet (Bakaloudi et al., 2021). Particularly, as high sources of magnesium are typically found in wholegrains, green leafy vegetables, nuts and seeds (Ilich & Kerstetter, 2000). Several studies including Kristensen et al. (2015), Schüpbach et al. (2017), Sobiecki et al. (2016) and Allès et al. (2017) reported the mean intakes of magnesium exceeded the dietary reference intake values adjusted for age and gender among vegans.

Summary

An individuals' achievement of peak bone mass and preservation of bone mineral density throughout life is largely influenced by a complex interplay of genetic, lifestyle and dietary factors. International research has made associations between the exclusion of dairy within a vegan diet and lower BMD in comparison to other dietary groups such as omnivores and vegetarians. Furthermore, the findings from international research may not be generalisable to the New Zealand vegan population due to differences in demographic characteristics, dietary patterns and accessibility of calcium-fortified foods.

Although, adequate intakes of calcium are proven as the most critical dietary component of bone health, it is recognised that several nutrients influence the development and maintenance of bone mineral density in adults. Currently, the dietary intakes of calcium, protein and measurements of bone parameters in NZ vegans have not been investigated. Pregnant and post-menopausal women following a vegan diet will be at a heightened risk of decreased bone mineral density if marginal intakes of calcium and protein are indicated. Importantly, by examining the measurements of bone density within the NZ vegan population this can assist in detecting whether the diet effects bone homeostasis. Therefore, this large study can provide valuable insight to guide future strategies for the development of dietary guidelines aimed at improving bone health among vegans, particularly within the context of New Zealand.

Chapter Three Manuscript

Abstract

Introduction: The evaluation of bone mineral density and nutrient intakes crucial for bone health have not been investigated in NZ vegans. International studies have reported suboptimal calcium intakes in vegans below the recommended dietary reference intake values (RDI) (Hansen et al., 2018; Sobiecki et al., 2016). Additionally, the association between low calcium intake and its effects on BMD is inconclusive. Hence, exploring these factors are important to gain insight on the bone health status of NZ vegan adults and inform strategies to prevent dietary deficiencies related to bone health.

Methods: Cross-sectional study of adults (N=212) (>18yrs), following a vegan diet for a minimum of 2 years. A 4-day food record was analysed for calcium, zinc, protein, magnesium, phosphorus and vitamin C intake and compared to the Estimated Average Requirement (EAR). Weight, height and BMI were obtained, bone mineral density (BMD) measured at the lumbar spine (LS) and femoral neck (FN) using dual x-ray absorptiometry (DXA). A linear regression examined associations between calcium intake, BMI, corrected calcium concentrations, serum PTH levels, and physical activity levels and BMD *Z* scores at the LS and FN.

Results: Overall calcium intake was 917 ± 347.23 (range 195 to 2,429 mg/day). Over half of participants (59%) met the EAR for calcium. Tofu and plant-based milks were the main sources of calcium. *Z* scores at the LS and FN were -0.29 ± 1.12 and -0.24 ± 0.89 respectively. Twelve participants had a Z-score of <-2.0 at the LS, and four at the FN. BMI was a sole predictor of Z-scores at the LS (*P* 0.004) and FN (*P* 0.003).

Conclusion: BMD-z scores (at the LS and FN), bone homeostasis markers and calcium intakes were normal in most vegans. Very low intakes in a subset of the study population indicated significant variations between participants. This highlights the importance of long-term dietary planning to ensure intakes of calcium are adequate in a vegan diet to optimise bone health. Longitudinal research is required to examine the long-term effect of a vegan diet on parameters of bone.

Key words: vegan diet; calcium; bone; adults.

Introduction

In recent times, there has been a growing a trend in the adoption of a plant-based diet, particularly in western countries. In the US, close to 10 million individuals in the past 15 years are following a vegan diet (Milfont et al., 2021). Despite the rise in the adoption of strict plant-based diets, results from the most recent New Zealand Health Survey (n=20,000) indicate that approximately 0.74% were vegans, whilst 93% of the population were meat eaters (Greenwell et al., 2024). This highlights the significance to understand bone health of the small vegan community early on before the trend of vegan diets grow globally. Particularly, since vegans may have a higher likelihood of experiencing nutrient deficiencies that can affect bone health (Ogilvie et al., 2022)

The Ministry of Health NZ advocates for the moderate inclusion of animal-based foods (dairy, seafood, eggs and poultry) and small quantities of red meat (McIntyre & Dutton, 2015). However, it is important to mention these recommendations do not align with the dietary choices of individuals following a vegan lifestyle. Particularly, as vegan diets exclude any form of meat, fish or animal product and are restricted to consuming plant-based foods (Linzey & Linzey, 2018). This highlights the potential gap in addressing the restrictive nature of following a vegan diet. Particularly with acquiring nutrients from non-animal food sources important for bone formation and maintenance, specifically, calcium, phosphorus, protein, vitamin C, zinc and magnesium (Beto, 2015; Iguacel et al., 2019).

High intakes of plant-based sources of vitamin C and magnesium in adult vegans have been well documented in research (Allès et al., 2017; Kristensen et al., 2015; Neufingerl & Eilander, 2022; Schüpbach et al., 2017; Sobiecki et al., 2016; Weikert et al., 2020). However, multiple international studies have reported suboptimal intakes of calcium in adult vegans below the daily reference intake values (Hansen et al., 2018; Ho-Pham et al., 2012; Schüpbach et al., 2017). Additionally, Aksoylu Özbek et al. (2023) and McClements and McClements (2023) have indicated the potential influence of phytic and oxalic acids in plant-based food sources known to inhibit calcium absorption.

However, the evidence regarding the duration of inadequate calcium intake that would contribute to the loss of bone mineral density is unclear. This is of particular

concern as inadequate calcium intake is a risk factor for the development of osteoporosis (Brown et al., 2007; Nguyen et al., 2004).

Osteoporosis is the primary cause for 82% of fractures in women and 64% in men over age 50 in New Zealand (Casey, 2015). It is estimated that the numbers of older adults admitted to health care services for fragility fractures will increase significantly in relation to the ageing population in New Zealand (Brown et al., 2011; Casey, 2015). Vegans may be placed at a greater risk of lower BMD and osteoporotic fracture incidence in the later decades of life in comparison to meat-eaters (Iguacel et al., 2019; Tucker, 2014). This can be attributed to the long-term deficiency of calcium and protein in the diet (Iguacel et al., 2019).

To date, there is no research conducted on the assessment of BMD and bone homeostasis markers in NZ vegans. International studies have reported associations between a vegan diet and low BMD (Ho-Pham et al., 2012; Iguacel et al., 2019; Karavasiloglou et al., 2020; Li et al., 2021a), however contradictory results exist with reports of no associations (Knurick et al., 2015; Menzel et al., 2021). This highlights the need for further research to understand bone health in NZ adult vegans.

This study aimed to describe calcium intake and bone health of adults following a vegan diet.

Methods

3.1.1 Study design

A cross-sectional study evaluating bone health in New Zealand male and female adults adhering to a vegan diet for a minimum of 2 years.

3.1.2 Participants and recruitment

Participants were recruited between June 2022 and February 2023 based on a single geographic location in Auckland. Recruitment occurred through advertisements on vegan Facebook pages, vegan community notice boards, The Vegan Society and word of mouth.

The inclusion criteria consisted of men and women aged >18yrs, who have been following a vegan diet for a minimum of two years, women who were not pregnant nor had any likelihood to be.

For this exploratory study, we aimed to recruit 220 participants in accordance with a recent study from a representative sample of New Zealand adults suggesting that <1% of the NZ population was vegan (Greenwell et al., 2024). Written and confirmed consent were obtained from the participants prior to data collection (Appendix 3). This research was approved by the Health and Disability Ethics Committee (HDEC): 2022 EXP 12312.

3.1.3 Data collection

An online screening questionnaire was obtained to confirm participant eligibility. The participants who met the inclusion criteria were contacted by the research assistant to arrange a visit to the Human Nutrition Research unit at Massey University in Auckland.

A demographic and lifestyle questionnaire (Appendix 5) based on a generic set of questions and adapted to this study were collected from participants to obtain information regarding age, gender, ethnicity, physical activity and medical history including previous bone fractures or family history of osteoporosis and corticosteroid medication use. Supplement use information was obtained from the dietary practice and supplement use questionnaire that was specifically developed for this study (Appendix 4). Information regarding the metabolic equivalent (MET-minutes), duration and frequency of physical activity were obtained from the New Zealand Physical Activity questionnaire (NZPAQ-SF) (Maddison et al., 2007).

Completion of a 4-day diet diary (4-DDD) including one weekend day and three weekdays detailing what was consumed during that timeframe was required from the participants (Appendix 6). Information regarding size or weight of the food eaten was included, alongside photographed Nutritional Information Panels on packaged, branded foods.

The dietary records were sent to the study coordinators upon completion who clarified any discrepancies in measurements and details of the type of food consumed with the participant. The food diaries were analysed for intake of calcium, zinc, protein, magnesium, phosphorus and Vitamin C including supplements using Foodworks 10 software programme (Xyris Software, Aust) (NZ FOODfiles 2010).

Intakes were compared against the Nutrient Reference Values (NRV), estimated average requirement (EAR) and recommended dietary intake (RDI) for Australian

and New Zealand adults. Results were converted to percentage of age specific EAR for the micronutrients. Intake of <100% of the EAR was defined as 'inadequate intake'. An evaluation of the dietary intake of vitamin D both at the individual and population level were not conducted due to the lack of vitamin D fortified foods in NZ. The total calcium content from pseudo cheese, pseudo milk, pseudo yoghurt and tofu were extracted from the 4-DDD and grouped into an excel spreadsheet. Subsequently, the total calcium content for each food source was divided by 212 participants. As a result, this estimated the average participant daily calcium contribution from these food sources.

Bone mineral density (BMD), expressed as g/cm^2 at the femoral neck (FN) and lumbar spine (LS), was measured using dual-energy x-ray absorptiometry (DXA) Hologic QDR series Horizon A (Wisconin, MA, USA) by a qualified operator. Quality controls were performed daily by standardisation of the densitometer to a standard phantom prior to the first participant scan. Participant information including weight, height, sex and date of birth were entered into the DXA database. The participant was asked to change into a gown and remove potential artifacts including wallets, keys, and jewellery that may interfere with the accurate assessment of BMD. They were positioned on the scan table as per the instructions for the machine.

The NHANES database was utilised as a reference to compare BMD to nationally representative data and were reported as Z-scores in relation to age and gender of the participant. A Z-score lower than -2.0 is considered low for age in people under the age of 50 years, whilst values above -2.0 is considered normal. T scores to assess BMD were excluded from the analysis as this diagnostic criterion is limited to post-menopausal women and men over the age of 50. The participants were under the age of 50, indicating this reference population would be inaccurate. Participants were notified if their results were below the normal criteria for BMD Z scores, reviewed by a consulting endocrinologist, and referred to their medical professional.

Weight was measured on an electronic scale without shoes. A difference of >0.1kg of weight indicated a third measurement. Height was measured using a portable stadiometer attached to a stable platform (SECA 510, SECA, Hamburg, Germany) to the nearest 0.1cm and calibrated weekly. BMI was calculated by dividing weight by

height squared (kg/m²) and reported by comparison to the reference ranges established from the Ministry of Health Guidelines (Ministry of Health, 2013).

The participant completion of the questionnaires, anthropometric measurements, DXA scans, and blood sampling were conducted during one required visit at the Human Nutrition Research unit at Massey University in Auckland.

3.1.4 Blood sampling and analysis

Venous blood samples were taken from each participant by a qualified phlebotomist to assess serum calcium (corrected and uncorrected values,), plasma intact PTH, and serum vitamin D status (25(OH)D). The analysis of serum ionised calcium concentrations as a gold-standard measure was excluded due to the practical limitations in cost for this study. Alternatively, serum calcium concentrations were corrected for albumin levels to determine calcium status.

The samples were centrifuged and plasma aliquoted and stored at -80ºC, prior to dispatch at North Shore Hospital Laboratory for analysis with standard protocols. Intact parathyroid hormone (PTH) in plasma was measured using an immunoassay (Atellica IM Analyzer, Siemens Healthineers, Germany). Precision of the assay was 0.1pmol/L with an intra-assay CV of ≤ 10% for samples between 1.1-2.12 pmol/L ≤ 8% CV for samples between 2.12-74.2 pmol/L, and ≤ 10% CV for samples >74.2 pmol/L.

3.1.5 Statistical analysis

The statistical analyses were completed using SPSS statistics, version 27.0, SPSS Inc., Chicago, IL. The sample size exceeded 30 indicating the applicability of the central limit theorem to assume normality. This theorem states as the sample size increases, the distribution sample means are likely to follow a normal distribution, regardless of the population distribution (Pallant, 2011). Histograms were used to identify outliers and skewness of the data. All values were reported as mean ± standard deviation (SD). Participants were categorised based on their bone status, with Z scores stratified as follows: low BMD (for age and sex) <-2.0 and normal BMD >-2.0 (World Health Organization, 2003). Differences in bone parameters between groups were analysed using multiple T-tests and were conducted at a 5% significance level. Bonferroni's corrections were applied for each of the groups bone

parameters to control for the type I error rate. To determine the relationship between plasma intact PTH, corrected serum calcium and 25(OH)D levels, Spearman's correlations were used. The density plots indicated that serum 25(OH)D and plasma PTH levels were not normally distributed and displayed a right-skewed pattern, therefore Pearson's correlation was not used. The variables known to impact calcium homeostasis including sex and age were controlled for in partial correlations (Bland, 2016).

The association between a vegan diet and determinants of BMD at the LS and FN was assessed using a multiple linear regression model. The dependent variables included Z-scores at the LS and FN, with the same set of independent variables applied to each, including calcium intake (mg) per 1kJ of energy, corrected serum calcium concentrations, physical activity scores and plasma intact PTH levels. Independent variables were deemed significant if the *P* value was less than 0.05. The assumptions for conducting a multiple linear regression analysis were met. The sample size of 212 participants in this study was suitable for a multiple regression model, given that >15 participants is required for each independent variable (Fidell & Tabachnick, 2003). Z-scores were tested for normality using Kolmogorov-Smirnov and Shapiro Wilk tests which demonstrated normally distributed and independent residuals (Durbin-Watson statistic 1.8). Multicollinearity did not exist within the data as residual values were above 0.10 (Pallant, 2011), at the LS (plasma PTH levels = 0.91, physical activity =0.96, Calcium intake (mg) per 1kJ of energy =0.95, corrected serum calcium concentrations and serum 25(OH)D =0.92 BMI =0.96), and at the FN (PTH levels =0.91, physical activity =0.96, calcium intake (mg) per 1kJ of energy =0.94, corrected serum calcium concentrations =0.95, serum 25(OH)D =0.92 and BMI =0.96, respectively. The Variance Inflation Factor Values (VIF) above 10 would suggest multicollinearity (Pallant, 2011), however the VIF values for independent variables were below 10 at the LS (plasma PTH levels =1.08, physical activity =1.03, calcium intake (mg) per 1kJ energy =1.06, corrected serum calcium concentrations =1.05, serum 25(OH) =1.08 and BMI =1.04), and the FN (plasma PTH =1.08, physical activity =1.03, calcium intake (mg) per 1kJ energy =1.06, corrected serum calcium concentrations =1.05, serum 25(OH)D =1.08 and BMI =1.04, indicating no significant multicollinearity issues. The variance of residuals was constant and appeared randomly scattered with minimal pattern of heteroscedasticity.

Results

3.1.6 Participants

The characteristics of 212 participants are shown in Table 3. The mean age of participants was 39.49 ± 12.38 years, with 73% female and 27% male. Overall, nearly two thirds of participants have been following a vegan diet for more than 5 years (Table 3).

Table 3. Participant characteristics (n=212)

Variable	**Total n= 212**	**Females n= 155 (73%)**	**Males n= 57 (27%)**	***P* value**
Age (years)	39 ± 12.38	39 ± 12.52	40 ± 12.09	0.55
Ethnicity, n (%)				
New Zealand European	178 (84%)	129 (83%)	49 (86%)	
Māori	8 (3.8%)	8 (5.1%)	0 (0%)	
Pacific Islander	1 (0.5%)	0 (0%)	1 (1.8%)	
Asian	16 (7.5%)	13 (8.4%)	3 (5.3%)	
Middle Eastern/Latin American/African	7 (3.3%)	4 (2.6%)	3 (5.3%)	
Missing	2 (0.9%)	1 (0.6%)	1 (1.8%)	
Duration of vegan diet (y), n (%)				
2 to 4 years	84 (39.6%)	62 (40%)	22 (39%)	
5 to 10 years	93 (43.9%)	65 (41.9%)	28 (49%)	
More than 10 years	35 (16.5%)	28 (18.1%)	7 (12%)	
Anthropometrics				
Weight (kg)	69.33 ± 12.37	65.78 ± 10.36	78.76 ± 12.42	**<0.001**
BMI (kg/m²)	23.92 ± 3.11	23.74 ± 3.15	24.41 ± 3.00	0.080
Physical activity level, n (%)				0.059
Low	35 (16.5%)	27 (17.5%)	8 (14%)	
Moderate	158 (74.5%)	120 (77.4%)	38 (66.7%)	
High	15 (7.1%)	7 (4.5%)	8 (14%)	
Missing	4 (1.9%)	1 (0.6%)	3 (5.3%)	

Data are presented as Mean ± SD (12.37 kg) and n (%), *P* values are for difference between males and females, BMI; body mass index, The following data is missing; physical activity levels; overall n=4, females n=3, males n=1

Bone parameters of participants are shown in Table 4. PTH blood samples were analysed for 150 participants. Normal serum intact PTH levels were assessed in comparison to the reference range of 1.6 – 7.0 pmol/L (Canterbury Health Laboratories, n.d). Additionally, normal corrected calcium concentrations were determined between 2.2 – 2.6 mmol/L for adults aged >18yrs (Canterbury Health Laboratories, n.d.-c). The reference range considered optimal for bone health in the assessment of vitamin D status used in this study were 50-150 nmol/L (Canterbury Health Laboratories, n.d.-c).

Table 4. Participant bone parameters (n=212)

Variable	**Total** **n= 212**	**Females** **n= 155 (73%)**	**Males** **n= 57 (27%)**	***P* value**
Corrected serum calcium (mmol/L)	2.21 ± 0.33	2.23 ± 0.33	2.15 ± 0.32	0.082
Uncorrected serum calcium (mmol/L)	2.19 ± 0.36	2.21 ± 0.36	2.14 ± 0.37	0.16
Serum 25(OH)D levels (nmol/L)	65.07 ± 23.36	64.81 ± 24.08	65.74 ± 21.62	0.62
PTH intact plasma (pmol/L)	5.16 ± 2.09	5.34 ± 2.21	4.71 ± 1.68	0.16
Missing	60	15	45	
LS BMD (g/cm²)	1.02 ± 0.15	1.01 ± 0.16	1.04 ± 0.15	0.22
LS Z score	-0.29 ± 1.12†	-0.24 ± 1.08†	-0.40 ± 1.23†	0.38
FN BMD (g/cm²)	0.79 ± 0.12	0.78 ± 0.11	0.82 ± 0.14	0.062
FN Z score	-0.24 ± 0.89†	-0.20 ± 0.86†	-0.35 ± 0.97†	0.17
Previous history of bone fractures	94 (44%)	62 (40%)	32 (55%)	

Data are presented as Mean ± SD, and n (%). *P* values are for difference between males and females, 25(OH)D; circulating vitamin D levels, PTH; parathyroid hormone, note: PTH samples were analysed for 150 participants, LS; lumbar spine, BMD; bone mineral density, FN; femoral neck, PTH; parathyroid hormone, 25(OH)D levels; circulating vitamin D levels, †normal BMD Z-scores for age and sex: >-2.0

3.1.7 Nutrient intake in relation to EAR

Table 5. includes the dietary intake of protein, calcium, magnesium, phosphorus, vitamin C and zinc in vegan adults in relation to the EAR and RDI. Overall calcium intake was adequate (917 ± 347 mg/day) which exceeded the EAR of 840 mg/day for adults aged 19-50 years. Over half of participants (59%) met the EAR for calcium.

Calcium intake was significantly higher in males (1,051 ± 364 mg/day) compared to females (867 ± 328 mg/day) ($p<0.001$). Variability in calcium intake was reported higher in females (range 194 to 2,428 mg/day) compared to males (range 382 to 2,267 mg/day) (Table 5)

Table 5. Daily intake of micro and macronutrients in relation to the EAR and RDI, based on a 4-day dietary record (n=212)

Nutrient	**Overall**	**Range**	**Male n=57**	**Range**	**Female n=155**	**Range**	***P* value**	**NZ EAR (M/F)**	**RDI (M/F)**
Protein (g)	77 ± 28	24, 185	99 ± 33	49, 185.	69 ± 20	24, 144	**<0.001**	37g / 52g	64g / 46g
Calcium (mg)	917 ± 347	195, 2,429	1,052 ± 364	382, 2,267.	867 ± 328	195, 2,429	**<0.001**	840 mg	1000 mg
Magnesium (mg)	569 ± 181	145, 1,328	666 ± 181	427, 1,328	532 ± 167	145, 1,163	**<0.001**	350 mg	400 mg / 310 mg
Phosphorus (mg)	1,472 ± 460	517, 3,612	1,774 ± 492	1,047, 3,612.	1,357 ± 392	517. 2,489	**<0.001**	4,000 mg	1000 mg
Vitamin C (mg)	144.55 ± 96.94	2.6, 814	149.6 ± 78.08	28.32, 410.16	142.6 ± 103.4	2.55, 814	0.29	30 mg	45mg
Zinc (mg)	10.5 ± 4	3,41	12.64 ± 3.51	7.80, 25.65	9.73 ± 3.90	3.1, 41	**<0.001**	12 mg / 6.5 mg	14 mg / 8 mg
Calcium supplement users, n (%)	6%								

All data are presented as Mean ± SD, range (lowest and highest intakes), n (%), *P* values are for difference between males and females, EAR = Estimated Average Requirement adapted from the Nutrient Reference Values for Australia and New Zealand, RDI = Recommended Dietary Intake, M/F; males and females

Overall, the intake of protein 77 ± 27.80 g/day including supplements met the EAR. Males had a significantly higher intake of protein (98.9 ± 33.20) g/day compared to females (69 ± 20.19) g/day (p<0.001) (Table 5).

Overall, intake of magnesium (569 ± 181.05) mg/day, and vitamin C (145 ± 96.94) mg/day met the EAR, excluding vitamin and mineral supplements. Overall intake of phosphorus (1,472 ± 459.98) mg/day and zinc (10.6 ± 4.01) mg/day did not meet the EAR. Use of calcium supplements were reported in 6% of all participants (Table 5)

3.1.8 Calcium sources in a vegan diet

The total daily mean contribution of dietary calcium per individual from food sources reported in the 4-day food diaries, included pseudo cheese (26 mg), pseudo milk (790 mg/day), pseudo yoghurt (18 mg/day) and tofu (416 mg/day).

3.1.9 Serum biomarkers of calcium status

Overall, serum 25(OH)D levels (65.07 ± 23.36) nmol/L were within the normal reference range of 50 – 150 nmol/L. Similarly, overall PTH intact plasma concentrations (5.16 ± 2.09) pmol/L (PTH range 1.6 – 7.0 pmol/L) were within the normal reference range. Additionally, overall serum calcium concentrations (corrected for albumin) (2.21 ± 0.33) mmol/L were within the normal reference range of 2.2-2.6 mmol/L. Males had serum calcium concentrations slightly below the reference range (2.15 ± 0.32) mmol/L. One hundred and nine participants (51%) had serum calcium corrected levels of <2.2 mmol/L, seventeen (8%) participants had serum calcium corrected levels <2.2 mmol/L & PTH >7.0 pmol/L, respectively.

The relationship between corrected serum calcium concentrations and PTH intact plasma concentrations was non-significant (*r*=-0.154, n=150, *P*(two-tailed) =0.060). This increased in significance after sex, age, and serum 25(OH)D levels were controlled for in a partial correlation (*r*=-0.17, n=150, *P*(two-tailed) =0.041).

The relationship between corrected serum calcium concentrations and serum 25(OH)D levels were non-significant (*r*= -0.08 n=202, *P*(two-tailed) =0.251). This remained the same after sex, age, and PTH intact plasma concentrations were controlled for (*r*= -0.10 n=202, *P*(two-tailed) =0.214).

Conversely, PTH intact plasma concentrations was negatively associated with serum 25(OH)D levels (*r*= -0.18 n=149, *P*(two-tailed) =0.026). The strength of this

relationship increased when sex, corrected serum calcium concentrations and age were controlled for (*r*= -0.20, n=149, *P*(two-tailed) = 0.014).

3.1.10 Association between Z scores and predictor variables

Table 6. illustrates non-statistically significant associations between Z-scores at the LS and PTH intact plasma concentrations, physical activity, calcium intake (mg) per 1kJ of energy, corrected serum calcium concentrations and serum 25(OH)D in a linear regression analysis. BMI was the only statistically significant predictor of Z scores of the LS (*P* 0.004). An increase in one unit of BMI suggests the Z-score at the lumbar spine will increase by 0.091 units.

F statistic (6,135) 1.95, *P* = .098, and the model accounted for 8.4% of the variation observed in BMD Z-scores at the LS.

Table 6. Predictors of Z scores at the lumbar spine (n= 212)

Model	Coefficient (B)	Standard error B	Standardised B	R2	*P* value
Model 1					0.075
Intercept	-1.963	1.777		0.084	0.098
Calcium intake (mg) per 1kJ of energy	-2.345	2.530	-0.080		0.356
Corrected serum calcium concentrations (mmol/L)	-0.113	0.289	-0.034		0.698
PTH intact plasma (pmol/L)	-0.042	0.049	-0.075		0.393
Physical activity score	8.168x10-6	0.000	0.005		0.950
BMI (kg/m²)	0.091	0.031	0.250		**0.004**
Serum 25(OH)D levels (nmol/L)	0.003	0.004	0.070		0.425

F statistic (6,135) = 1.9565, *P* = 0.098, PTH; parathyroid hormone levels, BMI; body mass index, 25(OH)D; circulating vitamin D levels, note: PTH samples were analysed for 150 participants

Table 7. Illustrates non-statistically significant associations between Z-scores at the FN and PTH intact plasma concentrations, physical activity, calcium intake (mg) per 1kJ of energy, corrected calcium concentrations and serum 25(OH)D levels in a linear regression analysis. Similarly, BMI was a statistically significant predictor of Z

scores at the femoral neck (*P* = 0.003) An increase of one unit of BMI suggests the Z score at the femoral neck will increase by 0.072 units.

F-statistic (1,135) 2.20, *P* = 0.021 and the model accounted for 9.3% variation observed in BMD Z- scores at the FN.

Table 7. Predictors of Z scores at the femoral neck (n=212)

Model	Coefficient (B)	Standard error B	Standardised B	R2	*P* value
Model 1					**0.047**
Intercept	-2.06	0.884		0.051	**0.021**
Calcium intake (mg) per 1kJ of energy	-2.516	1.901	-0.114		0.188
Corrected serum calcium concentrations (mmol/L)	0.137	0.217	0.054		0.531
PTH intact plasma (pmol/L)	0.000	0.037	-0.063		0.997
Physical activity score	-7.71x10-5	0.000	-0.063		0.464
BMI (kg/m²)	0.072	0.023	0.263		**0.003**
Serum 25(OH)D levels (nmol/L)	0.002	0.003	0.051		0.557

F statistic (1,135) = 2.204, *P* = 0.021*,* PTH; parathyroid hormone levels, BMI; body mass index, 25(OH)D; circulating vitamin D levels, note: PTH samples were analysed for 150 participants

3.1.11 Bone status

Table 8. represents differences in bone parameters amongst participants with low BMD Z-scores (for age and sex) and normal BMD Z-scores, at the FN.

A total of 208 participants exhibited normal BMD Z-scores, whilst 4 had low BMD (for age and sex) at the FN. Bone metabolism biomarkers did not differ significantly between the two groups. Z-scores in the Low BMD (for age and sex) group (-1.88 ± 0.77) differed significantly in comparison to the normal BMD group (-0.26 ± 1.11) **(***P* = 0.004). Mean protein intakes differed significantly between low BMD (48.5 g/day ± 14.11) and normal BMD groups (77.8 g/day ± 27.72), (*P* = 0.037**).**

Table 8. Participant bone parameters stratified by bone status at FN

Characteristic	**Low bone density (for age and sex) n= 4**	**Normal bone density n= 208**	***P* value**
Age (years)	42 ± 15.47	39 ± 12.35	
Corrected serum calcium (mmol/L)	1.97 ± 0.39	2.21 ± 0.32	
Serum 25(OH)D levels (nmol/L)	66 ± 38	65.1 ± 23.23	
PTH intact plasma (pmol/L)	6.75 ± 5.30	5.14 ± 2.05	
BMI (kg/m²)	21.67 ± 5.10	23.93 ± 3.13	
Protein (g)	48.5 ± 14.11	77.8 ± 27.72	***0.037**
Calcium (mg)	805.93 ± 124.59	919.75 ± 350.17	
Energy intake (kJ)	6761 ± 1258	9126 ± 2518	.
Z score	-1.88 ± 0.77	-0.26 ± 1.11	***0.004**
Physical activity, n			
Low	1	34	
Moderate	2	156	
High	1	14	
Vegan time, n			
2 to 4 years	1	83	
5 to 10 years	2	91	
>10 years	1	34	

Note. All data are presented as mean ± SD, *significant difference calculated by multiple t-tests, PTH; parathyroid hormone levels, FN; femoral neck, BMI; body mass index, BMD Z score; bone mineral density Z score, †normal BMD Z-scores for age and sex: >-2.0

Table 9 represents differences in bone parameters amongst participants with low BMD Z-scores (for age and sex) and normal BMD Z-scores, at the LS.

A total of 12 participants exhibited low BMD (for age and sex), whilst 200 had normal BMD, at the LS. Mean age differed significantly between the two groups (*P* = 0.004). Z-scores at the LS in the low BMD (for age and sex) group (-2.38 ± 0.42) differed significantly in comparison to the normal BMD groups (-0.16 ± 1.02) **(***P* = <0.001**).** In

the low BMD and normal BMD groups, significant differences were also observed in PTH intact plasma concentrations **(**6.71 pmol/L ± 3.57 vs. 5.04 pmol/L ± 1.89, (*P* = 0.010**),** and >10 years following a vegan diet (5 participants vs. 10 participants, *P* = 0.016), respectively.

Table 9. Participant bone parameters stratified by bone status at LS

Characteristic	**Low bone density (for age and sex) n= 12**	**Normal bone density n= 200**	***P* value**
Age (years)	49 ± 11.85	39 ± 12.18	***0.004**
Corrected serum calcium (mmol/L)	2.16 ± 0.27	2.21 ± 0.33	
Serum 25(OH)D (nmol/L)	72.9 ± 29.70	64.6 ± 22.92	
PTH intact plasma (pmol/L)	6.71 ± 3.57	5.04 ± 1.89	***0.010**
BMI (kg/m²)	23.74 ± 2.40	23.90 ± 3.21	
Protein (g)	70.4 ± 23	77.63 ± 28.1	
Calcium (mg)	997 ± 442.83	912.56 ± 341.54	
Energy intake (kJ)	8815 ± 2516	9093 ± 2525	
BMD Z score	-2.38 ± 0.42	-0.16 ± 1.02	***0.000**
Physical activity, n			
Low	3	32	
Moderate	7	151	
High	0	15	
Vegan time, n			
2 to 4 years	5	79	
5 to 10 years	2	91	
>10 years	5	30	**0.016**

Note. All data are presented as Mean ± SD, and n (%), *significant difference calculated by multiple t-tests PTH; parathyroid hormone, LS; lumbar spine, BMI; body mass index, BMD Z score; bone mineral density Z score, †normal BMD Z-scores for age and sex: >-2.0

Discussion

This study revealed that most adult vegans had normal BMD z-scores at the FN and LS for their age and sex. Moreover, overall serum and plasma markers of bone

health were within the normal reference range. Additionally, over half of participants (59%) met the EAR of calcium intake. We found a weak negative relationship between PTH intact plasma and serum 25(OH)D levels in vegans. Similarly, we also determined a weak relationship between corrected serum calcium and PTH intact plasma concentrations. No significant associations were found between the dietary intake of calcium, serum calcium concentrations and BMD z-scores at the LS or FN.

The participant socio-demographics in our study revealed a high prevalence of individuals with tertiary education, of NZ European ethnicity and over half were women (73%). Similarly, a NZ study by Greenwell et al. (2024) exploring the prevalence of veganism reported comparative socio-demographic factors from a representative sample of NZ adults. Considering the similarities of participant characteristics, it is possible to infer that our results are partially generalisable to the New Zealand vegan population.

3.1.12 Nutrient intake in relation to the EAR

Dietary calcium is regarded as a critical nutrient of concern for vegans (Koeder & Perez-Cueto, 2022; Menzel et al., 2021), particularly as the reported intakes are typically lower than omnivores and vegetarians (Melina et al., 2016). Mean daily intakes of zinc and phosphorus were under the EAR. Mean daily intakes of magnesium, protein, and vitamin C were adequate. However, variations in calcium intake were reported. Hence, this discussion will focus on calcium intake in vegans.

The mean daily intake of calcium exceeded the recommended EAR of 840 mg/day for adults aged 19-50 years. Similarly, Elorinne et al. (2016) reported sufficient intakes of calcium (1000mg/day) amongst vegans. Yet, recent research on the adequacy of calcium intakes have demonstrated conflicting results, particularly with low intakes (García-Morant et al., 2020; Hansen et al., 2018; Ho-Pham et al., 2012), whilst others have reported adequate calcium intakes (Elorinne et al., 2016; Weikert et al., 2020). Notably, our findings contrast with a study conducted in Vietnam by Ho-Pham et al. (2012), who reported very low mean calcium intakes of 300mg/day in vegans. One explanation for the observed differences in calcium intakes may be attributed to variations in cultural dietary practices. Moreover, traditional Vietnamese dietary practices may not typically include calcium-fortified plant-based products which are common in Western dietary patterns.

Additionally, the variations in calcium intakes reported across international studies may be linked with differences in the duration of self-administered dietary records. Specifically, as our study utilised a 4-DDD (including a weekend day) compared with 2 and 3-DDD used in other studies (Elorinne et al., 2016; Ho-Pham et al., 2012). It is also important to note that longer dietary records may be able to capture a more accurate estimation of the total daily intake of calcium whilst accounting for variability of intakes during weekends. Furthermore, in the analysis of dietary intakes, research by (Elorinne et al., 2016); Hansen et al. (2018); (Ho-Pham et al., 2012) utilised country-specific nutrient analysis databases whilst the Australia and NZ FoodWorks software program was used in our study. In short, this may influence the comparability of calcium intake data across international studies. Specifically, the nutrient analysis database used may reflect calcium content exclusive to locally consumed foods or calcium-fortified foods accessible within that country only.

Despite the overall daily intakes of calcium exceeding the EAR in this study, a large variation of intakes was also reported in females; ranging from 194 to 2,428 mg/day, and males; ranging from 382 to 2,267 mg/day. Moreover, forty-one percent of adult vegans reported calcium intakes below the recommended EAR (840mg/day). Similarly, the findings of Hansen et al. (2018) and Schüpbach et al. (2017) reported between forty to fifty-four percent of adult vegans with calcium intakes below the RDI of 800mg.

One reason for high calcium intakes may be attributed to the initial investigation of calcium in the vegan diet, which indicated plant-based milks (790mg/day) and tofu (416mg/day) were the primary sources of daily dietary calcium amongst participants. Furthermore, it would have been beneficial to quantify calcium absorption from food sources reported in the food diaries to determine the adequacy of calcium intake in relation to the EAR. Additionally, the presence of calcium inhibitors in the food diaries which are found in wholegrains, legumes and nuts (McClements & McClements, 2023), were unaccounted for in their potential inhibitory effect on calcium absorption.

Another factor to consider is the absorption and bioavailability of calcium in tofu, due to its second largest contribution to total daily calcium (416 mg) amongst participants. Weaver et al. (2002) reported calcium absorption from cow’s milk as

identical to tofu, based on the addition of calcium sulphate as a coagulant in tofu production (Peng et al., 2022). However, we were unable to determine if the type of coagulant in tofu would differ in their calcium bioavailability across different brands. Nonetheless, these results contradict the common notion of soybean products like tofu being responsible for the inhibition of calcium absorption due to their high phytate and oxalate content.

Overall, despite mean daily intakes of calcium for New Zealand vegan adults reported as above the recommended EAR, under half of participants (41%) are not obtaining sufficient calcium from their diet. Hence, these results demonstrated similarities observed beyond the mean from the underlying hypothesis that a vegan diet can lead to a reduction in the intake of calcium (Ho-Pham et al., 2012; Ogilvie et al., 2022).

Moreover, based on the examples illustrated above, it is also evident that the calcium fortification practices of plant-based food products and availability can vary internationally, resulting in differences of calcium intakes between studies. Therefore, reducing its generalisability of findings to the global vegan population.

3.1.13 Serum and plasma biomarkers of calcium status

Overall, most bone homeostasis markers including serum 25(OH)D3, serum corrected calcium, and PTH intact plasma concentrations were normal. However, corrected serum calcium concentrations (2.21 ± 0.33) mmol/L were at the lower end of the normal reference range (2.2 – 2.6 mmol/L). These findings are closely supported by Hansen et al. (2018), indicating that the overall serum calcium concentrations (corrected) were within the normal range (2.3 mmol/L), despite low mean calcium intakes (860 mg/day, ranged from 610-990 mg/day) in adult Danish vegans. One reason for this is explained by the tight homeostatic control of serum calcium concentrations. This remains relatively constant despite changes to intakes of calcium (Beto, 2015).

Interestingly, male participants (n=57) in our study exhibited borderline low mean corrected serum calcium concentrations, of 2.15 ± 0.32 mmol/L, which are below the reference range (2.2 mmol/L) despite calcium intakes of 1,052 ± 364 mg/day. These results may be indicative of non-dietary factors influencing the mean serum calcium concentrations in males. However, we were unable to determine if male participants

had clinical conditions like hypothyroidism (Fong & Khan, 2012) or other medical reasons, that explained low serum calcium concentrations. It is important to note the small and disproportionate number of males (n=57) vs. females (n=155) in our study, which suggests the influence of very low values on the overall mean serum calcium levels.

Contrastingly, Ho-Pham et al. (2012) reported post-menopausal vegans (N=88) with sub-optimal total serum calcium concentrations (1.51mmol/L). It is important to note that the type of serum calcium measurement was not indicated as they differ in their accuracy and the reference range to determine normal values. Moreover, controversy in the literature exists regarding the most reliable method for measuring calcium levels, particularly as ionised calcium concentrations are preferred but costly (Payne, 2019).

Another point to consider is the influence of PTH intact plasma and serum 25 (OH) D3 levels on the regulation of corrected serum calcium concentrations. Surprisingly, despite low serum calcium levels in males, mean PTH intact plasma levels (4.71 pmol/L) were within the normal reference range of 1.6 – 7 pmol/L (Canterbury Health Laboratories, n.d). Similarly, these findings are supported by Weikert et al. (2020), Hansen et al. (2018) and Ho-Pham et al. (2012) who reported vegans with adequate PTH intact plasma levels. Furthermore, serum 25 (OH) D3 (65 nmol/L) remained within the normal range.

We did not find a relationship between corrected serum calcium and serum 25(OH)D3 levels (r= -0.08 n=202, P(two-tailed) =0.251). Also, we found a weak negative relationship between PTH intact plasma and serum 25(OH)D3 levels (r=-0.18 n=149, P(two-tailed) =0.026). After factors such as age and sex were accounted for, the correlations increased in significance (r=-0.20 P = 0.014). Adjusting for confounding variables revealed the complex inter-relationship between PTH intact plasma, serum 25(OH)D3, and serum calcium concentrations. Moreover, our findings align with evidence towards the impact of serum 25(OH)D3 levels in regulating PTH secretion. In our study, participants had adequate mean serum 25(OH)D3 levels (65 nmol/L) in comparison to Hansen et al. (2018) who reported low serum 25(OH)D3 levels (47 nmol/L) in Danish vegans. The factors contributing to differences in serum 25(OH)D3 concentrations may be attributed to the variations in

geographical latitude, age, skin colour and cultural practices. Although mean serum 25(OH)D3 levels were adequate, the total intake of dietary vitamin D was not investigated. However, the underlying rationale for this is ascribed to a limited availability of food containing vitamin D in plant-based foods, and sun exposure as the main factor influencing serum 25(OH)D3 levels (Laird et al., 2010; Mendes et al., 2018).

The effect of confounding variables (age and sex) on calcium regulation is supported by Minisola et al. (1993) who investigated interrelationships of age and sex on the parathyroid endocrine system in healthy non-vegan men and women, where a significant inverse correlation was found between serum ionised calcium and PTH intact plasma in male subjects ($r=-0.661$, $P < 0.001$) and in fertile females ($r=-0.353$, $P < 0.037$). In contrast, our study utilised serum calcium corrected concentrations which differ in their clinical sensitivity compared to serum ionised calcium concentrations (Baird, 2011).

Furthermore, research on the assessment of calcium status through measurement of serum calcium concentrations amongst vegans is scarce. Contrastingly, Weikert et al. (2020) utilised a 24/hr urine calcium analysis in German vegans and reported a mean calcium excretion of 1.4 mmol/L amongst vegans. Yet, information on the established reference interval was not reported, which vary internationally. In accordance with the Canterbury Health Laboratories, New Zealand test methods for 24-hr urinary calcium, between 2.5 – 7.5mmol/24hr is considered normal. However, multiple separate measurements of urinary calcium are required due to large intra-individual variations (Canterbury Health Laboratories, n.d.-b). It is also important to note that this measurement can be influenced by recent dietary calcium, protein and sodium intake (Langlois, 2008), and medications (Liu et al., 2022), and therefore may not be representative of long-term calcium status.

Despite suboptimal serum calcium concentrations in males, the remaining bone-related biomarkers for both males and females were normal. To date, the research exploring serum and plasma parameters of bone in vegans are limited. More importantly, our results provide insight into the regulation of serum calcium levels in vegans in the context of this study, which are not negatively affected by a plant-based diet.

3.1.14 Association between Z scores, predictor variables and overall bone status in vegans

In the present study, most participants (94-98%) exhibited normal BMD Z scores (>-2) for age and sex at the lumber spine and femoral neck. Furthermore, no significant associations were found between Z scores of the lumbar spine and femoral neck, and PTH levels, physical activity levels, daily calcium intake (mg) per 1kJ of energy, corrected serum calcium concentrations and serum 25(OH)D3. However, BMI was the sole statistically significant predictor of Z-scores (P = 0.004).

For this study, *Z*-scores, and raw BMD (g/cm^2) values were used to describe bone health in vegans. Furthermore, we primarily utilised Z-scores as the standardised and recommended measure for assessing BMD in adults under 50 (Carey & Delaney, 2010). This measurement indicated whether a participants' BMD varied or remained the same to an average of BMD for age and gender matched individuals. Therefore, this removed the requirement to control raw BMD (g/cm^2) for age and sex in the analyses.

Overall, the BMD Z-scores at the lumbar spine (-0.29) and femoral neck (-0.24) in adult vegans revealed normal BMD for their age and sex. Our findings are similar to a study by Hsu et al. (2023), who reported adult Taiwanese vegans with a Z-score of -0.49 at the spine. Similarly, Knurick et al. (2015) reported total body BMD Z-scores of 0.26 in American vegans. These findings indicate that Z-scores in adult vegans were relatively close to zero, which is representative of the mean for normal BMD for age and sex. Therefore, in the context of this study, it is plausible to conclude that following a vegan diet does not have adverse effects on BMD.

Within our study population, BMD Z-scores at the LS and FN were not associated with predictor variables known to influence BMD, apart from BMI. An explanation for the lack of significant associations may be linked with additional unexplored variables known to influence BMD (Hsu et al., 2023). Moreover, due to the nature of an observational cross-sectional study, we were unable to determine causation between predictor variables and BMD Z-scores.

The significant association between BMD Z-scores and BMI in adults is to be expected as low BMI is a well-established risk factor for osteoporosis (Emaus et al.,

2014a). A Taiwanese study by Hsu et al. (2023) found statistically significant differences between sex, age, BMI, alcohol, tea and coffee intake and BMD scores at the lumbar spine amongst vegans and non-vegans. However, it is important to note that calcium intake and serum 25(OH)D3 levels was not accounted for which may have confounded the associations of BMD between vegans and non-vegans.

The variation of predictor variables and their associations with BMD Z-scores observed in our study and international research highlights the complexity of understanding factors that influence bone density and homeostasis. Moreover, the reasons for variations in predictor variables selected in the analyses is likely due to differences in study design, categorisation of vegans, number and characteristics of participants.

To date, we are the first study in NZ to explore determinants of BMD and Z-scores in vegans. Our findings revealed most participants (94-98%) had normal BMD for their age and sex. Despite the absence of BMD Z score associations with several predictor variables in vegans, BMD and calcium homeostasis are multifaceted. In other words, it is not only influenced by the overall adequacy of nutrients in the diet but also by genetics, age, sex, physical activity and mineral homeostasis (Redondo-Cuevas et al., 2018), which an observational study is unable to capture at a single point in time.

Conclusion

As highlighted above, the present study is the first to explore BMD at the FN and LS using DXA, evaluate intake of bone-related nutrients, explore plant-based sources of calcium, analyse plasma and serum markers of calcium homeostasis in a New Zealand adult vegan population. Our findings revealed adequate daily nutrient intakes of calcium, protein, magnesium, vitamin C and protein in relation to the EAR. Furthermore, most adult vegans exhibited normal serum and plasma markers of bone homeostasis and BMD for their age and sex. Our results are consistent with previous studies reporting vegans with BMD for age and sex, despite few reports of low calcium intakes in some vegan populations. Furthermore, due to the limited number of longitudinal studies currently being conducted, especially in the New Zealand context, additional research is required to understand the long-term impact of a vegan diet on bone health.

Chapter Four Conclusion and Recommendations

Achievement of research aim and objectives

The overall aim of this study was to describe bone health and calcium intake in NZ vegans. Four objectives were determined to successfully achieve the aim, which comprised of: describing BMD of the FN and LS using standard Z-scores, measuring dietary intake of calcium, magnesium, phosphorus, protein, zinc, Vitamins C and D in relation to the EAR, assessing serum 25(OH)D3, PTH intact plasma and serum calcium concentrations, as well as exploring the relationship between dietary intake of calcium, BMD and calcium status in adult vegans. Many participants (94-98%) exhibited normal bone density at the hip and spine for their age and sex, whilst 2-6% had low bone density. Mean intakes of calcium, protein, magnesium and vitamin C were adequate in comparison to the EAR. However, very low intakes of calcium demonstrated significant variations between participants. Additionally, this study found no significant differences in calcium intakes, serum calcium concentrations and BMD Z-scores at the FN and LS. These findings contribute to our understanding of bone health in a vegan population, which specifically address the concern of poor BMD outcomes compared to non-vegans within the literature. Interestingly, BMD measurements, calcium intakes, plasma and serum markers of bone homeostasis in participants were not negatively affected by a vegan diet.

Research impact

To my knowledge, this is the first large observational, cross-sectional New Zealand study (n=212) that explores BMD and calcium intakes in a vegan population. Previous international studies have reported normal BMD for age and sex in adult vegans (Hsu et al., 2023; Knurick et al., 2015). Furthermore, overseas comparative studies have indicated vegans had lower BMD measurements in comparison to omnivores (Ho-Pham et al., 2009; Knurick et al., 2015). However, no significant correlations between BMD measures and dietary calcium existed amongst the two dietary groups suggesting that it may not be of clinical relevance. Moreover, it is important to highlight the differences in sample size, age, duration of vegan diet, characteristics and dietary habits of participants included in overseas research which

may influence BMD. Hence, these findings are not generalisable to the New Zealand population.

Our research contributes valuable new findings to the existing literature on bone health in vegans, particularly with a broader focus on comprehensively analysing bone related parameters, nutrient intake and BMD measures. Furthermore, these new insights concerning the bone health of vegans is important for the broader public to be informed on as plant-based diets continue to increase in popularity. Therefore, it is important to highlight the impact of lifestyle and appropriate dietary planning to support optimising bone health in vegan adults. Particularly, during life stages of increased nutrient demands including lactation, pregnancy and older adulthood.

Moreover, the findings from this study provide valuable data for future research to expand on and potentially explore cause-and effect relationships between a vegan diet and BMD in longitudinal studies.

Strengths

A notable strength of this study included its large sample size and diverse age range of participants, which increased the reliability and generalisability of our findings to a NZ vegan adult population. Also, another strength of this study involved the measurement of BMD using DXA, which is considered the gold standard method for the assessment of BMD in various skeletal sites (Link & Kazakia, 2020).

Previous research on exploring bone health in a vegan population have differed in their assessment of nutrient intakes, BMD measurements, non-dietary determinants of BMD and plasma markers of bone homeostasis. Given this, several measures of bone parameters were assessed in our study to demonstrate a comprehensive overview of bone health and calcium intake in vegans. To my knowledge, our study is also the first to identify the main sources of calcium in a vegan diet. Our exploration of plant-based foods rich in calcium provided information into their calcium bioavailability, as this can vary widely (Shkembi & Huppertz, 2021). Notably, this is predominately influenced by the presence of calcium inhibitors which reduce the intestinal absorption of calcium (Shkembi & Huppertz, 2021).

However, research on the assessment of calcium bioavailability in vegans is required to determine the amount of calcium absorbed from the diet. This will provide information regarding whether the use of a nutrient analysis database alone is accurate to estimate total calcium intakes. Overall, these findings can be used inform future dietary recommendations specifically designed for vegans to promote consumption of plant-based sources high in calcium.

Research on the assessment of BMD Z scores in vegans to determine low or normal BMD for their age and sex is limited. Overseas studies commonly examined raw BMD (kg/m^2) values, which can also pose challenges in the interpretation of BMD due to varying ages and sex of participants. Consequently, another strength of this study included the utilisation of a standard measurement of BMD Z-scores. This allowed for accurate comparisons of participant BMD to an age and sex matched individual in a study population under 50 years old. A Z-score lower than -2.0 is considered low for age in individuals under the age of 50 years, whilst values above -2.0 are considered normal. Furthermore, the bone parameter variables of participants were included in the two BMD Z-score groups to identify differences in the factors influencing BMD.

Limitations

This study had several limitations. Firstly, the participants were recruited from the use of a convenience sample based on a single geographic location in Auckland. These findings may not be applicable to vegan populations located in other regions of New Zealand. Secondly, participants were mainly pre-menopausal females (73%) and the lack of even distribution between male and females reduced the generalisability of our findings to the broader NZ vegan adult population. Moreover, the higher incidence of veganism in females may have impacted our results as sex influences BMD. Thirdly, the exclusion criteria did not include clinical conditions known to affect serum calcium concentrations, such as hypoparathyroidism. Half of the participants (51%) exhibited borderline low serum calcium concentrations in reference to the normal values. However, participants appeared presumably healthy and as a result, this may have confounded the results. It is also important to note other unknown clinical reasons resulting in low serum calcium concentrations that weren't accounted for in this study. Fourthly, the FoodWorks software program utilised in this study was unable to assess calcium from supplements indicated in a

small number of participant food diaries (6%). Therefore, the impact of supplementation on the achievement of the EAR for nutrients essential for bone health, such as calcium, remains uncertain. Moreover, the additional information provided by supplementation can provide findings on whether dietary intake alone is sufficient to meet the EAR of a specific nutrient.

This observational and cross-sectional study provides valuable insights towards understanding bone health and calcium intakes in a cohort of NZ adult vegans. However, we were unable to determine causative factors of BMD at one point in time. Therefore, longitudinal research is warranted to establish causality between predictors of BMD and a vegan diet.

Recommendations for future research

Longitudinal research on the measurement of BMD, calcium intakes and calcium homeostasis in long-term vegans is required to understand the impact of veganism overtime on bone health. Furthermore, total nutrient intakes from food diaries and supplements (Vitamin D and calcium) should be evaluated to increase the validity and reliability of research findings by providing an accurate representation of participant nutrient intakes. Researchers should also include an ethnically diverse and equal number of sexes in the study population to illustrate a representative sample of the NZ adult vegan population.

Comparative studies of vegans vs. non vegans in the NZ context would be necessary to identify if differences in BMD exist, and if they are associated with dietary patterns and/or lifestyle factors. Especially, since differences in lifestyle determinants of BMD between vegans and omnivores are reported in the literature. These included vegans with lower BMI, smoking, alcohol intake and higher physical activity levels (Greenwell et al., 2024; Spencer et al., 2003; Tong et al., 2020). However, the impact of these lifestyle factors on BMD from following a specific dietary pattern is unknown.

The assessment of calcium bioavailability is recommended for future research, as this can vary widely in a vegan diet due to the presence of oxaolates in many plant-based foods. Researchers could utilise calcium isotope studies to quantify the amount of calcium excreted to evaluate the absorption and bioavailability of calcium from the diet.

Additional research is required to understand the relationship between calcium homeostasis markers and bone mineral density in vegans. Researchers should also investigate bone related resorption (N-terminal and C-terminal telopeptide of type 1 collagen) markers alongside serum PTH, serum 25(OH)D3 and ionised serum calcium concentrations. Essentially, this method will provide a more accurate representation of calcium homeostasis and increase sensitivity in the detection of changes to bone.

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Appendices

Appendix 1: Recruitment poster

CALLING ALL VEGANS

TAKE PART IN AN EXCITING NEW STUDY

GET YOUR BODY COMPOSITION AND NUTRITION STATUS

EMAIL: VEGANSTUDY@MASSEY.AC.NZ
TEXT OR CALL: 021 220 0092

WWW.MASSEY.AC.NZ/VEGANSTUDY

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

Appendix 2: Participant Information sheet

Participant Information Sheet

Health and Vegan Diet

A clinical investigation project included in Phase 2 of The Vegan Health Research Programme

Lead Researcher: Professor Pamela von Hurst

Study Site: Human Nutrition Research Unit, Massey University, Albany

Contact phone number: 09 414 0800 ext 43657

Ethics committee ref.: 2022 EXP 12312

You are invited to take part in a study investigating the impact of a vegan diet on your health**.** Whether or not you take part is your choice. If you 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.

This form is 8 pages. 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 questions
- 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
- Be given access to a summary of the study 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?

Interest in the vegan lifestyle is growing, and NZ ranks the fifth most vegan country in the world. A vegan diet tends to have some health benefits, but at the same time it might be associated with nutrient deficiencies.

These deficiencies could have significant health consequences if they occur during critical period of life (for example, pregnancy or the rapid growth and developmental stages). Therefore, dietary guidelines stress that those who follow strict vegetarian or vegan diets may

need extra information and/or support to ensure that they meet their nutrient needs. Our search has not found any studies to date that have investigated nutritional status, nutrient/food intake, motivations and nutritional knowledge and their sources of NZ vegans.

The aims of this study are to investigate nutritional status, nutrient/food intake, reasons for becoming vegan, nutrition knowledge and sources of nutrition information, and gastrointestinal discomfort symptoms among NZ vegans.

HOW IS THE STUDY DESIGNED?

This study will involve 220 individuals aged 18 years or older, who have been on a vegan diet for at least two years. Participants will take part in online or telephone screening to check eligibility. If eligible they will visit the Human Nutrition Unit at Massey University, once for approximately 90 minutes,

Participants will be required to have bone density, body composition, and blood pressure measurements, complete online questionnaires regarding health, demographics, lifestyle, physical activity, motivations for following a vegan diet, dietary intake, nutrition knowledge, and sources of nutrition knowledge, and complete a 4-day diet record. In addition, participants will be asked to provide a non-fasted blood sample.

WHO CAN TAKE PART IN THE STUDY?

Individuals aged 18 years or older, who have been following a vegan diet for at least two years will be included in this study. Women who are pregnant or have any likelihood of being pregnant will be excluded from this study. Participants will complete a short screening questionnaire to ensure they meet inclusion criteria.

WHAT WILL MY PARTICIPATION IN THE STUDY INVOLVE?

If you decide to take part in this study, after you have read and had time to consider the information in this information sheet, you will be required to complete the screening questionnaire. Screening involves answering a few inclusion criteria questions, this can be done at home either online or on the phone, and takes approximately five minutes. Your answers to this questionnaire will help us to see if you are eligible to take part in this study or not.

If you are eligible to take part in this study, you will be required to visit Human Nutrition Unit at Massey University in Albany on one occasion for data collection. Prior to your visit to Massey University, we will send you a consent form, some questionnaires that need to be completed online, and a diet diary. For the online questionnaires, we will ask you to:

- Complete demographic, health, lifestyle, and physical activity questionnaires.
- Complete a questionnaire to assess motivations for following a vegan diet
- Complete a questionnaire to assess dietary intake
- Complete a questionnaire to assess nutritional information and their sources
- Complete a questionnaire to assess gastrointestinal discomfort symptoms

For the diet diary, we request that for 4 days you record everything you eat and drink. Instructions will be provided in more detail at your visit.

A researcher will make an appointment with you at your convenience You will be required to not have caffeinated drinks and not exercise for 2hrs prior to the visit. This visit will take approximately 90 minutes and you will be reimbursed for your travel.

At this appointment you will first be asked to hand in the signed consent form for participating in the study and you will have the opportunity to ask any questions you may have about the study. During this visit, we will ask you to

- Have weight, height, and waist and hip circumferences measured by a trained researcher.
- Have bone density and body composition measured using dual-energy X-ray absorptiometry (DXA). This machine uses very low dose X-rays to measure the bone density of your hip and spine, and also measures your body composition (fat mass, lean mass, and bone mass of your body).
- Have blood pressure measured using electronic blood pressure monitor by a trained researcher
- Provide a small venous blood sample (about 20ml which is equivalent to 4 teaspoons). This will be taken by a qualified phlebotomist. It will be used to measure levels of various nutrients in your blood, such as iron and vitamin D.

WHAT WILL HAPPEN TO MY BLOOD 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 minus 80 degree freezer until the study is completed after which time the biochemical analysis will be conducted. While waiting for data and bloods to be collected from all participants and analysed in one batch, samples will be kept in the freezer at the Nutrition laboratory at Massey University, Building 27, Oteha Rohe campus, Albany.

On completion of the study, samples will be sent to the Canterbury Health Labs to assess vitamins D, B_{12}, folate, iron, lipids, calcium and albumin.

One drop of whole blood sample will be analysed on site at Massey University to assess haemoglobin, and another drop will be applied to a special paper to be sent to CSIRO laboratory in Adelaide to assess polyunsaturated fatty acids.

Participants may ask to withdraw their samples at any time during the study up to the time the samples are analysed. The analysis results in the destruction of the sample.

There may be participants who identify as Māori and if specific concerns develop, the support of Dr Bevan Erueti (Taranaki, Te Ati Haunui-ā-Papārangi, Ngāti Tūwharetoa), Associate Dean Māori, will be afforded. Dr Erueti has expressed that he is happy to act in the capacity of advisor and if required will assist and facilitate the projects Māori agenda and ensure that relational aspects of trust and appreciation are upheld with Māori participants. We are also aware that a diversity of beliefs and cultural concerns regarding the removal, storage and transport of tissue samples and these should be discussed with your whānau (family) or take advisement from hapū and iwi leaders. Nonetheless, the right to decline or withdraw from the study can be done at any stage of the project.

WHAT ARE THE POSSIBLE RISKS OF THIS STUDY?

The DXA has X-ray beams of different energies and, while no dose of radiation is harmless, this dose is very low and unlikely to cause harm. The total effective dose of radiation to which you will be exposed to is 10.8 microsieverts (μSv), which is much lower than the range normally used in medical diagnostics. To place this in perspective, the amount of radiation an individual would receive from flying in an aircraft to the United Kingdom equates to an effective dose about six times that received from the study. The effective dose received by the

participants from the study is also equivalent to about 2 days of background radiation to which all New Zealanders are exposed. This procedure is quick, non-invasive and completely painless. The room is private, and the staff are experienced and certified.

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 trained phlebotomist. There may be social or cultural discomfort from having a blood sample, bone density, body composition, and blood pressure 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 you wish. Every effort will be made to ensure your comfort and respect your participation.

WHAT ARE THE POSSIBLE BENEFITS OF THIS STUDY?

- You will be contributing to a greater understanding of the health implications of a vegan diet.
- You will not be charged for any of the measurements conducted for the study
- You will be provided with your body composition results, blood test results and a nutrient analysis of your diet from your 4-day diet diary.
- You will get a summary of the study results.

WILL ANY COSTS BE REIMBURSED?

Participants will not incur any costs as part of being involved in the study and will receive reimbursement for travel ($20 in 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.

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.

Anonymised Information

The lead researcher may remove the code from your de-identified information – this is called 'anonymisation'. This makes it very difficult (but not impossible) to identify the information that belongs to you. The researcher may share this anonymised information with other researchers on request for the purpose of accumulating data from individual studies. The anonymous/anonymised data is unable to be accessed, corrected, or withdrawn; and return of individual results will not be possible.

Future Research Using Your Information

If you agree, your fully anonymous/anonymised information may be used for future research related to veganism. This is optional and you could still participate in the present study if you do not agree.

This future research may be conducted overseas. You will not be told when future research is undertaken using your information. Your information may be shared widely with other researchers. Your information may also be added to information from other studies, to form much larger sets of data.

You will not get reports or other information about any future research that is done using your information.

Your information may be used indefinitely for future research unless you withdraw your consent. However, it may be extremely difficult or impossible to access your information, or withdraw consent for its use, once your information has been shared for future research.

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 scan (body composition) during the study. You can't access other study-specific information (e.g. diet analysis and blood test results) during the study, because these data will be analysed when the data from all participants are collected and the study is over.

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 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. However, findings of any future research conducted using fully anonymised data collected in this project will not be made available to participants.

WHO IS FUNDING THE STUDY?

This study is funded by the Lottery Health Project Grant.

Participants will not incur any costs for taking part in the study and will be reimbursed for travel.

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 Central Health and Disability 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. Hajar Mazahery, study manager
Email: h.mazahery@massey.ac.nz

Rebecca Paul, research assistant
Phone: 022 1294112
Email: veganstudy@massey.ac.nz

The other members of the research team are: Professor Pamela von Hurst, Associate Professor Cathryn Conlon, Associate Professor Kathryn Beck, and Dr. Rachel Batty (College of Health, Massey University).

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 Bevan Erueti, Taranaki, Te Ati Haunui-ā-Papārangi, Ngāti Tūwharetoa, Associate Dean Māori
Phone: 06 356 9099 Ext 83087
Email: B.Erueti@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

Appendix 3: Participant consent form

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

Health Implications of a Vegan Diet

A clinical investigation project included in Phase 2 of The Vegan Health Research Programme

Please tick to indicate you consent to the following

I have read 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.	☐	
I consent to the research staff collecting and processing my information, including information about my health.	☐	
I consent to my fully anonymous / anonymised information being shared with other researchers on request for future research and the purpose of accumulating data from individual studies, and the results of future studies using my anonymous / anonymised information will not be made available to me.	Yes ☐	No ☐
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 4: Participant dietary practices and supplement use

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

Health and Vegan Diet

Dietary Practices and Supplement Use

Please answer all questions. All the information you give us is in confidence and will only be used of the purposes of this study. If you need any help to complete questions, please ask one of the research team.

VEGANISM MOTIVATION

1. How long have you been following a vegan lifestyle?

	2 to 4 years	**5 to 10 years**	**>10 years**
I have been a vegan	[ ]	[ ]	[ ]

2. On a scale of 1 (not important) to 7 (very important) rate the importance of each of the following reasons for you to EXCLUDE MEAT OR ANIMAL PRODUCTS from your diet.

		Not important		**Moderately important**			**Very important**	
		1	**2**	**3**	**4**	**5**	**6**	**7**
1	I want to be healthy							
2	Plant-based diets are better for the environment							
3	Animals do not have to suffer							
4	Animals' rights are respected							
5	I want to live a long time							
6	Plant-based diets are more sustainable							
7	I care about my body							
8	Eating meat is bad for the planet							
9	Animal rights are important to me							
10	Plant-based diets are environmentally-friendly							
11	It does not seem right to exploit animals							
12	Plant products have less of an impact on the environment than animal products							
13	I am concerned about animal rights							
14	My health is important to me							
15	I don't want animals to suffer							

3. There might be other reasons for following a vegan diet. On a scale of 1 (NOT IMPORTANT) to 7 (VERY IMPORTANT), rate the importance of each of the following reasons for you to EXCLUDE MEAT OR ANIMAL PRODUCTS from your diet.

		Not important		Moderately important			Very important	
		1	2	3	4	5	6	7
1	Cultural/religious beliefs							
2	Allergy/intolerance to animal-based foods							
3	Having a vegan partner or family member							
4	Having a vegan friend							
5	Having a vegan classmate							
6	Having a vegan co-worker/colleague							

DIETARY HABITS

4. On a scale of 1 (NOT AT ALL IMPORTANT) to 5 (EXTEREMELY IMPORTANT), rate the importance of other people's support in helping you following a vegan diet.

[ ] 1 (not important)
[ ] 2 (slightly important)
[ ] 3 (moderately important)
[ ] 4 (very important)
[ ] 5 (extremely important0

5. In a typical week, how often do you eat the following MEALS during the week?

	Never	Rarely (1/4 of the time)	Sometimes (1/2 of the time)	Usually (3/4 of the time)	Always
Breakfast	[ ]	[ ]	[ ]	[ ]	[ ]
Lunch	[ ]	[ ]	[ ]	[ ]	[ ]
Dinner	[ ]	[ ]	[ ]	[ ]	[ ]

6. In a typical week, how often you eat the following MEALS at the weekend?

	Never	Rarely (1/4 of the time)	Sometimes (1/2 of the time)	Usually (3/4 of the time)	Always
Breakfast	[ ]	[ ]	[ ]	[ ]	[ ]
Lunch	[ ]	[ ]	[ ]	[ ]	[ ]
Dinner	[ ]	[ ]	[ ]	[ ]	[ ]

7. In a typical week, where are most of your..?

	At home	Out	Don’t eat meal
Breakfast	[ ]	[ ]	[ ]
Lunch	[ ]	[ ]	[ ]
Dinner	[ ]	[ ]	[ ]

8. How much responsibility do you have for:

	Little or none	About half	Most or all
Food shopping?	[ ]	[ ]	[ ]
Planning meals?	[ ]	[ ]	[ ]
Preparing meals?	[ ]	[ ]	[ ]

9. What type of food do you preferentially buy? (you can choose more than one answer)

[ ] Pre-cooked meals
[ ] Fresh foods
[ ] Frozen foods
[ ] Canned foods
[ ] Other (please state)
[ ] I don't do food shopping

10. How often do you eat convenient/frozen meals?

[ ] Never
[ ] Less than once a week
[ ] Once per week
[ ] 2 or more times per week
[ ] Don't know

11. Concerning fat content in food products, how often you have the followings?

	Never	Rarely (1/4 of the time)	Sometimes (1/2 of the time)	Usually (3/4 of the time)	Always	Don't know
Non-fat products	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Low fat products	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Reduced fat products	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
High fat products	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]

12. How often do you use following cooking methods to cook the foods you eat?

	Never	Rarely (1/4 of the time)	Sometimes (1/2 of the time)	Usually (3/4 of the time)	Always	Don't know
Boiling/Steaming	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Stir-frying	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Deep-fat frying	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Baking/Microwave/Grill	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]

13. What type of oil do you usually use in cooking (e.g., for frying, roasting, etc.)? (You can choose more than one answer).

[ ] Coconut oil
[ ] Olive oil, canola oil, avocado oil, soybean oil, peanut oil, rice bran oil
[ ] Sunflower oil, corn oil, safflower oil, cottonseed oil, sesame seed oil, grapeseed oil
[ ] Other oil (please state)

[ ] I don't use oil in cooking
[ ] Don't know

14. How often do you add salt to your foods/meals?

	Never	Rarely (1/4 of the time)	Sometimes (1/2 of the time)	Usually (3/4 of the time)	Always	Don't know
Whilst cooking	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
At the table to meals/snacks	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]

15. Are you on a doctor-prescribed low sodium diet?

[ ] Yes
[ ] No

16. What type of milk do you usually have?

[ ] Soy milk (regular)
[ ] Soy milk (light)
[ ] Soy milk (unsweetened)
[ ] Soy milk (protein plus)
[ ] Coconut milk (regular)
[ ] Coconut milk (light)
[ ] Coconut milk (unsweetened)
[ ] Oat milk
[ ] Rice milk
[ ] Almond milk (regular)
[ ] Almond milk (high protein)
[ ] Almond milk (unsweetened)
[ ] Cashew milk
[ ] Peanut milk
[ ] Seeds milk
[ ] Other milk (please state)
[ ] I don't use/drink milk
[ ] Don't know

17. How often do you choose whole grain breads and cereals (e.g. whole grain or multigrain breads, porridge or oats, oatmeal, oat flakes, bran based breakfast cereals, brown rice, wholemeal pasta, quinoa, buckwheat, food made with wholegrain, whole wheat or rye flour; food made from wheat flakes, whole barley, bulgur wheat) rather than more refined breads and cereals? (e.g. white breads, cornflakes, rice bubbles, white rice, white pasta, food made with white flour)

[ ] Never
[ ] Rarely (1/4 of the time)
[ ] Sometimes (1/2 of the time)
[ ] Usually (3/4 of the time)
[ ] Always
[ ] Not applicable – I don't eat bread and cereals
[ ] Don't know

18. What type of spread do you usually use on bread?

[ ] Monounsaturated fat margarine (e.g. spreads based on olive oil, rice bran oil , canola oil)
[ ] Polyunsaturated fat margarine (e.g. spreads based on sunflower oil)
[ ] Light monounsaturated fat margarine (e.g. Olivio spread light)
[ ] Light polyunsaturated fat margarine (e.g. Flora spread light)
[ ] Plant sterol enriched margarine - both full and low fat varieties (e.g. ProActive, Logical)
[ ] Other (please state)
[ ] I don't use spreads on bread
[ ] Don't know

19. How often do you eat savory snacks such as potato chips?

[ ] Never
[ ] Less than once a week
[ ] Once per week
[ ] 2 or more times per week
[ ] Don't know

20. How often do you eat sweet snacks such as biscuits, cakes, sweets, lollies, chocolate or ice blocks or puddings (e.g., fruit pies, crumbles, sponge puddings, steamed puddings)?

[ ] Never
[ ] Less than once a week
[ ] Once per week
[ ] 2 or more times per week
[ ] Don't know

21. How often do you have the following drinks?cordials or fizzy drinks (do not include diet or low-calorie varieties)?

	Less than once a week	Once per week	2 or more times per week	Don't know
Cordials (do not include diet or low calorie variety	[ ]	[ ]	[ ]	[ ]
Diet or low calorie cordials	[ ]	[ ]	[ ]	[ ]
Fizzy drinks (do not include diet or low calorie variety)	[ ]	[ ]	[ ]	[ ]
Diet or low calorie fizzy drinks	[ ]	[ ]	[ ]	[ ]

22. How often do you eat processed/ultra-processed foods?

[ ] Never
[ ] Less than once a week
[ ] Once per week
[ ] 2 times per week
[ ] 3 times per week
[ ] 4 times per week
[ ] 5 times per week

[ ] 6 times per week
[ ] 7 or more times per week
[ ] Don't know

23. How often do you eat at a restaurant/café?

[ ] Never
[ ] Less than once a week
[ ] Once per week
[ ] 2 or more times per week
[ ] Don't know

24. How often do you eat at a fast food outlet?

[ ] Never
[ ] Less than once a week
[ ] Once per week
[ ] 2 or more times per week
[ ] Don't know

25. How often do you eat meal with friends?

[ ] Never
[ ] Less than once a week
[ ] Once per week
[ ] 2 or more times per week
[ ] Don't know

26. How often do you eat at establishments such as work/education canteen?

[ ] Never
[ ] Less than once a week
[ ] Once per week
[ ] 2 or more times per week
[ ] Don't know

27. How often do you eat convenient/frozen meals?

[ ] Never
[ ] Less than once a week
[ ] Once per week
[ ] 2 or more times per week
[ ] Don't know

SUPPLEMENT USE

28. For the following NUTRIENT supplements, please check [x] the *YES* column and fill in the dose and brand name for those you USUALLY use; then state how often you use the supplement. For those you do not use, check [x] the *NO column*.

Nutrient supplement	Yes	No	Dose	Brand name	How often you use the supplement (please provide more details)
Calcium					
Vitamin D					
Vitamin B12					
B vitamins					
Zinc					
Iron					
Folate					
Iodine					
Selenium					
Multivitamin/ supplement					
Mineral supplement					
Omega-3 fatty acids					
Amino acids (please specify)					
a)					
b)					
c)					
Others (specify below)					
a)					
b)					
c)					
"I do NOT use any nutrient supplements" [ ] ***True*** [ ] ***False***					

29. For the following FOOD/HERBAL/DIETARY supplements, check [x] the *YES* column and fill in the dose and brand name for those you USUALLY use; then state how often you use the supplement. For those you do not use, check [x] the *NO column*.

Food/herbal/dietary supplement	Yes	No	Dose	Brand name	How often you use the supplement (please provide more details)
Nutritional yeast					
Wheat germ					
Soy/vegetable protein powder					
Spirulina					
Chlorella					
Others (specify below)					
a)					
b)					
c)					
"I do NOT use any food/herbal/dietary supplements" [ ] ***True*** [ ] ***False***					

Appendix 5: Health and demographics

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

Health and Vegan Diet

Health and Demographic Information

Please answer all questions. All the information you give us is in confidence and will only be used of the purposes of this study. If you need any help to complete questions, please ask one of the research team.

1. Do you have or have you ever had any acute illness?

   [ ] Yes
   [ ] No

2. If yes, please provide more details (including the diagnosis, date of diagnosis, by whom you were diagnosed, and any details you may think is relevant) below:

   _____
   _____
   _____

3. Do you have or have you ever had any chronic illness?

   [ ] Yes
   [ ] No

4. If yes, please provide more details (including the diagnosis, date of diagnosis, by whom you were diagnosed, and any details you may think is relevant) below:

   _____
   _____
   _____

5. Have you ever been diagnosed with any bone fracture (broken bone)?

   [ ] Yes
   [ ] No

6. If yes, please provide more details (which bone, age when it happened, how did it happen)

   _____
   _____
   _____

7. Do you usually have elevated blood pressure (

[ ] Yes
[ ] No
[ ] Unsure

8. If yes, please provide more details (elevated systolic blood pressure, elevated diastolic blood pressure or both?)

_____
_____
_____

9. Have you ever been diagnosed with iron deficiency?

[ ] Yes
[ ] No
[ ] Unsure

10. If yes, please provide more details about your iron deficiency (self-diagnosed or diagnosed by a health care provider, date and treatment)

_____
_____
_____

11. Do you get nose bleeds?

[ ] Yes
[ ] No

12. If yes, how often do you get a nose bleed?
_____ Times a month or _____ Times a year

13. If yes, how heavy are your nose bleeds?

[ ] Light
[ ] Medium
[ ] Heavy

14. Have you had any blood loss (other than periods or nose bleeds) such as wounds, regular scratches from contact sports, blood in stools, or urine in the past year?

[ ] Yes
[ ] No

15. If yes, please describe below.

_____
_____
_____

16. Have you had any medical condition which has resulted in blood loss?

[ ] Yes
[ ] No

17. If yes, please describe and give approximate date below.

_____
_____
_____

18. Do you donate whole blood (i.e. not plasma)?

[ ] Yes
[ ] No

19. If yes, when did you last donate blood?

Date _____ / _____ /_____
Day Month Year

20. If yes, how many times have you donated whole blood in the past year?

_____ (times in the past year)

21. Have you ever had iron infusion?

[ ] Yes
[ ] No

22. If yes, please provide details (reasons and date of infusion)

_____
_____
_____

23. Have you had a blood transfusion in the last year?

[ ] Yes
[ ] No

24. If yes, please provide details (reason and date of transfusion)

_____
_____
_____

25. Do you currently smoke?

[ ] Yes
[ ] No

26. If yes, how often do you smoke

[ ] Occasionally
[ ] A few times per week

[ ] Daily

27. If no, have you ever smoked?

    [ ] Yes
    [ ] No

28. If yes, how often did you use to smoke?

[ ] Occasionally
[ ] A few times per week
[ ] Daily

29. Are you currently taking any medication (excluding nutritional supplements)?

    [ ] Yes
    [ ] No

30. If yes, please state what medication you are taking and why?

    _____

    _____

    _____

31. Has any of your first-degree family members (parents and grandparents) had osteoporosis?

    [ ] Yes
    [ ] No
    [ ] Unsure

32. Has any of your first-degree family members (parents and grandparents) had the following illnesses when they were younger than 50 years old?

	Yes	No	Unsure
Cardiovascular diseases (i.e. angina, heart attack, transient ischaemic attack, stroke)	[ ]	[ ]	[ ]
Hypertension	[ ]	[ ]	[ ]
Diabetes	[ ]	[ ]	[ ]
Hypercholesterolemia	[ ]	[ ]	[ ]

33. In general, would you say your health is..
    [ ] Excellent
    [ ] Very good
    [ ] Good
    [ ] Fair
    [ ] Poor

Questions specific to women

34. Which of the following BEST describes your current Menstrual/Menopausal status?

[ ] Never menstruated
[ ] Still menstruating
[ ] Going through menopause
[ ] Postmenopausal
[ ] Other (Please explain)

35. Have you had a period in the last 3 months? (not including postmenopausal women)

[ ] Yes
[ ] No

36. How regular are your periods (21-34 days)?

[ ] Regular
[ ] Irregular

37. How many days do you usually have between periods? (for instance, counting from the first day of your last period to the day you expect your next period to start)

_____ days

38. Do you know when your last period started?
39.

[ ] Yes
[ ] No

40. When did your last period start?

_____ / _____ /_____
Day Month Year

41. How many days does your period usually last?

_____ days

42. Have you been pregnant within the last year?

[ ] Yes
[ ] No

43. If yes, did the pregnancy result in any significant blood loss that you are aware of? (Please comment below)

_____

_____

44. Are you on hormonal contraceptives?

Demographics (7 questions)

48. When were you born?

_____ Day (DD) _____ Month (MM) _____ Year (YYYY)

49. What is your gender?

[ ] Female
[ ] Male
[ ] Gender diverse
[ ] Other (Please state)

50. Which ethnic group do you belong to? Tick whichever applies to you (you may check [x] more than one box)

[ ] New Zealand European
[ ] Māori
[ ] Samoan
[ ] Cook Islands Māori
[ ] Tongan
[ ] Niuean
[ ] Chinese
[ ] Indian
[ ] Others, eg DUTCH, JAPANESE, TOKELAUAN.
Please state below.
a) _____
b) _____

51. What is your HIGHEST level of EDUCATION?

[ ] Lower than high school
[ ] High school
[ ] Diploma/certificate
[ ] Bachelor's degree
[ ] Master's degree
[ ] Doctorate or PhD

52. Do you have tertiary education in the following fields? (you can choose more than one answer)

[ ] Medicine
[ ] Nutrition/Dietetics
[ ] Nursing
[ ] Midwifery
[ ] Other health related fields (Please specify)
[ ] Others (Please specify)
[ ] Not applicable

53. What is your current employment status?

[ ] Full time
[ ] Part time
[ ] Volunteer
[ ] Seeking opportunities currently
[ ] Retired
[ ] Other (e.g., caregiver, studying, homemaker). Please describe.

54. What is your marital status?

[ ] Married / cohabiting / civil union / de facto
[ ] Divorced / Separated
[ ] Widowed
[ ] Single
[ ] Other (please describe)

55. How many children have you given birth to? (If female)

[ ] No children
[ ] 1 child
[ ] 2 children
[ ] 3 children
[ ] 4 children
[ ] 5 or more children

Appendix 6: 4-day diet diary

StudyID: _____

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

Health and Vegan Diet

4 Day Food Record

Thank you very much for taking part in this study. We are extremely grateful for your time, effort and commitment

If you have any questions, please contact Rebecca Paul on 022 1294112 (Email: veganstudy@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 the food diary with you when you come in for assessment at Massey University.

StudyID: _____

4 day food diary - what to do?

- Record all of the food that you eat and drink on the following dates.
- **Please complete the diary on consecutive days for 1 weekend day and 3 week days at your convenience. For example, Sunday, Monday, Tuesday and Wednesday <u>OR</u> Wednesday, Thursday, Friday and Saturday.**
- 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.
- You can also save any packets such as muesli bar wrappers and bring them in with your food diary

<u>Describing Food and Drink</u>

- 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	2 Weetbix (Sanitarium) 1 cup So Good unsweetened almond milk 1 tsp Chelsea white sugar
Lunch – Meat Free Bacon Style Rashers sandwich and home-made fries	2 slices of wholegrain bread (Vogels) 2 slices Vegie Delights Meat Free Bacon Style Rashers 25g zenzo Dairy Free Vegan Cheddar Cheese Alternative 2 tsp Tablelands Dairy Free Buttery Spread ½ cup fries (home-made, deep fried in Pam's sunflower oil)

	½ Tbs vegan aioli (Heinz Mayonnaise Vegan Aioli) Water 1 cup to drink
Dinner – Vegan lentils spaghetti bolognese	½ cup lentil sauce (see attached recipe) 1 cup spaghetti pasta (Homebrand)
Snacks	Tam & Luke Snack Ball Salted Caramel (2 balls, 28g) 1 small banana 2 Salada crackers with 1 tsp peanut butter 20g Doritos Spicy Sweet Chili Flavored Tortilla Chips

- Give details of all the **cooking methods** used. For example, fried, grilled, baked, poached, boiled…

General description	**Food record description**
Potatoes	2 medium size potatoes cut in slices and fried in 2tbs canola oil 2 large potatoes with skin (boiled)
Black bean and kumara burger	85g black bean and kumara burger (recipe provided) pan-fried in 2tsp olive oil 85g black bean and kumara burger (recipe provided) oven baked

- When using foods that are cooked (eg. pasta, rice, 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 alternatives	1 cup of cooked lentil sauce or 5 oven baked chicken style strips (Fry's)
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 alternative strips	100g chicken alternative strips (100g includes batter); fried in 3 Tbsp Nuttelex buttery margarine

General description	Food record description
Milo	I x cup Milo made with plant based Milo powder and 150mls So Good unsweetened almond milk, 100 ml hot water. No sugar

- **Record recipes** of home prepared dishes where possible 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 – e.g. a 425g tin of baked beans, a 32g cereal bar.

- 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 alternatives, fruits, vegetables and cheese alternatives.

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

- Using comparisons – e.g. Meat alternative equal to the size of a pack of cards, a scoop of vegan chocolate 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 alternatives	1 heaped tablespoon of grated dairy free cheddar cheese 1 slice dairy free cheddar cheese (8.5 x 2.5 x 2mm)

	1 cube dairy free cheddar cheese, match box size

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

- ***Please try to eat as normally as possible – e.g., Don't adjust what you normally eat just because you are keeping a diet record and be honest! This record will give us important information about your diet, and help us identify any possible deficiencies which we can then help you correct.***

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 weetbix
" "	So good unsweetened almond 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 meat free bacon style rashers (zenzo), 1 Tbsp chopped spring onion, 3 Tbsp vegan mozzarella cheese)
1.00pm	Water	500ml plain tap water
3.00pm	Biscuits	2 x Lotus Biscoff biscuits
6.00pm	Lasagne	½ cup cooked Sunfed Bull free beef meat alternative mince, 1 cup cooked Budget lasagne shaped pasta, ½ cup homemade (recipe attached) vegan bechamel sauce made with soy milk (So Good, regular), ½ cup mixed vegetables (Pam's carrots, peas and corn), 4 Tbsp Veesey grated pizza blend cheese

	1 cube dairy free cheddar cheese, match box size

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

- ***Please try to eat as normally as possible – e.g., Don't adjust what you normally eat just because you are keeping a diet record and be honest! This record will give us important information about your diet, and help us identify any possible deficiencies which we can then help you correct.***

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 weetbix
" "	So good unsweetened almond 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 meat free bacon style rashers (zenzo), 1 Tbsp chopped spring onion, 3 Tbsp vegan mozzarella cheese)
1.00pm	Water	500ml plain tap water
3.00pm	Biscuits	2 x Lotus Biscoff biscuits
6.00pm	Lasagne	½ cup cooked Sunfed Bull free beef meat alternative mince, 1 cup cooked Budget lasagne shaped pasta, ½ cup homemade (recipe attached) vegan bechamel sauce made with soy milk (So Good, regular), ½ cup mixed vegetables (Pam's carrots, peas and corn), 4 Tbsp Veesey grated pizza blend cheese

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6.30pm	Vegan banana cake with chocolate icing (homemade, recipe attached)	1/8 of a cake (22cm diameter, 8 cm high), 2 Tbsp chocolate icing
" "	Tip Top Crave dairy free salted caramel fudge frozen dessert	1/2cup (g) (125g)