A retrospective and cross-sectional study to evaluate the effect of dietary acculturation on the dietary calcium intake among Filipino women recently immigrated to New Zealand

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Abstract

Filipinos in New Zealand have steadily grown in number over recent decades, and the majority undergo a dietary acculturation process, or the dietary adaptation of individuals in their host country. In the Philippines, the nutrient with the highest inadequacy in the diet is calcium, primarily contributed by fish and indigenous vegetables that are not readily available in New Zealand. The aim of this study is to determine the effect of dietary acculturation on the calcium intake of Filipino women recently immigrated to New Zealand and to explore the primary factors affecting their bone mineral status. Sixty-two (62) healthy pre-menopausal Filipino women (20–45 years old) were recruited. Current and previous dietary calcium intake, serum 25(OH)D (nmol/L) (n=61), physical activity data via an accelerometer, and bone mineral density (BMD) and body composition through dual-energy X-ray absorptiometry (DXA) were measured. Gross lean mass was calculated (total mass – [whole body total bone content + total fat mass]). Variables considered to be associated with bone mineral status were applied to a multiple regression analysis using the enter method. The median calcium intake for New Zealand [418 (260, 620) mg d^{-1}] after immigration was significantly lower than the intake in the Philippines [506 (358, 823) mg d^{-1}], Z= -2.41, p=0.02, medium effect size r=0.22. The significant predictor of bone mineral status among Filipino women was gross lean mass, whereas current and previous dietary calcium intake, physical activity and serum 25(OH)D were not found to be significant. However, a high prevalence (69%) of serum 25(OH)D <50nmol/L (mild–moderate deficiency) was detected. These findings illustrate the potential detrimental consequences of dietary acculturation on the essential nutrient intake of immigrants, but also provide an opportunity to correct previous dietary inadequacies by exposure to corresponding nutrient-dense foods from the host country.

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I dedicate this research to all immigrants worldwide. Through all of the difficulties in adjusting to a new culture, to finding safety and security in your new community, and to fighting for equality and a sense of belonging to a new society. May you find all these things in your new home.

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Abbreviations

25(OH)D	25-hydroxyvitamin D
ACC	Accident Compensation Corporation
ANOVA	analysis of variance
ANS	Adult Nutrition Survey
ATP	adenosine triphosphate
BMC	bone mineral content
BMD	bone mineral density
BMI	body mass index
C	Celsius
CT	computed tomography
CV	coefficient of variation
d^{-1}	per day
DMPA	depot-medroxyprogesterone acetate
DXA	dual-energy X-ray absorptiometry
EAR	estimated average requirement
FFQ	food frequency questionnaire
FNRI	Food and Nutrition Research Institute
FHFFQ	Fred Hutchinson Food Frequency Questionnaire
g	gram
g/cm^2	gram per square centimetre
GP	general practitioner
IOM	Institute of Medicine
ID-LC-MS/MS	isotope-dilution liquid chromatography-tandem mass spectrometry
IU	International Unit
IU/day	International Unit per day
kcal/day	kilocalorie per day
kg	kilogram
kj	kilojoule
m	month
MYFCD	Malaysian Food Composition Database
mg	milligram
HNRU	Human Nutrition Research Unit
ml	millilitre
MOH	Ministry of Health
MSc	Master's of Science
n	number
ng/ml	nanogram per millilitre
NIST	National Institute for Standards and Technology
nmol/L	nanomole per litre
NZ	New Zealand
PBM	peak bone mass
PH	Philippines
PIXI	peripheral instantaneous X-ray imaging
PTH	parathyroid hormone
RDI	recommended dietary intake
RENI	recommended energy and nutrient intake
RNI	recommended nutrient intake

SD	standard deviation
NHANES	National Health and Nutrition Examination Survey
US	ultrasound
USDA	United States Department of Agriculture
UV	ultraviolet
UVB	ultraviolet beta radiation
VDR	vitamin D receptor
vs	versus
WHR	waist-to-hip ratio
WHO	World Health Organization
y	year
µg	microgram
µSv	microsieverts

Chapter 1 **Introduction**

Scope and justification

In the recent decades, a steady increase in the number of Filipino immigrants has been observed in New Zealand. This increase of Filipino immigrants working as nurses, domestic workers and dairy farmers in New Zealand brings about certain public health implications (StatisticsNZ, 2013). As Filipinos immigrate, the changes in the environment, cultural exposure and the availability (or the lack) of certain food items create large shifts in dietary patterns. These shifts affect the dietary intake of certain nutrients. Specifically, dietary calcium intake may potentially be affected, since the major sources from the traditional Filipino diet (i.e. small fish and indigenous vegetables) are dramatically reduced.

It could be noted that between the two countries, the primary contributors for dietary calcium intake are the opposite for fish, meat and poultry (34.5% PH vs. 7.4% NZ) and milk products (4.4% PH vs. 40.2% NZ). In terms of the amount of intake, a large discrepancy is also observed, as Filipino adults consume an average of 370 mg of calcium daily, compared to the median daily calcium intake of New Zealanders, which is 919 mg for males and 745 mg for females (Food and Nutrition Research Institute, 2008b; Ministry of Health, 2011).

The available scientific evidence supports that adequate calcium intake is crucial to bone health. Cross-sectional studies, large-scale clinical trials and cohort studies have indicated that low bone mineral density is the primary underlying factor for osteoporotic fractures (Cashman, 2002). Optimal bone mineral density is mainly achieved by adequate calcium intake, especially during the growth and attainment of peak bone mass. Thus, Filipino women, who are already at risk of osteoporosis, can potentially have a higher risk of calcium deficiency, which may lead to the development of bone diseases and increase the incidence of fractures, as they immigrate to New Zealand.

Moreover, bone health is also influenced by several other non-dietary factors. Aside from dietary calcium intake, physical activity, lean muscle mass and vitamin D status have been widely shown to influence bone mineral status among women. These factors were investigated in the research, and will be further discussed in the proceeding chapters of this thesis.

Aims, objectives and hypotheses

The aim of this research is to determine the effect of dietary acculturation on the dietary calcium intake of Filipino women recently immigrated to New Zealand, and to explore the key factors that affect their bone mineral status.

The specific objectives of this research are to:

- compare the current (New Zealand diet) and previous (Philippines diet) dietary calcium intake of recently-immigrated (≤5 years) Filipino women in New Zealand
- investigate predictors of bone health in this population.

Study hypotheses

- That the previous dietary calcium intake of recently-immigrated Filipino women will be greater than their current intake in New Zealand.
- That dietary calcium intake and other lifestyle factors, including physical activity, lean muscle mass and vitamin D status, are predictive factors of bone health in the study population,

Thesis structure

This thesis will consist of the findings of the original research conducted during my Master's degree programme. The first chapter is an introduction, and is followed by a review of the related literature in Chapter 2. The study manuscript is included in Chapter 3, highlighting the main points of the research. Lastly, the final chapter summarises the significant results of the research, the conclusions drawn, and further recommendations for future research.

Researchers' contributions

Research team member	Contributions
Dr Pamela von Hurst, Massey Institute of Food Science and Technology, Auckland Campus	Main supervisor of the research; assisted in the conceptualisation of study design and methodological approaches; assembled the research team; main author of Ethics Committee application; revised and approved final thesis

Professor Marlena Kruger, Massey Institute of Food Science and Technology, Palmerston North Campus	Co-supervisor; consulted on the results, statistical analyses and discussion of the findings; revised and approved final thesis
Professor Barbara Burlingame, Massey Institute of Food Science and Technology, Wellington Campus	Co-supervisor; consulted on the results, statistical analyses and discussion of the findings; revised and approved final thesis
Owen Mugridge, Massey Institute of Food Science and Technology, Auckland Campus	Postgraduate teaching technician; conducted DXA scans and blood extractions; facilitated gathering of physical activity data through accelerometers; ensured logistics of the research via room and facilities bookings; major aid during recruitment of participants
Liana Norrish, Massey Institute of Food Science and Technology, Auckland Campus	Co-researcher/MSc student; helped design questionnaires; assisted in the planning and implementation of research and Ethics Committee approval; input and processing of raw data
Rosario Pillar Monzales, Massey Institute of Food Science and Technology, Auckland Campus	Researcher/MSc student; planned and managed implementation of the research; contributed on the Ethics Committee approval; recruited participants; encoded and analysed data; performed statistical analysis and interpretation of results; recipient of NZ Aid funding from NZ Ministry of Foreign Affairs and Trade

Chapter 2 **Literature review**

Introduction

Literature review structure

This review begins with a brief background on immigration and the dietary acculturation process of Filipino immigrants in New Zealand. Discussing the differences in the sources and adequacy of dietary calcium intake for both countries, several concerns were raised about bone health among Filipino women immigrating to New Zealand. In view of this, the literature on primary factors affecting bone health, such as calcium, vitamin D, other bone-related nutrients, physical activity, body composition, contraceptive use and smoking, were also deliberated.

The Philippines

The Philippines is a Southeast Asian nation with a growing population of approximately 100.9 million (Philippine Statistics Authority, 2016a). With a total area of 300,000 square kilometres (1,830 of which is water), and surrounded by countries such as Indonesia, Malaysia and Vietnam, among others, the Philippines was historically a cultural melting-pot and served as sites of trade and commerce in Southeast Asia (World Atlas, 2015).

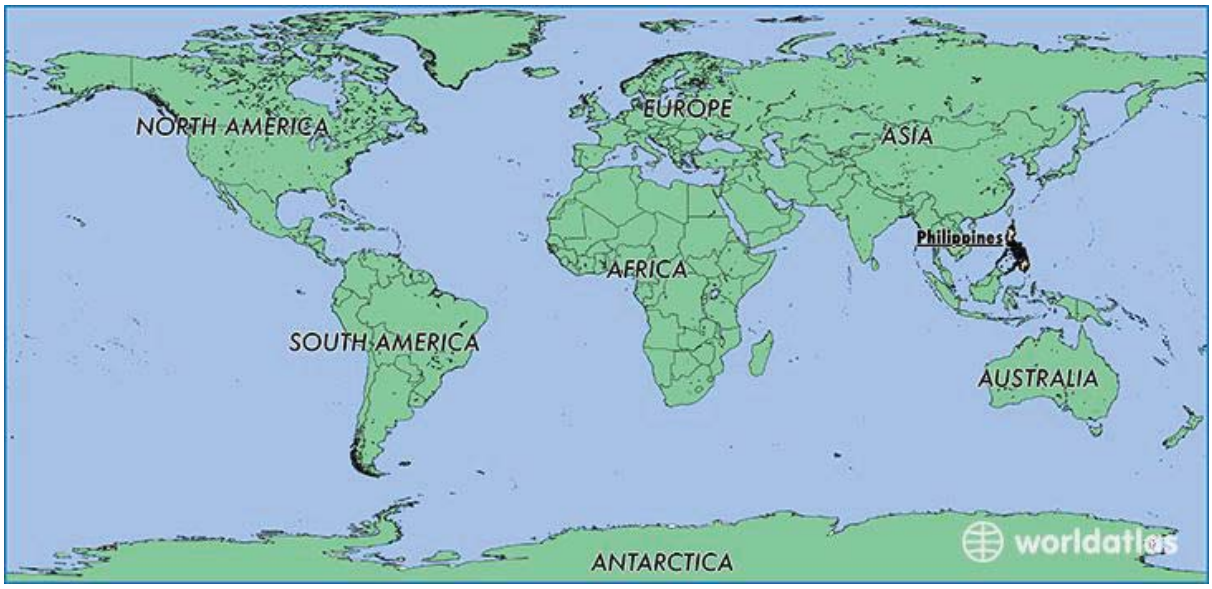

Figure 1. Philippines in the world map (World Atlas, 2015)

Apart from overpopulation, crime and political instabilities, the Philippines' major concern is the high poverty incidence among Filipinos, which was observed at 21.6% in 2015 (Philippine Statistics Authority, 2016b). Poverty in the Philippines is a longstanding problem, as it has been triggering a continuous flow of migration of Filipinos to more developed countries. As per the latest stock estimate, on December 2013, there are 10,238,614 Filipinos, including permanent residents, temporary contract workers and undocumented immigrants, living overseas (Commission on Filipinos Overseas, 2013).

Filipino immigration to New Zealand

In New Zealand, a steady increase in the number of Filipino immigrants has been observed over the past few decades. In the most recent census of ethnic groups in New Zealand, there are currently 40,347 Filipinos (median age = 30.8 years) living in New Zealand, more than half of whom (20,502) reside in Auckland (StatisticsNZ, 2013). This is a steep increase from the previous census of 2006, which reported only 16,938 Filipinos living in the country — a 138.2% increase, making Filipinos the fastest-growing ethnic group in New Zealand. Furthermore, the majority of Filipinos in New Zealand are working in health care and social welfare (23.3%), manufacturing (13.3%), retail (10.5%) and agriculture (5.6%) (StatisticsNZ, 2013).

Dietary acculturation of Filipinos in New Zealand

As Filipinos immigrate, they undergo a phenomenon known as dietary acculturation, which is defined as a 'multi-dimensional, dynamic and complex' adoption process of immigrants with the dietary patterns of the host country (Satia-Abouta, Patterson, Neuhouser, & Elder, 2002). Major differences in food availability, and the cultural and social landscapes between the two countries significantly impact the dietary patterns of immigrants. However, this is not a straightforward process, as several other factors — such as socioeconomic, demographic and cultural factors — affect the rate and extent of dietary acculturation of an individual (see Figure 2).

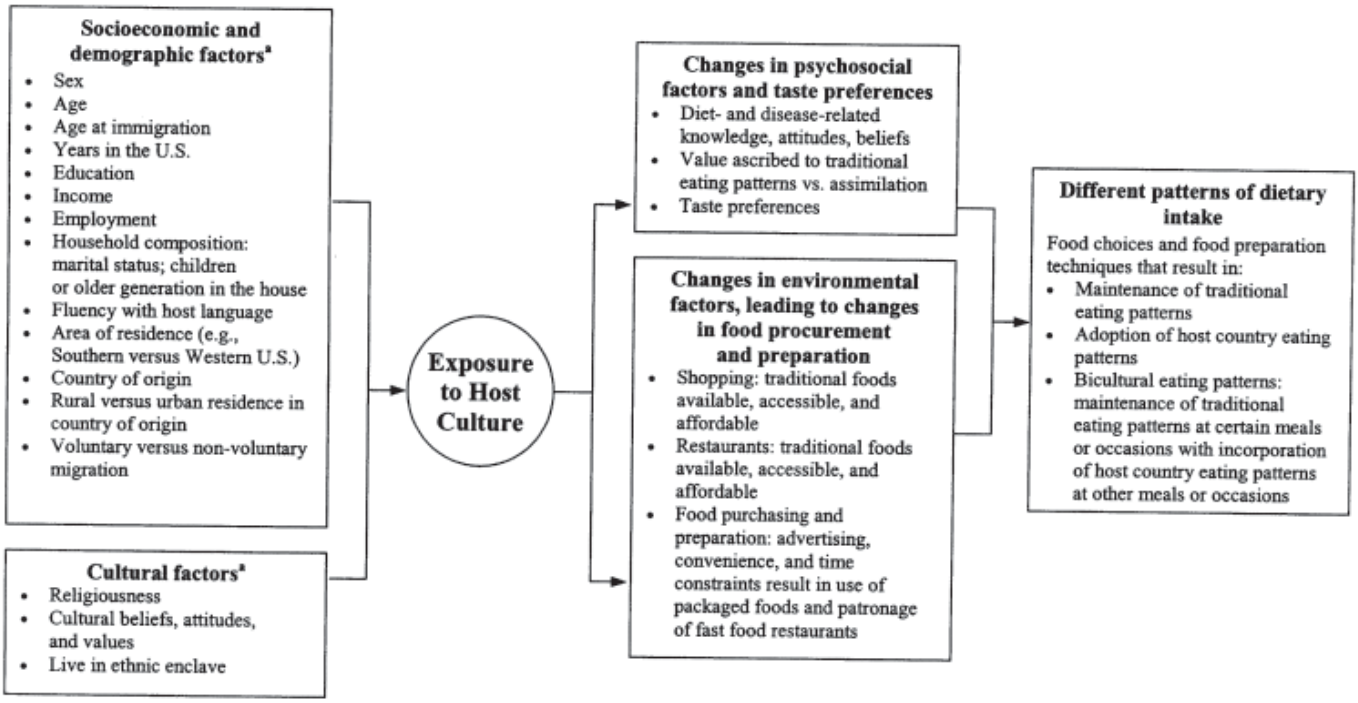

Figure 2. Dietary acculturation process of immigrants in the United States (Satia-Abouta, Patterson, Neuhouser, & Elder, 2002)

Due to its complexity, the change or adoption process of the host country's dietary pattern varies between individuals, depending on the above-mentioned factors. Accordingly, every immigrant's diet is acculturated at different rates and at different times. In a study of African migrant families in Australia, it was observed that parents and their children acculturate at different rates (Renzaho, 2007).

Changes in nutrient intake

Aside from the rate and extent of dietary acculturation among individuals, substantial changes in the nutrient composition of the immigrants' diets are also a major concern. East Asian immigrants (n=63) living in the United States were found to consume more saturated fats and sugar, and less meat and vegetables than they had in their country of origin (Pan, Dixon, Himburg, & Huffman, 1999). Similarly, an increased consumption of simple sugars and a lower intake of dietary fibre was observed among Latinos in the United States (Pérez-Escamilla & Putnik, 2007).

Filipino dietary acculturation studies have been recently emerging from over the past decade. Below is a summary of these studies, primarily conducted in the United States, where there is a huge population of Filipino immigrants.

Table 1. Dietary acculturation studies among Filipinos

Reference (author, year)	Subject group	Country	Type of study	Findings
(Cruz, Lao, & Heinlein, 2013)	First-generation, healthy Filipino Americans (*n*=30), 67% females, 25 years and older	United States (Southern California)	Exploratory descriptive study	Increased fruit, vegetable and fish intakes, but also a significant increase in meat, dairy and simple sugar intakes
(Serafica, Lane, & Ceria-Ulep, 2013)	Filipino immigrants (*n*=128), 77.3% females, 18 years old and above, born in the Philippines (95.3%)	United States (south-eastern part)	Cross-sectional study	High fat and sugar intakes were observed among Filipino Americans who scored high in the Western Dietary Acculturation Scale (path coefficient = 0.623 $p < .05$)
(Johnson-Kozlow et al., 2011)	Filipino American adults (*n*=35), 30–60 years, 60% females	United States (San Diego, California)	Observational (descriptive study)	Retention of basic food consumption pattern (white rice, savoury dish —fish/meat, vegetables, and fruit) for majority of participants
(Kim, Park, Grandinetti, Holck, & Waslien, 2008)	261 Filipinos out of the 1257 participants (Caucasian, Japanese and Hawaiian), 18–95 years, 61% women	United States (Hawaii)	Cross-sectional study	Adherence to traditional diet, such as consumption of rice, fishes and traditional ethnic foods; lower fruit and vegetable intakes compared to Japanese and Caucasian counterparts
(Vargas & Jurado,	First-generation Filipino Americans	Unites States	Cross-sectional	Acculturation with the Western diet indicated positive

2016)	(*n*=210), 63.8% females, 18–74 years	(New Jersey)	study	correlation with calorie (*p*=0.005) and fat intake (*p*=0.025) while a negative correlation was observed with carbohydrate intake (*p*=0.206)

These studies validate the non-linear and complex process of dietary acculturation. Immigrants may continue to consume traditional foods and find new strategies for incorporating them into their diet, or exclude traditional foods and start consuming completely new foods from the host culture (Satia, 2010). Filipino immigrants in the United States may increase or decrease their consumption of certain food items or nutrients, depending on their location and several other internal and environmental factors as mentioned in Figure 2.

Despite the increasing number of Filipino immigrants living in New Zealand, there are currently no dietary acculturation studies of this ethnic group. To further understand the dietary changes that Filipinos undergo in New Zealand, a brief background of the traditional Filipino diet is given below.

The Filipino diet

Dietary calcium intake of Filipino women in the Philippines

Food intake in the Philippines is primarily assessed by a national food survey completed every five years. The National Nutrition Survey, specifically conducted by the government-mandated institute, the Food and Nutrition Research Institute (FNRI), determines energy and nutrient intake from household food consumption surveys of 46,000 households selected through multi-stage stratified sampling of all regions and provinces in the Philippines (Philippine Statistics Authority, 2013).

The average Filipino diet is high in carbohydrates, and is mainly composed of rice as the staple food. It is also low in protein, with a macronutrient distribution of 70.5% carbohydrates, 12.1% protein and 17.3% fat (Food and Nutrition Research Institute, 2008a). In a recent national survey assessing the adequacy of nutrient intakes among individuals, it was indicated that dietary calcium intake had the lowest rate of adequacy (11.5%) in the diet

of the average Filipino household (see Table 2). This adequacy is extremely low compared with other nutrient intakes, such as niacin (89%) and protein (56.7%).

This indicates, therefore, that a large proportion of the population is at risk of the health consequences related to inadequate dietary calcium — especially bone health. Calcium is the main structural component of bone, and is the primary nutrient responsible for bone mass and strength. Apart from its bone-related functions, calcium is also utilised by the body for blood clotting, blood pressure stabilisation (Bristow, Gamble, Stewart, Horne, & Reid, 2015), relaxation and contraction of heart chambers (Catterall, 2011), cell signalling (Simms & Zamponi, 2014), activation of certain enzymes required for metabolic reactions (Patergnani et al., 2011), regulation of synaptic function (Dittrich et al., 2013) and muscular contraction (Huang et al., 2012).

Table 2. Recommended intake for women and the mean nutrient intake and adequacy of Filipino households

Nutrient	RENI[β]	Mean intake[μ]	Proportion of households meeting RENI (%)[α]
Energy (kj)	7782	7817	33.1[π]
Protein (g)	58	57.1	56.7[Ω]
Iron (mg)	27	9.7	13.5 [Ω]
Calcium (mg)	750	420	11.5 [Ω]
Retinol Eq. (μg)	500	451.6	21.5 [Ω]
Thiamin (mg)	1.1	0.85	34.5 [Ω]
Riboflavin (mg)	1.1	0.73	19.7 [Ω]
Niacin (mg)	14	21.3	89.0 [Ω]
Ascorbic acid (mg)	70	47.1	30.2 [Ω]

[α]Source: *7th National Nutrition Survey: Food Consumption and Nutrient Intake of Filipino Households* (Food and Nutrition Research Institute, 2008a)
[β]RENI (recommended energy and nutrient intake) values are based on 19- to 29-year-old female, weighing 51 kg
[μ] Values are mean (CVs for mean intake are all <15%)
[π] Meets 100% of RENI
[Ω] Meets 80% of RENI

Recommended energy and nutrient intakes (RENI) indicated in Table 2 is a dietary tool utilised to determine the adequacy of Filipino diets to maintain good health. It was obtained

using the following steps: (a) determination of the average requirement of a representative of a population, which is also described as an intake level which meets a specific adequacy criteria; and (b) evaluation of individual variation within the group (Barba & Cabrera, 2008). For step (b), if the distribution of the requirement values is unknown, the average plus two standard deviations (SD) were utilised to cover 97.5% of the population. Compared to other Asian countries, the Philippines has established a relatively low recommended intake for calcium (750 mg d^{-1}). The recommended daily calcium intake for Indonesian women is 800 mg d^{-1} (pre-pregnancy) and 950 mg d^{-1} (during pregnancy), while Malaysian women (19–50 years old) have a recommended nutrient intake (RNI) for calcium also set at 800 mg d^{-1} (Madanijah et al., 2016; Ministry of Health [Malaysia], 2005).

High prevalence of osteoporosis among Filipinos in the Philippines

This inadequate dietary calcium intake is demonstrated in several studies that indicate the pervasiveness of bone diseases among Filipinos. Several studies have observed the high prevalence and increased risk among Filipino women of developing osteoporosis and suffering hip fractures (M. M. M. Cruz, J. Sevilleja, M. E. L. Macalalag, A. E. Habana, & E. V. Macalalag, 2004; Kruger et al., 2013). National data, gathered through multi-stage cluster sampling of 2,850 adults over 50 years, indicated a high prevalence of fractures among females (11.3%) and males (9.0%) (Li-Yu et al., 2014).

Moreover, a recent study aimed to set baseline bone mineral density (BMD) data among postmenopausal Filipino women observed 21.2% were osteoporotic and 50.8% were osteopenic, from a cohort of 118 participants (Kruger et al., 2009). Another preliminary study, investigating the prevalence of bone disease among 285 adult (19- to 87-year-old) Filipino women through Lunar PIXI (peripheral instantaneous X-ray imaging) DXA analysis of the right heel, reported a prevalence of 40.7% of osteopenia among women below 40 years (M. M. M. Cruz, J. E. A. D. Sevilleja, M. E. L. Macalalag, A. E. Habana, & E. V. Macalalag, 2004)

A similar pattern was also observed among Filipinos overseas, as a study found discrepancies between the BMD of Filipino women and those of other ethnicities living in Hawaii. In this study which compared the bone mass of four ethnicities (Japanese, Hawaiian, Caucasian and

Filipino), lower BMD was observed among Filipino pre-menopausal women compared to their Caucasian and Hawaiian counterparts (Davis, Ross, Wasnich, & Novotny, 1994).

Primary contributors of calcium in the diet

The average calcium intake of a Filipino adult is 370 mg per day, which only covers 62% of the Philippine estimated average requirement (EAR = 80% of the RENI) (Food and Nutrition Research Institute, 2008b). The RENI for calcium intake for a 19- to 49-year-old Filipino woman is 750 mg daily intake whereas for a 19- to 50-year-old New Zealand woman, the recommended dietary intake (RDI) is set to 1000 mg per day. Moreover, the EAR for Filipino women (19- to 49-year-olds) is set to 600 mg daily, whereas New Zealand's EAR for the 19- to 50-year-old woman is 840 mg (Barba & Cabrera, 2008; National Health and Medical Research Council, Australian Government, Department of Health and Ageing, & New Zealand Ministry of Health, 2006).

Table 3 illustrates the food contributors of calcium in the Filipino diet per population group.

Table 3. Contributing food groups to calcium intake by population groups

Population group	Calcium intake (mg)	Contribution (%)				
		Cereals and products	Fish, meat and poultry	Milk products	Vegetables	Beverages and others
Children, 6m–5y	330	17.7	10.9	56.2	3.0	12.2
Children 6–12y	260	36.3	28.2	8.7	9.6	17.2
Adolescents	330	37.1	32.4	3.8	12.4	14.3
Adults	370	32.0	34.5	4.4	15.0	14.1
Elderly	330	27.5	28.9	12.9	17.2	13.5
Pregnant women	390	28.6	25.5	16.8	14.8	14.3
Lactating women	370	32.7	31.1	6.3	17.0	12.9

Source: *7th National Nutrition Survey: Individual Food and Nutrient Intakes- Food and Nutrition Research Institute* (Food and Nutrition Research Institute, 2008b)
Abbreviations: m = months; y = years

Among adults, the fish, meat and poultry category (34.5%) is the highest source of calcium in the Filipino diet. However, out of these three primary contributors, the majority of calcium comes from the fish, particularly the bones of small fish that are consumed by the locals. In the same national survey in Table 3, fish had a higher contribution (14.7%) to the mean of total daily food intake compared with the 10.9% contribution of all the other meats combined, such as pork, chicken and beef (Food and Nutrition Research Institute, 2008b). Likewise, a study investigating the sources of calcium among women in Davao, Philippines, found that indigenous small fish and plant foods were the primary sources of their dietary calcium (Miura et al., 2009).

Moreover, milk and dairy products contribute the least to the daily calcium intake of Filipinos out of all the food groups (only 4.4% of calcium coming from dairy). Previously, the milk of the carabao (scientific name: *Bubalus bubalis carabanesis*), a water buffalo native to the Philippines, and goat milk were common sources of calcium in rural areas of the Philippines, but these were gradually replaced by imported milk with the advent of the globalisation of cow's milk production (Miura, Yagi, Saavedra, & Yamamoto, 2010). The increase in availability, via export from dairy-producing countries, was initially observed in the 1960s among Asian countries such as China, Thailand and the Philippines, with dairy consumption in the latter increasing up to four times (Wiley, 2007). However, the high cost of milk and dairy products makes them inaccessible to a large percentage of the Filipino population, due to poverty. The effect of these changes can be observed in the decrease of an adequate calcium intake among adults, from 16.0% achieving the RENI in 2003, to 11.5% in 2008 (Food and Nutrition Research Institute, 2003, 2008b).

Dietary acculturation of Filipinos in New Zealand

Major calcium sources in the New Zealand diet

New Zealand is a major producer and consumer of cow's milk. In the recent Adult Nutrition Survey (ANS 2008/09), milk was indicated to provide the highest contribution of calcium to the diet (27%), followed by bread (10%), non-alcoholic beverages (10%), cheese (8%), vegetables (6%), dairy products (6%) and bread-based dishes (5%) (Ministry of Health [New Zealand], 2011).

Table 4 compares the major food groups that primarily contribute to the calcium intake of New Zealanders and Filipinos:

Table 4. Comparison of calcium intake per food group for Filipino and New Zealand adults

Food group	Filipino (%)[α, β]	New Zealand (%)[μ, π]
Fish, meat and poultry	34.5	7.4
Milk and dairy products	4.4	40.2
Cereals and products	32.0	29.3
Vegetables	15.0	5.7
Beverages and others	14.1	17.6

[α] *7th National Nutrition Survey: Individual Food and Nutrient Intakes — Food and Nutrition Research Institute* (Food and Nutrition Research Institute, 2008b)
[β] Values based on Filipino adults
[μ] Ministry of Health, (2011) *A Focus on Nutrition: Key findings from the 2008/09 NZ Adult Nutrition Survey (Nutrient Intakes and Dietary Sources: Micronutrients)*
[π] Values based on 31- to 50-year-old male adults

It can be noted from Table 4 that the percentage of calcium coming from cereals and products is comparable between the Philippines and New Zealand. However, the proportion of intake is opposite for fish, meat and poultry (34.5% PH vs. 7.4% NZ) and milk products (4.4% PH vs. 40.2% NZ).

In terms of adequacy, the New Zealand population has an estimated prevalence of inadequate dietary calcium intake of 59%, which indicates the percentage of population that is unable to meet the EAR (Ministry of Health, 2011). Despite the high prevalence, this is still considerably lower than the reported prevalence of inadequate calcium intake among Filipinos, which is 88.5% (as it was indicated in Table 2 that only 11.5% of Filipino households met the RENI for calcium) (Food and Nutrition Research Institute, 2008a). Also, compared with adults in the Philippines, New Zealand adults have a considerably higher consumption, with a median daily calcium intake of 745 mg for females and 919 mg for males, compared with the previously reported average daily calcium intake of Filipino adults of 370 mg per day (Food and Nutrition Research Institute, 2008b; Ministry of Health, 2011).

These large differences in the sources and the adequacy of calcium intake of the two countries prompted this investigation to determine how the acculturation process affects the dietary calcium intake of Filipino immigrants in New Zealand.

Factors affecting bone health

A. Dietary factors

Calcium

Roles and sources of calcium

Calcium is one of the most well-known nutrients associated with bone health. It is mainly found in milk, cheese and dairy products, but for some developing countries it is mainly supplied through indigenous small fish (Larsen, Thilsted, Kongsbak, & Hansen, 2000). The majority of calcium in the body (approximately 99%) is found in the bone as a fundamental structural component, in the form of hydroxyapatite (Flynn, 2003).

During growth, if dietary calcium intake is inadequate, bone resorption increases, which results to a negative balance between bone formation and resorption (Monsen, Rock, & Coulston, 2001). Calcium also plays an integral role in the maintenance of bone health throughout the different stages of the life cycle. A plethora of studies demonstrates that adequate calcium intake enhances bone health during pregnancy and lactation, and growth in young and middle adulthood (Ilich & Kerstetter, 2000). Vital functions of calcium include control of transporting ions across cell membranes, acting as an intracellular secondary messenger, activating blood coagulation factors and excitation of neuro-muscular junctions, and provision of rigidity and strength to bones and teeth (Marshall, 2012).

Clinical trials and prospective studies have demonstrated the significant protective effects of adequate dietary calcium in preventing fractures and bone diseases such as osteoporosis and osteopenia (Macdonald, New, Golden, Campbell, & Reid, 2004; Ponce, Fajardo, Zeni, & de Portela, 2005; Shivani Sahni et al., 2010; Vatanparast, Bailey, Baxter-Jones, & Whiting, 2007).

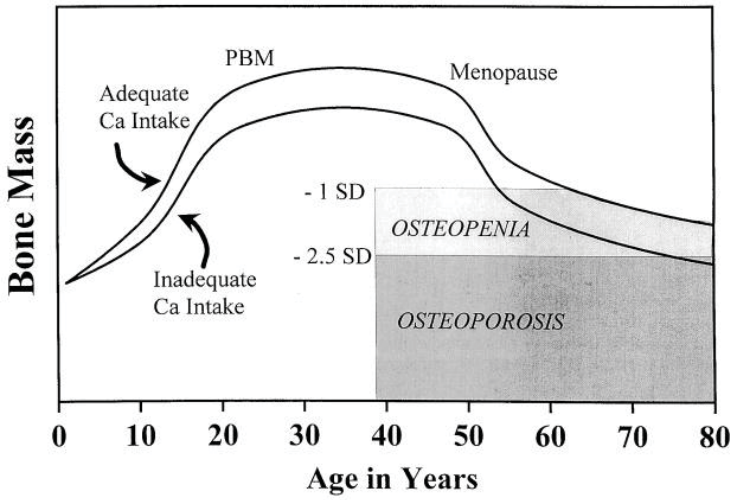

Figure 3. Change in bone mass of women with age (Ilich & Kerstetter, 2000)

Abbreviations: Ca = calcium; PBM = peak bone mass; SD = standard deviation

Figure 3 illustrates the substantial impact of inadequate dietary calcium intake on the risk of developing osteopenia and osteoporosis in later life. With inadequate calcium intake, lower peak bone mass (PBM) is achieved. (PBM is further explained in the section on concerns about the bone health of Filipino immigrants.) Thus, there is a higher risk of developing bone diseases, especially during the postmenopausal period. During this period, oestrogen hormone production is dramatically decreased. Oestrogen is a sex hormone which is widely known to have protective effects against bone loss. Oestrogen therapy is considered as one of the most effective treatments for osteoporosis prevention among postmenopausal women, due to its role in normalising the bone-remodelling process (Rossini et al., 2013).

Vitamin D

Role and metabolism of vitamin D

Vitamin D is a pro-hormone that plays an essential role in the regulation of the levels of calcium and phosphorus in the body, and in the mineralisation of bone (Wu-Wong, 2012). Sun exposure is the principal source of vitamin D among humans (Pearce & Cheetham, 2010). Through skin exposure to ultraviolet B radiation from the sun, 7-dehydrocholesterol is converted to pre-vitamin D_3 (Anderson & Garner, 2011).

The body's heat assists the conversion of pre-vitamin D_3 to vitamin D_3. This process, however, may occur over several days, and is dependent on the body's temperature (Feldman, Pike, & Glorieux, 2005). For vitamin D_3 or calciol to be made into its usable form, it undergoes two hydroxylation reactions in the liver and the kidneys, respectively (Whitney & Rolfes, 2015). As illustrated in Figure 4, calcitriol (1, 25-dihydroxyvitamin D_3) can originate from either sun exposure or through a dietary source. It then goes to its target organs, activating the vitamin D receptor (VDR) for uptake, so that it can perform its functions to maintain bone and metabolic health. VDR is a key receptor which is highly selective, and is only activated by the free form of calcitriol; it can be found in almost every tissue in the body (Ying et al., 2015).

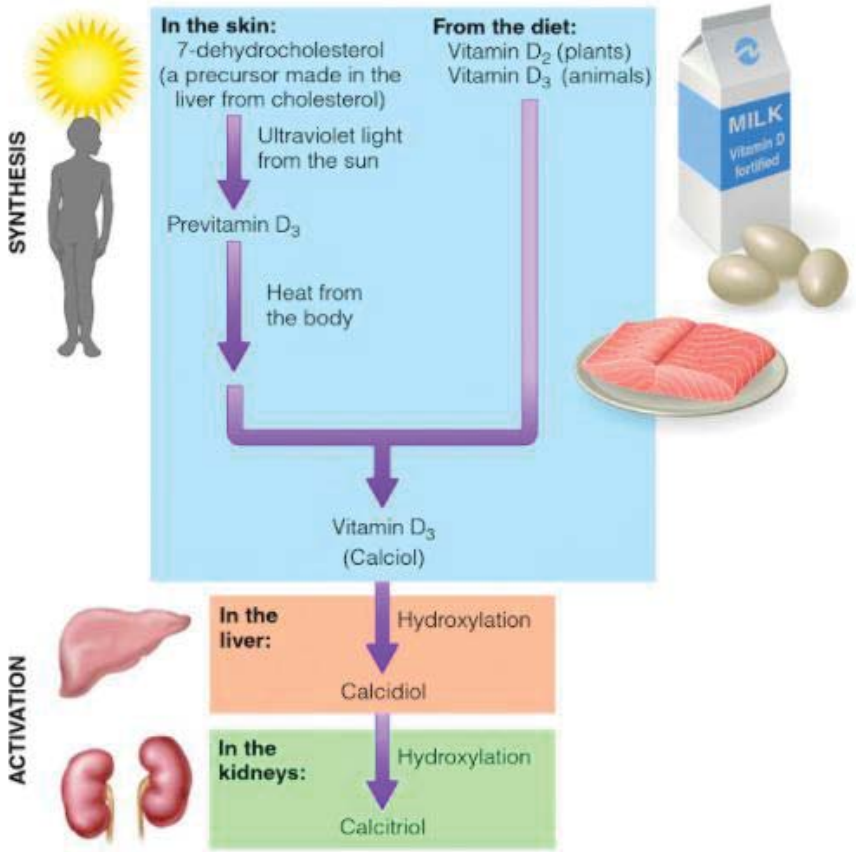

Figure 4. Vitamin D metabolism (synthesis and activation) (Whitney & Rolfes, 2015)

Vitamin D affects bone metabolism primarily in three different ways: (1) promoting adequate calcium and phosphorus absorption in the small intestines, thereby preserving calcium in the bones; (2) increasing the number of osteoclasts, by inducing cellular differentiation of

preosteoclasts into mature cells; and (3) stimulating the synthesis of osteocalcin, osteopontin and other bone matrix proteins (Holick, 2010).

Vitamin D also assists in the maintenance of bone health by working with other hormones. Parathyroid hormone (PTH), together with fibroblast growth factor 23 (FGF23) and 1,25(OH)2D, mainly regulates calcitriol synthesis in the kidneys (Bikle, 2014). The increase in calcitriol synthesis translates to an increase in blood calcium, through its action of inducing the intestines to be more efficient in absorbing dietary calcium. However, a negative feedback mechanism is activated once there is an adequate amount of calcium in the blood. PTH secretion is supressed once a normal serum calcium is achieved, upon detection by the calcium receptors in the parathyroid gland, as well as through the direct action of calcitriol in the same gland (Holick, 2010).

A meta-analysis of eight double-blind randomised controlled trials that investigated the efficacy of vitamin D supplementation in preventing fractures by reducing incidence of falls among older people indicated that the oral administration of vitamin D, ranging from 700 to 1000 IU/day, can reduce the risk of falls by 19% (Bischoff-Ferrari et al., 2009a). Similar findings were observed in another study, wherein the supplementation of vitamin D decreased the risk of developing hip and non-vertebral fractures among postmenopausal and osteoporotic women (Chapuy et al., 1992; Neer et al., 2001). Furthermore, positive association was found with vitamin D deficiency and the incidence of juvenile idiopathic osteoporosis and osteopenia among children (Bowden, Robinson, Carr, & Mahan, 2008). These studies demonstrate the importance of adequate vitamin D levels in the maintenance of bone health.

Dietary sources of vitamin D

Food, supplements and fortified foods

Vitamin D is mostly present in fatty fish, cod liver oil, egg yolk, meat, liver and wild mushrooms (Pearce & Cheetham, 2010). In New Zealand, foods that may be fortified with vitamin D include margarine and spreads, dairy products, plant-based dairy substitutes and liquid meal replacements (Ministry of Health, 2016). In the Philippines, vitamin D fortification is also not mandatory, but voluntary fortification may be done with its complementary health claim (Tulchinsky, 2015).

Table 5 specifies the amount of vitamin D obtained from dietary sources, and compares it with the quantity of vitamin D obtained from sun exposure. As reported in this table, sun exposure provides a significant amount of vitamin D compared with the primary dietary sources of vitamin D. The Institute of Medicine (IOM) recommends an intake of 400 IU/day for both men and women aged 19–30 years, and 600 IU/day for 30- to 50-year-olds of both sexes, assuming a minimum or low sun exposure (Institute of Medicine, 2011). Thus, supplementation may benefit some individuals who have minimal sun exposure or who have specific risk factors for low vitamin D status (which will be discussed further later on in this chapter).

Table 5. Comparison of dietary and non-dietary sources of vitamin D

Source	Amount	Vitamin D content (IU)[€]	Reference
Sun exposure	Short and regular exposure	10000[α]	(Feldman et al., 2005)
Cod liver oil[β]	1 Tbsp	1360	(Mahan, Raymond, & Escott-Stump, 2013)
Fatty fish (i.e. salmon)	100 g	400	(Lu et al., 2007)
Meat, liver[π]	1 kg	344	(Montgomery et al., 2000)
Meat, kidney[π]	1 kg	296	
Meat, top round steak[π]	1 kg	112	
Human breast milk[£]		5–136	(Holick, 2010)

[α] Obtained by adults during full skin surface exposure to the sun
[β]Cannell et al. (2008) claims a decline in the vitamin D content of modern cod liver oil, and cited a manufacturer marketing cod liver oil which contains only 3–60 IU of vitamin D per tablespoon
[π]Values from Continental–British crossbred cows (around 23 months of age); vitamin D content manually converted from µg/kg to IU
[£]Obtained from women consuming oral vitamin D between 600–700 IU/day; colostrum mean SD (15.9±8.6 IU/L)
[€] IU = International Units

Measurement of vitamin D

Compared to serum 1, $25(OH)_2D_3$ (the active form of vitamin D), serum 25(OH)D is more commonly used as a routine marker of vitamin D status. This is since serum 1, 25 $(OH)_2D_3$ is unstable and tends to decompose easily, and its levels do not necessarily reflect vitamin D intake due to other factors which affect its circulating levels (i.e. PTH) (Stolzt, 2006). Serum 25(OH)D is considered to be the best available measurement to reflect the contributions from

dietary vitamin D intake and the amount of vitamin D absorbed from the sun (Institute of Medicine, 2011).

Furthermore, levels of circulating serum 25(OH)D is interpreted through varying definitions. The Institute of Medicine (2011) defines vitamin D deficiency as 0–50 nmol/L serum 25(OH)D, vitamin D sufficiency as more than 50 nmol/L, and 250 nmol/L or more for vitamin D toxicity. On the other hand, three professional societies in Australia and New Zealand have presented a broader categorisation of vitamin D levels in their position statement, indicating: (1) <12.5 nmol/L as severe deficiency; (2) 12.5–29 nmol/L as moderate deficiency; (3) 30–49 as mild deficiency; and (5) 50 nmol/L or greater as sufficient vitamin D levels during the end-of-winter season (Nowson et al., 2012).

Factors affecting vitamin D status

The main factors that influence vitamin D status are sun exposure, adiposity and age. As previously mentioned, the majority of vitamin D in the body comes from the conversion of pre-vitamin D during sun exposure, hence the term 'sunshine vitamin'. Sun exposure is affected by several variables, such as latitude, season, level of air pollution, clothes, utilisation of sunscreen, and melanin distribution in the skin (Tsiaras & Weinstock, 2011).

The latitude is an area's location from the equator, with values ranging from 0° to 90° from the equator to the poles. Ultraviolet (UV) exposure is considered to be higher as the latitude moves closer to the equator, but this effect may be confounded by other factors, such as the height of the sun, cloud cover, altitude, ozone and ground reflection (World Health Organization, 2017). In a global meta-regression analysis of 394 studies, it was reported that there was no observed general trend on the 25(OH)D levels based on the latitude of the samples. However, in the same study, a separate analysis indicated a substantial decrease of vitamin D with an increase of latitude for Caucasians ($p = 0.02$), but was not observed for non-Caucasians ($p = 0.14$) (Hagenau et al., 2008). In New Zealand, the serum 25(OH)D levels of both men and women residing in the North Island is significantly higher than the levels of those from the South Island ($P<0.01$), thus indicating the potential effect of latitude (Rockell, Skeaff, Williams, & Green, 2006). These differences may have emerged due to the varying UV exposure of the islands, as the North Island sits at 34°S while the South Island is approximately located at 47°S (Walrond, 2005).

In the same study by Rockell et.al (2006), one of the major determinants of vitamin D status observed among New Zealanders was season, due to the differences between the 25(OH)D concentrations obtained during summer and spring, which ranged from 28–31 nmol/L for both men and women. With the change in the amount of UV rays coming in during seasons with low sun exposure, such as winter, there is a decreased cutaneous activation of vitamin D in the skin surface.

Another environmental factor which can affect vitamin D status is the level of air pollution in the atmosphere. A cross-sectional study on children of 9–24 months of age living in Delhi, India, demonstrated a 14.7 ng/ml difference in the mean serum 25(OH)D of children living in Mori gate (more polluted) and Gurgaon (less polluted) areas ($p<0.001$) (Agarwal et al., 2002). Areas with high levels of air pollution can substantially decrease the UV ground levels (Hosseinpanah et al., 2010). Therefore, this translates to a decline in the conversion of cutaneous pre-vitamin D into its usable form.

Furthermore, the Institute of Medicine (2011) cites the melanin distribution in the skin as a confounding factor which affects serum 25(OH)D concentration. Melanin is a coloured (black, brown, yellowish or reddish) skin pigment which is involved in the absorption of optical radiation through the epidermis (Borovansky & Riley, 2011). Higher skin pigmentation is inversely correlated with amount of sunlight absorbed to make vitamin D_3 (Holick, 2004).

Cultural factors — such as the wearing of traditional clothes which cover most of the skin, or the use of sunscreen — also affect the amount of UV radiation absorbed by the skin. A study of Arab women in the United States demonstrated severe vitamin D deficiency among the veiled and un-supplemented participants, with only 4 ng/mL serum 25(OH)D concentration in the blood (Hobbs et al., 2009). Similar findings were observed among Middle Eastern women in New Zealand, as the reported baseline median serum 25(OH)D concentration for all of the participants ($n=62$) in a supplementation study was 43.8 nmol/L (Mazahery, Stonehouse, & von Hurst, 2015). Moreover, a study among East Asian women in Australia indicated that higher sun-protection behaviour is associated with vitamin D deficiency (Brock et al., 2013). According to the researchers, the use of sunscreen among these participants reflects the traditional Asian culture which gives more value to fairer skin. Both of these cultural practices and behaviours lead to a marked decrease in the amount of UV absorbed in

the skin to help generate more active vitamin D to perform metabolic and bone health functions.

Body fat percentage also affects serum 25(OH)D concentration in individuals. This concept is further discussed below (under 'Low vitamin D status risks specific to Filipino women'). Moreover, the high prevalence of vitamin D deficiency is observed among older adults globally. Several studies indicate the association of the ageing process with vitamin D deficiency. Possible causes in the said association include: (1) decreased levels of 7-dehydrocholesterol in the skin, which decreases the efficacy of cholecalciferol synthesis upon sun exposure; (2) a decline in sun exposure due to immobility and isolation; and (3) increased adiposity during ageing (Oudshoom, van der Cammen, McMurdo, van Leeuwen, & Colin, 2009).

Other dietary factors affecting bone health

Protein

Protein is a vital macronutrient for the maintenance of bone health, through the production of hormones and growth factors that regulate bone cell formation, and for bone mineralisation (Palacios, 2006). The balance in dietary protein intake is an essential condition for optimal bone health. Excessive protein intake is associated with a negative calcium balance due to its effect on calcium excretion, whereas low protein intake is associated with an increased risk of fractures (Hannan et al., 2000). Low protein intake may adversely affect bone mineral metabolism through the disruption of calcium homeostasis. In a randomised controlled trial investigating the short-term effect of a restricted protein diet on mineral metabolism and parathyroid hormone (PTH) levels, it was found that the low protein diet significantly increased PTH levels and produced an adverse effect on calcium absorption (Kerstetter, O'Brien, & Insogna, 2003).

Phosphorus

Phosphorus is a mineral that is the second most abundant trace element in the body, secondary to calcium. Its primary function is for the proper mineralisation of bones. Low

dietary phosphorus is not a major concern for bones, as its association with the risk of developing of osteoporosis is not supported by robust scientific evidence (Palacios, 2006).

Instead, a high level of circulating phosphorus has been considered as a concern for bone health. Excessive dietary phosphorus results in an increase in serum phosphorus levels, which can lead to the decline in calcium concentration and an increase in PTH secretion and further bone resorption (Ilich & Kerstetter, 2000). According to the Institute of Medicine (2011), a low concentration of serum phosphorus promotes calcitriol synthesis, whereas high concentrations of phosphorus in the blood tend to decrease vitamin D production. A trial involving young women demonstrated that a high-phosphorus and low-calcium diet increased the PTH concentration among the young adults after four weeks of treatment (Palacios, 2006). A recent cross-sectional survey, conducted among 4935 Korean adults, found that a higher dietary calcium-to-phosphorus ratio intake is associated with higher bone mineral density for both men and women (Lee, Kim, Kim, Seo, & Song, 2014).

Magnesium

Compared to phosphorus and calcium, two-thirds of magnesium in the body is found in bones, with the remaining proportion found in soft tissues. Magnesium affects bone quality directly through its action in (1) minimising the hydroxyapatite crystalline size, thus inhibiting the formation of bigger crystals that lead to a more brittle bone, and (2) stabilising calcium phosphate and decreasing the rate of its conversion to hydroxyapatite (Orchard et al., 2014). It also affects bone metabolism indirectly due to its role in adenosine triphosphate (ATP) metabolism, and as a cofactor for a large number of enzymes utilised for bone metabolism (Palacios, 2006).

Animal and human supplementation studies have demonstrated that increased magnesium intake is associated with higher bone mineral density, and suppression of bone turnover (Aydın et al., 2010; Bae et al., 2011; Ryder et al., 2005). However, a large study among postmenopausal women (89,717 participants) found that participants with the highest magnesium intake had the highest risk for wrist fracture, and did not exhibit a protective effect on other forms of fractures (Ott, 2004). Hence, the evidence showing the association between magnesium and the risk of fractures remains inconclusive.

Vitamin C

Vitamin C's significance in bone metabolism is due to its role in collagen production and normal bone development (Sahni et al., 2009). In a study involving three-month-old female New Zealand White rabbits, it was demonstrated that vitamin C, in combination with selenium and vitamin E, has a positive effect on heparin-induced osteoporosis by restoring the structural damages in bone (Turan, Can, & Delilbasi, 2003). Moreover, a large prospective study (*n*=994) demonstrated that vitamin C supplementation has a protective effect against osteoporosis (radius, femoral neck and total hip) among postmenopausal women (Morton, Barrett-Connor, & Schneider, 2001).

Despite the presence of epidemiological studies indicating the association of vitamin C intake with an increase in BMD, human intervention trials (randomised controlled trials) are still required to validate these findings (Finck, Hart, Jennings, & Welch, 2014; Weber, 1999).

B. Lifestyle factors affecting bone health

Physical activity

A positive association between physical activity and the density, structure and quality of bones has been demonstrated by several experimental studies. High-performance activities were found to increase bone mineral content and density in the areas being subjected to tension (Vicente-Rodríguez et al., 2008). This was suggested as being due to the body's physiological responses in dealing with high mechanical demands, which include (1) higher calcium intestinal absorption, (2) lower urinary calcium excretion, and (3) a decline in PTH production (Anderson & Garner, 2011).

Additionally, physical activity is a key factor in stimulating the bone-remodelling process that helps maintain bone health. In bone remodelling, the mechanical load of physical activity initiates a response that leads to the repair of microfractures, and modifies bone structure through osteoclastic and osteoblastic activities, resulting in the bone activation, bone resorption and bone formation cycle (Hadjidakis & Androulakis, 2006). According to a review paper, physical activity, through biomechanical loading, stimulates bone cell activity, which results in an improvement of bone strength and prevents bone loss due to ageing (Robling, Castillo, & Turner, 2006).

A cross-sectional analysis of the NHANES (National Health and Nutrition Examination Survey) data demonstrated that screen-time (screen-based passive behaviours) was inversely associated with bone mineral content (Chastin, Mandrichenko, & Skelton, 2014). In animal studies, regular physical activity was found to increase alkaline phosphatase activity levels, and induce the formation of osteoblastic bone activity among Agouti rats (n=45) (Holy & Zerath, 2000). In this clinical trial, the rats that were assigned to the trained group had a strong positive correlation between the tibial bone volume (r=0.40, p<0.05) but no significant correlation among rats in the sedentary group animals (r=0.17, p=0.42).

Studies involving astronauts and cosmonauts indicate the negative impact of physical inactivity to bone density. A study on Russian cosmonauts (n=15) illustrated a significant mean bone loss during the first month (-1.7%) and second month (-1.9%) in space (Vico et al., 2000). As these cosmonauts are subjected to microgravity and weightlessness, the mechanical bearing on their joints is dramatically decreased, which results to decline in BMD. The diminution of bone density is persistent during these conditions, as both long-term and short-term explorations indicate decreased BMD measurements among astronauts and cosmonauts (Sibonga et al., 2007).

Body composition

Fat-free mass and percentage body fat

Several studies demonstrate the positive correlation of lean mass and bone density (Douchi et al., 1998; Okano et al., in press; Reid, Plank, & Evans, 1992). A recent study among 197 peri-menopausal women which investigated the link between several variables (physical fitness, body composition, metabolic markers, and consumption of a Mediterranean diet) and bone density, reported lean mass as the only variable to have an independent association with BMD (p<0.001) (Aparicio et al., 2016).

A study of Pacific Island premenopausal women in New Zealand reported that lean mass is positively correlated, whereas body fat percentage is negatively correlated to total body BMD. The authors cited the potential mechanism that having a greater mass will provide a greater mechanical loading on the skeleton which prompts the osteocytes to signal an increase in osteoblast activity or decrease in osteoclast activity (Casale et al., 2016).

Quite the reverse, a study on 921 ethnically-diverse premenopausal women in California indicated a positive association of both fat mass and lean mass with BMD. Both fat mass and lean mass were positively correlated with bone density in all of the skeletal sites measured (spine, femoral neck and whole body), with lean mass being identified as having a stronger independent association than that of fat mass (Wang et al., 2005). However, this study reported only an estimated measure of bone density for the spine and the femoral neck, called bone mineral apparent density (BMAD, g/cm^3) to derive an approximation for the volumetric bone mineral density.

Contraceptive use

Contraceptive use, particularly depot-medroxyprogesterone acetate (DMPA) is associated with reduced BMD among adolescent and pre-menopausal women (D. Scholes, LaCroix, Ichikawa, Barlow, & Ott, 2005). A population-based prospective cohort study among 457 women in America found that DMPA users had significantly decreased BMD levels ($p<0.01$) at the spine, femoral neck and greater trochanter areas compared with non-users, after multivariate adjustment for other risk factors related to BMD (Delia Scholes, Lacroix, Ott, Ichikawa, & Barlow, 1999).

Most of the studies cited above include the short-term use of DMPA. In a cross-sectional study of 185 long-term users of DMPA, it was reported that 153 users had low serum oestradiol levels and a decreased *Z*-score of lumbar spine mean BMD compared with the population mean (Gbolade, Ellis, Murby, Randall, & Kirkman, 1998). Nevertheless, the authors argued that there is no clinically significant adverse effect on BMD for the long-term users, and that bone-conserving treatments were not further required.

A controlled trial investigating the effect of DPMA on BMD among 155 women indicated an annual 2.74% BMD loss for DPMA users ($n=33$) compared with 0.37% annual BMD loss for non-users ($n=59$) ($p=0.01$) (Berenson, Radecki, Grady, Rickert, & Thomas, 2001). In the same study, non-hormonal oral contraceptive users exhibited BMD gains compared with the control group.

A cohort study among Filipino women in the Philippines who received contraceptives from the government-facilitated family planning programme ($n=1,728$) indicates that hormonal injectable contraceptive (DMPA) (35.5%) and non-hormonal pills (39.1%) were the most

commonly accepted forms of contraceptive among the participants (RamaRao, Lacuesta, Costello, Pangolibay, & Jones, 2003). However, at the follow-up interview, conducted almost a year later (*n*=1,460), the percentage use of DMPA had dramatically decreased to 11.9%, with only a slight decline among pill users (32.7%). This demonstrates the high acceptability of pill and DMPA contraceptives use, but also indicates low retention rates of injectable or DMPA use among Filipino women.

Smoking

There is strong scientific evidence that smoking has a negative effect on the bone mass density of individuals. In a meta-analysis that examined the results of 86 cross-sectional and prospective studies (a total of 40,753 cases), it was indicated that smoking has a dose-dependent effect on bone loss (Ward & Klesges, 2001). However, it was also shown that bone loss and fracture risk can be reversed through the cessation of smoking.

In a recent longitudinal study (12-month duration), smoking cessation was associated with increased muscle mass, muscle strength and bone density (Rom, Reznick, Keidar, Karkabi, & Aizenbud, 2015). Moreover, another longitudinal study, which observed female twins, demonstrated that the bone mass density of the smoking twin was lower than that of the non-smoking twin (Hopper & Seeman, 1994). The twin study also indicated that females smoking one pack of cigarettes every day in their entire adult life are more likely to have a mean deficit of 5–10% in bone mass density.

Potential mechanisms of how smoking contributes to the decline in bone mass density have been discussed in several published studies. However, there is still no clear mechanism and adequate scientific explanation on how smoking affects bone health (Wong, Christie, & Wark, 2007). Moreover, in a recent experimental study on mice, it was observed that exposure to smoking induces the reduction of bone marrow B cells, which further results in the loss of bone marrow lymphocytes and, eventually, in bone loss (Fusby et al., 2010). That study could potentially provide an explanation of how smoking contributes to the development of osteoporosis.

Among Filipinos, a recent national survey in the Philippines indicated that the 25.4% of Filipino adults (over 20 years of age) are currently smokers. However, the majority of the

smokers were classified as males, with 44.7% of active smokers identified as male, and only 7.8 % female smokers (Food and Nutrition Research Institute, 2013).

Concerns on bone health of Filipino immigrants

Peak bone mass

Peak bone mass (PBM) is the total weight of bone present at the end of the skeletal maturation process (Bonjour, Theintz, Law, Slosman, & Rizzoli, 1994). The quantity of bone during this period is at its maximum, and, once this is reached, bone growth is stabilised and bone mass begins to plateau. Referring to Figure 3, bone mass begins to decrease during the period near menopause, when the activity of osteoclast (bone tissue breakdown) becomes greater than that of osteoblast (bone-forming).

PBM is a crucial determinant of the risk for osteoporotic fracture (Sowers & Galuska, 1993). As mentioned above, an adequate dietary calcium intake results in a higher PBM. However, Filipino children and adolescents have the lowest dietary calcium intakes compared with other age groups. Mean daily calcium intakes of 330 mg for children six months to five years of age, 260 mg for children 6–12 years old and 330 mg for adolescents were reported in the Philippine National Nutrition Survey (Food and Nutrition Research Institute, 2008b). Despite the high calcium requirements for bone growth, these reported low mean intakes may contribute to the low PBM that is achieved by the majority of Filipino adults.

Other factors, such as genetics and environmental aspects, such as vitamin D intake, steroid hormones (i.e. oestrogen and testosterone) and high-impact physical activities, have been shown to influence peak bone mass (Klibanski et al., 2001). In a twin study, a substantial genetic contribution to bone density was observed in the spine and femur of the twin pairs (n=65 pairs) (Pocock et al., 1987). A longitudinal study in Ireland (n=460) demonstrated that genetics is a statistically significant predictor of spine BMD, accounting for 3.8% of the variance, as well as for the femoral neck BMD, accounting for 3.4% of the variance (McGuigan et al., 2002).

Furthermore, the previously mentioned study in Hawaii investigated the peak bone mass of Filipino, Hawaiian, Japanese and European women. This study included women aged 25–34 years, and assumed that their current BMD represents peak bone mass, as their average BMD remained stable with age in the spine, calcaneus and radius (Davis et al., 1994). This study

found very large ethnic differences (up to 11%) in the bone mass (measured via BMC and bone width), with Filipino women scoring the lowest in the spine and distal radius sites. Since Filipino women have environmental (low calcium intake) and possibly genetic risk factors in achieving lower PBM, they potentially have an increased risk of fracture during the later stages of life.

Low vitamin D status risks specific to Filipino women

Another important concern among Filipino women is the risk of low vitamin D status. Like the Philippines, neighbouring countries such as Malaysia and Indonesia have reported a high prevalence of vitamin D insufficiency among women (Green et al., 2008; Oemardi et al., 2007). The following factors are considered to be the main contributors to the potentially high prevalence of low vitamin D status among Filipino women.

Skin colour

Filipino women have generally a darker skin colour, which indicates a high amount of epidermal distribution of melanin (Taylor, 2002). As noted above, high concentration of melanin in the skin deters it from absorbing UV radiation. Thus, compared to ethnicities with lighter skin, Filipino women have less ability to make vitamin D_3 due to the higher concentration of melanin in their skins.

Adiposity

The alarmingly increasing prevalence of obesity among Filipinos due to lifestyle changes and the overconsumption of unhealthy food may also be contributing factors to the high prevalence of vitamin D insufficiency in the country. A recent National Nutrition Survey reported an upsurge in high waist circumferences and waist-to-hip ratios (WHR) among women over 20 years old. From 10.7% of women with a high waist circumference in 1998, the percentage increased dramatically to 19.9% in 2011, while high WHR also rose from 39.5% in 1998 to 65.5% in 2008 (Food and Nutrition Research Institute, 2013).

A large body of scientific literature supports the association of obesity with vitamin D deficiency. Despite this, the mechanism remains unclear, with some studies claiming vitamin D deficiency as a cause of obesity, whereas others state the reverse (obesity causes vitamin D deficiency) (Pourshahidi, 2014). In a recent clinical trial, two mechanisms were hypothesised

for the association: (1) adiposity decreases vitamin D bioavailability because of its sequestration in fat tissues, and (2) vitamin D maintains the homeostasis of calcium inside the cells, in which an increased calcium level activates lipogenesis and suppresses lipolysis (Shab-Bidar, Neyestani, & Djazayery, 2015).

A vitamin D supplementation study of Middle Eastern women living in New Zealand illustrated a significant negative correlation (-0.7, $p<0.01$) between vitamin D status and body fat percentage during the six-month supplementation of vitamin D (Mazahery et al., 2015). This signifies that lower body fat percentage translated to a larger change in the serum 25(OH)D concentration among participants during the supplementation period.

Sun avoidance

The political, economic and social value of having a light skin is evident in the Filipino culture (Glenn, 2009). Over the recent years, an increase in the marketing and use of skin-whitening products such as glutathione was observed in the Philippines (Handog, Datuin, & Singzon, 2016).

This preference over dark skin can still be observed among immigrants. A study of South Asian women living in New Zealand noted a high percentage of deliberate sun avoidance. Out of the 140 survey respondents, 19% noted that their sun avoidance is due to their not wanting to have a dark skin (von Hurst, Stonehouse, & Coad, 2010). This is likely to be true among Filipino immigrants in New Zealand, due to the perceived skin-darkening effect of the sun.

Likewise, an acculturation study on East Asian women living in Australia demonstrated that higher levels of acculturation are associated with higher sun exposure and higher vitamin D status (Brock et al., 2013). Participants who were less acculturated had less sun exposure, higher sun protection behaviour and lower vitamin D and calcium intake (dietary and supplements). The authors argued that the less-acculturated participants hold the values of Asian culture, which prefers fairer skin.

Measurement of bone mineral status

Bone mineral density measurement

Bone density measurement is one of the most common methods in assessing bone health. The primary techniques in bone densitometry are computed tomography (CT), ultrasound (US) and dual-energy X-ray absorptiometry (DXA). In terms of precision, the % coefficient of variation obtained from QUS (site: calcaneus, tibia and multi-site) range from 0.1%–5%, 1–2% for DXA (sites: spine, femur and total body) and 3% from CT (site: spine) (Fogelman & Blake, 2000). In a trial investigating the accuracy of DXA measurements in five ewes, the *ex vivo* measurements were closely related to *in vivo* DXA measurements for spine BMD (r=0.98) and BMC (r=0.97) (Pouilles et al., 2000). Thus, the standard for bone density measurement in terms of absorptiometry is DXA, which has the capacity to assess bone mineral and lean tissue. These two materials are measured by quantifying the X-ray transmission at two different energies (photon) (Guglielmi, 2013).

Globally, the World Health Organization (WHO) recognises DXA as a standard analysis in the diagnosis and risk assessment of fractures (Dimai, in press). It has the capability to adjust the differences of inter-device measurements (*T*-score) and compare the individual's value to the mean BMD expected for age and sex (*Z*-score). Among individuals, osteoporosis is defined as a BMD measurement with a *T*-score of less than 2.5 SD (Standard Deviations) (World Health Organization, 2007). On the other hand, a *Z*-score indicates the individual's fracture risk in comparison to the reference (a healthy person of the same sex and age), and cannot be utilised for osteoporosis diagnosis (Dimai, in press).

Moreover, apart from BMD measurement, DXA is also used for body fat percentage assessment. In a recent study assessing the validity and reliability of bioelectrical impedance analysis in estimating body fat proportion compared to DXA and air displacement plethysmography, DXA measurements were strongly related to the estimated true value (p=0.97 (0.96, 0.98)) and had highly correlated repeat measurements, which indicate an excellent reliability (von Hurst et al., 2010). Similar findings were observed with a study assessing the validity and reliability of DXA in assessing abdominal fat mass compared to computed tomography (CT) among adults aged 18–72 years (n=65) with a range of body raft mass (8.0–58.0 body fat percentage). In this study, an excellent correlation between CT and DXA measurements was demonstrated (r=0.858, p<0.001) (Glickman, Marn, Supiano, & Dengel, 2004)

Conclusion

In conclusion, Filipino women in New Zealand undergo a complex acculturation process which sequentially affects their dietary intake. Comparing the traditional Filipino and New Zealand diets, several changes in their dietary pattern could be predicted. One of the most noteworthy differences between the two diets is the quantity and food sources of dietary calcium intake. Thus, this research aims to investigate the changes in dietary calcium intake (in terms of adequacy and quality) of Filipino women who have recently immigrated in New Zealand.

Due to their ethnicity, age, predisposition to adiposity, low national average of dietary calcium intake and higher risk of vitamin D insufficiency, Filipino women can be considered as a vulnerable ethnic group with an increased risk of having fractures and bone diseases. Therefore, the secondary objective of this research is to determine the factors that affect the bone mineral status of Filipino women living in New Zealand. In this study, dietary calcium intake, vitamin D status, physical activity and gross lean mass will be investigated as predictors of bone mineral status.

Chapter 3 **Research study manuscript**

A retrospective and cross-sectional study to evaluate the effect of dietary acculturation on the dietary calcium intake among Filipino women recently immigrated to New Zealand and predictors of bone mineral density

Abstract

Filipinos in New Zealand have steadily grown in number over recent decades, and the majority undergo a dietary acculturation process, which is the dietary adaptation of individuals in their host country. In the Philippines, the nutrient with the highest inadequacy in the diet is calcium, which is primarily contributed by fish and indigenous vegetables that are not readily available in New Zealand. The aim of this study is to determine the effect of dietary acculturation on the calcium intake of Filipino women recently immigrated to New Zealand, and to explore the primary factors affecting their bone mineral status. Sixty-two (62) healthy pre-menopausal Filipino women (20–45 years old) were recruited. Current and previous dietary calcium intake, serum 25(OH)D (nmol/L) (n=61), physical activity data via an accelerometer, and bone mineral density (BMD) and body composition through dual-energy X-ray absorptiometry (DXA) were measured. Gross lean mass was calculated (total mass – [whole body total bone content + total fat mass]). The variables considered to be associated with bone mineral status were applied to a multiple regression analysis using the enter method. The median calcium intake for New Zealand [418 (260, 620) mg d^{-1}] after immigration, was significantly lower than the intake in the Philippines [506 (358, 823) mg d^{-1}], Z=-2.41, p=0.02, medium effect size r=0.22. The significant predictor of bone mineral status among Filipino women was gross lean mass, whereas current and previous dietary calcium intake, physical activity and serum 25(OH)D were not found to be significant. However, a high prevalence (69%) of serum 25(OH)D <50nmol/L (mild–moderate deficiency) was detected. These findings illustrate the potential detrimental consequences of dietary acculturation on the essential nutrient intake of immigrants, but also provide an opportunity to correct previous dietary inadequacies by exposing the participants to corresponding nutrient-dense foods from the host country.

Introduction

The number of Filipinos migrating to New Zealand has steadily increased in the past few decades. This ethnic group is identified as the fastest-growing ethnic minority in New Zealand (StatisticsNZ, 2013). Accordingly, this large number of Filipino immigrants undergo dietary acculturation, a complex adaptation process wherein immigrants adopt the dietary patterns of the host country. The traditional Filipino diet is primarily composed of rice, fish, meat and vegetables. Despite this variety, a large percentage of the Filipino population was not able to meet the recommended energy and nutrient intake (RENI) for the majority of the surveyed nutrients in the Philippine National Nutrition Survey (Food and Nutrition Research Institute, 2008b). Among these nutrients, a high prevalence of insufficient dietary calcium intake was observed among Filipinos, as a government-mandated national survey reported that only 11.5 % of Filipino households were able to meet the RENI for calcium intake (Food and Nutrition Research Institute, 2008a). Calcium plays a crucial role as the main structural component of bone, and is also involved in numerous metabolic activities, such as cell-signalling, activation of blood coagulation factors and neuromuscular junction excitation (Marshall, 2012).

The longstanding problem of inadequate dietary calcium intake among Filipinos has been demonstrated by a high prevalence of bone diseases among Filipino women. A preliminary study which investigated 285 adult Filipino women (19–87 years old) reported a 40.7% prevalence of osteopenia in women below 40 years old (Cruz et al., 2004). Due to the high prevalence of both inadequate dietary calcium intake and osteoporosis, Filipino women are considered at risk of bone-related diseases.

Furthermore, in New Zealand a large proportion of the population consume a significant amount of dietary calcium (mean 832 mg d^{-1}) compared with the average daily calcium intake of Filipinos (370 mg d^{-1}) (Food and Nutrition Research Institute, 2008b; Ministry of Health [New Zealand], 2011). There are also key differences in the quality of these diets, as most of the calcium in the traditional Filipino diet comes from fish, whereas milk and dairy products are the primary sources of calcium for New Zealanders. Due to the differences in dietary sources of calcium, Filipino women who are already at risk of osteoporosis can potentially have a higher risk of calcium deficiency, which may lead to the development of bone diseases and an increased incidence of fractures, following immigration to New Zealand.

Bone health is also influenced by several other non-dietary factors. Aside from dietary calcium intake, physical activity, gross lean mass, vitamin D status, and age have been widely shown to influence bone mineral status among women. Decreased physical activity, adiposity, an increased risk of having vitamin D insufficiency and the age of migrating Filipino women are significant variables that may potentially affect their bone health.

Hence, the aims of this research were to determine the effect of dietary acculturation on the dietary calcium intake of Filipino women recently immigrated to New Zealand, and to explore the key factors that affect their bone mineral status.

Methods

Study protocol

This was a retrospective and cross-sectional study conducted at Massey University Human Nutrition Research Unit (MHNRU) from September 2016 to March 2017 (spring and summer).

Participants

Filipino women who have recently immigrated to New Zealand (<5 years) were recruited from Auckland through posters, flyers, Filipino community gatherings (i.e. Sunday masses and cultural night markets), social media and word of mouth. The sample size calculation was based on the general requirement of a minimum of 10 participants for each investigated variable for a regression analysis (Harrell, 2001; Nunnally, 1978). In this study, 50 participants were required to investigate the 5 potential predictors of bone mineral status. Included in the study were 20-45-year-old women. Exclusion criteria were: (1) pregnancy or lactation; (2) chronic illness that may affect bone metabolism; (3) diagnosis of bone disease; (4) peri- or postmenopausal; and (5) on medications affecting metabolic health.

The conducting of this research was granted an ethical approval by the Massey University Human Ethics Committee (Southern A), Reference No. 16/31.

Data collection methods

Screened participants visited MHNRU twice. Written consent and a health and demographic questionnaire were completed. The employment details obtained from the health and demographic questionnaire was categorised into professions based on the Ministry of Health

classification (Ministry of Health [New Zealand], 2016). Anthropometric data (i.e. height and weight) were measured three times, using a stadiometer (SECA 213, Hamburg, Germany) and calibrated weighing scale (BIA Inbody230, South Korea). NHANES (National Health and Nutrition Examination Survey) anthropometry procedures served as the standard procedure for both anthropometric assessments (Centers for Disease Control and Prevention, 2012).

For this study, 'gross lean mass' was used as a proxy for muscle mass, since body fat percentage was the original measurement obtained by the DXA. Through body fat percentage, body fat mass (g) was calculated and, together with bone mineral content (g), was subtracted from the total mass to quantify fat-free, bone-free mass, which is referred to as 'gross lean mass', forthwith. Participants were sent home with fitted accelerometers (Actigraph Model GTX3, Actigraph Corp, Florida, USA) to be worn for at least 48 hours, and a physical activity diary which was completed to validate the readings of the accelerometer. Actigraph data was analysed using ActiLife v6.13.2. A wear-time validation algorithm (Troiano et al., 2008) was applied to remove periods when the actigraph was not worn. The remaining data was scored to calculate energy expenditure using Freedson VM3 Combination method (Sasaki, John, & Freedson, 2011).

Fasting venous blood samples were also collected. The two phases of FFQ were completed on two separate occasions with a minimum interval of 48 hours. DXA scans (whole body, hip total, femoral neck and lumbar spine) were obtained. The DXA scans reported on: bone mineral density (BMD), expressed as gram per square centimetre (g/cm^2); bone mineral content (BMC), expressed in grams (g); and *Z*-score (a comparison to the average BMD expected for the participant's age and sex), expressed as a standard deviation (SD).

The health and demographic questionnaire, physical activity diary and FFQ forms can be found in the supplementary Appendix B.

Data collection measures

Biochemical analyses

Serum 25(OH)D was analysed at the end of the collection phase of the study. An 18-minute competitive chemiluminescent immunoassay (ADVIA Centaur Vitamin D Total assay,

Siemens Healthcare Diagnostics Inc, II, USA) was utilised to determine the serum 25(OH)D of the participants.

Development of food frequency questionnaire

The food frequency questionnaire (FFQ) used in this study was constructed through an extensive literature review on the high calcium-containing food items commonly consumed in the Philippines and in New Zealand (Food and Nutrition Research Institute, 2008b; Ministry of Health [New Zealand], 2011; Miura et al., 2009). It was modelled on the FFQ format used in the study investigating the validity of the Fred Hutchinson Food Frequency Questionnaire (FHFFQ) among Filipino Americans (Johnson-Kozlow et al., 2011). The food items included in the FFQ were modified and obtained from the 2008/2009 New Zealand Adult Nutrition Survey, and from an investigative study on the average calcium intake of Filipino women in the Philippines, validated through the 2008 Philippine National Nutrition Survey (Food and Nutrition Research Institute, 2008b; Ministry of Health [New Zealand], 2011; Miura et al., 2009). The 48-item questionnaire was subdivided into five main categories: grains and cereal products; meat and poultry; dairy and products; fruits and vegetables; and other non-dairy beverages. Equal items of food (24 each) were included coming from the New Zealand and Philippine food sources. The average amounts (medium portion size) were based in the Food Exchange List published by Food and Nutrition Research Institute (FNRI) for the Philippine food sources, while the New Zealand average portions were based on the Food and Nutrition Guidelines for the 1997 National Nutrition Survey (Food and Nutrition Research Institute, 1998; Ministry of Health [New Zealand], 1996). However, the constructed FFQ in this study was not validated.

Participants completed the self-administered FFQ (either online or on paper) on two occasions, with a minimum of two days apart. The first phase was a retrospective assessment of dietary calcium intake while living in the Philippines. The second phase was an assessment of their current dietary calcium intake in New Zealand.

Frequency data was translated into servings through the General Nutrition Assessment FFQ Processing System of FHFFQ (Fred Hutchinson Cancer Research Center, 2010). The translated servings were processed into daily dietary calcium intake using the Malaysian Food Composition Database (MYFCD) for phase 1, and Foodworks7 Professional 2010 (Xyris Software, Queensland, Australia) for phase 2 (Institute for Medical Research, 1997).

MYFCD was utilised in preference to the Philippine and ASEAN food composition databases due to its accessibility to the researchers during the time of data analysis. There is also a similarity of food items in Malaysian and Filipino diets, and parallel trends were observed in the dietary pattern changes for both countries over recent decades (Lipoeto, Lin, & Angeles-Agdeppa, 2013). All unknown food items for both phases were supplied through the USDA Food Composition Databases (United States Department of Agriculture, 2017).

Statistical analysis

All data gathered was processed using IBM SPSS Statistics version 22 and 24 (IBM Corp., New York, USA). The baseline population was defined using descriptive statistics. For normally distributed data, mean and standard deviation were used, whereas median and 25th and 75th percentiles were used to describe data with skewed distribution. Kolmogorov–Smirnov and Shapiro–Wilk tests and histogram and Q–Q plots were used to assess the normality of the data. Categorical variables were expressed as proportions or *n* (%). A *p* value <0.05 was considered as statistically significant.

To identify differences between the groups or variables, non-parametric data was evaluated using the Wilcoxon Signed-Rank Test. The effect size was calculated using this formula: effect size $r=Z/\sqrt{n}$. A small effect is indicated by an effect size value of 0.1, a medium effect is indicated by a value of 0.3, and a large effect is indicated by a value of 0.5 or higher (Field, 2009). Multiple linear regression analysis using the enter method was conducted to determine the predictors of the bone mineral status of Filipino women living in Auckland.

Results

Sixty-two pre-menopausal were recruited, and their baseline characteristics are illustrated in Table 1. Based on the Ministry of Health classification, their professions were non-regulated health workers (*n*=33), students (*n*=9), registered nurses (*n*=6) and others (*n*=14) (Ministry of Health [New Zealand], 2016).

Table 1. Demographic and physical characteristics of all participants

Variable	Participants (*n*=62)
Age (years)	28.4 (26.2, 33.6)
Professions (%)	non-regulated health workers (53.2 %), students (14.5%), nurses (9.7%) and others (22.6%)
Months in NZ	17.5 (9.8, 24.0)
Smoking (% tobacco use)	6.5% (*n*=4)
Contraception (% use)	6.5% (*n*=4)
Height (cm)	156.0±5.1
Weight (kg)	60.5 (52.1, 69.3)
BMI (kg/m^2)	24.6 (21.9,28.7)
% body fat	34.8±4.7
Gross lean mass (kg)	36.66 (32.76, 42.55)
Physical activity (kcal/day)	334.6±155.7
Serum 25(OH)D (nmol/L)$^{\alpha}$	44.2±15.3 (range: 16.0–75.0)
Dietary calcium intake (mg d^{-1})	418 (260, 620)
Whole body BMD (g/cm^2)	0.961±0.071

Values are mean and standard deviations or medians and 25th and 75th percentiles
Abbreviations: BMI = Body Mass Index; NZ = New Zealand; BMD = Bone Mineral Density
$^{\alpha}$(*n*=61), due to the difficulty of extracting blood sample from one of the participants

The difference between dietary calcium intake in the Philippines and in New Zealand is presented in Table 2. A Wilcoxon Signed-Rank Test indicated that dietary calcium intake in the Philippines 506 (358, 823) mg d^{-1} was significantly higher than that in New Zealand 418 (260, 620) mg d^{-1}, *Z*=-2.41, *p*=0.02, medium effect size *r*=0.22. Table 2 also reports two relatively different estimated average requirements (NZ = 840 mg d^{-1}, PH = 600 mg d^{-1}) from both countries, thus, substantially affecting the % adequacy.

Table 2. Comparison of dietary calcium intake in the Philippines versus in New Zealand (*n*=62)

	Philippine intake	New Zealand intake	*P*-value
Dietary calcium intake (mg d^{-1})	506 (358, 823)	418 (260, 620)	0.02$^{\alpha}$
EAR (mg d^{-1})	600$^{\beta}$	840$^{\mu}$	–
% adequacy based on PH EAR	84.3	69.8	–
% adequacy based on NZ EAR	60.2	49.8	–

Values are medians and 25th and 75th percentiles
Abbreviations: EAR = estimated average requirement for the Philippines and New Zealand (Food and Nutrition Research Institute, 2015; National Health and Medical Research Council et al., 2006); NZ = New Zealand; PH = Philippines
$^{\alpha}$Differences between the Philippine intake and the New Zealand intake ($p<0.05$) Wilcoxon Signed-Rank Test
$^{\beta}$Values based on 19–49-year-old female adults
$^{\mu}$ Values based on 19–50-year-old women

To determine the changes in diet quality, a comparison of the contributing food groups for the Philippine and New Zealand intakes are illustrated in Table 3. Calcium from fish and meat intake in the Philippines 138 (94, 197) mg d^{-1} was significantly higher than the New Zealand intake of 59 (33, 86) mg d^{-1}, Z=-6.59, p<0.001, large effect size r=0.59. Similar results were observed with the fruit and vegetables category, with a substantially higher dietary calcium contribution from fruit and vegetables for the Philippine intake, 35 (20, 80) mg d^{-1} than the New Zealand intake, 12 (6, 25) mg d^{-1}, Z=-6.31, p<0.001, large effect size r=0.57. It is also noteworthy that the contribution of the milk and dairy food group to the New Zealand intake is higher than its contribution to the Philippine intake; however, the reported difference was not statistically significant (p=0.20).

Table 3. Comparison of contributing food groups to previous and current dietary calcium intakes

Food group	Philippine intake (mg d^{-1})	New Zealand intake (mg d^{-1})	*P*-value[α]
Cereals	125 (74, 156)	99 (71, 161)	0.33
Fish and meat	138 (94, 197)	59 (33, 86.)	<0.001
Milk and dairy products	149 (51, 350)	213 (89, 345)	0.20
Fruit and vegetables	35 (20, 80)	12 (6, 25)	<0.001
Beverages	6 (1, 17)	9 (4, 17)	0.15
Supplements	0 (0, 0)	0 (0, 0)	0.09

Values are medians and 25th and 75th percentiles
[α]Differences between the Philippine intake and the New Zealand intake (p<0.05) Wilcoxon Signed-Rank Test

Table 4 describes the bone mineral status of the participants in the hip (total), the femoral neck, the lumbar spine and for the whole body. Three (3) participants did not have data for the hip total and femoral neck areas due to some technical problems with the DXA machine.

Table 4. Current bone mineral status of Filipino women living in New Zealand

Area	*T*-score	*Z*-score	BMD (g/cm^2)	BMC (g)
Hip (total) (*n=59*)	-0.82±0.94	-0.78±0.94	0.842±0.115	23.173±6.513
Femoral neck (*n=59*)	-1.01±1.11	-0.93±1.09	0.737±0.123	3.288±0.779
Lumbar spine (*n=62*)	-0.82±0.95	-0.73±0.96	0.957±0.104	49.225±7.737
Whole body (*n=62*)	-1.91±0.96	-1.91±0.92	0.961±0.071	1,737.184±222.694

Values are mean and standard deviations
Abbreviations: BMD = bone mineral density; BMC = bone mineral content

The multiple linear regression analysis (enter method) was conducted to determine the predictors of the bone mineral status of Filipino women in New Zealand, by building a model with the following selected predictors: previous dietary calcium intake, current dietary calcium intake, physical activity, serum 25(OH)D and gross lean mass. Dietary calcium intake (Bischoff-Ferrari et al., 2009b; Macdonald et al., 2004) and serum 25(OH)D (Kuchuk, van Schoor, Pluijm, Chines, & Lips, 2009) were selected as covariates, since robust scientific evidence supports their association with bone mineral status.

Furthermore, positive correlations were observed between physical activity and hip *Z*-score (r=0.35, n=58, p=0.004), with lumbar *Z*-score (r=0.48, n=61, p<0.001) and with whole body *Z*-score (r=0.36, n=61, p=0.002). Similarly, gross lean mass was also positively correlated with hip *Z*-score (r=0.44, n=58, p<0.001), with lumbar *Z*-score (r=0.53, n=61, p<0.001) and with whole body *Z*-score (r=0.49, n=61, p<0.001). Due to the said correlations, physical activity and gross lean mass were included in the model. However, body fat percentage was not included in the model due to its potential collinearity with gross lean mass. On the other hand, previous and current dietary calcium intakes were both included in the model, as their correlation is relatively low (r<0.9) to produce collinearity in the model. Thus, the final covariates included in the model were calcium intake (previous and current), serum 25(OH)D concentration, physical activity and gross lean mass. Assumptions for regression analysis, such as normality of dependent variable, independence (Durbin-Watson test) and normality of residuals, homoscedasticity and multicollinearity, were all met by the three models (Tables 5a–5c).

All the regression models for hip (total), spine and whole body *Z*-scores demonstrated that the gross lean mass is a significant predictor of bone mineral status of Filipino women in New Zealand. These models also demonstrated that previous dietary calcium intake, current dietary calcium intake, physical activity and serum 25(OH)D did not reach significance as predictors of bone mineral status as measured by *Z*-score.

Upon analysis of variance (ANOVA), it was shown that the regression models (hip (total) *Z*-score (p=0.02), spine (p<0.01) and whole body (p=0.004)), are all statistically significant models. In terms of the predictor's strength, gross lean mass was the strongest predictor of bone mineral status for all the three models, as indicated by their standardised β-coefficients.

Table 5a. Hip total: predictors of hip *Z*-score among Filipino women living in New Zealand

Model	Coefficient (*B*)	Standard error *B*	95% CI *B*	Standardised β	R^2	*P* value
Model 1					0.218*	0.02
Intercept	-3.463	0.890	-5.249, -1.678	-		0.000
NZ calcium intake (mg d^{-1})	0.000	0.000	-0.001, 0.001	0.058		0.679
PH calcium intake (mg d^{-1})	7.294E-5	0.000	-0.001, 0.001	0.030		0.829
Physical activity (kcal/day)	0.001	0.001	-0.001, 0.003	0.118		0.440
Serum 25(OH)D (nmol/L)	0.007	0.008	-0.009, 0.022	0.108		0.401
Gross lean mass (g)	0.053	0.021	0.010, 0.096	0.385		0.016

Abbreviations: NZ = New Zealand; PH = Philippines
*Enter method, *F* (5,57) = 2.905, 95% confidence interval

Table 5b. Spine: predictors of lumbar *Z*-score among Filipino women living in New Zealand

Model	Coefficient (*B*)	Standard error *B*	95% CI *B*	Standardised β	R^2	*P* value
Model 1					0.380*	<0.001
Intercept	-4.138	0.792	-5.726, -2.551	-		0.000
NZ calcium intake (mg d^{-1})	7.097E-5	0.000	-0.001, 0.001	0.022		0.856
PH calcium intake (mg d^{-1})	0.000	0.000	0.000, 0.001	0.183		0.138
Physical activity (kcal/day)	0.001	0.001	0.000, 0.003	0.230		0.085
Serum 25(OH)D (nmol/L)	0.010	0.007	-0.004, 0.024	0.154		0.167
Gross lean mass (g)	0.057	0.019	0.018, 0.096	0.398		0.005

Abbreviations: NZ = New Zealand; PH = Philippines
*Enter method, *F* (5, 60) =6.748, 95% confidence interval

Table 5c. Whole body: predictors of whole body *Z*-score among Filipino women living in New Zealand

Model	Coefficient (*B*)	Standard error *B*	95% CI *B*	Standardised β	R^2	*P* value
Model 1					0.264*	0.004
Intercept	-4.493	0.823	-6.142, -2.845			0.000
NZ calcium intake (mg d^{-1})	0.000	0.000	-0.001, 0.001	-0.078		0.556
PH calcium intake (mg d^{-1})	0.000	0.000	0.000, 0.001	0.119		0.370
Physical activity (kcal/day)	0.001	0.001	-0.001, 0.002	0.109		0.449
Serum 25(OH)D (nmol/L)	0.004	0.007	-0.010, 0.019	0.072		0.549
Gross lean mass (g)	0.055	0.020	0.015, 0.095	0.406		0.008

Abbreviations: NZ = New Zealand; PH = Philippines
*Enter method, *F* (5, 60) =3.949, 95% confidence interval

Discussion

Differences in dietary calcium intake

This study demonstrates a significantly higher dietary calcium intake in the participants' previous intake in the Philippines than in their current intake in New Zealand (p=0.02). The values for the Philippine calcium intake obtained from this study are higher than the values from a cross-sectional study conducted in the Philippines aimed at determining the daily calcium intake and physical activity status of Filipino women. That study presented a median of 289 (225, 434) mg d^{-1} compared with this study's reported median of 506 (358, 823) mg d^{-1} (Miura et al., 2009). These differences may have been brought about by the variations in the methods used in gathering data (i.e. a food frequency questionnaire [FFQ] was used in this study vs. direct analysis of food duplicates collected within three days for the Philippine-based study). Additionally, the FFQ used in this study was not validated with another dietary assessment tool, and its major limitation of relying heavily on the participant's memory made it more prone to estimation errors. Since the previous dietary recall data is dependent on the long-term memory of participants, the relatively higher values of dietary calcium intake obtained from this study may also be a reflection of inaccurate reporting as accuracy of dietary recall data diminishes over time. Compared with the study by Miura et al. (2009), which was set in a poor urban area in the Philippines, this study consisted of Filipino women from different parts of the Philippines, and some may have come from the rural parts of the country, where rich sources of calcium are more widely available, such as fish and indigenous green leafy vegetables. Furthermore, participants of this study are presumably not socioeconomically disadvantaged as they had the capacity to immigrate, which implies that they would have greater food security than the poor women in the surveillance study. 46 of the 58 women (79%) in the surveillance study had an income below $1.35 per person per day, which is the absolute poverty threshold in Asia (Miura et al., 2009). Likewise, many of the participants were regulated and non-regulated health workers (62.9 %), suggesting a higher level of education and greater health-seeking behaviour, hence, a higher intake of nutrient-dense foods.

With the differences in dietary calcium intake, the proportion of those reaching adequacy was also substantially affected. Only 49.8% of participants were able to meet the estimated average requirement (EAR) for daily calcium intake in New Zealand, as it was set at 840 mg per day compared with the Philippine EAR, which was set at 600 mg daily. Similarly, the

nutrient reference values of calcium for New Zealand were also set higher, as the recommended dietary intake (RDI) is established at 1000 mg daily, whereas the Philippines' recommended energy and nutrient intake (RENI) for calcium is 750 mg per day (Food and Nutrition Research Institute, 2015; National Health and Medical Research Council et al., 2006).

In addition to the difference in amount of dietary calcium intake, differences in the quality of diet were also observed. Moreover, the results in Table 3 are consistent with the findings of the Philippine and New Zealand nutrition surveys. Compared with a 7.4% contribution in the New Zealand diet, 34.5% of total calcium intake in the Filipino diet comes from fish, meat and poultry. Vegetables also exhibited a higher contribution, with 15.0% for the Filipino diet compared with a 5.7% contribution to the New Zealand diet (Food and Nutrition Research Institute, 2008b; Ministry of Health [New Zealand], 2011).

The similarity in the dietary patterns of the current dietary calcium intake of Filipino women in the study and the average New Zealand diet implies the significant effects of dietary acculturation among the recently-immigrated Filipino immigrants. Notwithstanding the relatively short duration (17.5 (9.8, 24.0) months) of the participants' stay in New Zealand, their dietary pattern, in terms of calcium intake, is already moving towards to that of the New Zealand diet. Nonetheless, calcium intake [418 (260, 620) mg d^{-1}] was still much lower than the average New Zealand diet, which has a median daily intake of 745 mg for women (Ministry of Health [New Zealand], 2011).

Despite these similarities, the contribution of milk and dairy to the dietary calcium of the Philippine intake was not consistent with the previous national survey. Milk and dairy was the highest contributor of calcium for both Philippine [149 (51, 350) mg d^{-1}] and New Zealand [213 (89, 345) mg d^{-1}] dietary calcium intakes in this study. This is substantially different from the Philippine survey, where fish and meat were the greatest contributors of calcium in the diet, and milk and dairy only contributed 4.4% to the total mean calcium intake (Food and Nutrition Research Institute, 2008b). This could be reflective of the participants' socioeconomic status prior to immigration as mentioned previously, since milk and dairy are imported commodities with high market prices (Miura et al., 2010).

Predictors of bone mineral status

A surveillance study aimed to establish normative BMD values for Filipino women in the Philippines reported mean lumbar and femoral neck BMDs (Torralba et al., 2004). To compare the mean BMD values of participants from the current study and the surveillance study, the current study's participants were stratified by age, based on the grouping indicated in the surveillance study (Table 6).

Table 6. Comparison of lumbar and femoral neck bone mineral density (BMD) values between the participants from a previous Filipino study (Torralba et al., 2004) versus the current study participants

	Torralba ,et al., 2004 study participants (g/cm^2)$^{\alpha}$	**Current study participants (g/cm^2)$^{\beta}$**
	Age (20–29 y)	
N	116	Lumbar (*n*=37), Femoral neck (*n*=35)
Lumbar BMD (L1–L4)	1.088±0.11	0.960±0.09
Femoral neck	0.890±0.11	0.759±0.12*
	Age (30–39y)	
N	40	19
Lumbar BMD (L1–L4)	1.132±0.12	0.938±0.12
Femoral neck	0.887±0.11	0.730±0.10
	Age (40–49y)	
N	70	Lumbar (*n*=6), Femoral neck (*n*=5)
Lumbar BMD (L1–L4)	1.075±0.13	0.998±0.12
Femoral neck	0.875±0.10	0.609±0.17

Values are mean and standard deviations
Abbreviations: BMD = bone mineral density; FWHS = Filipino Women's Health Study (current study); y = years
*Non-normally distributed, expressed as mean±SD
$^{\alpha}$Values based on the representative mean BMDs of Filipino women by age group (Torralba et al., 2004)
$^{\beta}$ Values based on the spine and femoral neck BMD, stratified per age group

The BMD values gathered from this study are comparatively lower than the values from the surveillance study of Filipino women in the Philippines by Torralba et al. (2004). In terms of the number of participants, there was a total of 226 participants in the surveillance study compared with the 59 (femoral neck and hip total) and 62 (spine and whole body) participants included in the analysis of the current study. The Torralba study was more

representative of Filipino women than our study, as this study involved self-selected participants with higher socioeconomic status, higher education and a higher level of health-seeking behaviour. Conversely, participants in the Torralba study were not specifically recruited as health workers, and the study recruited participants across the socioeconomic and academic spectrum.

Mean serum 25(OH)D from this study was reported as 44.2±15.3 nmol/L, which is categorised as mild vitamin D deficiency based on the cut-off points developed by the Australian and New Zealand Bone and Mineral Society, the Endocrine Society of Australia and Osteoporosis Australia (Nowson et al., 2012). Based on the same cut-off values, 19 (31%) were identified with sufficient serum 25(OH)D (≥50nmol/L), 30 (49%) had mild deficiency (30–49 nmol/L), and 12 (20%) had moderate serum 25(OH)D deficiency (12.5–29 nmol/L) among the participants (Nowson et al., 2012). However, the Endocrine Society suggested higher cut-off values for vitamin D sufficiency (deficiency: <50 nmol/L, insufficiency: ≥50–72.5 nmol/L and sufficiency: 75–250 nmol/L), whereas the Institute of Medicine cut-off points (deficiency: <40 nmol/L, sufficiency: ≥50 nmol/L and upper levels: 125 nmol/L) were comparable to the values developed in Australia and New Zealand (Holick et al., 2011; Institute of Medicine, 2011).

This demonstrates the susceptibility of Filipino women to vitamin D deficiency due to specific risks. Adiposity, darker skin colour and sun avoidance due to preference for whiter skin are general characteristics of Filipino women that increase their tendency to have lower vitamin D levels (Food and Nutrition Research Institute, 2013; Glenn, 2009).

The regression models in the areas of hip *Z*-score, spine *Z*-score, and whole body *Z*-score consistently indicate gross lean mass as a significant and strongest predictor of bone mineral status among Filipino women living in New Zealand. A similar study among Pacific Island women in New Zealand demonstrated that lean mass (bone-free, fat-free lean mass) was positively associated with whole body BMD, accounting for 21% of its variation ($F(1, 82)=21.5, p<0.001$) (Casale et al., 2016). The authors explained that this is potentially due to the protective effect of lean mass contributing to an increased total mass, which in turn provides the bones with greater mechanical loading, and thus triggers the osteocytes to increase bone formation.

Physical activity had consistently positive correlations with all sites measured, although it was not a significant predictor in the regression models. This could be explained by the

body's physiological responses in dealing with higher mechanical demands, which include increased intestinal calcium absorption, decreased urinary calcium excretion, and a decline in PTH production (Anderson & Garner, 2011). Aside from these well-recognised effects, physical activity was also found to increase the rate of bone mineral accrual during growth (Vicente-Rodriguez, Ara, Perez-Gomez, Dorado, & Calbet, 2005). These results agree with the findings of Mazess & Barden (1991), which indicated that physical activity (measured as kilojoule/hour) was not associated with BMD. The cited study observed that an initial effect of physical activity on the bone density disappeared once body weight was controlled for (Mazess & Barden, 1991). This implies the significance of the mechanical loading effect of the total weight on bone mineral status, as mentioned above.

Previous and current dietary calcium intake and serum 25(OH)D were also not found to be significant predictors of bone mineral status. Dietary calcium intake is a well-recognised factor which affects bone health. A meta-analysis of 59 randomised controlled trials reported a direct relationship between dietary calcium intake and bone mineral density in the total body, lumbar spine and femoral neck sites (Tai, et.al, 2015). However, similar results were not observed in this study due to limitations in the dietary intake reporting such as the lack of validation tool for the FFQ. Furthermore, this could also be explained by the low vitamin D levels observed among the study participants. Vitamin D contributes to increased bone mineral status through the promotion of calcium and phosphorus absorption in the intestines, inducing cellular differentiation of preosteoclasts into mature cells, thereby increasing the number of osteoclasts and the stimulation of the synthesis of several bone matrix proteins (Holick, 2010). However, in this study, serum 25(OH)D did not predict bone mineral status, possibly because the participants' levels may be too low to be able to detect a difference. The collection of samples covered the period from spring to summer, which may have affected the serum 25(OH)D concentrations due to the low amount of sunshine before summer, thus decreasing the rate of subcutaneous pre-vitamin D activation. A study in New Zealand investigating 21,987 adults (17,265 women) demonstrated similar effects of seasonal variation on the serum 25(OH)D, since 48% of the participants had suboptimal levels (<50nmol/L) during the time of testing and was predicted to increase to 63% during winter and spring (Bolland et al., 2008).

Limitations of the study

This study has the following limitations identified: (1) the physical activity data gathered via accelerometer covered only two days compared with the recommended three to five days, due to logistical limitations and the limited availability of equipment; (2) comparison of dietary intake was made across two different time periods: one relied on relatively short term memory (current intake in NZ), whereas the other was dependent on the long-term memory (previous intake in the Philippines) of participants. Since the accuracy of dietary recall diminishes over time, this is a potential major confounder in this study; (3) the FFQ was not validated against another dietary assessment tool, such as food record and food recall, and some of the food items lacked specificity (some food included in the FFQ were described broadly and were not well-specified; i.e. the methods of preparation and the proportion of ingredients in combination dishes were not indicated), which made it prone to estimation errors and; (4) analysis of the FFQ used three different food databases, which can potentially affect the accuracy of the dietary calcium intake measurement.

Conclusion and recommendations

The dietary acculturation which recently-immigrated Filipino women undergo in New Zealand significantly impacts on the amount and quality of their dietary calcium intake. Apart from calcium, further studies on other micronutrient intakes, such as iron, iodine and vitamin A, may also be explored, as these nutrients have a high prevalence of deficiency among Filipinos.

Gross lean mass strongly and significantly predicted the bone mineral status of these Filipino women in Auckland. Although 25(OH)D was not a statistically significant predictor of bone mineral status, the factors that affect vitamin D status of Filipino women warrant a more in-depth investigation due to the high prevalence of mild-moderate vitamin D deficiency observed in this study, and the existence of specific risks for low vitamin D status in this ethnic group.

Due to the significant contributions of Filipino immigrants not only in the health workforce but also to vital industries in New Zealand, such as aged care, agriculture and construction, the health status of this ethnic group must be proactively deliberated. If left unresolved, there is a potential for increased costs to the government health budget due to the health

consequences brought about by this disparity in the adequate intake of specific nutrients and in bone health indicators among Filipino women.

Chapter 4 **Conclusion and recommendations**

Conclusion

This study was conducted to determine the effect of dietary acculturation on the dietary calcium intake of Filipino women who have recently immigrated in New Zealand. Through this study, it was demonstrated that the dietary calcium intake of the participants significantly decreased as they immigrated to New Zealand. The change in dietary calcium intake was not limited to quantity, as a difference in diet quality was also observed. The amount of calcium contributed by fish, meat, fruit and vegetables in the Philippine intake was markedly decreased in the New Zealand intake. This could be due to the decreased availability of, and accessibility to, the fish, meat and other indigenous foods that are rich sources of calcium. Furthermore, both previous and current dietary calcium intakes are well below the recommended intake of both countries.

The secondary objective of this study was to explore the key predictors of bone mineral status of Filipino women in New Zealand. Gross lean mass consistently predicted hip, total body and spine *Z*-scores. This highlights the importance of maintaining healthy body composition (increasing lean mass), as it may have a protective effect against low bone mineral status through biomechanical loading, which induces metabolic reactions that trigger bone formation. However, previous and current dietary calcium intake, physical activity and serum 25(OH)D were not reported as significant predictors of bone mineral status. This was expected, since the study only measured recent physical activity and did not indicate the intensity of activities, which may potentially have a stronger association with bone mineral status. Serum 25(OH)D was also not a predictor due to the possible influence of season, since samples were obtained from spring to summer season, and because the values were too low to be able to detect a difference.

Strengths and limitations of the study

The main strength of this research is its high level of participant adherence. All of the participants returned for their second visit, completed the physical activity diary, and returned the accelerometer. Missing data was limited to serum 25(OH)D (n=1) when a difficulty was encountered in extracting blood from a participant, and total hip and femoral neck BMD

(*n*=3) due to technical errors from the DXA machine. Another strength of this research is its study population. To our knowledge, this is the first research which determined the effects of dietary acculturation among Filipinos in New Zealand. As a growing ethnic minority, this provided insights on the current situation in terms of the decline in their dietary calcium intake, their low vitamin D status (69% mild-moderate deficiency) and low bone mineral status. The median length of stay in New Zealand of the participants is also the study's strong point, because it was relatively short [17.5 (9.8, 24.0) months], which makes the estimation of the previous Philippine intake less prone to errors. Moreover, a methodological strength of the study is the separate setting of the first (previous intake) and second (current intake) FFQ. The two FFQs were administered at least two days apart to decrease bias in answering the questionnaires.

There were four identified limitations of this study. (1) The physical activity data gathered from the accelerometers only covered at least 48 hours for all the participants. In monitoring physical activity with accelerometers, a period of three to five days is recommended to get a reliable estimate for adults (Trost, McIver, & Pate, 2005). However, due to the limited availability of the accelerometers, each participant only had a chance to wear the accelerometers for two days. Due to this, weekday and weekend variations were also not noted. This information is critical, as the work shift pattern of the participants may vary and can substantially impact the amount of energy recorded per day. (2) Another limitation of this study is the lack of another dietary intake tool, such as a food record or food recall, to validate the results of FFQ. As the FFQ is focused only on determining calcium intake, other nutrients that affect bone health such as phosphorus, magnesium, protein, vitamin C and fibre, may interact with calcium and produce different effects (Lee et al., 2014; Orchard et al., 2014; Palacios, 2006; Sahni et al., 2010). The single-nutrient approach can be insufficient in quantifying the complex nutrient–nutrient interactions in studies involving free-living subjects, and the potential collinearity with some other nutrients may make it more difficult to isolate the effect of a single nutrient (Hu et al., 1999). Furthermore, some of the food items lacked specificity (some food included in the FFQ were described broadly and were not well-specified; i.e. the methods of preparation and the proportion of ingredients in combination dishes were not indicated), which made it prone to estimation errors. (3) The analysis of the FFQ is also considered as a limitation. In the first phase of the FFQ (the Philippine intake), the calcium content of food was determined through the Malaysian food composition database, as the Philippine food database was not accessible during the time of the analysis.

Moreover, as both the previous and the current FFQs contain some food items that are unique to each country, the calcium content of these items was obtained from the USDA Food database for both analyses. Due to this, potential errors in the estimation of dietary calcium intake for both FFQs may arise. Lastly, the comparison of previous and current FFQ data is a major study limitation due to the diminishing accuracy in gathering dietary recall data over time. This is because there may be a significant difference in the accuracy of FFQ results for phases 1 and 2 since one relied on long-term memory and the other was dependent on relatively short-term memory, respectively (4) A potential recruitment bias may be present, as participants were self-selected. The majority of the participants were working in the health sector, which translates to a higher health-seeking behaviour and greater nutrition knowledge. This can provide further explanation of the higher dietary calcium intake of Filipino women compared with a previous study and the Philippine national average.

Final recommendations

Apart from calcium, further studies on other micronutrients, such as iron and vitamin A, may also be explored, as the prevalence of nutrient deficiencies is high among Filipinos (Food and Nutrition Research Institute, 2008b, 2013).

To quantify the effect of dietary acculturation, a longitudinal cohort study design is recommended. In this study, the measurements were done only once, and the comparison of the changes in dietary calcium intake relied heavily on the participants' memories.

If a study of the same nature is conducted again, a dietary assessment tool (i.e. a food record or food recall) should be used to validate the findings of the FFQ. Several limitations of the FFQ, such as reliance on participants' memory and a single-nutrient approach, will be able to be eliminated by this cross-validation.

Due to the demonstrated high prevalence of low vitamin D status among recently-immigrated Filipino women, a more in-depth investigation is warranted. Predictors of serum 25(OH)D among this ethnic group must be further explored.

Finally, community or government-led programmes must be employed to protect this ethnic minority from the further health consequences of the low calcium intakes reported in this study. Nutrition education is a recommended programme to increase the awareness of

recently-immigrated Filipino immigrants on the possible alternative sources of calcium that are widely available and highly accessible in New Zealand.

Appendix A- supplementary methods

Sample size

Sample size calculation was based on the requirement of the regression analysis used to investigate predictors of bone health in this population. The general requirement is to have a minimum of 10 participants for each variable to be investigated (Harrell, 2001; Nunnally, 1978). The formula used is illustrated below:

No. of variables to be investigated x 10 = required samples

5 variables x 10 = 50 trial participants required

Blood sampling

Blood samples were taken by venepuncture from an antecubital vein in the forearm. Within 30 minutes after blood collection, samples were centrifuged at 2000g rpm for 10 minutes. Serum aliquots were immediately stored in polypropylene tubes at -80°C.

Serum 25(OH)D analysis

In this study, competitive chemiluminescent immunoassay (ADVIA Centaur Vitamin D Total assay) was utilised to determine serum 25(OH)D of the participants. It is an 18-minute assay which used three primary reagents: (1) anti-fluorescein antibody bound to paramagnetic particles; (2) acridinium ester-labelled anti-25(OH) vitamin D antibody; and (3) fluorescein-labelled vitamin D analog (Freeman, Wilson, Spears, Shalhoub, & Sibley, 2014). The amount of vitamin D present in the sample is determined by analysing the quantity of relative lights unit detected by the system, as these variables have an established inverse relationship.

The ADVIA Centaur Vitamin D Total assay is a certified procedure of the Vitamin D standardisation programme, as its reported results have had a strong agreement with the results of the programme's reference measurement procedure (Isotope-Dilution Liquid Chromatography-tandem Mass Spectrometry or ID-LC-MS/MS) (Freeman et al., 2014). It demonstrated a mean bias of 0.3% compared with the acceptable criteria of ±5.0%, and a mean imprecision of 5.5% compared with the criterion which allows less than 10.0%. The

recent vitamin D standardisation programme developed a reference method of determining 25(OH)D featuring LC-MS/MS to compare with other assays to be able to obtain uniform results in assays conducted in nutrition surveys, clinical laboratories, as well as from assay manufacturers (Phinney et al., 2012). The standardisation programme is a collaborative effort from institutions in the United States; namely, the National Institutes of Health, the Centers for Disease Control and Prevention, and the National Institute for Standards and Technology (NIST) with its reference laboratories located at NIST and the University of Ghent in Belgium (Chen et al., 2012).

Table 1. Measurements and methods validity

Domain	Measures/methods	Reference	Equipment	Concept captured
Anthropometry	Anthropometric assessment (height and weight) utilising NHANES protocol and standards	(Pi-Sunyer, 1998); (Lyznicki, Young, Riggs, & Davis, 2001)	Stadiometer and calibrated weighing scale	Body mass index
Total body composition Bone health indicator	DXA (dual-energy X-ray absorptiometry) scans Hip, spine, and whole body	(Maddalozzo, Cardinal, & Snow, 2002)	Dual-energy X-ray absorptiometry (DXA) Hologic QDR Discovery A, Hologic Inc, Bedford, MA. Integrated with APEX v.3.2 software	Bone mineral density and percent body fat
Biochemical	Competitive chemiluminescent immunoassay (Procedures attached)	(Chen et al., 2012)	ADVIA Centaur Vitamin D Total assay, Siemens Healthcare Diagnostics Inc, II, USA	Vitamin D status
Physical activity pattern	Provision of accelerometers	(Hagströmer, Oja, & Sjöström, 2007)	Actigraph MTI model 7164 (Manufacturing Technology Inc., Fort Walton Beach, FL)	Physical activity
Calcium intake	Food frequency questionnaire (FFQ)	Developed for this research	Utilisation of Foodworks7 Professional 2010 (Xyris Software Australia) Queensland,	Dietary calcium intake: amount and sources

	Australia, Malaysian Food Composition Database and USDA Food Composition Database

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ADVIA Centaur®
ADVIA Centaur® XP
Immunoassay Systems

Vitamin D Total (VitD)

Current Revision and Date	Rev. C, 2012-08	
Product Name	ADVIA Centaur® VitD assay (100 tests)	REF 10491994
	ADVIA Centaur VitD assay (500 tests)	REF 10631021
Materials Required but Not Provided	ADVIA Centaur VitD Calibrator, 2-pack	REF 10493589
	ADVIA Centaur VitD Calibrator, 6-pack	REF 10630911
	ADVIA Centaur VitD QC, 3-pack	REF 10632229
	ADVIA Centaur VitD Diluent, 2-pack	REF 10494100
	ADVIA Centaur VitD Diluent, 1 bottle	REF 10632114
Specimen Type	Human serum and plasma (EDTA, lithium-heparin, sodium-heparin)	
Sample Volume	20 µL	
Assay Range	4.2–150 ng/mL (10.5–375 nmol/L)	

Intended Use

The ADVIA Centaur Vitamin D Total (VitD) assay is for *in vitro* diagnostic use in the quantitative determination of total 25 (OH) vitamin D in human serum and plasma (EDTA, lithium-heparin, sodium-heparin) using the ADVIA Centaur and ADVIA Centaur XP systems. The ADVIA Centaur VitD assay is intended as an aid in the determination of vitamin D sufficiency.

Summary and Explanation

Vitamin D is a steroid hormone involved in the intestinal absorption of calcium and the regulation of calcium homeostasis. Vitamin D is essential for the formation and maintenance of strong, healthy bones.

Vitamin D deficiency can result from inadequate exposure to the sun, inadequate alimentary intake, decreased absorption, abnormal metabolism, or vitamin D resistance.[1] Recently, many chronic diseases such as cancer,[2,3,4] high blood pressure,[5] osteoporosis,[6,7] and several autoimmune diseases[9,10] have been linked to vitamin D deficiency. Whether consumed or produced, both forms of vitamin D (D_2 and D_3) are metabolized by the liver to 25(OH)D, and then converted in the liver or kidney into 1,25-dihydroxyvitamin D.[11] Vitamin D metabolites are bound to a carrier protein in the plasma and distributed throughout the body. The most reliable clinical indicator of vitamin D status is 25(OH)D because serum and plasma 25(OH)D levels reflect the body's storage levels of vitamin D, and 25(OH)D correlates with the clinical symptoms of vitamin D deficiency.[12]

Principles of the Procedure

The ADVIA Centaur VitD assay is a one-pass, 18-minute antibody competitive immunoassay that uses an anti-fluorescein monoclonal mouse antibody covalently bound to paramagnetic particles (PMP), an anti-25(OH) vitamin D monoclonal mouse antibody labeled with acridinium ester (AE), and a vitamin D analog labeled with fluorescein.

An inverse relationship exists between the amount of vitamin D present in the patient sample and the amount of relative light units (RLU) detected by the system.

Reagents

Reagent	Description	Storage	Stability
ADVIA Centaur VitD ReadyPack® primary reagent pack	**Lite Reagent** 5.0 mL/reagent pack: anti-VitD (monoclonal mouse) antibody labeled with acridinium ester (~0.8 µg/mL) in buffer with bovine serum albumin, mouse IgG, and sodium azide (< 0.1%)	Store the reagents upright at 2–8°C. Protect reagent packs from all heat and light sources.	Until the expiration date on the pack label. Onboard stability– 28 days.
ADVIA Centaur VitD ReadyPack primary reagent pack	**Solid Phase** 10.0 mL/reagent pack: anti-fluorescein (monoclonal mouse)-coated paramagnetic particles (PMP) (~0.60 mg/mL) in buffer with bovine serum albumin, surfactant, and sodium azide (< 0.1%)	Store the reagents upright at 2–8°C. Protect reagent packs from all heat and light sources.	Until the expiration date on the pack label. Onboard stability– 28 days.
ADVIA Centaur VitD ReadyPack primary reagent pack	**Ancillary Well Reagent** 5.0 mL/reagent pack: vitamin D-analog conjugated to fluorescein (~0.2 µg/mL) and 1-anilinonaphthalene-8-sulfonic acid in buffer with bovine serum albumin and sodium azide (< 0.1%)	Store the reagents upright at 2–8°C. Protect reagent packs from all heat and light sources.	Until the expiration date on the pack label. Onboard stability– 28 days.
ADVIA Centaur VitD ReadyPack ancillary reagent pack	**VitD Ancillary Pack Reagent** 25.0 mL/reagent pack: releasing agent in buffered saline with sodium azide (< 0.1%) and stabilizers	Store the reagents upright at 2–8°C. Protect reagent packs from all heat and light sources.	Until the expiration date on the pack label. Onboard stability– 28 days.
ADVIA Centaur VitD diluent ancillary reagent pack	**VitD Diluent Ancillary Reagent Pack** 25.0 mL/reagent pack: phosphate buffer with BSA, cholesterol and sodium azide (< 0.1%)	Store the reagents upright at 2–8°C. Protect reagent packs from all heat and light sources.	Until the expiration date on the pack label or 28 consecutive days after accessing the ancillary reagent pack.

Reagent	Description	Storage	Stability
ADVIA Centaur Wash 1* WASH 1	1500 mL/pack phosphate-buffered saline with sodium azide (< 0.1%) and surfactant	Store the reagents upright at 2–25°C.	Until the expiration date on the vial. Onboard stability–1 month.
ADVIA Centaur Wash 1* WASH 1	2500 mL/pack phosphate-buffered saline with sodium azide (< 0.1%) and surfactant	Store the reagents upright at 2–25°C.	Until the expiration date on the vial. Onboard stability–1 month.

* See *Materials Required but Not Provided*.

Note Discard reagent packs at the end of the 28-day onboard stability interval. Do not use reagents beyond the expiration date.

Protect reagent packs from all heat and light sources. Reagent packs loaded on the system are protected from light. Store unused reagent packs at 2° to 8°C away from heat and light sources.

Store reagent packs upright.

Warnings and Precautions

Safety data sheets (MSDS/SDS) available on www.siemens.com/diagnostics.

CAUTION

This device contains material of animal origin and should be handled as a potential carrier and transmitter of disease.

Note Some components of this product contain sodium azide as a preservative. Sodium azide can react with copper or lead plumbing to form explosive metal azides. On disposal, flush reagents with a large volume of water to prevent the buildup of azides. Disposal into drain systems must be in compliance with prevailing regulatory requirements.

Dispose of hazardous or biologically contaminated materials according to the practices of your institution. Discard all materials in a safe and acceptable manner and in compliance with prevailing regulatory requirements.

For *in vitro* diagnostic use.

Preparing Reagents

Reagents are liquid and ready to use. Remove all of the reagents from the refrigerator and mix all primary reagent packs by hand before loading them onto the system. Visually inspect the bottom of the reagent pack to ensure that all particles are dispersed and resuspended. For detailed information about preparing the reagents for use, see the system operator's guide.

Ensure that the system has sufficient primary and ancillary reagent packs. Load the primary reagent packs in the primary reagent area. You can use the arrows on the end label as a placement guide. The system automatically mixes the primary reagent packs to maintain homogeneous suspension of the reagents. Load the ancillary reagent pack in the ancillary reagent entry. For detailed information about loading reagents, refer to the system operating instructions or to the online help system.

For detailed information about preparing the system, refer to the system operating instructions or to the online help system.

Specimen Collection and Handling

The following recommendations for handling and storing blood samples are furnished by the Clinical and Laboratory Standards Institute (CLSI):[13]

Collecting the Specimen

- Collect all blood samples observing universal precautions for venipuncture. Handle all samples as if capable of transmitting disease.
- Human serum and plasma (EDTA, lithium-heparin, sodium-heparin) are the recommended sample types for this assay.
- Allow samples to clot adequately before centrifugation.
- Keep tubes stoppered and upright at all times.
- Test samples as soon as possible after collecting.
- Do not use samples that have been stored at room temperature for longer than 24 hours.
- Do not use specimens with obvious microbial contamination.

Storing the Specimen

- Tightly cap and refrigerate specimens at 2° to 8°C up to 7 days if the assay is not completed within 24 hours. Specimens may be stored on the clot up to 6 days.[14]
- Freeze samples at or below -20°C if the sample is not assayed within 7 days.[14]
- Freeze samples up to 4 times, and mix thoroughly after thawing.[14]
- Do not store in frost-free freezer.

Materials Provided

REF	Contents	Number of Tests
10491994	1 ReadyPack primary reagent pack containing ADVIA Centaur VitD Lite Reagent, Solid Phase, and Ancillary Well Reagent 1 ReadyPack ancillary pack containing ADVIA Centaur VitD Ancillary Reagent ADVIA Centaur VitD Master Curve card	100
10631021	5 ReadyPack primary reagent packs containing ADVIA Centaur VitD Lite Reagent, Solid Phase, and Ancillary Well Reagent 5 ReadyPack ancillary packs containing ADVIA Centaur VitD Ancillary Reagent ADVIA Centaur VitD Master Curve card	500

Materials Required but not Provided

Item	Description	
REF 10493589	ADVIA Centaur VitD Calibrator, 2-pack	2 vials of low calibrator CAL L 2 vials of high calibrator CAL H
REF 10630911	ADVIA Centaur VitD Calibrator, 6-pack	6 vials of low calibrator CAL L 6 vials of high calibrator CAL H
REF 10632229	ADVIA Centaur VitD Control, 3-pack	3 vials of control 1 CONTROL 1 3 vials of control 2 CONTROL 2
REF 10494100	ADVIA Centaur VitD Diluent	2 ancillary reagent packs of diluent VITD DIL
REF 10632114	ADVIA Centaur VitD Diluent	1 bottle containing 25 mL VITD DIL
01137199 (112351)	ADVIA Centaur Wash 1 WASH 1	2 x 1500 mL/pack
or		
03773025	ADVIA Centaur Wash 1 WASH 1	2 x 2500 mL/pack

Assay Procedure

For detailed instructions on performing the procedure, refer to the system operating instructions or to the online help system.

Before placing samples on the system, ensure that samples have the following characteristics:

- Samples are free of fibrin or other particulate matter. Remove particulates by centrifugation at 1000 x g for 10 to 15 minutes.
- Samples are free of bubbles.

This assay requires 20 µL of sample for a single determination. This volume does not include the unusable volume in the sample container or the additional volume required when performing duplicates or other tests on the same sample. For detailed information about determining the minimum required volume, refer to *Sample Volume Requirements* in the system operating instructions or to the online help system.

The ADVIA Centaur and ADVIA Centaur XP systems automatically perform the following steps:

1. Dispenses 20 µL of sample into a cuvette, and incubates for 15 seconds.
2. Dispenses 200 µL of Ancillary Pack Reagent, and incubates for 4.5 minutes at 37°C.
3. Dispenses 50 µL of Lite Reagent, and incubates for 5.5 minutes at 37°C.
4. Dispenses 100 µL of Solid Phase reagent, and 50 µL of ancillary well reagent, and incubates for 2.75 minutes at 37°C.
5. Separates the Solid Phase from the mixture, and aspirates the unbound reagent.
6. Washes the cuvette with Wash 1.
7. Dispenses 300 µL each of Acid Reagent and Base Reagent to initiate the chemiluminescent reaction.

The ADVIA Centaur systems report results according to the selected option, as described in the system operating instructions or in the online help system.

Calibrating the Assay

The ADVIA Centaur VitD assay requires a Master Curve calibration when using a new reagent lot number. For each new lot number of Lite Reagent and Solid Phase, use the bar-code reader or keyboard to enter the Master Curve values on the system. The Master Curve card contains the Master Curve values. For detailed information about entering Master Curve values, refer to the system operating instructions or to the online help system.

Calibrate the assay at the end of the 28-day calibration interval. Additionally, this assay requires a two-point calibration when:

- Changing lot numbers of primary reagent packs.
- Replacing system components.
- Quality control results are repeatedly out of range.

For detailed information about entering calibration values, refer to the system operating instructions or to the online help system.

Using Bar-Code Labels

Calibrator bar-code labels are lot-number specific. Do not use bar-code labels from one lot of calibrators with any other lot of calibrators.

Use the ADVIA Centaur VitD Calibrator bar-code labels to identify the Low and High Calibrator sample cups when performing the ADVIA Centaur VitD assays. Place the bar-code label on the sample cup so that the readable characters on the side of the label are vertical on the sample cup.

Performing a Calibration

Each lot of calibrators contains a Calibrator Assigned Value card to facilitate entering the calibration values on the system. Enter the values using the bar-code scanner or the keyboard.

Perform the calibration procedure using the following steps:

Note This procedure uses calibrator volumes sufficient to measure each calibrator in duplicate.

1. Schedule the calibrators to the worklist.
2. Label two sample cups with calibrator bar-code labels: one for the low and another for the high.
3. Gently mix the Low and High Calibrators and dispense at least 0.5 mL into the appropriate sample cups.

4. Load the sample cups in a rack.
5. Place the rack in the sample entry queue.
6. Ensure that the assay and ancillary reagents are loaded.
7. Start the entry queue, if required.

Note Dispose of any calibrator remaining in the sample cups after 10 hours. Do not refill sample cups when the contents are depleted; if required, dispense fresh calibrators.

Performing Quality Control

Follow government regulations or accreditation requirements for quality control frequency.

To monitor system performance and chart trends, as a minimum requirement, 2 levels of quality control material should be assayed on each day that samples are analyzed. Quality control samples should also be assayed when performing a two-point calibration. Treat all quality control samples the same as patient samples.

For quality control of the ADVIA Centaur VitD assay, use ADVIA Centaur VitD quality control material. Refer to the Expected Value card for the suggested expected values specific for the lot number of the controls.

For detailed information about entering quality control values, refer to the system operating instructions or to the online help system.

Using Bar-Code Labels

Control bar-code labels are lot-number specific. Do not use bar-code labels from one lot of controls with any other lot of controls.

Use the ADVIA Centaur VitD quality control bar-code labels to identify the positive and negative sample cups when performing the ADVIA Centaur VitD assay. Place the bar-code label on the sample cup so that the readable characters on the side of the label are vertical on the sample cup.

Perform the quality control procedure using the following steps:

Note This procedure uses control volumes sufficient to measure each control in duplicate.

1. Schedule the quality control samples to the worklist.
2. Label two sample cups with quality control bar-code labels: one for the positive and another for the negative.
3. Gently mix the quality control materials and dispense at least 250 µL into the appropriate sample cups.
4. Load the sample cups in a rack.
5. Place the rack in the sample entry queue.
6. Ensure that the assay reagents are loaded.
7. Start the entry queue, if required.

Note Dispose of any quality control materials remaining in the sample cups after 10 hours. Do not refill sample cups when the contents are depleted; if required, dispense fresh quality control materials.

Taking Corrective Action

If the quality control results do not fall within the Expected Values or within the laboratory's established values, do not report results. Take the following actions:

1. Determine and correct the cause of the unacceptable control results:
   a. Verify that the materials are not expired.
   b. Verify that required maintenance was performed.
   c. Verify that the assay was performed according to the instructions for use.
   d. Rerun the assay with fresh quality control samples, and confirm that quality control results are within acceptable limits before running patient samples.
   e. If the quality control results are not within acceptable limits, recalibrate the assay, and repeat step d.
   f. If necessary, contact your local technical support provider or distributor for assistance.
2. Repeat testing of patient samples before reporting results.

Perform corrective actions in accordance with your established laboratory protocol.

Results

Results should always be interpreted in conjunction with the patient's medical history, clinical presentation, and other findings.

The system reports serum and plasma VitD results in ng/mL (common units) or nmol/L (SI units), depending on the units defined when setting up the assay. The conversion formula is 1 ng/mL = 2.5 nmol/L.

For detailed information about how the system calculates results, refer to the system operating instructions or to the online help system.

Dilutions

Dilute and retest serum samples with vitamin D levels greater than 150 ng/mL (375 nmol/L) to obtain accurate results. Manually dilute the patient samples with ADVIA Centaur Vitamin D Diluent, and then load the diluted sample in the sample rack, replacing the undiluted sample. The recommended dilution is 1:2.

Ensure that results are mathematically corrected for dilution. If a dilution factor is entered when scheduling the test, the system automatically calculates the result.

Limitations

Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays.[15] Patients routinely exposed to animals or to animal serum products can be prone to this interference and anomalous values may be observed. Additional information may be required for diagnosis.

Do not use hemolyzed samples. Hemoglobin at concentrations ≥ 155 mg/dL will cause falsely depressed values.

Expected Values

From a review of the available literature,[1,16,17,18] the recommendations for 25(OH)D levels are:

Vitamin D Status	Range
Deficiency	< 20 ng/mL (50 nmol/L)
Insufficiency	20–30 ng/mL (50–75 nmol/L)
Sufficiency	30–100 ng/mL (75–250 nmol/L)
Toxicity	> 100 ng/mL (250 nmol/L)

Data using the ADVIA Centaur VitD assay was obtained on serum samples collected from 542 adults: 258 adults not taking supplements containing vitamin D, and 284 adults taking supplements containing vitamin D. The samples were collected in different seasons and different geographical regions of the United States. Samples with abnormal values for PTH, calcium, magnesium, phosphorus, and TSH were excluded from this study. Based on the 95% confidence interval, the following values were established following CLSI guideline C28-A2.[19]

The following values were obtained:

Observed Values	
Median 25 OH Vitamin D	21.1 ng/mL (52.8 nmol/L)
Observed Range 2.5th to 97.5th Percentile	10.6–43.4 ng/mL (26.5–108.5 nmol/L)

As with all *in vitro* diagnostic assays, each laboratory should determine its own reference range(s) for the diagnostic evaluation of patient samples.[19]

Performance Characteristics

Assay Range

The ADVIA Centaur VitD assay measures 25(OH) vitamin D from concentrations of 4.2 to 150 ng/mL (10.5 to 375 nmol/L). The low end of the assay range is defined by the limit of quantitation (LoQ).

Specificity

The ADVIA Centaur VitD Total assay shows high specificity for 25(OH) vitamin D_2 and 25(OH) vitamin D_3. The following compounds were tested with total 25(OH) vitamin D concentrations of 35 and 115 ng/mL. Percent change is calculated as:

Percent cross-reactivity = (corrected assay value / amount of compound spiked) x 100

The following results were obtained:

Compound	Concentration (ng/mL)	Cross-Reactivity (%)
1, 25 $(OH)_2$ Vitamin D_2	100	4.0
1, 25 $(OH)_2$ Vitamin D_3	100	1.0
25 OH Vitamin D_2	30	104.5
25 OH Vitamin D_3	30	100.7
Paricalcitol	24	0.1
3-epi-25-OH Vitamin D_3	100	1.1
Vitamin D_2	1000	0.5
Vitamin D_3	1000	0.3

Sensitivity

The limit of blank (LoB), limit of detection (LoD), and the limit of quantitation (LoQ) were determined as described in CLSI Document EP17-A.[20] The ADVIA Centaur VitD assay had an LoB of 1.7 ng/mL (4.3 nmol/L), an LoD of 3.20 ng/mL (8.0 nmol/L), and an LoQ of 4.2 ng/mL (10.5 nmol/L). The LoD is defined as the lowest concentration of 25(OH) vitamin D that can be detected with 95% probability.

The functional sensitivity of the ADVIA Centaur VitD assay is 3.33 ng/mL (8.33 nmol/L). The functional sensitivity was determined using multiple samples in the range of 2 to 10 ng/mL (5 to 25 nmol/L). All samples were assayed twice a day in replicates of 4 over 10 days using 2 lots (n = 320 for each sample) of ADVIA Centaur VitD reagents.

Linearity

Linearity was evaluated according to the CLSI protocol EP6-A.[21] A sample containing high levels of total 25(OH) vitamin D was mixed in various proportions with a sample containing low levels of total 25(OH) vitamin D. The resulting sample mixtures were assayed for total vitamin D. On the ADVIA Centaur system, the VitD assay is linear from 4.2 to 150 ng/mL.

Precision

Precision was evaluated according to the CLSI protocol EP5-A2.[22] Six samples were assayed twice a day in replicates of 4, over 20 days (n = 160 replicates per sample) using the ADVIA Centaur VitD assay. The following results were obtained:

Mean	Within-Run		Total	
(ng/mL)	SD	%CV	SD	%CV
11.7	0.81	7.0	1.30	11.1
18.0	1.20	6.6	1.74	9.6
32.4	1.87	5.8	3.17	9.8
49.9	2.22	4.5	4.07	8.2
55.8	2.66	4.8	4.38	7.8
132.1	3.53	2.7	6.33	4.8

Specimen Collection Comparison

The ADVIA Centaur VitD assay was evaluated using different specimen matrices and tube collection types. A specimen collection study was performed using 231 matched specimens drawn in different tube types including serum red top, serum separator tube, EDTA, lithium-heparin, and sodium-heparin. Vitamin D values ranged from 11.9 to 136.9 ng/mL (29.8 to 342.3 nmol/L). Linear regression analysis was performed using the following tube types:

- serum (x) vs. Serum Separator Tube (y_1)
- serum (x) vs. EDTA (y_2)
- serum (x) vs. lithium-heparin (y_3)
- serum (x) vs. sodium-heparin (y_4)

No significant difference between tube types was observed. The following results were obtained:

Tube Types*	Slope	Intercept	R
Serum vs. Serum Separator Tube	1.01	-0.33	0.994
Serum vs. EDTA	1.09	-0.17	0.993
Serum vs. Lithium Heparin	1.04	0.18	0.992
Serum vs. Sodium Heparin	1.04	0.90	0.992

* This study was performed using Becton Dickinson tubes.

Method Comparison

For 195 samples in the range of 6.2 to 150 ng/mL (15.3 to 375 nmol/L), the relationship between the ADVIA Centaur VitD assay (y) and the IDS 25-Hydroxy Vitamin D EIA assay is described using Deming regression as:

ADVIA Centaur VitD = 1.00 (IDS 25-Hydroxy Vitamin D EIA) + 2.22 ng/mL, r = 0.96

For 580 samples in the range of 4 to 150 ng/mL (10 to 375 nmol/L), the relationship between the ADVIA Centaur VitD assay (y) and liquid chromatography coupled with tandem mass spectrometry (LC/MS/MS) (x) is described using Deming regression as:

ADVIA Centaur VitD = 1.15 (LC/MS/MS) + 0.70 ng/mL, r = 0.91

Dilution Recovery

Eight serum samples in the range of 154 to 237 ng/mL (385 to 592.5 nmol/L) of total 25 (OH) vitamin D were diluted 1:2, with ADVIA Centaur VitD diluent and assayed for recovery and parallelism. The recoveries ranged from 91 to 109% with a mean of 99.6%.

Sample	Dilution	Observed ng/mL (nmol/L)	Expected ng/mL (nmol/L)	Recovery %
1	1:2	76.9	77.2	100
2	1:2	93.0	95.6	97
3	1:2	83.5	89.0	94
4	1:2	124.8	118.6	105
5	1:2	126.2	116.0	109
6	1:2	96.3	93.1	103
7	1:2	84.2	85.6	98
8	1:2	71.9	79.0	91
Mean				**99.6**

Interferences

Interfering substances were tested as described in CLSI Document EP7-A2[23] using the ADVIA Centaur VitD assay.

Specimens That Are	Demonstrate ≤ 10% Change in Results Up To
hemolyzed	155 mg/dL of hemoglobin
lipemic	540 mg/dL of triglycerides
icteric	40 mg/dL of conjugated bilirubin
icteric	40 mg/dL of unconjugated bilirubin

Specimens That Contain	Demonstrate ≤ 10% Change in Results Up To
cholesterol	350 mg/dL
uric acid	20 mg/dL
human immunoglobulin	12 g/dL

Standardization

The ADVIA Centaur VitD assay is standardized using internal standards which are traceable to LC/MS/MS. The relationship between the ADVIA Centaur VitD assay (y) and liquid chromatography coupled with tandem mass spectrometry (LC/MS/MS) (x) is described using linear regression as:

ADVIA Centaur VitD = 1.01 (LC/MS/MS) + 8.9 ng/mL, r = 0.99

Technical Assistance

For customer support, contact your local technical support provider or distributor.

www.siemens.com/diagnostics

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US Pats 5,609,822; 5,788,928

Origin: US

Siemens Healthcare Diagnostics Inc.
Tarrytown, NY 10591-5097 USA

EC REP Siemens Healthcare Diagnostics Ltd.
Sir William Siemens Sq.
Frimley, Camberley, UK GU16 8QD

Understanding the Symbols

The following symbols may appear on the product labeling:

Symbol	Definition	Symbol	Definition
	*In vitro* diagnostic medical device		Catalog number
	Manufacturer		Authorized Representative in the European Community
	CE Mark		CE Mark with identification number of notified body
	Consult instructions for use		Caution! Potential Biohazard
	Do not freeze (> 0°C)		Temperature limitation (2–8°C)
	Lower limit of temperature (≥ 2°C)		Upper limit of temperature (≤ -10°C)
	Keep away from sunlight		Use by
	Store upright		Shake the reagent pack vigorously. Refer to *Preparing Reagents* in the assay-specific ADVIA Centaur product instructions for detailed information.
	Batch code		Contains sufficient for (n) tests
2010-01	Date format (year-month)		Printed with soy ink
	Green dot		Recycle

Appendix B — Supplementary results

Table 2. Correlations between predictor variables and bone mineral status

	Measurements	1	2	3	4	5	6[β]	7	8
1	FFQ 2[α]	1.00	0.42***	-0.05	–0.13	-0.17	-0.01	<0.001	-0.11
2	FFQ 1[α]	0.42***	1.00	0.09	–0.19	0.12	0.08	0.23*	0.13
3	Physical activity[α]	-0.05	0.09	1.00	0.06	0.57***	0.35*	0.48***	0.36*
4	Serum 25(OH)D[α]	-0.13	-0.19	0.06	1.00	-0.10	0.07	0.09	0.03
5	Gross lean mass[α]	-0.17	0.12	0.57***	–0.10	1.00	0.44***	0.53***	0.49***
6	Hip *Z*-score[β]	0-.01	0.08	0.35*	0.07	0.44***	1.00		
7	Lumbar *Z*-score[α]	<0.001	0.23*	0.48***	0.09	0.53***		1.00	
8	Whole body *Z*-score[α]	-0.11	0.13	0.36*	0.03	0.49***			1.00

Abbreviations: FFQ 2 = Food Frequency Questionnaire Phase 2 (New Zealand intake); FFQ 1 = Food Frequency Questionnaire Phase 1 (Philippine intake); gross lean mass = bone mass and fat mass subtracted from total mass

Correlation coefficients (*r*) from Pearson correlation (one-tailed)

P values: **p*<0.05; ***p*<0.01; ****p*<0.001

[α]*n*=61

[β]*n*=58

Based on Table 1, the following correlations were observed:

- There was a significant positive correlation between physical activity and the hip *Z*-score [*r*=0.35, *n*=58, *p*=0.004]

- There was a significant positive correlation between gross lean mass and the hip *Z*-score [*r*= 0.44, *n*=58, *p*<0.001]

- There was a significant positive correlation between FFQ1 and the lumbar *Z*-score [*r*=0.23, *n*=61, *p*=0.036]

- There was a significant positive correlation between physical activity and the lumbar *Z*-score [*r*=0.48, *n*=61, *p*<0.001]

- There was a significant positive correlation between gross lean mass and the lumbar *Z*-score [r=0.53, n=61, p<0.001]

- There was a significant positive correlation between FFQ1 and FFQ2 [r=0.42, n=61, p<0.001]

- There was a significant positive correlation between physical activity and gross lean mass [r=0.57, n=61, p<0.001]

- There was a significant positive correlation between physical activity and the whole body *Z*-score [r=0.36, n=61, p=0.002]

- There was a significant positive correlation between gross lean mass and the whole body *Z*-score [r=0.49, n=61, p<0.001]

Gross lean mass associations with bone mineral status

Observable positive correlations were indicated in the scatterplots comparing gross lean mass with the Z-scores measured in the hip total, lumbar and whole body areas.

Hip *Z*-score

Figure 1. Scatterplot diagram between gross lean mass and hip (total) *Z*-score

Abbreviations: ffbfmass = fat-free bone-free mass or gross lean mass

Lumbar *Z*-score

Figure 2. Scatterplot diagram between gross lean mass and lumbar *Z*-score

Abbreviations: ffbfmass = fat-free bone-free mass or gross lean mass

Whole body *Z*-score

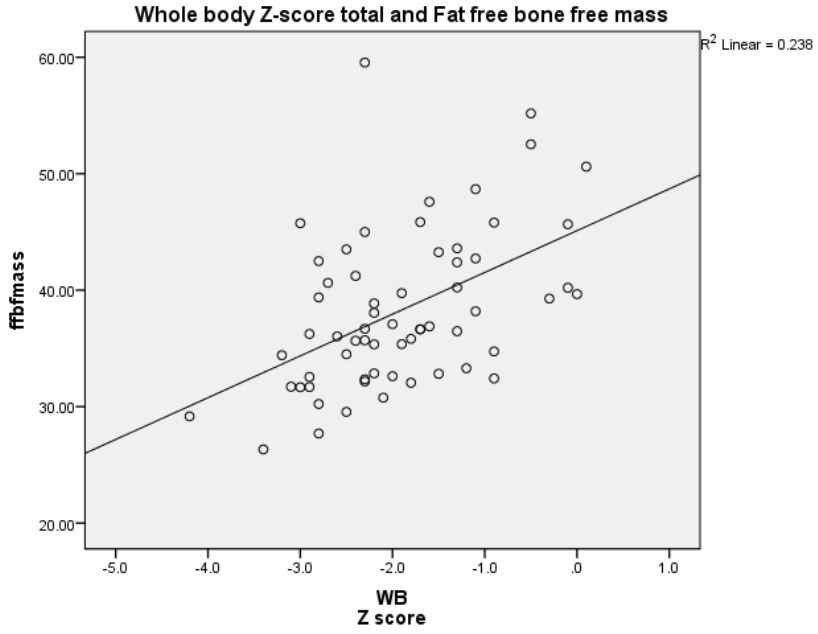


Figure 3. Scatterplot diagram between gross lean mass and whole body *Z*-score

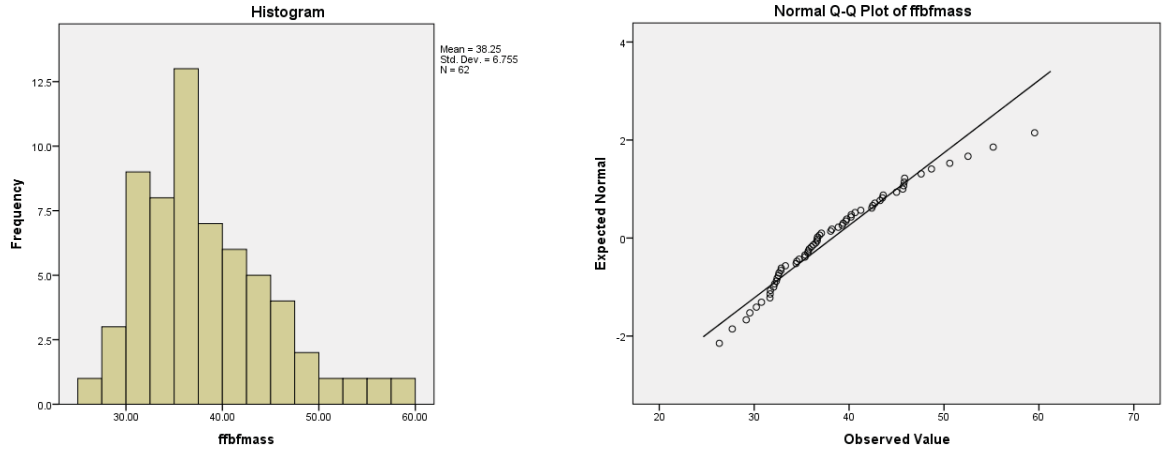
Abbreviations: ffbfmass = fat-free bone-free mass or gross lean mass

Associations between gross lean mass and body fat percentage

Gross lean mass was derived from the body fat percentage obtained from the DXA scans. To determine whether the two variables were correlated, the following tests were done:

Determination of the normality of the two variables

Testing for the normality of the body fat percentage and gross lean mass, the body fat percentage indicated a normal distribution as both Kolmogorov–Smirnov and Shapiro–Wilk tests had $p>0.05$. FFBF mass or gross lean mass did not indicate normality ($P<0.05$) for both tests, implying non-normally distributed data. Therefore, Spearman's correlation was utilised.



Figures 4 and 5. Histogram and Q–Q plots for gross lean mass

Abbreviations: ffbfmass = fat-free bone-free mass or gross lean mass

The histogram chart of gross lean mass illustrates a distribution skewed to the left. The Q–Q plot also showed a non-normal distribution, as the data points do not follow the designated line.

Table 3. Testing for correlations between body fat percentage and gross lean mass

	Variables	1	2
1	Gross lean mass [α]	1.00	0.40**
2	Body fat percentage [α]	0.40**	1.00

Correlation coefficients (r) from Pearson correlation (two-tailed)
**p=0.001
[α]n=62

Body fat mass and gross lean mass were significantly correlated with p=0.001 significance.

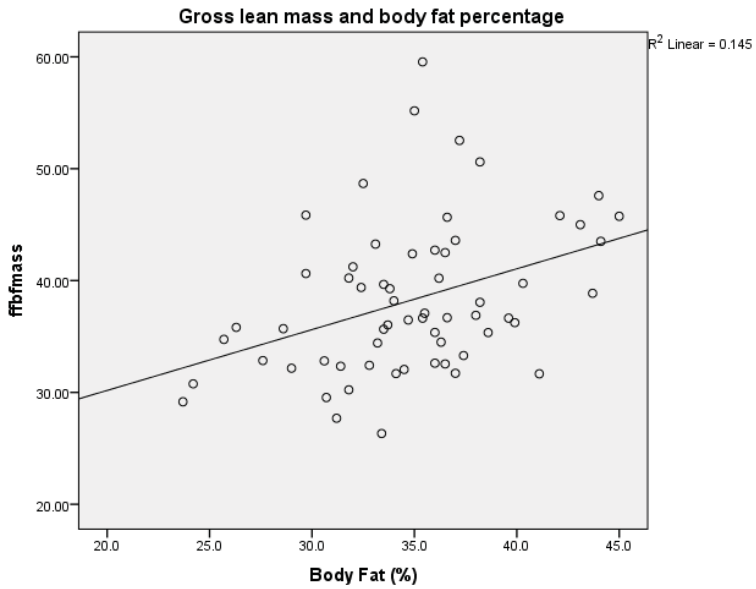


Figure 6. Scatterplot diagram between gross lean mass and body fat percentage

Abbreviations: ffbfmass = fat-free bone-free mass or gross lean mass

Figure 6 illustrates the positive correlation between gross lean mass and body fat percentage, as values were clustered along the best fit line with an upward slope. This indicates that, as body fat percentage increases, gross lean mass also tends to increase. Therefore, because of this significant and strong correlation, only gross lean mass will be applied in the study's regression model, as its collinearity with body fat percentage may substantially affect the model.

Appendix C— Questionnaires and materials used in conducting the research

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA

Massey Institute of Food Science and Technology

Massey University

Private Bag -102-904

North Shore Mail Centre

Filipino women's health study

An investigation on the effects of changing diets on calcium intake among Filipino immigrants in New Zealand

Information for Study Participants

Please read this information carefully and ask questions about anything you want to clarify before deciding to take part in the research.

You have been invited to take part in a university research project that will mainly 1) examine the changes in calcium intake because of changing diets from migration; and 2) find out the factors that affect bone mineral status among recently-immigrated Filipino women living in Auckland. The research is being conducted by a team of investigators at the Human Nutrition Research Unit (HNRU) in Massey University with the following contact details:

Dr. Pamela von Hurst (Principal Investigator) Ph: (09) 213 6657 Email: p.r.vonhurst@massey.ac.nz	Rosario Pillar Monzales (MSc Student) Ph: +64220284112 Email: riomonzales@gmail.com	Liana Norrish (MSc Student) Ph: +64277807362 Email: ltnorrish@gmail.com	Owen Mugridge (Postgraduate Teaching Technician) Ph: (09) 414 0800 ext. 43650 Email: O.Mugridge@massey.ac.nz
School of Food and Nutrition, College of Health, Massey University Auckland			

What is the purpose of this research?

Calcium, which we mostly get from milk, small fishes and green leafy vegetables, is an important nutrient for bone health. A new survey suggests that the Filipino diet lacks calcium. This situation may be worsened or improved once Filipinos migrate overseas. Lack of calcium in the diet is known to increase risk of osteoporosis and incidence of fractures as we age.

Currently, there is no information on the effect of dietary changes on the calcium intake of Filipino women living in New Zealand. Therefore, this study aims to find out the effect of these changes on the calcium intake of Filipino women living in Auckland and other factors affecting their bone health.

The information obtained from this research will be used to raise awareness among the Filipino community and to communicate with government authorities that protect migrants' health and rights for potential programmes that promote good health among Filipino women living in New Zealand.

Why have I been invited to participate in this research?

You are invited to participate in this research because you are a 20-45-year-old Filipino woman who has recently immigrated (within the last five years) to New Zealand. However, if you are pregnant, breast-feeding, or are a smoker, you are not eligible.

What is going to happen?

If you agree to participate in this research, you will be asked to visit HNRU on the Massey, Albany Campus, twice.

HNRU Visit 1

A. Study orientation, informed consent and questionnaires

You will be briefed about the study and asked to fill out health, family history and general questionnaires and a consent form. Training on using accelerometer (a device that measures physical activity) will also be done. After the visit, you will be asked to wear the accelerometer for 2 days and fill in a physical activity diary.

B. Body measurements

We will measure your height, and weight.

C. Blood sample

Blood sample will be obtained through the forearm. You will be required to fast 10-12 hours before the blood is taken, but please make sure that you have had adequate water. From this sample, we will measure your vitamin D status.

D. Food Frequency Questionnaire (Phase 1)

You will fill-up an online questionnaire about your food intake while still in the Philippines.

HNRU Visit 2

Aside from returning your physical activity diary and accelerometer, the following measurements will also be conducted.

A. DXA scan

With this test, you will be asked to wear a robe supplied by the researchers. DXA measures the density of your bones, and also estimates the difference between lean and fat tissue accurately through X-ray beams at different energies. We will be conducting a full body scan, as well as smaller scans of your spine and one hip. While there is no dose of radiation that is considered harmless, the dose for this scan is very low and unlikely to cause harm. The total effective dose of radiation is around 10.8 microsieverts (μSv), a much lower dose than the range normally used in medical diagnostics (for example: 50 μSv for a dental X-ray).

Because we don't want to expose unborn babies to even this small dose of X-rays, women are asked to book a time within the first 14 days from the first day of their last menstrual period to be completely sure they are not pregnant. Researchers who will undertake these measurements of body composition are fully trained and accredited.

You will need to remove all jewellery and body-piercings for the tests. To reduce risk of loss, we advise that you do this at home.

B. Food Frequency Questionnaire (Phase 2)

You will be asked complete this questionnaire about your food intake in New Zealand via paper or online.

The total amount of time involved for the 2 visits will be approximately 1 and ½ hour (90 minutes).

What are the benefits and risks of taking part in this research?

You will get a full report of your results and suggestions for further action (if required) will be sent to you via mail or email. Results that will fall within the range of abnormal values will be screened and a signed referral letter will be provided for your GP.

A summary of findings of the research will also be sent to you by February 2017. Most importantly, the major benefit in participating in this research is that you are able to contribute to research knowledge which will potentially further benefit the health of Filipino immigrants in New Zealand.

A petrol or food voucher will also be provided for each visit.

Will my taking part in the research be maintained confidential?

All study participants will be assigned a code to prevent identification and maintain confidentiality.

Access to your information is limited to the primary researcher and the supervisors and all data will be stored in a secure location. Your identity will not be revealed and your confidentiality will be protected in any reports of this study which may be published or presented at seminars or conferences. No personal information, results or answers to questionnaires will be shared with any other institutions, or government authorities. All data remains anonymous and completely confidential.

Upon the completion of this research, your name and assigned code will be destroyed while any raw data which results of the research depend will be maintained in a secure storage for 10 years and after which, will also be destroyed.

Who is funding the research?

The research made possible through the funding of Massey University Graduate Research Fund and the New Zealand Aid Scholarship Post-Graduate Research and Thesis Allowance

Participants' rights

You are under no obligation to accept this invitation. If you decide to participate, you have the right to:

- decline to answer any particular question;
- withdraw from the study (at any time);
- ask any questions about the study at any time during participation;
- provide information on the understanding that your name will not be used unless you give permission to the researcher;
- be given access to a summary of the project findings when it is concluded.

Massey Human Ethics Committee Approval Statement

This project has been reviewed and approved by the Massey University Human Ethics Committee: Southern A, Application 16/31. If you have any concerns about the conduct of this research, please contact Mr Jeremy Hubbard, Chair, Massey University Human Ethics Committee: Southern A, telephone 04 801 5799 x 63487, email humanethicsoutha@massey.ac.nz.

Project contacts

For further information, questions or concerns about the project, kindly contact the principal investigators or the project supervisor (details on page 1)

Compensation for Injury

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

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

MASSEY UNIVERSITY
COLLEGE OF HEALTH
TE KURA HAUORA TANGATA

Filipino Women's Health study

An investigation on the effects of changing dietary patterns on calcium intake among Filipino immigrants in New Zealand

PARTICIPANT CONSENT FORM

I have read the Information Sheet and have had the details of the study explained to me. My questions have been answered to my satisfaction, and I understand that I may ask further questions at any time.

I agree to participate in this study under the conditions set out in the Information Sheet.

I am willing to be contacted in connection with future research projects within the Massey Institute of Food Science and Technology and for this purpose I agree to my contact information being retained on condition that it is in no way linked to the data I have supplied for this research project.

Signature: ..... **Date:** .....

Full Name – Printed .....

Please send me a referral letter to my General Practitioner should any of my results fall outside the normal range.

(Put a line through this statement if you do NOT wish to be informed or receive a referral letter)

MASSEY UNIVERSITY
COLLEGE OF HEALTH
TE KURA HAUORA TANGATA

Filipino Women's Health study

Visit 1

Informed consent and detail check _____

General health & demographic questionnaire _____

On line questionnaire

FFQ link (Phase 1) _____

Height _____

Weight _____

Blood Sampling (initials/time) _____/_____

Take home pack

Accelerometer/Time started _____/_____

Physical Activity Diary _____

Return envelope _____

Voucher no. _____

Thank you very much for your participation!

The Filipino Women's Health Research team

MASSEY UNIVERSITY
COLLEGE OF HEALTH
TE KURA HAUORA TANGATA

Filipino Women's Health study

Visit 2

DXA _____

On line questionnaire

FFQ link (Phase 2) _____

Returned

Accelerometer and Physical Activity Diary _____

Voucher no. _____

Thank you very much for your participation!

The Filipino Women's Health Research team

MASSEY UNIVERSITY
COLLEGE OF HEALTH
TE KURA HAUORA TANGATA

Filipino Women's Health study

Anthropometry & blood pressure

Data sheet

Body Composition INDICATOR	MEASUREMENT	FINAL VALUE
Height	1.	
	2.	
	3.	
RECORDER:		
Weight	DXA	
RECORDER:		

PHASE 1-Food Questionnaire on Usual Calcium Intake in the Philippines

Name: _____ Date: _____

Your usual food intake **while you are still in the Philippines** will be evaluated in this questionnaire. Kindly follow the instructions below and if you have any questions, please do not hesitate to send us an email or contact us via phone.

General Instructions

1. Complete each question as much as you can. Estimate if you are unsure. A guess is better than leaving it blank.
2. Mark the column with ✓ to show how often, on average, you ate the foods below.

	HOW OFTEN DID YOU EAT THESE FOODS?									
	Medium serving size	**NEVER or less than once per month**	**1** per month	**2–3** per month	**1** per week	**2** per week	**3–4** per week	**5–6** per week	**1** per day	**2+** per day
White breads, including toast, sandwich, bagels, rolls and English muffins	2 slices or 1 medium	☐	☐	☐	☑	☐	☐	☐	☐	☐

FOR EXAMPLE: This person ate white bread every Sunday. She usually ate about 2 slices.

I. GRAINS AND CEREAL PRODUCTS

	HOW OFTEN DID YOU EAT THESE FOODS?									→
	NEVER or less than once per month	**1** per month	**2–3** per month	**1** per week	**2** per week	**3–4** per week	**5–6** per week	**1** per day	**2+** per day	**Medium serving size**
1. Rice	☐	☐	☐	☐	☐	☐	☐	☐	☐	½ cup
2. Corn grits	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 cup
3. Bread	☐	☐	☐	☐	☐	☐	☐	☐	☐	2 slices or 2 pieces
4. Bihon, green bean noodles, etc.	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 cup
5. Instant noodles, spaghetti, etc.	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 cup
6. Cereal snacks	☐	☐	☐	☐	☐	☐	☐	☐	☐	½ cup
7. Mung bean	☐	☐	☐	☐	☐	☐	☐	☐	☐	½ cup
8. Sweet potato	☐	☐	☐	☐	☐	☐	☐	☐	☐	½ piece
9. Bread-based dishes	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 cup
10. Cakes and muffins	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 slice
11. Breakfast cereals	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 cup
12. Sugar and sweets	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 tablespoon or 1 piece
13. Potatoes, kumara and taro	☐	☐	☐	☐	☐	☐	☐	☐	☐	½ piece
14. Pies and pastries	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 small piece
15. Savoury sauces and condiments	☐	☐	☐	☐	☐	☐	☐	☐	☐	¼ cup
16. Puddings and desserts	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 piece or 1/3 cup
17. Biscuits	☐	☐	☐	☐	☐	☐	☐	☐	☐	2 pieces
18. Snack bars	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 piece

II. MEAT AND POULTRY

	HOW OFTEN DID YOU EAT THESE FOODS?									→
	NEVER or less than once per month	**1** per month	**2–3** per month	**1** per week	**2** per week	**3–4** per week	**5–6** per week	**1** per day	**2+** per day	**Medium serving size**
19. Small fish with bone and head eaten	☐	☐	☐	☐	☐	☐	☐	☐	☐	¼ cup or 2 pieces
20. Fried fish and dried fish with bone and/or head	☐	☐	☐	☐	☐	☐	☐	☐	☐	1/3 cup or 1 piece
21. Paksiw, escabetche, fried large fish, etc.	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 slice or 1/3 cup
22. Meat adobo, etc.	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 slice matchbox size
23. Processed meat (chorizo, sausage, roasted, etc.)	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 piece medium or 3 slices
24. Canned sardines	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 piece
25. Boiled/fried egg	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 piece
26. Fish soup	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 cup
27. Meat soup	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 cup
28. Poultry	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 slice or 1 medium piece
29. Beef and veal	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 medium slice
30. Lamb and mutton	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 medium slice
31. Other meat	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 medium slice
32. Fats and oils	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 tablespoon

III. DAIRY AND PRODUCTS

	HOW OFTEN DID YOU EAT THESE FOODS?									→
	NEVER or less than once per month	**1** per month	**2–3** per month	**1** per week	**2** per week	**3–4** per week	**5-6** per week	**1** per day	**2+** per day	**Medium serving size**
33. Powdered milk	☐	☐	☐	☐	☐	☐	☐	☐	☐	¼ cup
34. Milk	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 cup (250 ml)
35. Cheese	☐	☐	☐	☐	☐	☐	☐	☐	☐	2 cm cube or 1 tablespoon
36. Yogurt	☐	☐	☐	☐	☐	☐	☐	☐	☐	¼ cup
37. Butter and margarine	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 tablespoon

IV. FRUITS AND VEGETABLES

	HOW OFTEN DID YOU EAT THESE FOODS?									→
	NEVER or less than once per month	**1** per month	**2–3** per month	**1** per week	**2** per week	**3–4** per week	**5–6** per week	**1** per day	**2+** per day	**Medium serving size**
38. Lao-uy /Sinabawang gulay (vegetable soup with small fish)	☐	☐	☐	☐	☐	☐	☐	☐	☐	½ cup
39. Fruit soup (i.e. sinigang)	☐	☐	☐	☐	☐	☐	☐	☐	☐	½ cup
40. Vegetable dishes	☐	☐	☐	☐	☐	☐	☐	☐	☐	½ cup
41. Banana/banana cue	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 piece
42. Guso/sea grasses	☐	☐	☐	☐	☐	☐	☐	☐	☐	½ cup
43. Fruits except banana and fruit soup	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 piece
44. Nuts and seeds	☐	☐	☐	☐	☐	☐	☐	☐	☐	10 pieces

V. OTHER NON-DAIRY BEVERAGES

	HOW OFTEN DID YOU EAT THESE FOODS?									→
	NEVER or less than once per month	**1** per month	**2–3** per month	**1** per week	**2** per week	**3–4** per week	**5–6** per week	**1** per day	**2+** per day	**Medium serving size**
45. Carbonated soft drink intake (cup)	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 small bottle or 1 can
46. Coffee	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 cup
47. Alcoholic beverages	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 ½ cups
48. Supplements providing energy	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 cup

Additional information:

Do you use vitamin D or calcium supplements (Yes/No)? _____

If yes, please indicate brand name and frequency of intake: _____

PHASE 2-Food Questionnaire on Usual Calcium Intake in NZ

Name: _____ Date: _____

Your usual food intake currently **in New Zealand** will be evaluated in this questionnaire. Kindly follow the instructions below and if you have any questions, please do not hesitate to send us an email or contact us via phone.

General Instructions

3. Complete each question as much as you can. Estimate if you are unsure. A guess is better than leaving it blank.
4. Mark the column with ✓ to show how often, on average, you ate the foods below.

	HOW OFTEN DID YOU EAT THESE FOODS?									
	Medium serving size	**NEVER or less than once per month**	**1** per month	**2–3** per month	**1** per week	**2** per week	**3–4** per week	**5–6** per week	**1** per day	**2+** per day
White breads, including toast, sandwich, bagels, rolls and English muffins	2 slices or 1 medium	☐	☐	☐	☑	☐	☐	☐	☐	☐

FOR EXAMPLE: This person ate white bread every Sunday. She usually ate about 2 slices.

I. GRAINS AND CEREAL PRODUCTS

	HOW OFTEN DID YOU EAT THESE FOODS?									→
	NEVER or less than once per month	**1** per month	**2–3** per month	**1** per week	**2** per week	**3–4** per week	**5–6** per week	**1** per day	**2+** per day	**Medium serving size**
1. Rice	☐	☐	☐	☐	☐	☐	☐	☐	☐	½ cup
2. Corn grits	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 cup
3. Bread	☐	☐	☐	☐	☐	☐	☐	☐	☐	2 slices or 2 pieces
4. Bihon, green bean noodles, etc.	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 cup
5. Instant noodles, spaghetti, etc.	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 cup
6. Cereal snacks	☐	☐	☐	☐	☐	☐	☐	☐	☐	½ cup
7. Mung bean	☐	☐	☐	☐	☐	☐	☐	☐	☐	½ cup
8. Sweet potato	☐	☐	☐	☐	☐	☐	☐	☐	☐	½ piece
9. Bread-based dishes	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 cup
10. Cakes and muffins	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 slice
11. Breakfast cereals	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 cup
12. Sugar and sweets	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 tablespoon or 1 piece
13. Potatoes, kumara and taro	☐	☐	☐	☐	☐	☐	☐	☐	☐	½ piece
14. Pies and pastries	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 small piece
15. Savoury sauces and condiments	☐	☐	☐	☐	☐	☐	☐	☐	☐	¼ cup
16. Puddings and desserts	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 piece or 1/3 cup
17. Biscuits	☐	☐	☐	☐	☐	☐	☐	☐	☐	2 pieces
18. Snack bars	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 piece

II. MEAT AND POULTRY

	HOW OFTEN DID YOU EAT THESE FOODS?									→
	NEVER or less than once per month	**1** per month	**2–3** per month	**1** per week	**2** per week	**3–4** per week	**5–6** per week	**1** per day	**2+** per day	**Medium serving size**
19. Small fish with bone and head eaten	☐	☐	☐	☐	☐	☐	☐	☐	☐	¼ cup or 2 pieces
20. Fried fish and dried fish with bone and/or head	☐	☐	☐	☐	☐	☐	☐	☐	☐	1/3 cup or 1 piece
21. Paksiw, escabetche, fried large fish, etc.	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 slice or 1/3 cup
22. Meat adobo, etc.	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 slice matchbox size
23. Processed meat (chorizo, sausage, roasted, etc.)	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 piece medium or 3 slices
24. Canned sardines	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 piece
25. Boiled/fried egg	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 piece
26. Fish soup	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 cup
27. Meat soup	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 cup
28. Poultry	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 slice or 1 medium piece
29. Beef and veal	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 medium slice
30. Lamb and mutton	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 medium slice
31. Other meat	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 medium slice
32. Fats and oils	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 tablespoon

III. DAIRY AND PRODUCTS

	HOW OFTEN DID YOU EAT THESE FOODS?									→
	NEVER or less than once per month	**1** per month	**2–3** per month	**1** per week	**2** per week	**3-4** per week	**5–6** per week	**1** per day	**2+** per day	**Medium serving size**
33. Powdered milk	☐	☐	☐	☐	☐	☐	☐	☐	☐	¼ cup
34. Milk	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 cup (250 ml)
35. Cheese	☐	☐	☐	☐	☐	☐	☐	☐	☐	2 cm cube or 1 tablespoon
36. Yogurt	☐	☐	☐	☐	☐	☐	☐	☐	☐	¼ cup
37. Butter and	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 tablespoon

IV. FRUITS AND VEGETABLES

	HOW OFTEN DID YOU EAT THESE FOODS?									→
	NEVER or less than once per month	**1** per month	**2–3** per month	**1** per week	**2** per week	**3–4** per week	**5–6** per week	**1** per day	**2+** per day	**Medium serving size**
38. Lao-uy /Sinabawang gulay (vegetable soup with small fish)	☐	☐	☐	☐	☐	☐	☐	☐	☐	½ cup
39. Fruit soup (i.e. sinigang)	☐	☐	☐	☐	☐	☐	☐	☐	☐	½ cup
40. Vegetable dishes	☐	☐	☐	☐	☐	☐	☐	☐	☐	½ cup
41. Banana/banana cue	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 piece
42. Guso/sea grasses	☐	☐	☐	☐	☐	☐	☐	☐	☐	½ cup
43. Fruits except banana and fruit soup	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 piece
44. Nuts and seeds	☐	☐	☐	☐	☐	☐	☐	☐	☐	10 pieces

V. OTHER NON-DAIRY BEVERAGES

	HOW OFTEN DID YOU EAT THESE FOODS?									→
	NEVER or less than once per month	**1** per month	**2–3** per month	**1** per week	**2** per week	**3–4** per week	**5–6** per week	**1** per day	**2+** per day	**Medium serving size**
45. Carbonated soft drink intake (cup)	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 small bottle or 1 can
46. Coffee	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 cup
47. Alcoholic beverages	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 ½ cups
48. Supplements providing energy	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 cup

Additional information:

Do you use vitamin D or calcium supplements (Yes/No)? _____

If yes, please indicate brand name and frequency of intake: _____

Guidelines in using the Accelerometer

Please wear the accelerometer on the following dates

_____
_____
_____
_____
_____

- Please put on the accelerometer as soon as you wake up.

- Please take off the accelerometer when you go to bed.

- Take the accelerometer off when showering, bathing and swimming (example: take off when in water).

- The accelerometer should be worn on the waist (just above the hip) using the elastic belt supplied. The accelerometer should be held snugly against the body.

- The accelerometer can be worn either above or below clothing. It is not necessary for the accelerometer to make skin contact.

- You will return the accelerometer together with your food diary when you come for your second appointment.

- Please take good care of the accelerometer as they are very expensive to replace.

Accelerometer Diary

Use this diary to record the time you woke up, the time you put the accelerometer on, the time you took the accelerometer off, times that you did not wear the accelerometer and the activity you were doing at these times.

Day and Date	Day 1	Day 2	Day 3
At what time did you wake up?			
At what time did you put the accelerometer on?			
At what time did you take the accelerometer off at the end of the day?			
At what time did you go to bed?			
At what times did you not wear the accelerometer during the day?			
What activity were you doing during these times?			
Please record the duration of any weight-training, resistance exercise or cycling you undertook whilst wearing the accelerometer			

MASSEY UNIVERSITY
COLLEGE OF HEALTH
TE KURA HAUORA TANGATA

Filipino Women's Health study

Personal Information, Health and Demographics Questionnaire

First name: _____

Family name: _____

Name you would like to be called by: _____

Address: _____

Phone number: _____ Date of birth: _____

Medical Practitioner:_____

Address: _____

Phone: _____

What is your first language?

English ☐

Other ☐

If other, please state:_____

I would like to receive a brief report summarising the main findings of the project:

Yes □ No □

I am willing to be contacted in connection with future research projects within the Massey Institute of Food Science and Technology and for this purpose I agree to my contact information being retained on condition that it is in no way linked to the data I have supplied for this research project:

Yes □ No □

Health and Demographic information

How long have you been living in New Zealand? _____

Do you have children? Yes □ No □

- How many children do you have? _____

- When was your youngest child born? __ / __ / ____ (DD/MM/YYYY)

When did your last period start? (Day / month / year) **_____**

Do you currently use birth control? Yes □ No □

If yes, please specify: _____

Do you have any surgical or cosmetic implants? Yes □ No □

Is your menstrual cycle regular? Yes □ No □

Are you currently in paid employment? Yes □ No □

If yes,

Full time Yes □ No □

Part time Yes □ No □

On-call Yes □ No □

If yes, specify hours per week:_____

If applicable, specify how many night shifts per week:_____

Describe your job or paid employment or work:

TITLE / DESCRIBE HOURS PER WEEK

_____

_____

Do you follow a specific diet for health reasons? Yes □ No □

Please explain

_____

_____

Do you follow any diet for cultural or religious reasons? Yes □ No □

If yes, what type of diet do you follow? _____

_____

_____

Have you been diagnosed with bone disease (osteoporosis, osteomalacia, rickets, etc.)?

Yes □ No □

Please specify

_____

_____

Have you had any fractures?

Yes □ No □

Please specify how many times and which area

_____

_____

Are you taking any form of medication, including traditional or homeopathic medicine and contraception? Please bring any medications with you when you come for your testing.

Yes □ No □

Please specify the condition, the medication and the dosage in the table provided.

Condition	Medication	Dosage	Frequency

Are you taking any form of supplements, including tablets or drinks? Yes □ No □

Please bring any supplements with you when you come for your testing.

If yes, what are the name, brand and dosage of the supplements you are taking? _____

Supplement	Brand	Dosage	Frequency

Family History

Do you have a family history of	Yes	No
High blood pressure		
Heart disease		
Diabetes		
Bone disease and fractures		

Do you smoke cigarettes? Yes □ No □

If yes, approximately how many cigarettes per day:_____

Do you drink alcohol? Yes □ No □

If yes, approximately how many standard drinks per week:_____

[1 standard drink = a glass of wine (120ml), 1 bottle/can of beer, 1 tot of spirits (45mL)]

Do you have any allergies? Yes □ No □

Please specify _____

_____

Please tell us how you found out about the Filipino Women's Health study. Did you found out from:

- A friend?
    - If yes, what is him/her name?.....
      .....
- An email list?
    - If yes, what is the name of the email list?.....
      .....
- At an event?
    - If yes, which event?.....
      .....
- Flyer on noticeboard?
    - If yes, where was the noticeboard?
      .....
- Other……..……
  ……

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