VITAMIN D INTAKE AND STATUS IN A SAMPLE OF HEALTHY YOUNG ADULTS OF DIFFERENT ETHNICITY LIVING IN CANADA

A Thesis Submitted to the College of
Graduate Studies and Research
In Partial Fulfillment of the Requirements
For the Degree of Master of Science
In the College of Pharmacy and Nutrition
University of Saskatchewan
Saskatoon, Saskatchewan, Canada

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ABSTRACT

Vitamin D plays an important role in over-all health. Few data exist on vitamin D deficiency related with intake for a Canadian population. The purpose of this study is to assess vitamin D intake and status in healthy young adults of diverse ancestry during the wintertime.

One hundred and seven young healthy adults living in Southern Ontario were recruited during the late winter of 2007. Their serum 25-hydroxyvitamin D [25(OH)D], skin melanin and anthropometric measures were determined. They completed a food frequency questionnaire (FFQ) (twice) and a 7-day food diary. Correlation analyses and *t*-test were used to validate the FFQ against the 7-day diary and 25(OH)D; one way ANOVA was used to determine ethnic group differences in vitamin D intake and status.

The results indicated that the FFQ used in this study was valid. Vitamin D deficiency [25(OH)D<50 nmol/L] was widespread and more apparent in the East and South Asian groups than in the European group (P<0.05). The dairy products were the greatest food source of vitamin D for each of the three groups and the European group exhibited higher total vitamin D intake ($P<0.05$). There was a trend for the European group to have higher consumption of dairy products, especially cow's milk ($0.05<P<0.10$). Combining subjects in the three ethnic groups, vitamin D intake but not BMI was closely related with serum 25(OH)D concentrations ($r= 0.520$, $P<0.001$; $r=-0.018$, $P>0.05$, respectively). The 25(OH)D levels were inversely related with parathyroid hormone (PTH) levels ($r= -0.273$, $P= 0.009$). With adequate calcium

intake (≥1000 mg/d), PTH levels were significantly lower when vitamin D was not deficient ($P<0.05$).

This study provides evidence that vitamin D deficiency is prevalent in healthy young adults living in Canada during wintertime, and non-European groups have a higher prevalence of this deficiency. Vitamin D intake varies with ethnicity, and dietary intake plays an important role in maintenance of serum vitamin D in wintertime. Compared with calcium intake, serum vitamin D levels may be a more important factor suppressing PTH levels.

ACKNOWLEDGEMENTS

I would like to express my gratitude to all those who gave me the opportunity to engage in this research and who supported the completion this thesis.

I am deeply indebted to my supervisor Dr. Susan Whiting whose help, stimulating suggestions and encouragement helped me in all the phases of research and in all the phases of the thesis writing.

I would like to thank Dr. Esteban Parra, Ms. Agnes Gozdzik and all other colleagues of the University of Toronto who worked in the vitamin D pigment study and provided the raw data for our analyses.

I would like to extend my sincere gratitude to my advisory committee, Dr. Gord Zello, Dr. Phyllis Paterson and Dr. Wendy Dahl. Thank you for your time, suggestions and expertise.

I would like to thank Dr. Hassanali Vatanparast for his help and valuable hints. I would like to thank Ms. Tracy McLennan for her assistance in preparing the thesis.

I extend my sincere gratitude to the College of Pharmacy and Nutrition for providing the financial assistance, so that I could pursue this research.

I would especially like to give my thanks to my special friend, Dr. Glenn Haley whose patient love enabled me to complete this work.

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ABBREVIATIONS

AI	Adequate Intake
BMI	Body Mass Index
CSFII	Continuing Survey of Food Intakes by Individuals
DRIs	Dietary Reference Intakes
EAR	Estimated Average Requirement
FFQ	Food Frequency Questionnaire
IU	International Unit. 1 IU vitamin D = 0.025 μg
κ_w	Weighted Kappa
LL-37	Cathelicidin
NHANES	National Health and Nutrition Examination Survey
PC-SIDE	A software for estimation of usual intake distribution
PTH	Parathyroid Hormone
RCT	Randomized Controlled Trial
RDA	Recommended Dietary Allowance
SDD	Standard Vitamin D Dose
SPF	Sun Protection Factor
TB	Tuberculosis
UL	Tolerable Upper Intake Level
UV	Ultraviolet
UVB	Ultraviolet B
VDR	Vitamin D Receptor
y	Year
$1,25(OH)_2D$	1,25-dihydroxyvitamin D
25(OH)D	25-hydroxyvitamin D

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND

Humans get vitamin D from exposure to sunlight, from their diet, and from dietary supplements (Holick, 2007). Cholecalciferol (Vitamin D_3) is synthesized from the action of ultraviolet B (UVB; 295-310 nm) on its precursor in the skin or obtained from dietary sources. Vitamin D is hydroxylated in liver to its circulating form, 25-hydroxyvitamin D [25(OH)D]. 25(OH)D is converted in the kidney into the biologically active form, 1,25-dihydroxyvitamin D [$1,25(OH)_2D$]. The production of $1,25(OH)_2D$ in the kidney is homeostatically controlled, mainly by the action of parathyroid hormone (PTH) in response to serum calcium and phosphorus levels (Holick, 2003).

The principle role of vitamin D is to ensure adequate intestinal absorption of calcium and phosphorus and regulate bone mineralization (Gibson, 2005). Impaired calcium metabolism, due to low serum 25(OH)D, triggers secondary hyperparathyroidism, increased bone turnover, and progressive bone loss (Holick, 2006). Vitamin D also has an important role in a number of cellular functions in tissues other than bone. It is a notable modulator of the immune response and the regulation of cell differentiation, proliferation, and apoptosis (Omdahl et al., 2002). Vitamin D plays a role in decreasing the risk of many chronic illnesses, including common cancers, autoimmune disease, infectious diseases, and cardiovascular diseases (Holick, 2007).

Factors related to decreased sun exposure affect skin production of vitamin D, which is the major source of vitamin D for most humans (Webb, 2006). The available UVB for vitamin D synthesis is greatly decreased in the winter season and in high latitude regions. Skin pigmentation genetically affects vitamin D production in the skin as melanin is a natural sunscreen. Since very little vitamin D is naturally present in foods, vitamin D deficiency is common because in situations when skin production is compromised, dietary vitamin D intake cannot meet the compensatory requirement. Several studies identified a high prevalence of vitamin D deficiency in Canadian populations (Vieth et al., 2001; Rucker et al., 2002; Roth et al., 2005; Weiler et al., 2005; Vecino-Vecino et al., 2006; Weiler et al., 2007; Ward et al, 2007). However, very few studies have demonstrated the effects of ethnicity on vitamin D status. Two studies showed that white women had lower prevalence of low 25(OH)D than non-white women (Vieth et al., 2001) or Aboriginal women (Weiler et al., 2007). One study showed vitamin D-deficiency rickets was persistent in Canada, particularly among children residing in the north and among infants with darker skin (Ward et al., 2007).

The major dietary sources for vitamin D in Canada include fortified foods and supplements. This is because high levels of fatty fish, the richest natural source of vitamin D, are not consumed by the Canadian population. It has been found that even in countries with mandatory staple food fortification, vitamin D intakes are low in some groups due to their unique dietary patterns, such as low milk consumption, vegetarian diet, limited use of dietary supplements, or loss of traditional high fish intakes (Calvo et al., 2005). However, information is still unavailable for vitamin D

intake related with vitamin D deficiency in ethnic groups in Canada, which is a country with high ethnic diversity.

Vitamin D intake estimations require information on the vitamin D content of foods and supplements and the frequency, types, and amounts of foods and supplements consumed. It is challenging to develop an effective tool for obtaining vitamin D intake information from a multiethnic perspective. In order to assess vitamin D intake in a large cohort study, a method such as the food frequency questionnaire (FFQ) is useful, as it has low respondent burden and the results are easy to collect and process. The FFQ results can be taken to represent usual intakes over a period of time (Gibson, 2005).

1.2 HYPOTHESIS AND OBJECTIVES

It is hypothesized that total dietary vitamin D intake of Canadians, with regard of ethnicity, is not sufficient to maintain desirable vitamin D status in winter. The purpose of the study is to assess the vitamin D status and dietary vitamin D intake during the winter season in a sample of healthy young adults of diverse ancestry living in Southern Ontario. The objectives of the study are as follows:

1. To validate an FFQ for measuring vitamin D and calcium intakes in subjects of varying ethnicity;
2. To determine the relationship between the variety of major sources of vitamin D intake and the differences in vitamin D status among ethnicities;
3. To evaluate the roles of vitamin D status and calcium intake in the maintenance of PTH level.

CHAPTER 2

LITERATURE REVIEW

2.1 VITAMIN D

2.1.1 Vitamin D production and metabolism

Vitamin D is a generic term for vitamin D_2 (ergocalciferol), vitamin D_3 (cholecalciferol), and their metabolites (Gibson, 2005). Vitamin D_2 is derived from the yeast and plant sterol, ergosterol, and is the form widely used in pharmaceutical preparations. Vitamin D_3 is mainly produced from exposure to sunlight. During exposure to sunlight, UVB photons (290-315 nm) penetrate into the viable epidermis and dermis where they are absorbed by 7-dehydrocholesterol (7-DHC). The absorption of UVB radiation causes 7-DHC, a steroid, to open its B ring, forming precholecalciferol (pre-D_3). Pre-D_3 undergoes rearrangement of its double bonds to form vitamin D_3 (Holick, 2003).

Vitamin D is metabolized in the liver to 25-hydroxyvitamin D [25(OH)D], which is the major circulating and storage form that is delivered to tissue for further activation. Some 25(OH)D is converted in the kidney to a biologically active hormonal form, 1,25-dihydroxyvitamin D [1,25$(OH)_2$D]. A variety of factors, including serum phosphorus and PTH, regulate the renal production of 1,25$(OH)_2$D. The 1,25$(OH)_2$D regulates calcium metabolism through its interaction with the major target tissues, the bone and the intestine (Holick, 2003). Besides the small intestine and the osteoblast, nuclear vitamin D receptor (VDR) of 1,25$(OH)_2$D has been identified in brain, heart, skin, pancreas, breast, colon, and immune cells. The

1,25(OH)2D helps regulate cell growth and maturation, stimulate insulin secretion, inhibits renin production, and modulates the functions of activated T and B lymphocytes and macrophages (Norman, 1998; DeLuca & Cantorna, 2001; Andorini, 2002). Thus 1,25(OH)2D has other important functions in addition to calcium homeostasis.

2.1.2 Vitamin D and health

The role of vitamin D in bone health has long been established. Vitamin D deficiency and the attendant secondary hyperparathyroidism cause defective mineralization of the bone matrix lain down by osteoblasts (Holick, 2005). This results in rickets in children due to the skeletal deformation, and leads to osteoporosis and osteomalacia in adults due to the skeletal wasting.

Vitamin D-deficiency rickets is a persistent problem among infants and children in Canada. There were 104 cases between 2002 and 2004 reported in a recent study through the Canadian Paediatric Surveillance Program (Ward et al., 2007). The condition was most frequently observed among darker-skinned, breast-fed infants and children, with the highest incidence among children from north (Yukon Territory, Northwest Territories and Nunavut). Maternal characteristics of skin colour, lack of sun exposure and inadequate vitamin D intake or supplementation were contributing factors.

In adults, osteoporosis leads to increased bone fragility and risk of fracture, particularly of the hip, spine and wrist (Osteoporosis Canada, 2007). Seventy percent of hip fractures are osteoporosis-related. Hip fractures result in death in up to 20 percent of cases, and disability in 50 percent of those who survive. Osteoporotic

fractures are multi-factorial and may result from a variety of causes, either singly or in combination (Brown & Josse, 2002). However, low vitamin D is an established risk factor.

Osteomalacia can cause isolated or generalized aching in the bone as well as muscle pain and muscle weakness. A recent study showed 93% of 140 primary care outpatients with nonspecific muscle aches and bone pain had vitamin D deficiency (Plotnikoff & Quigley, 2003). These patients were from six broad ethnic groups, both sexes, and aged 10 to 65 years. It has been reported that muscles have VDRs (Demay, 2003). Vitamin D deficiency causes muscle weakness and therefore increases risk of the elderly to fall and thereby fracture. A meta-analysis based on five double-blind randomized, controlled trials (RCTs) involving 1237 participants found that vitamin D supplementation reduced falling among the elderly by 22% (Bischoff-Ferrari et al., 2004).

In vitro and in vivo studies indicate that vitamin D may have anti-cancer benefits, including protection against progression and metastasis of a wide spectrum of cancers (Giovannucci, 2005). The evidence that higher 25(OH)D levels through increased sunlight exposure or dietary or supplement intake inhibit colorectal carcinogenesis is substantial (Grant & Garland, 2004). The biologic evidence for an anti-cancer role of 25(OH)D is also strong for prostate cancer (Beer et al., 2003).

The $1,25(OH)_2D$ plays a role in reducing the risk of developing common autoimmune diseases. The $1,25(OH)_2D$ interacts with its VDR in immune cells and it is a potent immunomodulator. It is recognized that living at a latitude above 37° increases risk of developing multiple sclerosis throughout life by 100% (Ponsonby et

al., 2002). Furthermore, taking a multivitamin that contains 400 IU (10 μg) of vitamin D reduces risk of developing multiple sclerosis by 40% (Embry et al., 2000). Women taking 400 IU (10 μg) of vitamin D in a multivitamin decreased their risk of rheumatoid arthritis by 40% (Merlino et al., 2004). An observation showed that children in Finland who received 2000 IU (50 μg) of vitamin D a day from one year of age on and who were followed for the next 25 years had an 80% decreased risk of developing type 1 diabetes, whereas children who were vitamin D deficient had a four-fold increased risk of developing this disease later in life (Hypponen et al., 2001).

It is known that $1,25(OH)_2D$ is one of the most potent hormones for down-regulating the blood pressure hormone renin in the kidney (Li et al., 2004). Inappropriate activation of the renin-angiotensin system is thought to play a role in some forms of hypertension, and increasing vitamin D levels may be important for improving cardiovascular health.

The role of sunlight in preventing microbial infections has been known for over a century. Recent studies have investigated the antimicrobial action of vitamin D at the molecular level. One of the better studied antimicrobial peptides, cathelicidin (also called LL-37), contains sites for VDR (Gombart et al., 2005). Low vitamin D levels may partly explain the high prevalence of tuberculosis in Aboriginal Canadians, who suffer from tuberculosis at a rate that is 8-10 times higher than overall Canadian rates (Health Canada, 2007).

2.1.3 Determination of vitamin D status

The measurement of the major circulating form of vitamin D, 25(OH)D is well recognized as the standard to determine the vitamin D status (Bischoff-Ferrari et al., 2004, 2006; Heaney, 2005; Giovannucci et al., 2006). Most agree that a 25(OH)D concentration < 50 nmol/L (20 ng/mL) is an indication of vitamin D deficiency whereas 51-74 nmol/L (21-29 ng/mL) indicates insufficiency, and 75 nmol/L (30 ng/mL) is considered to be sufficient (Holick, 2008). Available data including studies on bone mineral density, fracture prevention, lower extremity function, and cancer prevention support a target serum level of 75 nmol/L (30 ng/mL) 25(OH)D (Bischoff-Ferrari, 2007). The vitamin D intake needed to bring to this desirable 25(OH)D level or above has not been defined. However, researchers have started to estimate vitamin D requirements for meeting this desirable 25(OH)D level. Several studies indicate that mean intake of vitamin D needs must be at least 500 IU (12.5 μg) for young Caucasian adults in summer to maintain desirable 25(OH)D and prevent wintertime bone loss (Heaney et al., 2003; Meier et al., 2004). In situations of prolonged absence of sun exposure, the mean requirement for vitamin D is estimated to be at high as 2000 IU (50 μg) for older Caucasian and African American adults (Aloia et al., 2005; Heaney, 2006).

2.1.4 Major factors affecting cutaneous vitamin D synthesis

The major source for vitamin D for most humans is exposure to sunlight (Holick, 2003). In the presence of adequate sunlight that provides UVB in the wavelength range of 290-315nm, sufficient vitamin D (as D_3) is produced in the skin. A number

of environmental and personal factors affect the availability and absorption of suitable UV photons and therefore influence cutaneous vitamin D synthesis (Webb, 2006). These factors include season, latitude, skin type, clothing and sunscreen barriers.

The available UVB for vitamin D production greatly decreases during the winter season and decreases in high latitude regions. When the sunlight travels through atmosphere, the major attenuation process is called Rayleigh scattering. In Rayleigh scattering the shorter wavelengths will scatter more than the longer wavelengths. In the winter or at high latitude, the radiation traverses a longer path through the atmosphere, which greatly attenuates shorter wavelengths and the remaining energy is spread over a large surface area. Both the intensity and the electromagnetic spectrum of the incident radiation at the surface decrease (Webb, 2006). The short UVB wavelengths required for vitamin D synthesis are therefore strongly scattered by this process.

Clothing is an obvious physical barrier between solar radiation and the skin. Another barrier is sunscreen. A sunscreen having the sun protection factor (SPF) of 8, reduces cutaneous production of previtamin D_3 by >95%; SPF15 reduces the capacity by >99% (Webb & Engelsen, 2006).

Skin type genetically affects vitamin D production in the skin (Webb, 2006). It is associated with the amount of melanin pigment in the skin. Melanin is the primary determinant of human skin colour. It absorbs UV radiation and prevents the UV photons from entering other skin cells. The more melanin there is in the skin the lower the amount of previtamin D_3 synthesized for a given dose of UVB. Generally

there are six skin types based on melanin content (Table 2.1). A person with skin type V or VI requires 10-50 times the exposure to sunlight to produce the same amount of vitamin D_3 in their skin as does a person with skin type II or III (Holick, 2004).

The skin color of indigenous races generally darkens as latitude decreases from the poles to the equator, and the ambient UV increases. Within the limits of individual genetic makeup, more radiation results in greater amounts of skin-darkening melanin produced by the skin for protection. Yet with the effects of human migrations, people in one place can show tremendous variation in skin tone. Migrant populations or the descendants of migrant populations, who have moved significantly pole-ward, have skin types that do not match the ambient UV environment with relatively lower radiation intensity (Webb, 2006). Therefore, dark-skinned people nearer the poles have a greater risk of sub-optimal vitamin D status.

Table 2.1 General characteristics of skin types

Skin type	Colour	Reaction to sunlight	Reaction to UV radiation
Type I	Caucasian; blonde or red hair, freckles, fair skin, blue eyes	Always burns easily, never tans; very fair skin tone	Very sensitive
Type II	Caucasian; blonde or red hair, freckles, fair skin, blue or green eyes	Usually burns easily, tans with difficulty; fair skin tone	Very sensitive
Type III	Darker Caucasian, light Asian	Burns moderately, tans gradually; fair to medium skin tone	Sensitive
Type IV	Mediterranean, Asian, Hispanic	Rarely burns, always tans well; medium skin tone	Moderately sensitive
Type V	Middle Eastern, Latin, light-skinned black, Indian	Very rarely burns, tans very easily; olive or dark skin tone	Minimally sensitive
Type VI	Dark-skinned black	Never burns, deeply pigmented; very dark skin tone	Least sensitive

Adapted from Webb, 2006

Table 2.2 Sun exposure time (in minutes) for production of 1 standard vitamin D dose (SDD) as a function of latitude, season and skin type *

Skin type** by latitude	1200 h	
	21 December	21 June
42.5° N		
I	70	4
II	94	5
III	127	6
IV	999	8
V	999	11
VI	999	19
62.5° N		
I	999	7
II	999	9
III	999	11
IV	999	16
V	999	21
VI	999	36

* All calculations represent clear sky conditions. SDD is defined corresponding to the UV equivalent of an oral dose of 1000 IU (25 µg) vitamin D. 1200 h is local solar noon. 999 signifies that an SDD was not available.

** Refer to Table 2.1 for skin type.

Adapted from Webb, 2006

Table 2.2 shows sun exposure time needed upon exposure of 25% of body surface area to gain 1 standard vitamin D dose (SDD) under the standard atmospheric conditions. SDD is defined corresponding to the UV equivalent of an oral dose of 1000 IU (25 µg) vitamin D (Holick, 2004). Latitude 42.5° N and 62.5° N are given in the table because major cities in Canada are located in this range. Sun exposure time is categorized in terms of six skin types and two seasons. The table shows that longer sun exposure time is needed for darker skin types to produce 1 standard vitamin D dose in the summer. The UV exposure time required for a healthy vitamin D status is

impractical in the winter. It is not possible to synthesize vitamin D_3 by almost all of the skin types in typical Canadian latitude ranges in winter.

2.1.5 Sub-populations at risk of vitamin D deficiency

The nutritional vitamin D status of the human fetus and neonate is dependent on the vitamin D stores of the mother; thus, if the mother has vitamin D deficiency, her fetus will experience depleted vitamin D exposure throughout the developmental period (Hollis & Wagner, 2004). Further, because there is very little vitamin D in human milk, infants are at high risk of developing vitamin D deficiency and rickets if they are not given a vitamin D supplement (Kreiter et al., 2000).

Aging decreases the amount of 7-dehydrocholesterol produced in the skin by as much as 75% by the age of 70 y (Holick, 2004). The elderly are at risk for vitamin D deficiency because of poor dietary vitamin D intake and decreased exposure to sunlight. A study showed that more than 30% of free-living elderly were vitamin D deficient at the end of August in Boston (Holick, 2002).

It has been assumed that young and middle-aged adults are not at risk of vitamin D deficiency because of their outdoor activities and dietary intake. However, young and middle-age adults who very seldom spend any time outdoors or always wear sun protection outdoors are also at high risk of vitamin D deficiency. It was recognized that 42% of African American women aged 15-49 years throughout the United States were vitamin D deficient at the end of the winter (Nesby-O'Dell et al., 2002). It was observed that 32% of healthy adults 18-29 years of age were vitamin D deficient at the end of the winter in Boston (Tangpricha et al., 2002).

Obesity is associated with a trend towards lower vitamin D status (Nesby-O'Dell et al., 2002). A study on 410 healthy women between 20 and 80 years of age with body mass index (BMI) ranging from 17 to 30 kg/m² indicated an inverse relation between percentage body fat and serum 25(OH)D concentration (Arunabh et al., 2003). NHANES III data indicated an inverse relation between measures of body fat or BMI and serum 25(OH)D concentrations in women as well (Looker, 2005). Some researchers attribute this trend to that, whether vitamin D is ingested in the diet or obtained from exposure to sunlight, it is deposited in the large body fat stores and is not bio-available (Wortsman et al., 2000). New Zealand researchers found that the negative effect of obesity on vitamin D status was shown in women but not in men (Rockell et al., 2006). They concluded that whether this was due to obese women having less UV light exposure or the sequestering of 25(OH)D by adipose tissue was yet to be determined.

Ethnicity difference in vitamin status is recognized as skin pigmentation affects the cutaneous production of vitamin D. In NHANES III, 42% of African American women had serum 25(OH)D ≤ 37.5 nmol/L but only 4.2% of Caucasian females of comparable age had such low levels (Nesby-O'Dell et al., 2002). In both NHANES III and the 1999-2000 NHANES surveys, non-Hispanic whites generally had higher 25(OH)D levels than Hispanic and black (Zadshir et al, 2005; Yetley et al., 2008). It was observed that 30%, 42%, and 84% of free-living white, Hispanic, and black elderly were vitamin D deficient at the end of August in Boston (Holick, 2002). A study in New Zealand showed that a lower mean 25(OH)D and a higher prevalence of vitamin D insufficiency was found in Māori and Pacific peoples than in New

Zealand Europeans (Rockell et al., 2006). All of these studies were reports of vitamin D insufficiency patterns reflected by 25(OH)D concentrations; none of them related vitamin D intake with vitamin D status.

2.1.6 Vitamin D status in Canada

Worldwide, vitamin D status is suboptimal relative to circulating levels of 25(OH)D needed to prevent a variety of chronic conditions (Brot et al., 2001; Nowson & Margerison, 2002; Brock et al., 2004; Anderson et al., 2005; Rockell et al., 2006; Woo et al., 2008). Several studies identified a high prevalence of vitamin D insufficiency in healthy adults living in North America (Vieth et al., 2001; Rucker et al., 2002; Tangpricha et al., 2002; Looker et al., 2002; Nesby-O'Dell et al., 2002). These studies confirmed the dominant effect of seasonality and latitude on serum 25(OH)D.

Canada is far from the equator, which results in reduced amounts of UVB available for biosynthesis of vitamin D in wintertime. Dark skinned individuals living in regions with low UV radiation, such as Canada, are at greater risk of vitamin D deficiency. Table 2.3 shows recent studies of vitamin D status in Canadian population. These studies reported that vitamin D deficiency was prevalent in Canada and that the higher rate of vitamin D deficiency was found in non-white and Aboriginal people.

Because of the well-documented effects of sunlight on skin aging and the promotion of skin cancer, it is not advisable for individuals to prolong their exposure to sunlight to produce the needed higher concentrations of vitamin D (Gilchrest,

2007). Therefore it becomes important for dietary and supplementary vitamin D compensation to maintain serum 25(OH)D levels when cutaneous synthesis is limited. Higher levels of vitamin D administrated as dietary supplements has been demonstrated in the studies to effectively improve 25(OH)D status (Trivedi et al., 2003). A study also showed that consuming milk fortified with vitamin D at higher levels over 24 months significantly raised serum 25(OH)D levels (Chee et al., 2003).

Very little vitamin D is naturally present in our food. Some foods are fortified with vitamin D (e.g., milk, margarine, cereal) in some countries (Calvo et al., 2005). For example, vitamin D-fortified foods are predominantly fluid milk and ready-to-eat cereals in the United States. Other foods that may be fortified with vitamin D in the United States include milk products, grain products and pastas, margarine, calcium-fortified juices and juice drinks. A study examined the dietary intake of vitamin D for the entire US population using two nutrition surveys, NHANES III and CSFII 1994-1996, 1998 (Moore et al., 2004). It was shown that dairy products were the primary sources of vitamin D in the United States.

Fortified foods provide an essential source of vitamin D in Canada (Table 2.4). Vitamin D intake has not been analyzed for the effect of fortification. Nevertheless, Canadian intakes are not adequate to meet the needs of the population living at high latitudes (Calvo & Whiting, 2003). A study showed that consumption of vitamin D at the dietary guideline for young adults (5 μg) was not effective in maintaining vitamin D status at the 80 nmol/L level for 25(OH)D in winter in Toronto (Vieth et al, 2001). In a study in three Canadian long-term care facilities, only 30% of patients got

adequate amounts of vitamin D (AI of 600 IU / 15 μg for older than 70 years) through diet alone (Lee et al., 2002).

Table 2.3 Selected studies of vitamin D status in Canadian population

Authors	Studied population	Results
Vieth et al, 2001	Young women in Toronto (*n*=796)	Prevalence of low 25(OH)D (< 40 nmol/L) was lower in white women (14.8%) than non-white and non-black women (25.6%) through the whole year.
Rucker et al, 2002	Adults in Calgary (*n*=188)	34% of low 25(OH)D (< 40 nmol/L) through the whole year
Roth et al, 2005	Children and adolescents in Edmonton (*n*=90)	34% of low 25(OH)D (< 40 nmol/L) at the end of winter; the risk was higher among older children
Weiler et al, 2005	Mother-infant pairs in Winnipeg (*n*=50 pairs)	46% of mothers had 25(OH)D < 37.5 nmol/L; 36% of infants had 25 (OH)D < 27.5 nmol/L
Vecino-Vecino et al, 2006	Older adults in Quebec (*n*=256)	32% of females had 25(OH)D <20 nmol/l as compared to 51% of the males; the lower levels of 25(OH)D happening in early spring with a recovery at the end of the summer
Weiler et al, 2007	Aboriginal women in Manitoba (*n*=355)	32% of rural Aboriginal, 30.4% of urban Aboriginal, and 18.6% of urban white women had serum 25(OH)D concentrations < 37.5 nmol/L
Ward et al, 2007	Canadian children with rickets (*n*=2325)	Vitamin D-deficiency rickets is persistent in Canada, particularly among children who reside in the north and among infants with darker skin who are breast-fed without appropriate vitamin D supplementation.

Table 2.4 Selected vitamin D food sources in the average Canadian diet

Food Source	Serving Size	Vitamin D (μg)	Vitamin D (IU)
Cow's milk * (homogenized, 2%, 1% and skim)	250 mL	2.7	102
Margarines *	15 mL	1.9	76
Canned sockeye salmon (with bones)	100g	19.5	737
Atlantic herring	100g	4.2	159
Canned tuna (white)	100g	2.0	76
Egg	1 large	0.6	23
Soy beverage (fortified)	250 mL	2.2	83
Meat and poultry (Health Canada, 2008)	75g	0.1~1.7	4~68

* Cow's milk and margarines in Canada are fortified with vitamin D.

Canada is different from most other Western countries in that immigrants comprise a much larger share of its population. In 2001, 18% of Canada's population was foreign-born. Canada's immigrant population is estimated to between 7.0 million and 9.3 million by 2017 (Statistics Canada, 2005). This represents an increase of 24% to 65% over 2001, when immigrants numbered 5.4 million. Over the same period, the non-immigrant population in Canada is predicted to grow modestly at a rate ranging from 4% to 12%. Immigrants will account for 22% of the total population by 2017. Although two studies demonstrated that the presence of darker skin pigmentation, as found in Asian and Aboriginal people in Canada, was associated with a lower circulating level of 25(OH)D (Vieth et al, 2001; Weiler et al., 2007), reliable information is still unavailable for multi-ethnic groups making up the Canadian population.

2.1.7 Vitamin D and calcium

Most of the human body's calcium is stored in bone. Bone provides mechanical strength to the skeleton and serves as a reservoir for maintaining normal plasma calcium. Low calcium and vitamin D are established risk factors for osteoporosis (Francis, 2006). Data indicate that 1.4 million Canadians suffer from osteoporosis. One in four women over the age of 50 has osteoporosis. At least one in eight men over 50 also has the disease. The cost of treating osteoporosis and the fractures it causes is estimated to be $1.9 billion each year in Canada alone (Osteoporosis Canada, 2007).

Vitamin D is a key factor in calcium absorption (Brown & Josse, 2002). Without vitamin D, the small intestine absorbs no more than 10-15% of dietary calcium (Holick, 2004). In a person with vitamin D sufficiency, the small intestine absorbs 30% of dietary calcium; during growth, lactation, and pregnancy, the efficiency increases to 80% (Holick, 2004). When 25(OH)D levels are low, calcium absorption is insufficient to satisfy the calcium requirements. The body responds by increasing the production and release of PTH into the circulation. The increase in PTH restores calcium homeostasis by enhancing the production of $1,25(OH)_2D$, increasing tubular re-absorption of calcium in the kidney, and increasing calcium mobilization from the bone (Holick, 2006). Vitamin D has an inverse relationship with PTH with regard to calcium homeostasis (Passeri et al., 2008). The suppression effect of vitamin D on PTH is partly mediated by its effect in promoting calcium absorption; and is also mediated by a more direct mechanism involving metabolism of 25(OH)D to $1,25(OH)_2D$ within parathyroid tissue (Dawson-Hughes et al., 2005).

In a vitamin D status study in older adults, it was found that lower levels of vitamin D status with higher levels of PTH happened in the winter while the opposite was seen during sunny season with high levels of vitamin D and normalization of PTH levels (Vecino-Vecino et al, 2006). A study of healthy adults suggested that vitamin D sufficiency could ensure ideal serum PTH values even when the calcium intake level was less than 800 mg/d, while high calcium intake (>1200 mg/d) was not sufficient to maintain ideal serum PTH, as long as vitamin D status was insufficient (Steingrimsdottir et al., 2005).

2.2 DIETARY ASSESSMENT: METHODOLOGY

2.2.1 Dietary assessment methods

Dietary assessment is used to evaluate food consumption and nutrient intake in individuals or groups of people. Dietary assessment methods are available for national, household and individual levels. Two types of methods are used to measure the food consumption of individuals. The first type consists of recalls or records, designed to measure the quantity of every food (including beverages) consumed in one day. Using this method, estimates of the usual intakes of individuals can be obtained by increasing the number of measurement days. The second type of method includes the dietary history and food frequency questionnaire. They both obtain retrospective information on the pattern of food use during a longer time period. The usual intake of individuals is also estimated. These methods are summarized in Table 2.5.

2.2.1.1 Selection of appropriate methods

The selection of a dietary assessment method depends on what information is desired from the study. To determine the mean nutrient intake of a group, a single 24-h recall or a 1-d food record can be used for measuring the food intake of each subject in the group (Gibson, 2005). The size of the group necessary to characterize the group mean usual nutrient intake depends on the degree of precision required and the day-to-day variation between subjects in the nutrient intakes.

To calculate the population percentage 'at risk' of inadequate nutrient intakes, repeated 24-h recalls, or replicate weighed or estimated 1-d food records are the methods to estimate the usual intakes of the subjects (Gibson, 2005). Once a series of replicate observations on at least 30 individuals have been obtained, an adjustment needs to be made to the observed distribution of intake to remove the within-subject variation, i.e., to remove the variability introduced by day-to-day variation in nutrient intakes within an individual (Health Canada, 2006). This adjustment can be performed using the program PC-SIDE (Iowa State University, 2008). The adjustment process provides estimates of the usual nutrient intake for a specified group.

To find the correlation with biomarkers, large numbers of measurement days for each individual are required, using 24-h recalls or estimated or weighed food records (Gibson, 2005). A semi-quantitative food frequency questionnaire or a dietary history can be used too.

Table 2.5 Measuring food consumption of individuals *

Methods	Advantages	Disadvantages
24-h recall	- Quick; easy; low respondent burden - Eating pattern would not be changed - Can be repeated to measure daily variation and improve precision	- Prone to underestimate intake due to omissions (memory mistakes) - Single observation provides poor measure of usual intake
Estimated food record	- Prospective observation of current diet - Provide actual information	- Higher respondent burden and lower cooperation - Require literacy and numeracy skills - Consumption pattern may change due to awareness of recording
Weighed food record	- Prospective observation of current diet - Precision of portion sizes - Provide actual information	- Time consuming for participants - Consumption pattern may change due to awareness of recording - Subjects need to be well motivated
Dietary history	- Assess usual diet over a relatively long time period - Less expensive than prospective methods because it is a single interview	- Time consuming - Labor intensive therefore costly - Require skills of interviewer - Over or under-reporting of foods (believed to be healthy or unhealthy)
Food frequency questionnaire	- Quick; inexpensive; suitable for large-scale surveys - Low respondent burden and high response rate - Short version can focus on specific nutrients with few food sources	- Relatively low accuracy - Requires validation in relation to reference measure - Require literacy and numeracy skills if self-completed

* Information taken from Gibson, 2005

When the study objective is ranking individuals within a group and for the purpose of linking dietary intakes with risk of chronic disease, the preferred approach is to obtain multiple observations on each individual (Gibson, 2005). Repeated 24-h recalls, food records, or a semi-quantitative food frequency questionnaire may also be used in this case.

2.2.1.2 Two commonly used methods: food diary and FFQ

The two most commonly used methods for dietary assessment are the estimated food records (food diary) and the food frequency questionnaire (FFQ). The 7-day records (7-day diary) are recommended as a preferred method for estimating usual intakes of individuals, but is time consuming for the researchers and the subjects. The FFQ aims to assess the frequency with which food items or food groups are consumed during a specified time period. It can be completed in 20-30 minutes and coded quickly by the researchers (Gibson, 2005).

For the estimated food record (food diary), the participant is asked to record, at the time of consumption, all foods and beverages (including snacks) eaten in household measures, for a specified time period. Nonconsecutive days are prefered; and weekend days are always be proportionately included. The FFQ consists of a list of foods and an associated set of frequency-of-use categories. With the addition of portion-size estimates, the method has become semi-quantitative. The FFQ has less respondent burden than most of the other dietary assessment methods. The results are easy to collect and process, and are taken to represent usual intakes over an extended period of time. Specific combinations of foods in FFQ can be used as predictors for intakes of certain nutrients. These semi-quantitative FFQs are often used in

epidemiological investigation to study associations between dietary habits and disease (Gibson, 2005).

2.2.1.3 Measurement errors and underreporting of energy intake

The existence of measurement errors in dietary assessment can have serious consequences when interpreting dietary data. It will weaken correlations between nutrient intake and the outcome parameters, and important associations between diet and disease may be unclear. The major sources of errors are summarized in Table 2.6.

Studies that have examined underreporting of energy intake have found variable bias between persons (Black & Cole, 2001). It is found that biased underreporting is characteristic of some persons. Repeat measurements do not necessarily provide valid measures of individual intake. Subjects most prone to reporting bias may be repeatedly misclassified in the distribution. This is a challenge for the design of the surveys and the handling of flawed data.

2.2.2 Evaluation of dietary assessment methods

2.2.2.1 Validity of dietary assessment methods

Validity describes the degree to which a dietary method measures what it is intended to measure. Absolute validity is usually assessed in institutional settings and is limited. Relative validity, rather than absolute validity, is more assessed by evaluating the 'test' dietary method against another 'reference' dietary method. This reference method is chosen for its accuracy and ability to measure similar parameters over the same time frame. Errors in the reference method should be independent of any errors present in the test method. For example, various FFQs exist for use among

Table 2.6. Sources of errors in the methods estimating food consumption

Sources of errors	24-h recall	Dietary history	Weighed record	Estimated record	FFQ
Omitting foods	●	●	◘	◘	●
Adding foods	●	●	○	○	●
Estimating food weights	●	●	○	●	●
Estimating frequency of food consumption	○	●	○	○	●
Day-to-day variation	●	○	●	●	○
Changes in diet	○	○	●	◘	○
Coding errors	●	●	●	●	○

●: error is likely;
◘: error is possible;
○: error is unlikely.
Adapted from Gibson, 2005

different population and for different purposes. The reference dietary method always used for validating an FFQ is multiple food records. Biomarkers are also used to validate dietary assessment methods. This is an approach using an external variable to measure the relative validity of the dietary assessment method. Most biomarkers have a strong direct relationship with intakes of dietary components (Gibson, 2005).

Statistical methods used to measure validity include paired *t*-tests for comparing means (Wilcoxon's signed rank test for comparing medians) of intakes from test and reference method. Correlation analysis is the most commonly used method to measure the strength of the relationship between the intakes from the test and the reference method, and between the intake from the test method and the biomarker. The strength of the relationship is indicated by the Pearson correlation coefficient. However, correlation analysis does not measure the extent of the agreement between two dietary methods; therefore other analyses such as percentage agreement and

Cohen's weighed kappa should be described together (Gibson, 2005). It is not possible to produce recommendations on an ideal mean difference, limits of agreement or correlation. However, for lower correlations, say below 0.3 or 0.4, it will be difficult to detect associations (Cade et al, 2001).

2.2.2.2 Reproducibility of dietary assessment methods

Reproducibility refers to the extent to which a specific dietary method used repeatedly in the same situation gives similar results. Reproducibility (also called reliability) of a dietary assessment method depends on the time frame of the method, the population group under study, the nutrient of interest, and the technique used to measure the foods consumed. True reproducibility cannot be measured because nutrient intakes vary daily. Reproducibility is estimated using test-retest design. The extent of agreement is assessed between the intakes obtained on two separate measures by the same method. Statistical methods used to measure validity include paired *t*-tests for comparing means (Wilcoxon's signed rank test for comparing medians) of intakes. Correlation analysis is used as well. For reproducibility of an FFQ, correlation coefficients between the two administrations of 0.5 to 0.7 were common (Cade et al, 2001). Percentage of misclassification is calculated to test agreement (Gibson, 2005).

2.2.3 Evaluation of dietary intake

2.2.3.1 Components of DRI

The Dietary Reference Intakes (DRIs) are a comprehensive set of nutrient reference values for healthy populations that can be used for assessing and planning

diets. The DRIs reflect the current state of scientific knowledge for nutrient requirements (Otten et al., 2006).

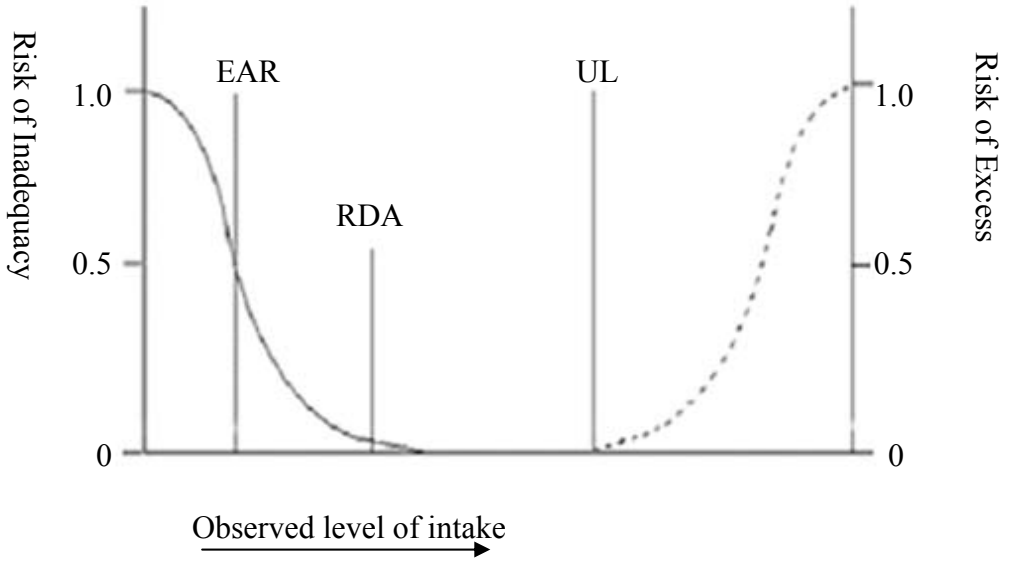


Figure 2.1 Relationship of DRI values to risk of nutrient inadequacy and risk of adverse health effects. EAR = Estimated Average Requirement; RDA = Recommended Dietary Allowance; UL = Tolerable Upper Intake Level

Adapted from Health Canada website

Figure 2.1 illustrates relationships among the different types of DRIs. The Estimated Average Requirement (EAR) is the intake at which the risk of inadequacy is 50% for an individual. The Recommended Dietary Allowance (RDA) is the intake at which the risk of inadequacy to the individual is very small (only 2% to 3%). The Adequate Intake (AI) does not bear a consistent relationship to the EAR or the RDA because it is set without being able to estimate the requirement, and as such, cannot be placed on the figure. At intakes between the RDA and the Tolerable Upper Intake

Level (UL), the risks of inadequacy and of excess to the individual are both close to zero. At intakes above the UL, the potential risk of adverse effects to the individual increases. Little is known about the shape of the risk curve, so the proportion of the population experiencing adverse effects at any specific intake above the UL cannot be estimated accurately.

2.2.3.2 Use of the DRIs in Dietary Assessment

The EAR is the best estimate of an individual's requirement. Usual intake below the EAR very likely needs to be improved (the probability of adequacy is 50% or less). Usual intake between the EAR and the RDA probably needs to be improved. Usual intake at or above the RDA has a low probability of inadequacy. Usual intake of the nutrient at or above the AI has a low probability of inadequacy. Usual intake below the AI cannot be assessed, as the AI has not been set using the probability method used to set RDA. Usual intake above the UL places an individual at potential risk of adverse effects from excessive intake of the nutrient (Barr, 2006; Health Canada, 2006).

Dietary assessment at the group level typically involves comparing usual nutrient intakes with nutrient requirements to assess the prevalence of inadequacy among the group members. Two approaches have been developed: 1) the probability approach, which involves determining the risk of inadequacy to each individual in the group and summing the probabilities (weighted average of the risk of inadequacy); 2) the EAR cut-point method, a simplified version of the probability approach that can be used when specific conditions are met, to approximate the proportion of the group with intakes below requirements. The RDA should not be used as a cut-point for

assessing nutrient intakes of groups because a serious overestimation of the proportion of the group at risk of inadequacy would result. For nutrients with an AI, the best that can be done is to look at mean and median intake relative to the AI. When the AI has been set based on the mean intake of a healthy group, similar groups with mean intakes at or above the AI can be assumed to be adequate. When mean intakes of groups are below the AI, nothing can be inferred about the adequacy of the group's intake. The UL can be used as a cut-point against which to measure usual intakes in order to estimate the proportion of a group at potential risk of adverse effects from excessive intake of a nutrient. (Barr, 2006; Health Canada, 2006)

2.3 SUMMARY OF THE LITERATURE

In summary, it is now recognized that the function of vitamin D is far beyond that required for calcium homeostasis. Vitamin D is important to over-all health and well-being. Vitamin D deficiency is common because environmental and personal factors affect cutaneous vitamin D synthesis. Further, dietary sources of vitamin D are quite limited (Holick, 2008).

Although there is a high prevalence of vitamin D deficiency in Canada, very few studies had evaluated vitamin D status from a multiethnic perspective. The reports of vitamin D deficiency related with vitamin D intake are still unavailable. To target the sub-populations at higher risk of vitamin D deficiency in Canada, ethnic group studies with reliable dietary assessment are needed. Thus appropriate approaches can be suggested for improving vitamin D supplementation in terms of high ethnic diversity in Canadian population.

The FFQ can be an effective tool for measuring the food consumption in the large cohort study. Comparing with other methods commonly used for dietary assessment, the FFQ imposes less burden on respondents and the results are easy to collect and process. Once the validity of the FFQ for estimating food intakes is identified, the nutrient intake information will be obtained from analyzing the FFQ results.

CHAPTER 3

MATERIALS AND METHODS

3.1 SUBJECT RECRUITMENT AND DATA COLLECTION

The study originated at the University of Toronto Mississauga campus, directed by Dr. Esteban Parra. Recruitment took place at the University of Toronto at Mississauga, Ontario during January-March of 2007. Ethical approval was obtained from University of Toronto Health Sciences Research Ethics Board. Participant eligibility for the study was assessed using a questionnaire that was completed prior to study enrollment. The following were exclusion criteria: age (< 18 years, > 30 years, except for one 17 years old male subject; in terms of age categorization in DRI for young adults); diseases associated with impaired vitamin D absorption or metabolism, bone or kidney or liver function, and intestinal absorption (including familial hypophosphatemia, fanconi syndrome-related hypophosphatemia, hyperparathyroidism, rickets, osteomalacia, osteopenia, hypercalcemia, kidney stones, gastrointestinal disease, anemia, Crohn's disease, Inflammatory Bowel Syndrome, Ulcerative Colitis); person taking drugs that affect vitamin D metabolism (examples include steroids, anticonvulsants, cholesterol-lowing drugs, osteoporosis medications and diuretics); recent (< three months) exposure to large amounts of UV radiation (tanning salons, visits to equatorial locations, etc).

A total of 107 subjects were eligible and agreed to participate. Most of the participants were either students or employees of the university. Participants met with the researchers twice during the study. During the first visit, their ancestries were

assessed based on responses to a personal questionnaire, which asked questions pertaining to the birthplace, migration history, native languages and self-reported ethnicity of the participants, their parents and grandparents. Anthropometric measurements (height and weight) were taken and melanin content was measured in the inner upper arm using a narrow band reflectometer (Dermaspectrometer, Cortex Technology, Hadsund, Denmark) (Gozdzik et al, 2008). One blood sample was drawn so that concentrations of serum 25(OH)D, calcium and PTH were measured. Participants completed a first food frequency questionnaire (FFQ) in the initial visit; and they returned their 7-day food diary and completed the second FFQ in the second visit (mostly within two weeks of first interview). Among the 107 participants, 105 of them completed both FFQs and the 7-day diary, representing a response rate of 98.1%.

The starting point of my work was analyzing raw data sent from University of Toronto. It included compiling vitamin D intake from the FFQ and coding the 7-day food diary using ESHA Food Processor software (version 8.0 and its revisions, ESHA Research Inc., OR, USA), which included Canadian Nutrient File 1997 from Health Canada.

3.2 DEVELOPMENT AND ADMINISTRATION OF THE FFQ

The FFQ administered in this study was based on a questionnaire developed for vitamin D and calcium intake assessment (Shrestha et al., 1994), which had been only validated against 4-day food records. The FFQ was expanded to include newly fortified foods and foods that might be consumed by persons from different

ethnicities. It consisted of 37 items of specific foods or food groups with 9 response options ranging from ‘never or less than 1 per month’ to ‘2+ per day’ for the frequency of consumption. The serving sizes were based on household measures (e.g. cups, spoons) and natural units (e.g. 1 slice). Additional open-ended questions on the FFQ collected information on nutritional supplement use.

Participants were asked to complete the FFQ twice: in the first visit, and then after two weeks, in the second visit. Subjects were asked to recall their frequency of consumption of food items over the preceding one month. Average daily intake of vitamin D was calculated by expressing the response to the food item as a portion of daily use, which was then multiplied by the amounts of the specified portion sizes and by the vitamin D content of the food. Average daily intake of calcium was obtained from the similar calculation. Vitamin D and calcium contents for each food item in the FFQ were determined using ESHA Food Processor (Appendix A & B). Dietary intake was organized as Excel spreadsheet report. Supplement intake reported in the FFQ was added into the spreadsheet. Vitamin D content of the supplements was obtained from the label declarations (Appendix C).

3.3 SEVEN-DAY DIARY AS A REFERENCE METHOD FOR VALIDATION OF THE FFQ

Detailed instructions were provided for the participants during the initial visit to complete food records on 7 consecutive days. The starting day was randomly allocated in the initial recording and then with the rest of the 7 days for the subsequent recording so that weekdays and weekend days were appropriately

recorded. Participants were asked to record, at the time of consumption, all foods and beverages (including snacks) eaten in household measures. Brand names of products and recipes used were included. Any nutritional supplements consumed were also recorded. Participants returned their 7-day food diaries during the second visit.

Among the 105 participants who provided food diaries, 97% of them had 7 days of records (*n*=102); 1% (*n*=1) had 6 days and 2% (*n*=2) had 5 days of records due to incomplete recording. ESHA Food Processor was used for food records analysis. Each food item and its amount recorded in the diary were determined according to the information available in the database of Food Processor. Appropriate food ingredients and amount were carefully determined when the records were in recipe format, and/or in non-Western dietary patterns. Dietary intake was organized as Excel spreadsheet report. Supplement intake recorded in the diaries was added into the spreadsheet to give total intake.

3.4 SERUM 25(OH)D AS THE BIOMARKER FOR VALIDATION OF THE FFQ

An aliquot of whole blood was centrifuged and the serum fraction was removed after clotting and stored at -80°C. 25(OH)D concentrations were determined by DiaSorin "25-OH Vitamin D TOTAL" competitive chemiluminescence immunoassay on the automated LIAISON ® analyzer (Stillwater, MN) (Gozdzik et al., 2008). This analysis was completed at the University of Toronto.

3.5 VALIDATION OF THE MODIFIED FFQ

Additional FFQ and 3-day diary records were collected from a small group of healthy young adults (*n*=39) at the University of Saskatchewan. This was approved by U of S Behavioral Ethics Research Board. Participants were the undergraduate students who enrolled in NUTR 221 in 2007-2008 Term 2. They had practiced recording a 3-day diary and coding it by themselves as their coursework. At the beginning of a class, the students were divided into two groups matched for age and gender. One of two FFQs was administered to each student. One FFQ was the original FFQ and the other was the proposed modified FFQ, i.e., with serving size and category modification (Table 3.1). FFQ vitamin D intake results were compared with the 3-day diary results, which had been verified by the course instructor.

Table 3.1 Comparison of the two FFQs used in the validation of the modified FFQ

	Original FFQ	Modified FFQ
The definition of 'large serving'	2 * medium serving	1.5 * medium serving
Options in 'orange juice' category	- Fortified with calcium - Fortified with Vitamin D and calcium	- Unfortified - Fortified with calcium - Fortified with Vitamin D and calcium

3.6 STATISTICAL ANALYSIS

Analyses were based on subjects (*n*=105) who had completed all three assessment methods [FFQ, 7-day diary and biomarker 25(OH)D]. Intake data were presented as

mean ± SD. Analyses were performed using SPSS version 15.0 (SPSS Inc, IL, USA). A P value < 0.05 was considered significant. Histogram displays were examined to determine the shape of the spread required by inferential statistics (Appendix G). Significant departures from the assumption of normality were found for some variables including serum 25(OH)D, melanin index, and all intake variables (except energy intake). Therefore, square-root transformations were performed to normalize the distribution. Paired *t*-test was used to examine mean vitamin D intakes on FFQ and food diary, and on the first and second FFQ. Correlation analysis was used to measure the strength of the relationship between the intakes from the two dietary methods, and between the two methods and the biomarker, 25(OH)D. Cross-classification and weighted kappa (κ_w) were used to test the relative agreement between FFQ and 7-day diary, and between the first and second FFQ (Gibson, 2005). ANOVA was used to evaluate group difference for serum measurements and intake levels. The LSD method was used in post-hoc analysis for multiple comparisons.

When transformed data did not satisfy normality, the non-parametric test Kruskal-Wallis was used to evaluate group difference in supplement use and soy milk consumption. The Mann-Whitney test was used when there were less than 20 cases for two of the calcium intake stratified sub-groups. Fisher's exact test results were reported when using Chi-square test to evaluate the ethnic difference in the distribution of vitamin D status, vitamin D intake levels and supplement use because some frequencies (*n*) were less than 10.

CHAPTER 4

RESULTS

4.1 CHARACTERISTICS OF THE STUDY SAMPLE

The characteristics of the study sample are highlighted in Table 4.1. The subjects in this study were between 17 and 28 years old, with mean age at 20 years. Among the subjects, 46 of them (43.8%) had BMI below 18.5 kg/m², whereas 10 of them (9.5%) were classified as overweight (BMI ≥ 25 kg/m²) and 3 of these were obese (BMI ≥ 30 kg/m²). Compared with female students, male students were one year older (21.3 y vs. 20.2 y) and had higher BMI (21.3 kg/m² vs. 18.8 kg/m²). Subjects self-identified their ethnicities as European (*n*=31), East Asian (*n*=27), South Asian (*n*=32), African (*n*=7), and others (*n*=8). The 'others' include subjects who self-identified more than one ethnicity and who reported being of other ethnicities. Most of the analyses were performed using the three major ethnic groups European, East Asian and South Asian only (*n*=90). Gender differences were found in age and BMI ($P<0.05$) but not in melanin index, PTH, and 25(OH)D. Gender differences were found in average energy intake ($P< 0.05$), but not in vitamin D and calcium intakes in terms of either FFQ or 7-day diary results.

Table 4.1 Characteristics of the study sample in total and by gender

Variables	Total participants (*n*=105)	Male (*n*=47)	Female (*n*=58)
Age (y) *	20.7 ± 2.0	21.3 ± 2.3	20.2 ± 1.6
BMI (kg/m²) *	19.9 ± 3.9	21.3 ± 3.4	18.8 ± 3.9
Melanin index	35.1 ± 8.5	36.0 ± 10.7	34.4 ± 6.2
25(OH)D (nmol/L)	42.8 ± 16.5	42.1 ± 15.2	43.4 ± 17.6
PTH (pmol/L)	3.4 ± 1.2	3.5 ± 1.1	3.2 ± 1.2
Vit D intake (IU/d):			
7-d diary	172 ± 145	146 ± 129	193 ± 158
FFQ	258 ± 177	235 ± 170	276 ± 181
Calcium intake (mg/d):			
7-d diary	916 ± 379	946 ± 403	892 ± 360
FFQ	1032 ± 627.9	1078 ± 645	995 ± 617
Energy intake (kcal/d)*	1989 ± 555	2141 ± 610	1866 ± 476

* $P<0.05$. Variables were presented as mean ± SD. 7-day diary results were original. FFQ results were the modified first FFQ results. Energy intake values were from 7-day diary results.

4.2 VALIDITY AND REPRODUCIBILITY OF THE FFQ

4.2.1 Modification of original FFQ results

When we compared intakes from 7-day diary and the first FFQ, it was apparent that the subjects had overestimated their vitamin D intake in the first FFQ. The participants who claimed large as serving size in their FFQs overestimated their milk consumption. Furthermore, there was no 'Unfortified' but only 'Fortified with calcium' and 'Fortified with calcium and vitamin D' options in the 'Orange juice' category in the FFQ food list. It was observed that participants who claimed fortified orange juice consumption in their FFQs had not recorded this in their 7-day diaries.

Overestimation of milk intake and inappropriate classification of juice intake into fortified sub-categories seemed to account for the higher vitamin D intake results of

FFQ. Ethnic and gender differences were not found in these reporting behaviors. Therefore we re-calculated the vitamin D intake by assuming the error in description of large serving size and categorization of orange juice. In the analysis using the modified FFQ, all fortified orange juice reporting was excluded as the analysis of the 7-day diary demonstrated that none of the subjects had consumed it.

4.2.2 Meat-adjustment of 7-day diary results

The analysis of the 7-day diary was further reconciled to account for vitamin D in meat using Canadian Nutrient File 2007b which contains updated meat vitamin D values. The output spreadsheets for each subject's intake derived from the 7-day diary were reviewed. Meat items were re-assessed for their vitamin D amounts in terms of vitamin D values provided in the new 2007 Canadian nutrient database.

From the 7-day diary, vitamin D from meat intake was 16±13 IU/d, only 8.5% of total vitamin D intake. There was no ethnic difference in meat intake ($P>0.05$). The 7-day diary values with and without accounting for meat were highly correlated ($r=0.996$, $P<0.001$). Correlations of the original or meat-adjusted intakes from 7-day diary with serum 25(OH)D levels showed significant correlation ($r=0.513$ vs. $r=0.515$, $P<0.001$). This indicates that our analyses of vitamin D intake using the 1997 Canadian Nutrient File (i.e., lacking intake values for meat) was not underestimating intake in these subjects by a significant amount. All results presented in this report are those without meat values.

4.2.3 Validity assessment of the FFQ

The Pearson correlation coefficients between FFQ and the reference method, as well as between the FFQ and the biomarker are shown in Table 4.2. The correlation coefficients between FFQ and 7-day diary were good, i.e. higher than 0.50. FFQ was significantly related with the biomarker ($P<0.001$). For relative agreement between FFQ and 7-day diary, more than 65% were correctly classified into the same third of intake and less than 20% were misclassified into the opposite third. The weighted kappa showed close to moderate agreement. The correlations and agreement tended to be stronger after using modified FFQ results.

4.2.4 Reproducibility assessment of the FFQ

Mean vitamin D intakes measured from the first and second FFQ and the mean differences between these two measures are presented in Table 4.3. Mean intakes from the first FFQ were significantly higher than the second FFQ ($P<0.05$), indicating a change in reporting behaviour. For relative agreement, less than 40% were correctly classified into the same third of intake and more than 35% were misclassified into the opposite third. However, the Pearson correlation coefficients between these two FFQs were higher than 0.65, indicating reliability. The correlations and agreement tended to be stronger after using modified FFQ results.

Table 4.2 Pearson correlation coefficients between each of the three methods, percentages of subjects classified into the same and opposite tertiles of intakes, and weighted kappa (κ_w) of 1st FFQ and 7-day diary (n=105)

	Pearson correlation coefficients		Cross-classification (FFQ vs. 7-d diary)		
			% in tertiles:		
	FFQ vs. 7-d diary	FFQ vs. 25(OH)D	Same	Opposite	κw
Original FFQ	0.529 *	0.481 *	69	12	0.33
Modified FFQ	0.602 *	0.520 *	66	17	0.37

* P<0.001. 7-day diary results were original. FFQ results were the first FFQ results.

Table 4.3 Pearson correlation coefficients, percentages of subjects classified into the same and opposite tertiles, and weighted kappa (κ_w) of 1st and 2nd FFQ (n=105)

	1st FFQ (IU/d)	2nd FFQ (IU/d)	P	1st FFQ – 2nd FFQ	Correlation		% in tertiles:		κw
					r	P	Same	Opposite	
Original	311 ± 217	264 ± 241	0.013	47 ± 189	0.663	0.001	29	46	-0.06
Modified	258 ± 177	221 ± 175	0.005	37 ± 130	0.725	0.001	37	37	0.03

* Intake values are presented as mean ± SD.

4.2.5 Validation of the modified FFQ using a U of S student sample

There were 39 students participating in this small survey; 20 students filled the unmodified FFQ while 19 students filled the modified FFQ. Mean vitamin D intakes measured from 3-day diary and FFQ are presented in Table 4.4. There was no significant difference in either 3-day diary or FFQ results between the two groups. For students who filled unmodified FFQ, their FFQ results were higher than the 3-day diary results ($P<0.05$) but these two results were highly correlated ($r=0.641$, $P=0.002$). For students who filled modified FFQ, their mean values of 3-day diary and FFQ results were not significant different ($P>0.05$) but not correlated ($r=0.287$, $P=0.233$).

Table 4.4 Vitamin D intakes measured from 3-d diary and FFQ of the U of S student sample ($n=39$)

Group	Vit D intake (IU/d; 3-d diary)	Vit D intake (IU/d; FFQ)	*P*	*Correlation*	
				r	*P*
Unmodified FFQ ($n=20$)	191 ± 91	257 ± 174	0.043*	0.641	0.002*
Modified FFQ ($n=19$)	215 ± 113	248 ± 134	0.347	0.287	0.233

*$P<0.05$. Intake values were presented as mean ± SD.

4.3 VITAMIN D INTAKE AND STATUS BY ETHNICITY

4.3.1 Variables of the study sample by ethnicity

We focused on 90 participants of these three groups: East Asian ($n=27$), European ($n=31$) and South Asian ($n=32$). Ethnic differences were found in melanin index and serum 25(OH)D concentrations ($P<0.001$). Ethnic differences were also found in vitamin D intake and calcium intake ($P<0.05$) (Table 4.5).

Table 4.5 Variables of the study sample by ethnicity

Variables	East Asian	European	South Asian
n (male, female)	27 (10, 17)	31 (15, 16)	32 (13, 19)
Age (y)	20.5 ± 1.8	20.9 ± 2.3	20.6 ± 2.2
BMI (kg/m²)	18.6 ± 4.1	20.6 ± 4.0	19.9 ± 3.7
Melanin index *	32.0 ± 3.0 [b]	28.6 ± 2.5 [a]	38.3 ± 5.0 [c]
25(OH)D (nmol/L) *	37.7 ± 11.9 [a]	56.0 ± 19.4 [b]	35.7 ± 11.1 [a]
PTH (pmol/L)	3.1 ± 0.9	3.1 ± 1.1	3.5 ± 1.2
Vit D intake (IU/d):			
7-d diary *	133 ± 102 [a]	231 ± 173 [b]	164 ± 143 [ab]
FFQ *	225 ± 196 [a]	336 ± 197 [b]	228 ± 127 [a]
Calcium intake (IU/d):			
7-d diary *	754 ± 294 [a]	1074 ± 397 [c]	958 ± 396 [bc]
FFQ *	801 ± 639 [a]	1293 ± 734 [b]	1009 ± 526 [ab]
Energy intake (kcal/d; 7-d diary) *	1779 ± 444 [a]	2187 ± 618 [b]	1941 ± 509 [ab]

*P<0.05. Variables are presented as mean ± SD. Values with same letters were not significantly different in multiple comparisons. Meat intake was not considered in the analyses. FFQ results were the modified first FFQ results.

4.3.2 Serum vitamin D levels

Median serum 25(OH)D concentration was as low as 38 nmol/L when combining subjects in the three ethnic groups. In all, 71.1% (n=64) had vitamin D deficiency according to the definition [serum 25(OH)D < 50 nmol/L]. Median serum (OH)D was lower in the East Asian (35 nmol/L) and the South Asian (33 nmol/L) groups than in the European group (54 nmol/L) (P<0.05). Much larger percentages of subjects in the East Asian and the South Asian groups had vitamin D deficiency [25(OH)D<50 nmol/L] than that in the European group (P<0.001). Each ethnic group had very few subjects or even no subjects who reached the desirable 25 (OH)D level of 75 nmol/L (Table 4.6 & Figure 4.1).

Table 4.6 Serum 25(OH)D by ethnic group

25(OH)D		East Asian (*n*=27)	European (*n*=31)	South Asian (*n*=32)
Median (nmol/L) (25, 75%)		35 (30, 40)	54 (41, 63)	33 (27, 44)
% of subjects within each group	< 50 nmol/L *	85.2% [b] (*n*=23)	41.9% [a] (*n*=13)	87.5% [b] (*n*=28)
	< 75 nmol/L	96.3 % (*n*=26)	87.1 % (*n*=27)	100% (*n*=32)

*P<0.05. Values with same letters were not significantly different in multiple comparisons.

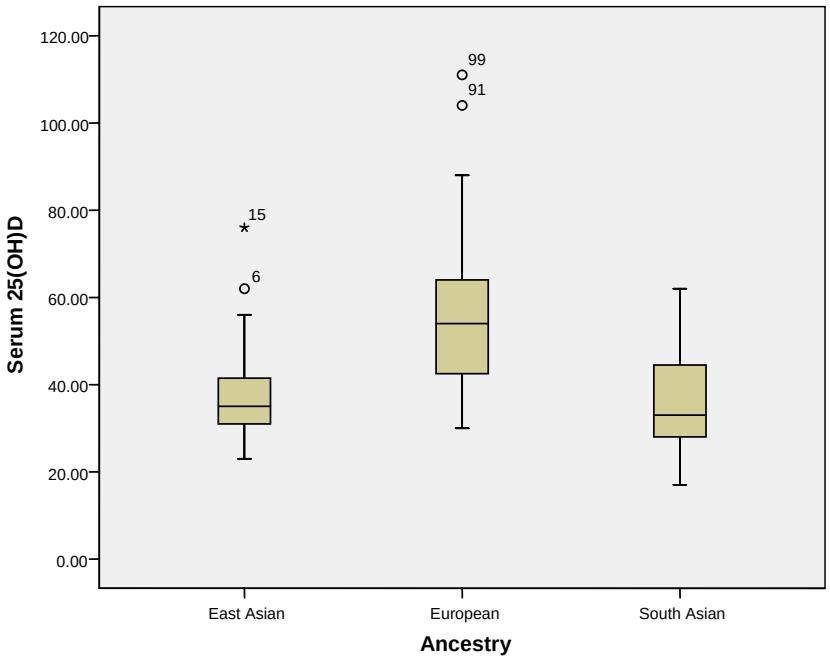


Figure 4.1 Serum 25(OH)D (nmol/L) according to ethnic group

* The boxplot shows minimum, 25%, median, 75% and maximum values of 25(OH)D levels for each ethnic group. The four dots were outliers with their ID numbers. The dots with mark ‘ º ’ were classified as outliers; the dot with mark ‘ * ’ was an extreme outlier (Hinton et al., 2004).

4.3.3 Vitamin D intake levels

The European group had higher total vitamin D intake than the other two groups ($P<0.05$. Table 4.5). Percentages of subjects in each group who had vitamin D intake lower than the AI value of 200 IU/d and lower than 400 IU/d are presented in Table 4.7. There was no significant ethnic difference among these percentages ($P>0.05$).

Table 4.7 Distribution of vitamin D intake below 200 and 400 IU/d by ethnic group

	East Asian (*n*=27)	European (*n*=31)	South Asian (*n*=32)
	7-d diary		
% below 200 IU/d (AI)	77.8% (*n*=21)	51.6% (*n*=16)	71.9% (*n*=23)
% below 400 IU/d	96.3% (*n*=26)	83.9% (*n*=26)	93.8% (*n*=29)
	FFQ		
% below 200 IU/d (AI)	55.6% (*n*=15)	29.0% (*n*=9)	50% (*n*=16)
% below 400 IU/d	85.2% (*n*=23)	64.5% (*n*=20)	87.5% (*n*=28)

* 7-day diary results were original. The percentages were from the modified first FFQ results.

4.3.4 Sources of vitamin D intake

Sources of vitamin D intake were categorized from FFQ as dairy products, non-dairy products and supplement (Table 4.8). Intakes of some major vitamin D foods were also presented in the table. There was a trend that the European group was relatively higher in dairy products intake ($P=0.089$) and in cow's milk consumption ($P=0.074$). The East Asian group had higher intake of soy milk than the other two groups ($P=0.001$).

Although there was ethnic difference in total vitamin D intakes, significant difference was not found in mean intakes from specific foods vs. supplements among

Table 4.8 Sources of vitamin D intake (IU/d & % of total intake) by ethnic group

		East Asian (*n*=27)	European (*n*=31)	South Asian (*n*=32)
Total vit D intake (IU/d) *		225 ± 196 [a]	336 ± 197 [b]	228 ± 127 [a]
Dairy products **	(IU/d)	133 ± 155 [a]	194 ± 151 [b]	159 ± 108 [ab]
	(%)	59%	58%	70%
Non-dairy products	(IU/d)	48 ± 40	54 ± 59	33 ± 26
	(%)	21%	16%	15%
Supplements	(IU/d)	44 ± 96	87 ± 152	36 ± 83
	(%)	20%	26%	16%
Major food sources (IU/d)				
Cow's milk **		106 ± 152 [a]	162 ± 152 [c]	139 ± 96 [b]
Margarines		8 ± 9	10 ± 16	6 ± 12
Fish		21 ± 30	27 ± 58	15 ± 20
Egg		16 ± 14	14 ± 16	9 ± 9
Soy milk (fortified) *		19 ± 35 [b]	8 ± 23 [a]	4 ± 18 [a]

Intake values are presented as mean ± SD. Values were from the modified first FFQ results. Intake values with same letters were not significantly different in multiple comparisons. The percentages refer to distribution of vitamin D provided by dairy, non-dairy and supplement sources.
* $P<0.05$; ** $0.05<P<0.10$.

Table 4.9 Food vs. supplement sources of vitamin D intake (IU/d) by ethnic group

	East Asian (*n*=27)	European (*n*=31)	South Asian (*n*=32)
7-d diary			
Foods	97 ± 66	142 ± 94	130 ± 104
Supplement	37 ± 92	89 ± 148	34 ± 83
FFQ			
Food	181 ± 160	248 ± 165	192 ± 112
Supplement	44 ± 96	88 ± 152	36 ± 83

* Intake values are presented as mean ± SD. Intakes were the modified first FFQ results.

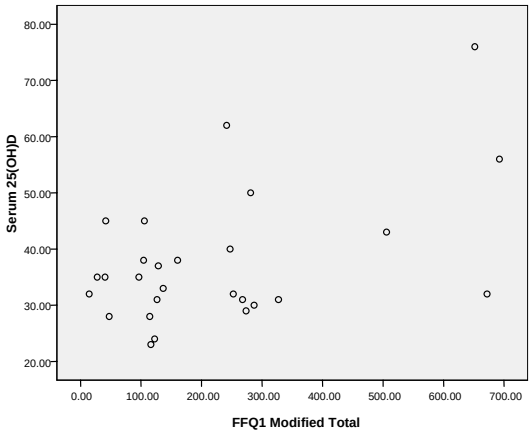
the three groups (P>0.05) (Table 4.9). In the European group, 29% (9 of 31) took vitamin D supplements, while only 22.2% (6 of 27) in the East Asian group and 25% (8 of 32) in South Asian group took them. There was no significant ethnic difference among these percentages.

4.3.5 Effect of vitamin D intake, melanin and BMI on serum vitamin D status

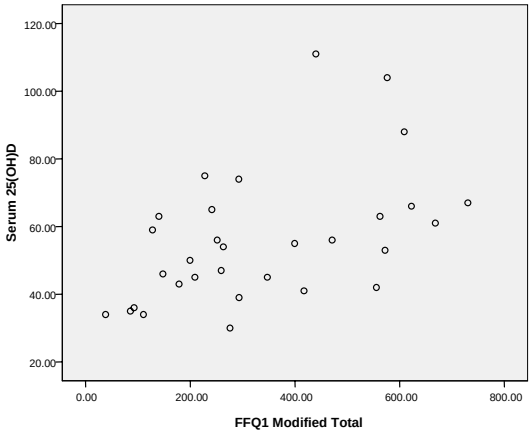
Vitamin D intake (from the first modified FFQ results) was significantly positively related to serum 25(OH)D concentrations in each of the three ethnic groups, while melanin content was not (Figure 4.2 & 4.3). Melanin content was negatively related with serum 25(OH)D when data from all three ethnic groups were combined (r=-0.270, P=0.01). After controlling for vitamin D intake, partial correlation test showed the P values became marginal (r=-0.206, P=0.052). Vitamin D intake was always related with serum 25(OH)D when data were combined from all three ethnic groups either before or after being controlled for melanin content (r=0.520, P<0.001 vs. r=0.511, P<0.001). The relationships among vitamin D intake, melanin and serum vitamin D status are demonstrated in Figure 4.4. It can be seen that the effect of dietary vitamin D intake on serum 25(OH)D concentrations was greater than the effect of melanin content on serum 25(OH)D concentrations.

BMI was not related with either serum 25(OH)D concentrations (r=-0.018, P=0.869) or with vitamin D intake (from the modified first FFQ results; r=-0.087, P=0.414). There were no gender and ethnic differences for these observations (P>0.05).

A East Asian (n=27) r=0.498, P=0.008



B European (n=31) r=0.493, P=0.005



C South Asian (n=32) r=0.421, P=0.016

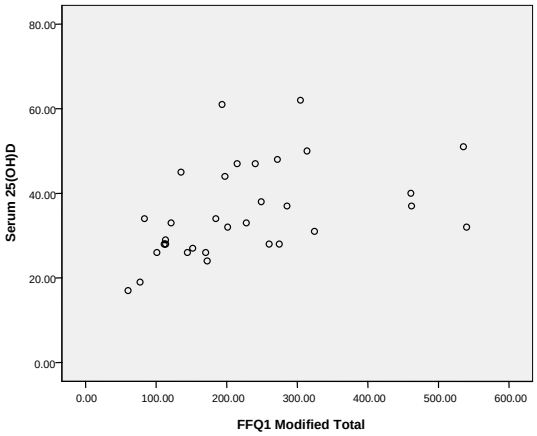
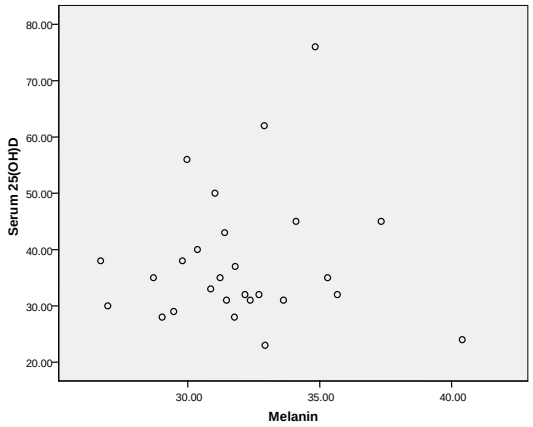
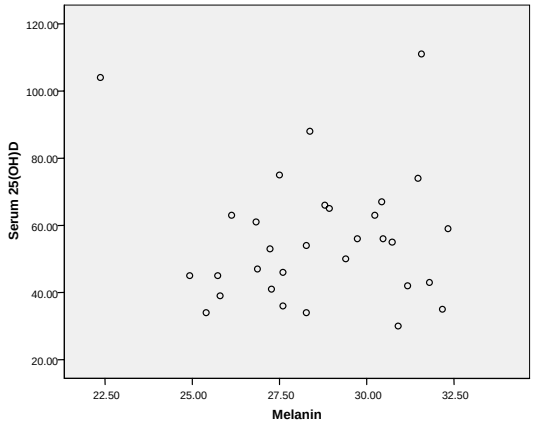


Figure 4.2 Relations between mean vitamin D intake and serum 25(OH)D by ethnic group. Vitamin D intake values were from the modified first FFQ results.

A East Asian (*n*=27) *r*=0.066, *P*=0.745



B European (*n*=31) *r*=-0.030, *P*=0.874



C South Asian (*n*=32) *r*=0.340, *P*=0.057

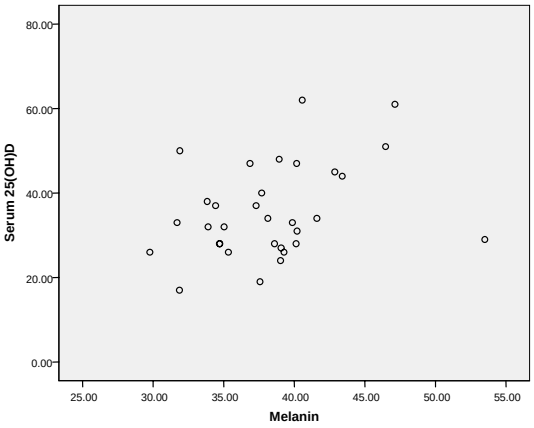


Figure 4.3 Relations between mean melanin index and serum 25(OH)D by ethnic group

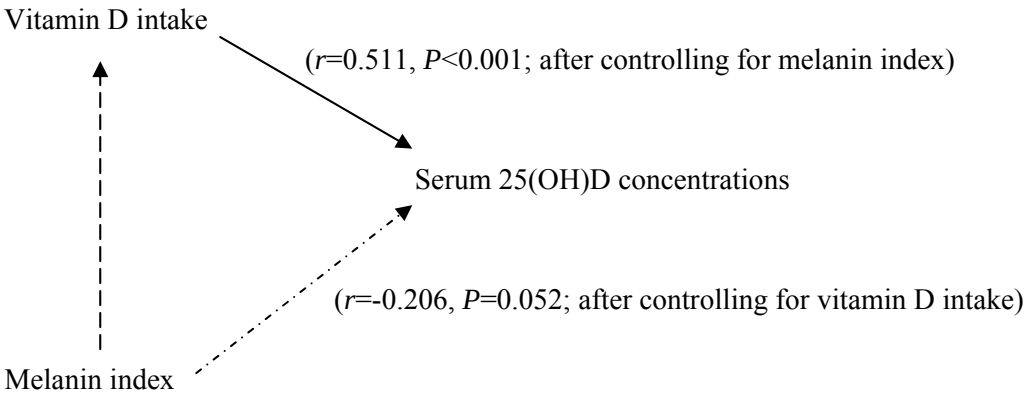


Figure 4.4 Relationship among vitamin D intake, melanin and serum 25(OH)D. Vitamin D intake values were the modified first FFQ results.

4.4 EFFECT OF SERUM 25(OH)D LEVELS AND CALCIUM INTAKE ON PTH LEVELS

In Table 4.5, it was shown that there was an ethnic difference in mean calcium intake ($P<0.05$). The European group's calcium intake was significantly higher than the East Asian group ($P<0.05$). However, there was no ethnic difference in PTH levels ($P>0.05$).

There was an inverse relationship between serum 25(OH)D concentrations and PTH levels ($r=-0.273$, $P=0.009$) when the three ethnic groups were combined. When 25(OH)D concentrations were categorized as ≥ 50 nmol/L and < 50 nmol/L, and calcium intakes were categorized as ≥ 1000 mg/d (AI) and < 1000 mg/d, PTH levels were significant lower when vitamin D status was sufficient ($P<0.05$) but did not show a difference by calcium intake levels ($P>0.05$; Table 4.9). Table 4.10 shows that with categorized 25(OH)D levels, there was no difference in PTH levels by

calcium intake levels (P>0.05). However, with higher calcium intake (≥ 1000 mg/d), PTH levels were lower for subjects who had sufficient 25(OH)D levels (P<0.05).

Table 4.10 PTH levels by serum 25(OH)D levels or calcium intake (n=90)

	Serum 25(OH)D	
	< 50 nmol/L (*n*=64)	≥ 50 nmol/L (*n*=26)
PTH (pmol/L) *	3.43 ± 1.07	2.77 ± 0.98

	Calcium intake	
	< 1000 mg/d (*n*=52)	≥ 1000 mg/d (*n*=38)
PTH (pmol/L)	3.22 ± 1.12	3.26 ± 1.04

* P<0.05. PTH levels were presented as mean ± SD. Calcium intakes were the modified first FFQ results.

Table 4.11 PTH levels by calcium intake and serum 25(OH)D (n=90)

	Calcium intake	
	< 1000 mg/d	≥ 1000 mg/d
25(OH)D < 50 nmol/L		
n	39	25
PTH (pmol/L)	3.34 ± 1.12	3.57 ± 1.01
25(OH)D ≥ 50 nmol/L		
n	13	13
PTH (pmol/L)	2.88 ± 1.11	2.67 ± 0.85

	25(OH)D levels	
	< 50 nmol/L	≥ 50 nmol/L
Calcium intake < 1000 mg/d		
n	39	13
PTH (pmol/L)	3.34 ± 1.12	2.88 ± 1.11
Calcium ≥ 1000 mg/d		
n	25	13
PTH (pmol/L) *	3.57 ± 1.01	2.67 ± 0.85

* P<0.05. PTH levels were presented as mean ± SD. Calcium intakes were the modified first FFQ results.

CHAPTER 5

DISCUSSION

5.1 Discussion

This study provides evidence that vitamin D intake and serum vitamin D status vary with ethnicity in healthy young adults in Canada in wintertime. We demonstrate a high prevalence of vitamin D deficiency in our study sample and suggest that dietary intake has an important role in predicting serum vitamin D levels in wintertime.

We used a cross-cultural semi-quantitative FFQ for estimating vitamin D intake in a young adult population. The subjects of our study were recruited from the same population in which a larger study (n=600) of vitamin D intake and status assessment will be carried out. We piloted the FFQ on a sufficient and representative sample of the population to which the final FFQ will be applied. However, we cannot rule out the possibility that those who participated in this study had a greater interest in and awareness of vitamin D intake than those who would be included in nutrition epidemiological studies using the FFQ alone. Although the subjects were well-motivated to complete the food diary for seven days, there was the possibility that the food diary was not representative of long-term dietary habits that we expected.

We assessed the validity and reproducibility of the FFQ used in our study. The FFQ was found to be valid as the FFQ results were highly correlated with the reference dietary method (7-day diary) and the biomarker [25(OH)D], and the agreement between the FFQ and 7-day diary results were satisfactory as well.

Comparison of means revealed a tendency for higher estimation of intake on the FFQ than the 7-day diary. We therefore attempted to eliminate this bias through modification of the FFQ. After modification, the strength of correlation and extent of agreement were both enhanced. The first FFQ highly correlated with the second FFQ; a significant difference in mean intakes between the two time periods, however, indicated a bias. The agreement between the first and second FFQ also was not satisfactory. The large within-subject variation may be due to the short time frame between the two FFQs. Having become aware of the study purpose and having learned from food diary recording, the subjects might have adjusted their estimation of intake amounts in the second FFQ.

The small study of FFQ modification in the University of Saskatchewan student sample did not support the notion that modification produced a better FFQ. However, the reference method used for comparison was a 3-day diary and the subjects were recruited from a different population. The students in this sample had obtained training in food recording and diet reporting in their nutrition class. The small sample size might also affect the statistic results

The Canadian Nutrient File recently added vitamin D values for meat. We investigated whether or not having these additional contents would be a problem on our 7-day diary. Meat foods contain relatively low vitamin D. The meat intake level was not high in our study sample and there was no ethnicity difference in meat vitamin D intake. While updated vitamin D values from meat intake in 7-day diary results did not change intake results, meat-containing foods should be added into the FFQ list for future use.

Vitamin D deficiency [25(OH)D < 50 nmol/L] was widespread in wintertime among the subjects in this study (71.1%). Further, more than 85% of the subjects did not reach the desirable 25(OH)D level (75 nmol/L). This low vitamin D status in young healthy adults in our study was consistent with the findings of other studies on children, adolescents, and older adults in Canada in wintertime (e.g., Roth et al., 2005; Vecino-Vecino et al., 2006). In our study vitamin D deficiency was more apparent in the East and South Asian groups than in the European group. This result was parallel with studies which showed Caucasian or non-Hispanic whites had lower prevalence of vitamin D deficiency than other darker-skinned ethnic groups such as African Americans or Pacific islanders (Holick, 2002; Nesby-O'Dell et al., 2002; Zadshir et al, 2005; Rockell et al., 2006; Yetley et al., 2008). The significance of low 25(OH)D in wintertime is not fully understood. There are indications of seasonal incidence of diseases that have been related to vitamin D status. For example, epidemiological evidence indicates vitamin D deficiency is the 'seasonal stimulus' of influenza (Cannell et al, 2006).

There were four subjects in our sample who had much higher 25(OH)D levels than others in their ethnic groups (62 and 76 nmol/L in East Asian group; 104 and 111 nmol/L in European group), which were shown as 'outliers'. However, it is physiologically acceptable that individuals have high 25(OH)D levels if they consume more vitamin D and/or have experienced more sun exposure. Vitamin D intoxication typically does not occur until 25(OH)D concentration are higher than 375 nmol/L (Holick, 2008). Further, statistical tests showed similar results either with

or without these values. Therefore we included these four outliers in our analysis procedure.

The ethnic difference in total vitamin D intake was parallel with what we found in vitamin D status. This is consistent with the recent finding that there is no racial difference in response of serum 25(OH)D per 1 μg of vitamin D intake (Aloia et al, 2008). In our study the European group had higher total vitamin D intake than the East and South Asian groups. The dairy group was the greatest food source of vitamin D for each of the three groups. This was similar with the vitamin D intake observed in the United States (Moore et al., 2004). There was a trend that the European group had higher consumption of dairy products, especially in cow's milk (*P* values between 0.05 and 0.10). We believe this is evidence that consumption of dairy products and in particular cow's milk is higher in European subjects' diets than in East and South Asian groups. The East Asian group had a significantly higher consumption of fortified soy beverage but this made little difference to overall mean intake of vitamin D. Thus we suggest that fortified soy beverage could be an important source of vitamin D for some ethnic groups if consumed more often. There was no significant ethnic difference in supplement use. We observed that using supplements was not common for subjects in any of the three groups.

In this study, vitamin D intake was well related with serum 25(OH)D levels. The data in our study were collected in late wintertime, when skin production of vitamin D was not possible and previous serum vitamin D stores had been depleted or nearly depleted. This timing allowed for an examination of the importance of dietary intake on vitamin D status in the winter. The close correlation between melanin content and

serum 25(OH)D concentrations became marginal after vitamin D intake was controlled. An explanation could be that melanin content was consistent with ethnic grouping, and, as we showed, there was an ethnic difference in vitamin D intake. Thus we conclude that melanin's relation with serum vitamin D levels reflected the ethnic difference in vitamin D intake in our study.

NHANES data have indicated an inverse relation between measures of BMI and serum 25(OH)D concentrations (Yetley, 2008). However, this relationship between BMI and serum 25(OH)D was not observed in our study. Our sample subjects had relatively low BMIs and there were few obesity cases. Among 105 subjects, only 13 of them had BMI higher than 25 kg/m², but 46 of them had BMI lower than 18.5 kg/m². In another words, the variance of this variable (BMI) might not be sufficient in our study for the analysis to observe the effect of body fat on 25(OH)D levels that others have reported (Arunabh et al., 2003; Looker et al., 2005; Rockell et al., 2006).

An inverse relationship between 25(OH)D and PTH was observed in this study. This is consistent with the demonstration that PTH levels are elevated with vitamin D deficiency (Passeri et al., 2008). The mean PTH levels were significantly higher for subjects who had vitamin D deficiency [25(OH)D < 50 nmol/L]. Further, with adequate calcium intake (≥1000 mg/d), PTH levels were significantly lower only when vitamin D was sufficient. The results of our study suggest that for suppressing PTH levels, it is more important to keep sufficient vitamin D status than to raise calcium intake levels. A previous study involving more subjects (n=2310) used two cutoffs of calcium intake, 800 and 1200 mg/d, to demonstrate the relationship between serum PTH levels, vitamin D sufficiency and calcium intake

(Steingrimsdottir et al., 2005). We only used the calcium intake at 1000 mg/d, the AI for young adults, as the cutoff in our study due to our smaller sample size.

5.2 Limitations

When Gibson (2005) described the reproducibility in dietary assessment, she noted that in repeated dietary assessment, subjects might show a sequence effect that might result in changing reported nutrient intakes over time. This effect could be severe if subjects completed the repeated assessment too close in time. Although the two-week time frame of our study brought us fairly high response rate of the participants, the subjects likely changed the description of their intake habits in the second FFQ, which was completed soon after the food diary recording.

Although the sample size of our study did not limit the validation assessment of the FFQ, the relatively small number of subjects in each ethnic group affects the power of statistical analysis for vitamin D intake and status assessment. A main consequence is that some relationships may not be observed or may not be strong enough among the variables. In our study, the fairly small size of some groups (e.g., African group) also limits the opportunity to look into other ethnicities than East Asian, South Asian and European.

Another limitation is that most of the subjects in our study were either students or employees of the University of Toronto, and thus, were likely to have different socio-economic status than the general population. Therefore we cannot generalize our findings to the whole population of young healthy adults living in Canada.

5.3 Future Research

Since there is a wider variety of ethnicity in Canada, the findings of our study should encourage large studies to further investigate vitamin D intake and status with more subjects and in more ethnic groups. For example, I propose the following study. Ranked after Ontario, British Columbia had the second highest proportion of foreign born population in Canada in 2006 (BC Stats, 2008). Of those who reported a single ethnic origin, the largest proportion was Chinese (17.7%), which was higher than the second, Canadian (12.4%). The other single ethnic origins reported were English, East Indian, German, Scottish, Filipino, Irish, Italian, and Ukrainian. It has been reported that vitamin D deficiency is common in non-pregnant women in Hong Kong and Beijing in late winter and early spring (Woo et al., 2008). It would be interesting to investigate if food fortification in Canada and the change of life style will affect vitamin D intake and status of immigrated Chinese living in BC. To target other ethnic groups at risk of vitamin D deficiency would also be important.

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APPENDIX A

SAMPLE OF FOOD DIARY

Direction for using the food diary

1. It is important to keep your food diary current. List foods immediately after they are eaten. **Please print or write legibly all entries**.

2. Record only one food item per line in this record booklet.

3. Be as specific as possible when describing the food item eaten. We need to know the way it was cooked (if it was cooked) and the amount that was eaten.

4. Include brand names and restaurant names whenever possible.

5. Report only the food portion that was actually eaten – for example: chicken leg, **3 oz. baked with skin**. (Do not include the weight of the bones and indicate part of food eaten.)

6. Record amounts as weights or volumes. You may use household measures – for example: **tablespoons, cups, slices** or **units**, as in **one cup of skim milk**. Whenever possible please use the measuring tools we have provided for you to describe the portion size you have eaten.

7. Include the method that was used to prepare the food item – for example: **fresh, frozen, stewed, fried, baked, canned, broiled, raw** or **braised**.

8. For canned foods, include the type of liquid in which it was canned: **sliced peaches in heavy syrup, fruit cocktail in light syrup** or **tuna in water**.

9. Do not alter your normal diet during the period you keep this diary.

10. Remember to record the amounts of fats used in mixed dishes (such as sandwiches). These fats include oils, butter, salad dressings, margarine.

PLEASE LIST EVERY FOOD and DRINK as you consume it *DAY 1* *Date*: _____

Time	Food Items	Type & Preparation	Amount	Brand Name or Where Bought	Code
Morning					
Mid-morning					
Noon Meal					
Midday					
Evening Meal					
Before Bed					

Was this intake usual? Circle one: Yes No (if No, explain why not _____)

Did you take any vitamins/minerals during this time? Circle one: Yes No (if Yes, list names on next page)

BRAND NAME OF SUPPLEMENT	NUTRIENTS	AMOUNT

Recipe information:

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

APPENDIX B

SAMPLE OF FOOD FREQUENCY QUENSTIONNAIRE

Subject ID _____

FOOD FREQUENCY QUESTIONNAIRE

Today's date: _____

Please list **nutritional supplements** used in past month:

BRAND NAME OF SUPPLEMENT	NUTRIENTS	AMOUNT

1. We want to know how often you eat or drink certain foods **each month**.

2. Think about a **typical month** not just what you ate this week which might be different.

3. **Medium** portion sizes are given to help you determine the usual size of the food or drink, and to compare to small and large.

4. If you drink or eat *much less* (*approximately half*) than the medium portion size described, then check small. If you drink a large glass of milk every day (*approximately twice the size of medium*) then check large.

5. Fill out the form similar to this example:

- If you drink a carton of chocolate milk (500 mL) Monday through Friday, then choose L (large) because it is 2 times the size of the medium portion.

Types of Food or Drink	Never or less than 1 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**	Your Serving Size		
											S	M	L
Milk: whole, 2%, 1% or skim										1cup (8 oz or 250 mL)			
Chocolate Milk: whole, 2%, 1% or skim							X			1 cup (8 oz or 250 mL)			X

Types of Food or Drink	Never or less than 1 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**	Your Serving Size		
											S	M	L
Milk: whole, 2%, 1% or skim										1cup (8 oz or 250 mL)			
Chocolate Milk: whole, 2%, 1% or skim										1 cup (8 oz or 250 mL)			
Soy Milk: plain or flavored										1 cup (8 oz or 250 mL)			
Soy Drink: not fortified										1 cup (8 oz or 250 mL)			
Other plant milks										1 cup (8 oz or 250 mL)			
Milk in coffee or tea										1 tablespoon			
Milk on cereal (if not included above)										½ cup			
Milk shake										1 cup (8 oz or 250 mL)			
Milk dessert (ice cream, pudding, custard)										½ cup (one scoop, 1 container)			
Yogurt (regular or soy; frozen)										½ cup (125g, 1 container)			
Cheese: soft or spread										1 tablespoon			
Cheese; hard										1 cube 2” (2 slices)			
White bread, roll, bun, biscuit bagel, nan, tortilla										1 slice, 1 small roll, ½ bagel			
Dark bread, roll, bagel										1 slice, 1 small roll, ½ bagel			
Taco chips, nacho chips										50 g (1 cup slightly packed)			
Waffle, pancake, French toast										1 piece (1/4 waffle, 4” round)			
Butter (in any foods eaten)										1 pat; teaspoon			
Margarine (in any foods eaten)										1 pat; teaspoon			

Types of Food or Drink	Never or less than 1 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**	Your Serving Size		
											S	M	L
Tofu										1 cube (2")			
Macaroni with cheese										1 cup			
Canned salmon										2 tablespoon or 1 cup salmon casserole			
Canned tuna										2 tablespoon or 1 cup tuna casserole			
Canned sardines										2 fish (1/2 can)			
Salmon steak										90 g (3 oz)			
Other fish: white										90 g (3 oz)			
Other fish: oily										90 g (3 oz)			
Cream soups made with milk										1 cup (250 mL)			
Eggs: eaten alone or in other foods										1 large egg			
Potatoes; mashed with milk and margarine										½ cup (1 scoop)			
Orange juice; fortified with calcium										1 cup (8 oz, 250 mL)			
Orange juice; fortified with calcium and vitamin D										1 cup (8 oz, 250 mL)			
Broccoli, kale, greens										1 cup raw or 1/3 cup cooked			
Seafood: shrimp, prawn, lobster, crab										1 cup meat			
Shellfish: mussels, oyster										½ cup			

APPENDIX C

VITAMIN D VALUES PER MEDIUM SERVING PER DAY IN THE FFQ

Type of Food or Drink	Medium Serving / week	Comments	Vit D / week (IU)	Vit D / day (IU)
Milk: whole, 2%, 1% or skim	1 cup (8 oz or 250 mL)		100	14
Chocolate Milk: whole, 2%, 1% or skim	1 cup (8 oz or 250 mL)		100	14
Soy Milk: plain or flavored	1 cup (8 oz or 250 mL)		100	14
Soy Drink: not fortified	1 cup (8 oz or 250 mL)		0	0
Other plant milks	1 cup (8 oz or 250 mL)		0	0
Milk in coffee or tea	1 tablespoon	15 mL	6	0.9
Milk on cereal (if not included above)	½ cup		50	7
Milk shake	1 cup (8 oz or 250 mL)		60	8.6
Milk dessert (ice cream, pudding, custard)	½ cup (one scoop, 1 container)		0	0
Yogurt (regular or soy; frozen)	½ cup (125g, 1 container)		20	2.9
Cheese: soft or spread	1 tablespoon		0	0
Cheese; hard	1 cube 2" (2 slices)		14	2
White bread, roll, bun, biscuit bagel, nan, tortilla	1 slice, 1 small roll, ½ bagel		0 (commercial) or 5.6 (prep w/ milk)	0 or 0.8
Dark bread, roll, bagel	1 slice, 1 small roll, ½ bagel		0	0
Taco chips, nacho chips	50 g (1 cup slightly packed)		0	0
Waffle, pancake, French toast	1 piece (1/4 waffle, 4" round)	1/6 (1c milk+1egg)	1/6 (100+25)=21	3
Butter (in any foods eaten)	1 pat; teaspoon		1.2	0.2
Margarine (in any foods eaten)	1 pat; teaspoon		25	3.5
Tofu	1 cube (2")		0	0
Macaroni with cheese	1 cup	1/4 c milk +1tsp margarine	25+25=50	7
Canned salmon	2 tablespoon or 1 cup salmon casserole	35 g	218	31
Canned tuna	2 tablespoon or 1 cup tuna casserole	35 g	13	1.9
Canned sardines	2 fish (1/2 can)	76 g (38g / fish)	70	10
Salmon steak	90 g (3 oz)		245	35
Other fish: white	90 g (3 oz)	Tilapia	0	0
Other fish: oily	90 g (3 oz)	Herring	195	28
Cream soups made with milk	1 cup (250 mL)	1/2 c milk	50	7
Taco or burrito made with cheese	1 regular taco; ½ burrito		0	0
Pizza made with cheese	1 slice		0	0
Lentils, beans, peas	½ cup cooked		0	0
Eggs: eaten alone or in other foods	1 large egg		25	3.5
Potatoes; mashed with milk and margarine	½ cup (1 scoop)	½ (1tbsp milk +1 tsp margarine)	½*(6+25) = 15.5	2.2
Orange juice; fortified with calcium	1 cup (8 oz, 250 mL)		0	0
Orange juice; fortified with calcium and vitamin D	1 cup (8 oz, 250 mL)		100	14
Broccoli, kale, greens	1 cup raw or 1/3 cup cooked		0	0
Seafood: shrimp, prawn, lobster, crab	1 cup meat	All are either 0 or --.	0	0
Shellfish: mussels, oyster	½ cup	Mussels: or Oyster:	8.8 / 0	1.3 / 0

APPENDIX D

CALCIUM VALUES PER MEDIUM SERVING PERDAY IN THE FFQ

Type of Food or Drink	Medium Serving / week	Comments	Ca / week (mg)	Ca / day (mg)
Milk: whole, 2%, 1% or skim	1 cup (8 oz or 250 mL)		300	43
Chocolate Milk: whole, 2%, 1% or skim	1 cup (8 oz or 250 mL)		300	43
Soy Milk: plain or flavored	1 cup (8 oz or 250 mL)		300	43
Soy Drink: not fortified	1 cup (8 oz or 250 mL)		0	0
Other plant milks	1 cup (8 oz or 250 mL)		0	0
Milk in coffee or tea	1 tablespoon	15 mL	18	2.6
Milk on cereal (if not included above)	½ cup		150	21
Milk shake	1 cup (8 oz or 250 mL)		180	26
Milk dessert (ice cream, pudding, custard)	½ cup (one scoop, 1 container)		80	11.4
Yogurt (regular or soy; frozen)	½ cup (125g, 1 container)		197	28
Cheese: soft or spread	1 tablespoon		12	1.7
Cheese; hard	1 cube 2" (2 slices)		360	51
White bread, roll, bun, biscuit bagel, nan, tortilla	1 slice, 1 small roll, ½ bagel		35	5
Dark bread, roll, bagel	1 slice, 1 small roll, ½ bagel		20	2.9
Taco chips, nacho chips	50 g (1 cup slightly packed)		70	10
Waffle, pancake, French toast	1 piece (1/4 waffle, 4" round)	1/6 (1c milk+1egg)	1/6 (300+25)=54	7.7
Butter (in any foods eaten)	1 pat; teaspoon		1	0.14
Margarine (in any foods eaten)	1 pat; teaspoon		1	0.14
Tofu	1 cube (2")		130	19
Macaroni with cheese	1 cup	1/4 c milk +1tsp margarine	75+1=76	11
Canned salmon	2 tablespoon or 1 cup salmon casserole	35 g	80	11.4
Canned tuna	2 tablespoon or 1 cup tuna casserole	35 g	3.9	0.56
Canned sardines	2 fish (1/2 can)	76 g (38g / fish)	276	39
Salmon steak	90 g (3 oz)		6.3	0.9
Other fish: white	90 g (3 oz)	Tilapia	12.6	1.8
Other fish: oily	90 g (3 oz)	Herring	95	14
Cream soups made with milk	1 cup (250 mL)	1/2 c milk	150	21
Taco or burrito made with cheese	1 regular taco; ½ burrito	taco	221	32
Pizza made with cheese	1 slice		300	43
Lentils, beans, peas	½ cup cooked		19	2.7
Eggs: eaten alone or in other foods	1 large egg		25	3.6
Potatoes; mashed with milk and margarine	½ cup (1 scoop)	½ (1tbsp milk +1 tsp margarine)	½*(18+1) = 9.5	1.4
Orange juice; fortified with calcium	1 cup (8 oz, 250 mL)		300	43
Orange juice; fortified with calcium and vitamin D	1 cup (8 oz, 250 mL)		300	43
Broccoli, kale, greens	1 cup raw or 1/3 cup cooked		22	3.1
Seafood: shrimp, prawn, lobster, crab	1 cup meat	All are either 0 or --	60 / 93 / 118	8.6 / 13 / 17
Shellfish: mussels, oyster	½ cup	Mussels: or Oyster:	26 / 12	3.7 / 1.7

APPENDIX E

VITAMIN D AND CALCIUM AMOUNTS

IN BRAND NAME SUPPLEMENTS IN THE STUDY

Brand name	Vit D (IU/tablet)	Ca (mg/tablet)
Amway Ca	---	250
Amway multivitamin	400	200
Avon Vitadvance Men's complete	400	160
Avon Vitadvance Women's complete II	400	200
Caltrate Ca	---	600
Citracal Caplets+D	200	315
Centrum	400	175
Centrum Forte	400	175
Exact cod liver oil	200	---
Exact multivitamin	400	150
Flintstones	400	160
GNC Mega Men	200	200
Jamieson multivitamin	400	150
Jamieson Ca	---	650
Jamieson Ca+Mg	---	650
Kirkland multivitamin	400	162
Life Kid's	400	200
One-A-Day women's	200	400
Sisu Supreme	200	---
Progressive	400	200

Product information was collected in 2007 from Canadian supplement market.

APPENDIX F

EVALUATION OF REPORTED ENERGY INTAKE IN THE 7-DAY DIARY

Underreporting of energy intake on 7-day diary (%; n=105):

		Cumulative %
Whole group		13.3
Gender_specific *	Male	23.4
	Female	5.2
Ancestry_specific	East Asian	12.9
	European	18.5
	South Asian	9.4

*P<0.05. Values were from the original 7-day diary results.

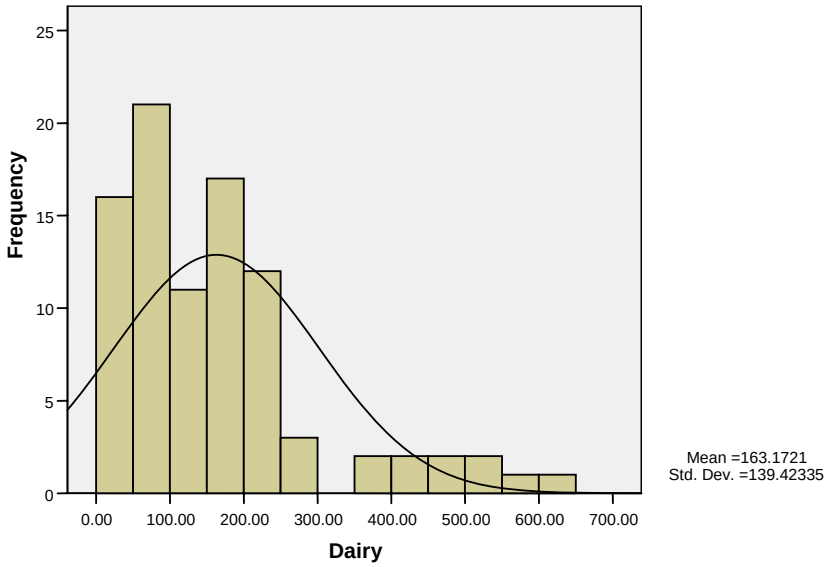
The reported energy intake was compared with resting energy expenditure (REE) (Lee & Nieman, 2003):

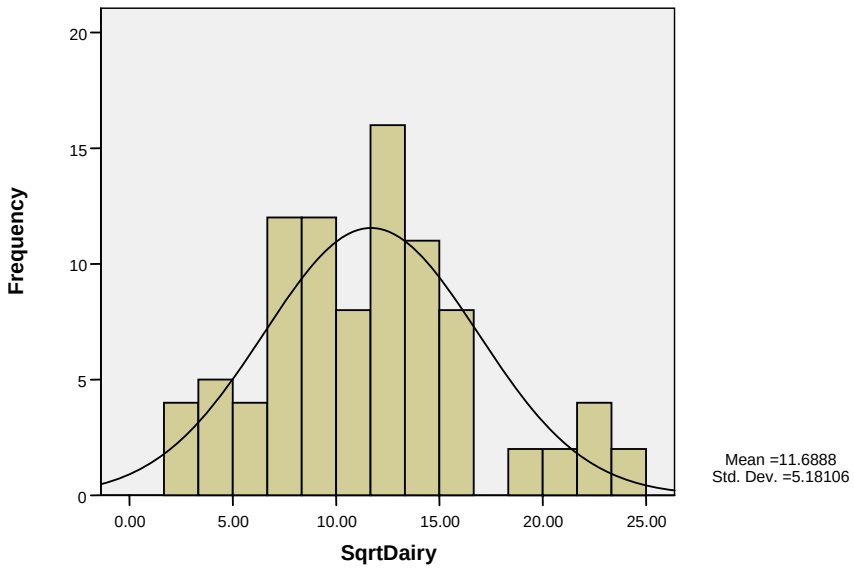
1. REE was calculated using WHO formula for 18-30 years old.
2. Those subjects who had energy intake lower than -1 deviation of REE were determined.

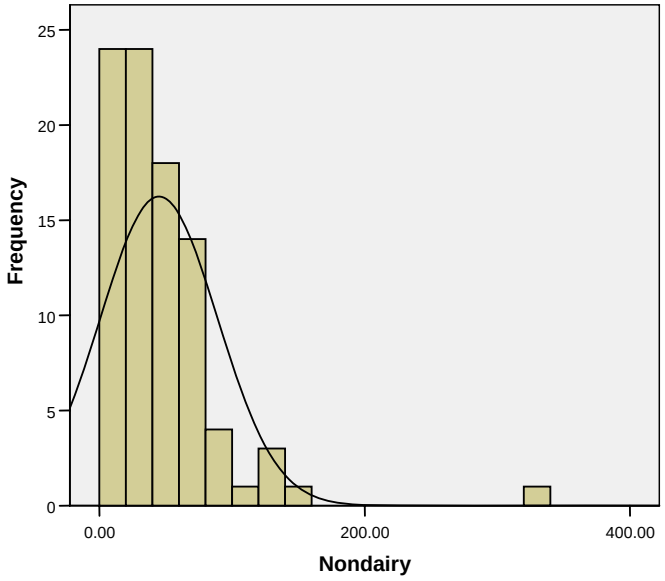
APPENDIX G

HISTOGRAMS OF VITAMIN D INTAKE DATA BEFORE AND AFTER SQUARE-ROOT TRANSFORMATION

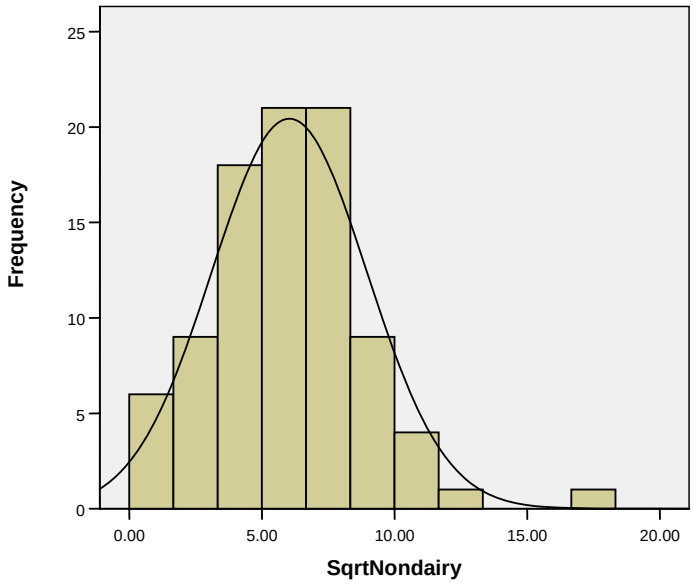
(The East Asian, South Asian and European groups; *n*=90 *)



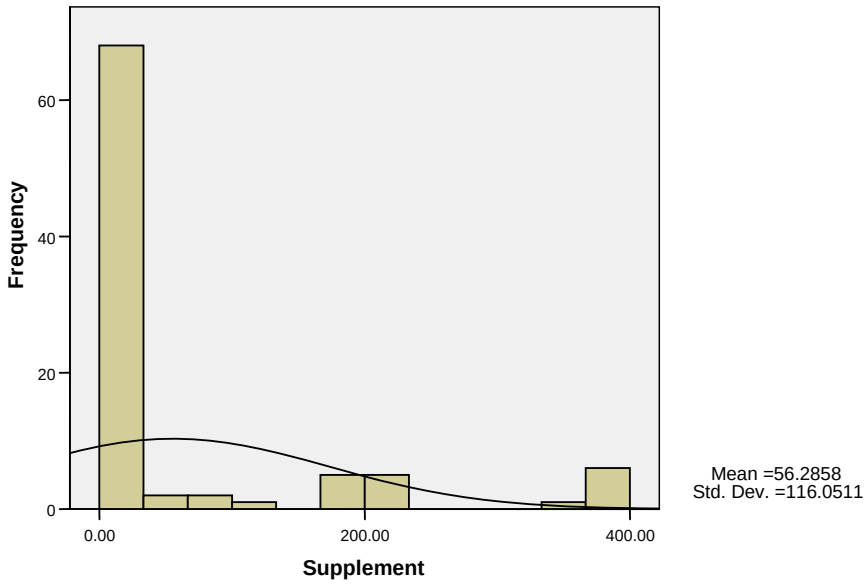




Mean =44.9072
Std. Dev. =44.20997



Mean =6.0357
Std. Dev. =2.92786


Frequency
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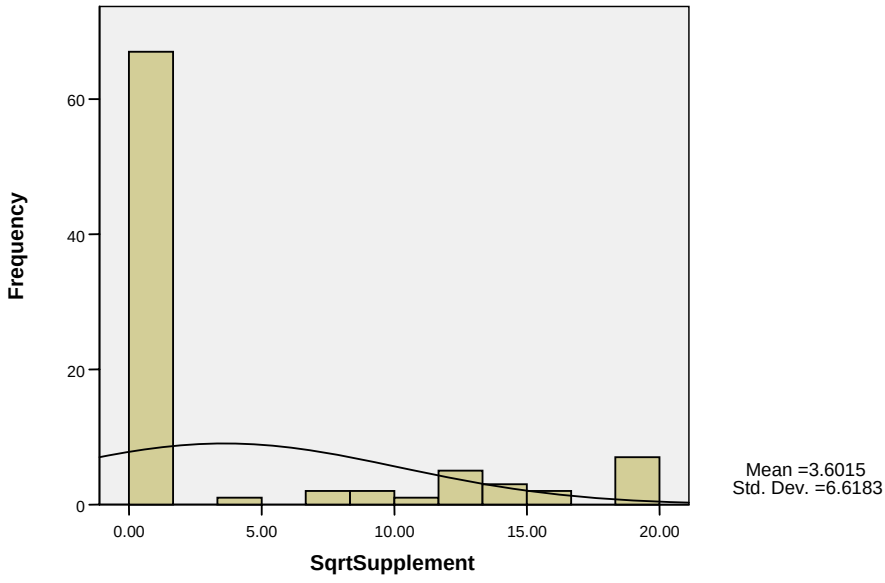
40
20
0
0.00
200.00
400.00
Supplement
Mean =56.2858
Std. Dev. =116.0511


Frequency
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40
20
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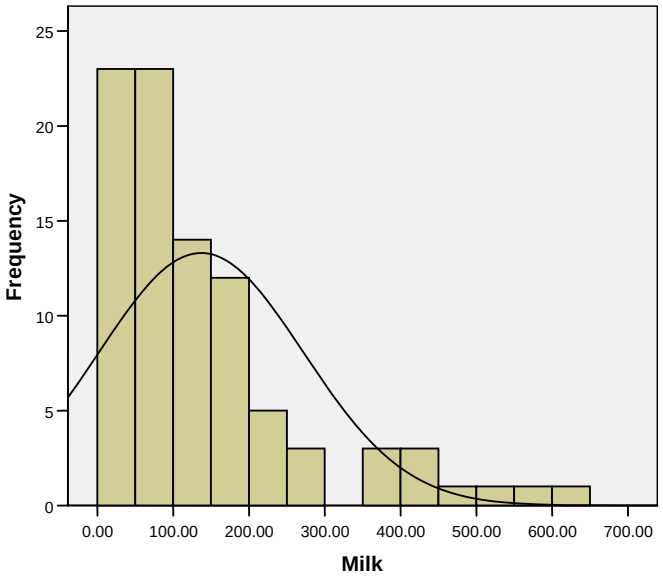
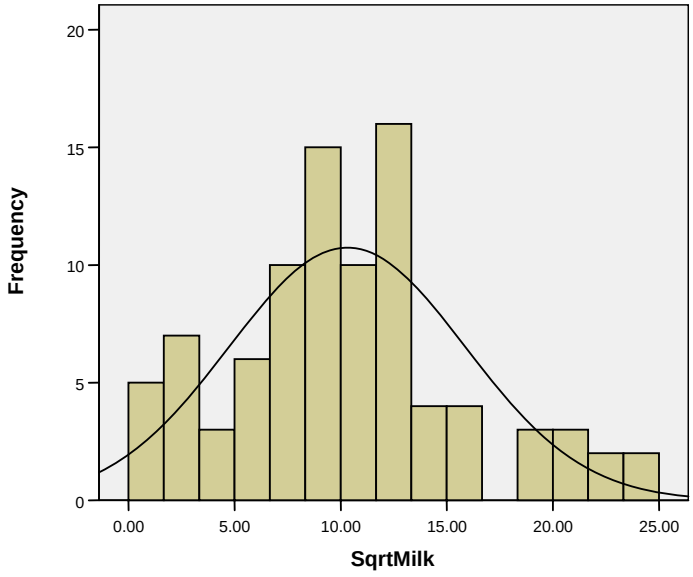
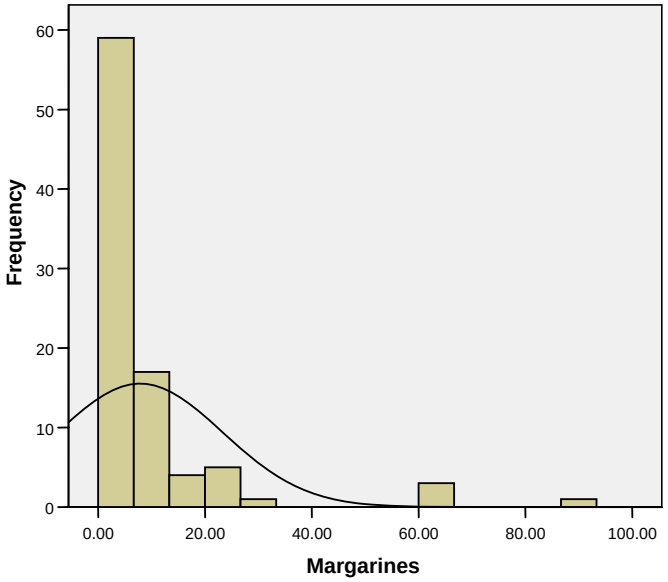
0.00
5.00
10.00
15.00
20.00
SqrtSupplement
Mean =3.6015
Std. Dev. =6.6183



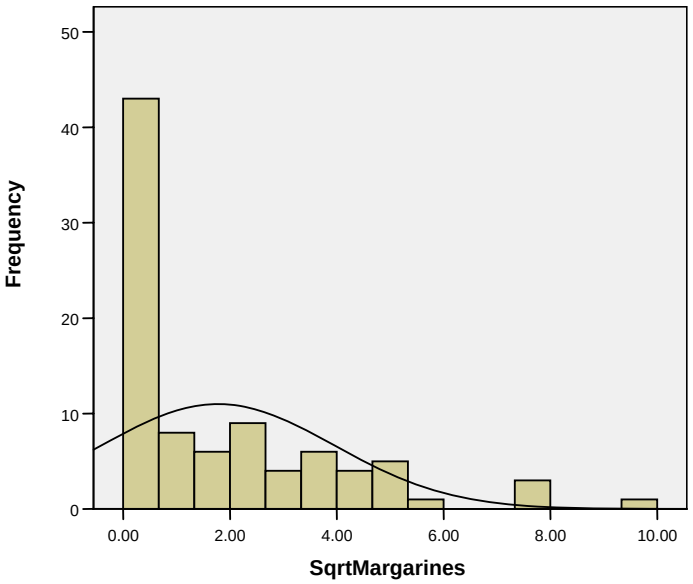
Mean =136.9612
Std. Dev. =134.92949



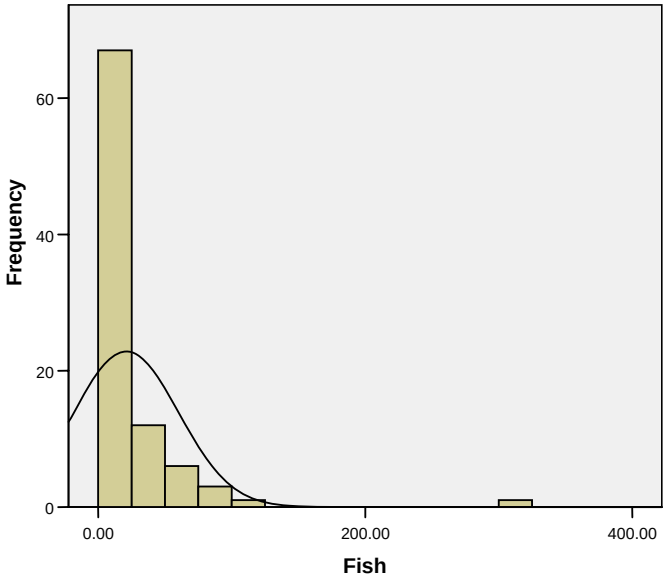
Mean =10.3081
Std. Dev. =5.57226



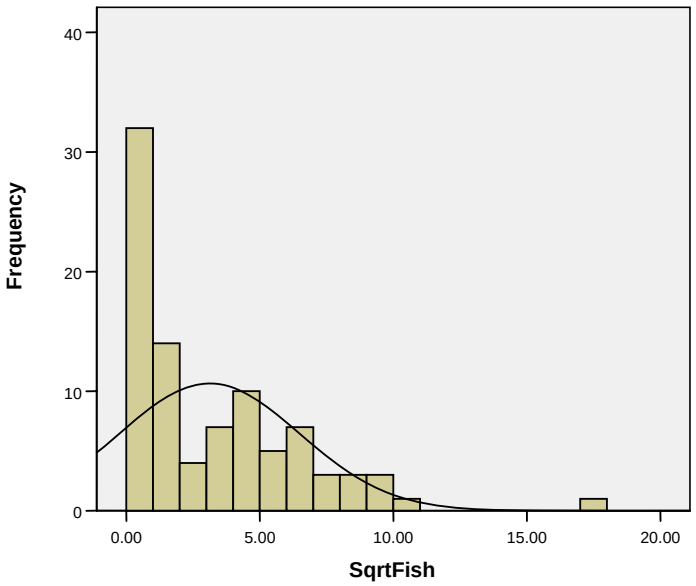
Mean =7.842
Std. Dev. =15.43455



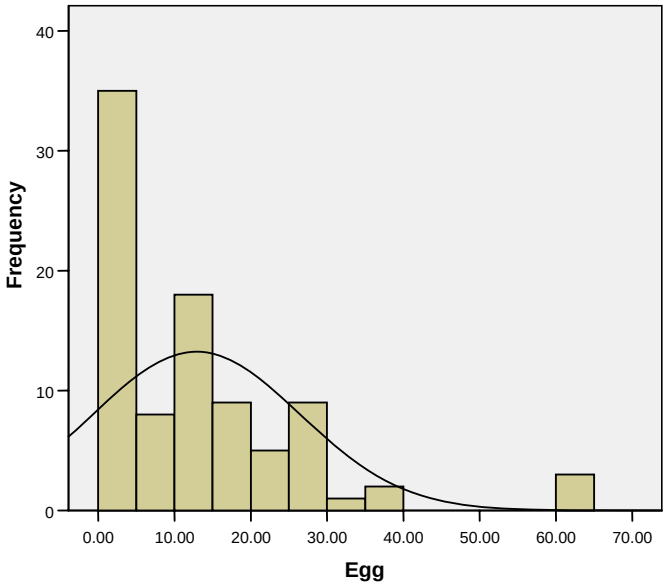
Mean =1.7738
Std. Dev. =2.17909



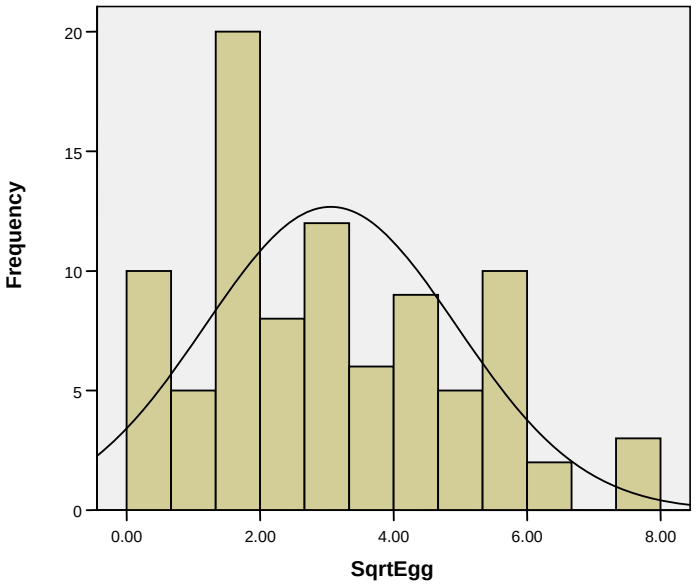
Mean =20.9488
Std. Dev. =39.34449



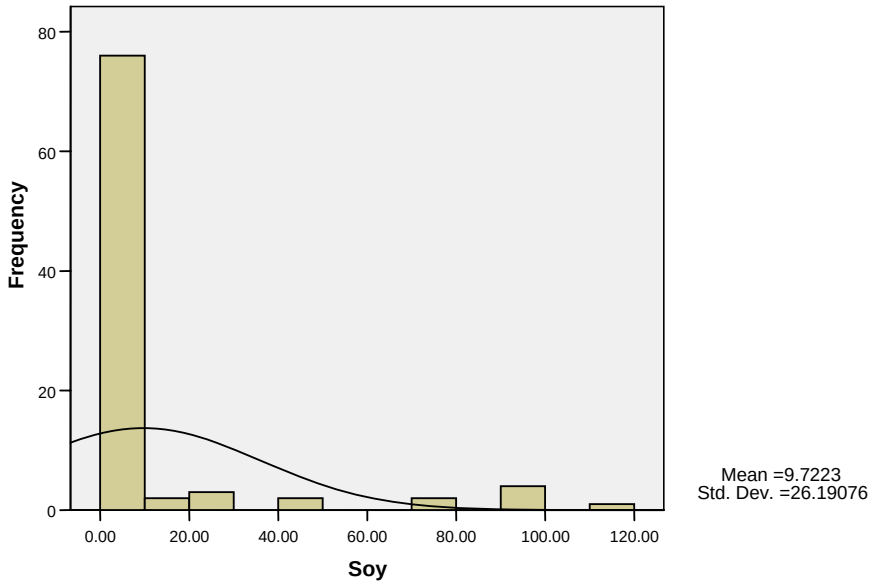
Mean =3.1123
Std. Dev. =3.37477

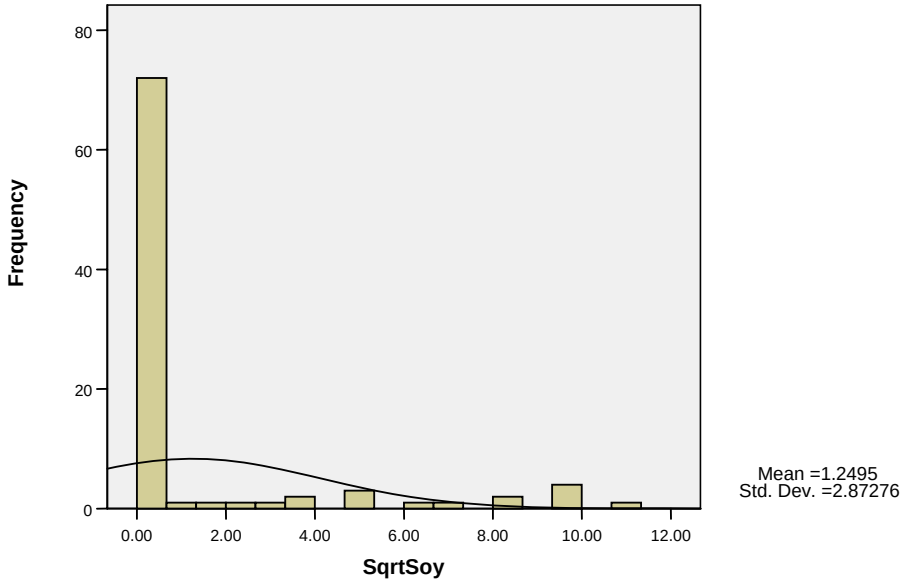


Mean =12.8808
Std. Dev. =13.5628



Mean =3.0587
Std. Dev. =1.88803





* The data were from the modified first FFQ results.