Nutrient intake of lactating women in Montreal with emphasis on calcium, vitamin D and omega fatty acids

Samira Bou Raad

School of Dietetics and Human Nutrition

McGill University

Montreal, Canada

August, 2010

A thesis submitted to McGill University in partial fulfillment of the requirements of the degree of Master of Science

Table of Contents

Acknowledgements

This thesis would not have been possible had I not been surrounded by inspiring and encouraging individuals. I owe my deepest gratitude to everyone who saw me through this process.

First and foremost I would like to thank my research supervisor Dr Hope Weiler for giving me the opportunity to be part of this research. Her guidance, support and encouragement are unmatched. I greatly appreciate her dedication and time. I would also like to thank my committee members Dr. Grace Marquis and Dr. Kristine Koski for their input and assistance.

In addition I would like to thank Catherine Vanstone who made available her support in every possible way. She is truly patient, professional and an excellent listener. I cannot forget to acknowledge Sina Gallo for all her help with statistics and her ability to come to the rescue whenever needed. I want to thank my lab members for their understanding and empathy.

Last but not least I would like to thank all my friends and family. Your belief in me when I felt like there was no light at the end of the tunnel made this thesis possible. Finally I would like to thank my parents for teaching me the value of education and for their unfaltering support and endless love and guidance. Words cannot explain how blessed I am to have you.

Abstract

The objective of this study was to examine whether calcium, vitamin D and n-3 and n-6 fatty acid consumption in lactating women living in Montreal meets recommended values. A sample of 70 predominantly lactating women were assessed using one 24-hour recall administered at each of 1 (baseline), 3 and 6 months postpartum and analyzed using the Canadian Nutrient File. Only 52%, 46%, 15%, 31% and 11% of the women attained adequate intake (AI) levels for calcium, vitamin D, linoleic acid, alpha-linolenic acid and docosahexaenoic acid (DHA) respectively from food alone. Supplement intake increased the percentage of women achieving the AI for calcium, vitamin D and DHA to 82%, 90% and 13% respectively. However, supplements had no impact on total intake of linoleic acid and alpha-linolenic acid. Therefore, while supplementation improved the proportion of women reaching the AI for calcium and vitamin D, intakes of n-3 and n-6 fatty acids remain low. These data support the development of a larger and random sample to establish if improvements in the intakes of calcium, vitamin D and n-3 and n-6 fatty acids are needed for this population.

Résumé

L'objectif de cette étude était d'examiner si les quantités de calcium, de vitamine D et d'acides gras oméga-3 et oméga-6 consommées par les femmes; allaintantes, habitant à Montréal, sont en accord avec les valeurs recommandées. L'alimentation d'un échantillon de 70 femmes qui allaitaient de manière prédominante a été évaluée par des rappels de 24 heures faits à 1 (Initial), 3 et 6 mois après l'accouchement et analysée utilisant le fichier Canadien sur les éléments nutritifs. Un niveau supérieur à l'apport suffisant (AS) a été atteint seulement par 52% des femmes pour le calcium, 46% pour la vitamine D, 15% pour l'acide linoléique, 31% pour l'acide alpha-linolénique et 11% pour l'acide docosahexanoique (DHA). La prise de suppléments a augmenté le pourcentage de femmes atteignant l'AS pour le calcium, la vitamine D et DHA à 82%, 90% et 13% respectivement. Cependant, les suppléments n'ont pas eu d'impact pour l'acide linoléique et l'acide alpha-linolénique. Presque la moitié des femmes ayant pris part à cette étude n'ont pas atteint l'AS pour le calcium et la vitamine D et plus de la moitié des femmes n'ont pas atteint l'AS pour les acides gras oméga.

Contribution of Authors

The author of this thesis was responsible for collecting maternal 24-hour recalls. She was also responsible for analyzing maternal dietary data and performing all statistical analyses included in this thesis. Other graduate students who are also registered dietitians, Saja Al-Saleh, Sina Gallo, Kate Trussler and Sonia Jean Phillippe, working on different aspects of the main Vitamin D dose response study (this thesis is a part of the Vitamin D dose response study) also assisted with the administration of the maternal 24-hour recalls.

Catherine Vanstone was the research assistant on the umbrella study and was responsible for the coordination of the whole project.

Sina Gallo was the doctoral student working on the umbrella study and was responsible for the recruitment of women as well as management of all data.

Dr. Celia Rodd was the co-investigator on the vitamin D dose response umbrella study.

Dr. Hope Weiler was the principle investigator on the whole vitamin D dose response study. Dr Hope Weiler was Samira Bou Raad's thesis supervisor.

List of Tables

List of Figures

List of Abbreviations

$1,25(OH)_2D$	1,25-dihydroxyvitamin D
25(OH)D	25-hydroxyvitamin D
AA	Arachidonic Acid
AI	Adequate Intake
ALA	Alpha Linolenic Acid
AMDR	Acceptable Macronutrient Distribution Range
BMC	Bone Mineral Content
BMD	Bone Mineral Density
BMI	Body Mass Index
COMA	Committe on Medical Aspects of Food Policy
COX-II	Cyclo-oxygenase-II
D_2	Ergochalciferol
D_3	Cholecalciferol
DHA	Docosahexaenoic acid
DPA	Docosapentaenoic Acid
DRI	Dietary Reference Intake
EAR	Estimated Average Requirement
EPA	Eicosapentaenoic Acid
FFQ	Food Frequency Questionnaire
GFR	Glomerular Filtration Rate
IL	Interleukin
IOM	Institute of Medicine
IRMA	Immunoradiometric Assay

ISSFAL	International Society for the Study of Fatty Acids and Lipids
IU	International Units
LA	Linoleic Acid
LCPUFA	Long Chain Polyunsaturated Fatty Acid
NHMRC	National Health and Medical Research Council
NO	Nitric Oxide
NPNL	Never Pregnant Never Lactated
PG	Prostaglandin
PRI	Popoulation Reference Intake
PTH	Parathyroid Hormone
PTHrP	Parathyroid Related Peptide Hormone
PUFA	Polyunsaturated Fatty Acid
RA	Rheumatoid Arthritis
RBC	Red Blood Cell
RDA	Recommended Dietary Allowance
RDI	Recommended Daily Intake
SPF	Sun Protection Factor
TNF	Tumor Necrosis Factor
UV	Ultraviolet
UVB	Ultraviolet B
VDDR	Vitamin D Deficiency Rickets
WHS	Women's Health Study

1.0 Introduction

Exclusive breastfeeding for six months has become universally recommended by organizations and scientific bodies [1, 2] because human milk is the preferred food for the term infant [3]. Lactating mothers have increased nutritive demands because they have to maintain adequate milk supply and be able to provide necessary nutrients for infant growth and development via their breast milk.

Dietary practices of lactating women may have significant effects on the nutritional status of both their children and themselves [4, 5]. The amount of ingested nutrients channelled into milk biosynthesis may depend on maternal nutrient stores and maternal intake. These same stores may be utilised to supply nutrients for milk biosynthesis and it is highly probable that the extent of nutrient mobilization is based upon dietary intake [4, 5]. Of specific importance in Canadian lactating women are the micronutrients calcium, and vitamin D, as well as other nutrients such as n-3 and n-6 fatty acids due to the fact that these are most affected when maternal dietary habits are inadequate.

Recent research suggests that lactating women may restrict intake of certain foods such as milk, thinking that it is a fat dense food, therefore putting themselves at risk for calcium and vitamin D deficiency [6]. Furthermore, Vatanparast [7] illustrated that generally Canadian women of childbearing age (19-45) do not meet the recommendation for calcium even when supplements are

added to their diet. There is concern that this observation may also occur in pregnant and lactating women. Vitamin D intake is of particular importance in the Canadian population due to minimal sun exposure as a result of long winters in addition to polar latitudes [6]. Furthermore, postpartum women often feel the pressing need to lose weight accumulated throughout pregnancy and attempt to do so by decreasing intakes of fat and dairy products hence cutting down on consumption of polyunsaturated and monounsaturated fatty acids as well as calcium and vitamin D [6, 8].

Information on the intake of n-3 and n-6 fatty acids, in lactating women in North America, particularly Canada, is meagre. However, data on polyunsaturated fatty acid intakes in non-pregnant, non-lactating women may be extrapolated to gain some understanding of the dietary pattern in the lactating population. A survey of 1544 Canadians using 24-hour dietary recalls reflected intakes of 10.6% and 5.0% energy from monounsaturated and polyunsaturated fat respectively for women 18-34 years [9, 10]. These figures are comparable to a group of 55 Canadian pregnant women studied by Innis et al [10]. In this study 1 in 6 women consumed <67 mg docosahexaenoic acid (DHA) per day during the latter part of gestation, 60% had an intake of <150 mg DHA/d and 16% consumed >300 mg DHA/d. Mean intakes of linoleic acid (LA), alpha-linolenic acid (ALA), arachodonic acid (AA), eicosapentaenoic acid (EPA) and DHA were 11.2 g, 1.6 g, 121 mg, 78 mg and 160 mg per day, respectively.

The most important omega-3 and omega-6 fatty acids for breastfed infants are DHA and AA respectively. DHA in particular is very important in the central nervous system, visual acuity and perhaps bone mineral accrual in breastfeeding infants [11]. Concentration of DHA in breast milk varies depending on maternal diet thereby, rendering maternal intake of DHA very important [12]. Weiler et al [11] found that cord blood long chain polyunsaturated fatty acid (LCPUFA) was correlated with femur and lumbar spine bone mineral content (BMC) in human infants at birth. No studies have been conducted on the effect of polyunsaturated fatty acids (PUFA) in breast milk on bone mineral accrual in breastfeeding infants. However since BMC in newborn infants is associated with cord blood or maternal PUFA status, then it is logical to postulate that breast milk PUFA may have an important influence on bone mineral accumulation in infants postnatally.

As for direct adverse effects of low maternal LCPUFA intake on the mother herself, none have been reported apart from a claim stating that this decrease may exacerbate postpartum depression and that supplementation with LCPUFA may alleviate the problem [13]. However, there is recent evidence suggesting a link between omega fatty acids and bone health. Emerging evidence from human and animal research support the hypothesis that dietary lipids influence bone modeling and remodelling [14]. It is established that osteoporosis associated with a decline in estrogen production in postmenopausal women results in increased production of osteoclastogenic cytokines such as interleukin 1 (IL-1), interleukin 6 (IL-6) and (tumour necrosis factor alpha) TNF-α. These cytokines

induce the expression of cyclo-oxygenase-II (COX-II) in osteoblastic and stromal cells, resulting in the production of PGE_2 [15]. PGE_2 along with TNF-α through specific pathways enhance the differentiation of osteoclast progenitors into mature osteoclasts. This process leads to osteoporosis. Since the period of lactation is one of low estrogen production (similar to menopause) due to inhibited ovarian function, adequate (as per recommendations) n-3 fatty acid intake in this population should be encouraged in order for lactating mothers to provide sufficient omega fatty acids for both themselves (as an alternate route of bone protection) and their infants.

Recommended intakes for many nutrients during lactation exist; in fact dietary recommendations for lactating women have been drafted by several countries. Calcium, vitamin D, and n-3 and n-6 fatty acids are no exceptions. The National Health and Medical Research Council (NHMRC) in Australia [16], the Committee on Medical Aspects of Food Policy (COMA) in the UK [17], France's "apports nutritonels conseillés" [18], the Institute of Medicine (IOM) [19] and Health Canada [20] advise on recommended dietary intake for lactating women (Table 1). These recommendations differ between the organizations due to discrepancies in research and unavailability of exact data reflecting the needs of lactating women due to differences and uniqueness of the populations. In addition, there are different fortification policies in place for these populations.

Table 1**:** International recommended nutrient intakes for lactating women

	Calcium, (mg/day)	**Vitamin D, (µg/day)**	**Omega fatty Acids, (g/day)**
NHMRC [16]	RDI: 1000 EAR: 840	5-10	n-3: 1.2 n-6: 12
COMA [17]	RNI: 700 + 550	10	Max: 1.0 Min: 0.2
Apports Nutritionels [18]	1000	10	-
IOM [19]	1000	5	n-3: 1.3 n-6: 13
Health Canada [20]	1000	5	n-3: 1.3 n-6: 13

Despite the presence of all the above international recommendations, research on evaluating dietary practices and nutrient adequacy of food in lactating women, especially micronutrient intake, based on these recommendations is very limited. Recent studies reflecting low vitamin D intake during pregnancy have channelled attention towards whether Canadian lactating mothers are meeting current nutrient recommendations [21].

On an international level there is very little published data concerning actual nutrient intakes of lactating women. Some studies focused solely on sufficiency of macronutrient intake with minimal attention to micronutrient intake [5, 22]. Few studies emerging from the United States, Mexico and New Zealand concentrated on measuring the sufficiency of micronutrient intake along with macronutrient consumption of lactating women [3, 4, 6, 8].

In Canada, data on nutrient intake of lactating women are more limited than other populations despite the fact that breastfeeding initiation is successful in Canada. Surveys have shown that 85% of Canadian females initiate breastfeeding, with geographic variations ranging from 31% in eastern Newfoundland to 83% in Ontario [23, 24]. The possibility that this proportion of women (Canadian lactating women) may not be attaining adequate intakes of calcium, vitamin D, n-3 and n-6 fatty acids has raised the question of whether supplementation during lactation is a necessary solution for inadequate intake [6]. The purpose of this thesis is therefore to determine whether calcium, vitamin D and n-3 and n-6 fatty acids consumption in a specific group of lactating women in Montreal meet the

dietary reference intakes (DRI). This will be done primarily by quantifying the number of lactating women who are consuming at or above the adequate intake for the above nutrients from diet alone and with supplements. An additional objective is to determine predictors of dietary intake (such as age, prepregnancy BMI, weight gain during pregnancy and postpartum BMI) of the above mentioned nutrients amongst lactating women using three 24-hour recalls.

The following chapter, is a detailed examination of previous research focused on investigating lactating women's intakes of calcium, vitamin D and n-3 and n-6 fatty acids in Canada as well as internationally. Chapter two also includes an examination of research that looked at impact of inadequate calcium, vitamin D and n-3 and n-6 fatty acids on lactating women and their infants. Chapter three is a description of the study conducted for this thesis, presented in manuscript format followed by chapter four, an extended discussion.

2.0 Review of the Literature

2.1 Intakes of Calcium, Vitamin D, and Omega Fatty Acids in Lactating Women World Wide

2.11 Calcium Intake

Calcium intake of lactating women takes on different facets; some women have good intake while others do not achieve dietary recommendations. Data from the United States (1173 mg/d) and Mexico (1,606 mg/d) show that mean intake of calcium for lactating women is at and above dietary recommendation [3, 4]. On the other hand, a study from New Zealand found that lactating women 3

months postpartum had mean intakes of 923 mg of calcium per day which is slightly below the New Zealand-adopted Australian Recommended Daily Intake (RDI) (1000 mg/day) but higher than the Australian Estimated Average Requirement (EAR) (840 mg/day) [8]. In Spain, Sanchez et al [25] divided a group of lactating women into calcium restrictors defined as calcium intake <1200 mg/day and non restrictors (>1200mg/day). At the time of that study, calcium population reference intake (PRI) for lactating women as recommended by the Commission of the European Communities was 1200 mg/day. The authors observed a higher number of restrictors as compared to non restrictors (25 women vs. 14 women). The mean calcium intake in the restrictor group was 812.4 mg/day while the non restrictors exhibited an average intake of 1619.5 mg of calcium per day [25]. In Canada, a study out of Calgary depicted a median intake of 1326 mg/day of calcium without supplements provided that the women did not restrict milk intake [6]. However, women who did restrict milk intake had a median intake of 895 mg/day. Furthermore, data compiled for 47 Canadian indigenous communities presented variable results on calcium intake with some communities having low mean intakes (438 and 487 mg/d) and others having a mean intake of 1036 mg/day [26]. Doran et al [27] found a median intake of 928 mg of calcium per day amongst lactating women in a low income community in Ontario. These Canadian studies however, are not reflective of Canadian lactating women as a whole; women from Calgary only vs. indigenous communities vs. women from low economic status in Ontario. Lactating women in other areas of Canada may have different values. Other studies have found regional differences in the intakes

of macro and micro nutrients [4, 22]. Schofield et al [22] compared energy, protein, fat and fibre intake between a group of lactating women from London and a group of lactating women from Edinburgh. Women living in London had a higher intake of these nutrients when compared with women living in Edinburgh. Similar results were seen in Mexico; lactating women living in Northwest Mexico had higher intake of many nutrients when put side by side with lactating women from Mexico City [4]. In Italy, discrepancies in intake of various macronutrients were seen between northern, central and southern regions [5]. In summary, lactating women living in different regions of Canada may have different intakes of calcium which may or may not be in line with dietary recommendations.

2.12 Vitamin D Intake

There is a large variability in the intake of vitamin D amongst lactating women. Some groups of women have adequate intake yet others have insufficient intakes. Vitamin D intake in lactating women from Calgary who did not restrict milk was at a mean of 6.3 and 2.2 μg/day for the women that did restrict milk, [6] as compared with 7.5 μg/day in non-restrictors of milk in the United States. Spanish lactating women have suboptimal vitamin D intake ranging from almost no vitamin D to about 3.5 μg/day. British lactating women seem to have the same inclination towards low vitamin D intake. Black et al [28] described an intake of 3.2 μg of vitamin D per day. Low maternal vitamin D can result in nutritional rickets in breast fed children. This nutritional deficiency was once thought to have been eradicated but has re-emerged in Canada and the United States [6]. Lebrun et

al [29] investigated the vitamin D status of 80 mother-child pairs in a Manitoba community with a high incidence of rickets. The authors observed that 70% of the mothers said that they drank no milk while another 24% declared that they were milk intolerant. Moreover, 24% of the mothers did not take any supplements and those that did admitted to taking them occasionally.

Mothers with an adequate intake or supplementation of vitamin D have higher concentrations of the vitamin in their breast milk [12]. Berti et al [26] looked at the diet of lactating Indigenous Canadian arctic women. They observed that intakes ranged between 9.4 µg to 20.4 µg across 47 communities which is much higher than the recommended adequate intake of 5 µg for lactating women. However, indigenous communities may have higher consumption of fish as part of their traditional food patterns and their intake of vitamin D may not be reflective of other non-indigenous communities.

2.13 n-3 and n-6 Fatty Acids Intake

Detailed data on n-3 and n-6 fatty acid intake of women around the world is very scarce, with limited information even in industrialized countries. In addition, whatever little information there is pertains to pregnant women and there is almost no mention of lactating women. A current analysis of household food availability in Brazil reported that the relative contribution of fish to total energy consumption was very low [30]. It is reasonable to believe that this population in general, and pregnant and lactating women in particular are far from achieving the current recommendations for fatty acids [30]. In addition, Brazilians

predominantly use vegetable oils especially soybean oil, as a dietary source of polyunsaturated fatty acids (PUFA) [30, 31]. Vegetable oils are rich in essential fatty acids such as LA and ALA, but limited in LCPUFA. LA and ALA are converted into AA, EPA and DHA but the literature points towards the fact that endogenous DHA synthesis is insufficient and ingestion of preformed DHA is advisable [32, 33]. Brazilian lactating women were found to have poor DHA status as determined by a lower 22:6 n-3/22:5 n-6 (DHA/Docosapentaenoic acid (DPA)) and higher 22:5 n-6/22:4 n-6 (DPA/Docosatetraenoic acid) values in the erythrocyte membrane. All these findings are consistent with the dietary pattern of Brazilian mothers, with higher intake of n-6 PUFA and low intake of preformed n-3 LCPUFA, especially DHA due to low consumption of fish [30].

The above mentioned papers from Brazil seem to be the most detailed of their kind with regard to lactating women's intake of n-3 and n-6 fatty acids, nevertheless, studies from Holland and Mexico illustrated a clear picture of intake of PUFA's for lactating women. The relative consumption of LA, ALA, EPA and DHA amongst Dutch lactating mothers was 14 g, 1 g, 46 mg and 86 mg per day respectively [34]. In Mexico, a difference was found between the intakes of PUFA amongst two subgroups of lactating women. Northwest Mexican mothers ingest about 26.2 g of PUFA per day, whereas mothers from Mexico City reflected an intake of 10.3 g per day [4]. This was due to the fact that Northwest Mexican mothers had a higher consumption of fried foods (that may have been fried in soybean oil), leafy vegetables, eggs, beans, and wheat tortillas. Italian

recommended dietary allowance (RDA) for PUFA is 2.5 -15% of total energy. Northern Italian mothers have an intake of 3.9% while central and southern Italian mothers have an intake of 3.0% [5]. Information on the intake of n-3 and n-6 fatty acids of lactating women in North America, particularly Canada, is meagre. However, data on PUFA intakes in non-pregnant, non-lactating women may be extrapolated to the lactating population in order to gain some understanding of the dietary pattern in this population. A survey of 1544 Canadians using 24-hour dietary recalls reflected intakes of 10.6% and 5.0% energy from monounsaturated and polyunsaturated fat, respectively for women 18-34 years [9, 10]. These figures are comparable to a group of 55 Canadian pregnant women studied by Innis and Elias [10]. In this study 1 in 6 women consumed <67 mg DHA/d during the latter part of gestation, 60% had an intake of <150 mg DHA/d and 16% consumed >300 mg DHA/d. Mean intakes of LA, ALA, AA, EPA and DHA were 11.2 g, 1.6 g, 121 mg, 78 mg and 160 mg per day, respectively. Denomme et al [35] looked at nutrient intake of 20 pregnant Canadian women. Findings reflected very low intakes of DHA. The International Society for the Study of Fatty Acids and Lipids recommends an intake of 300 mg/d of DHA yet only 10% of the participants in this study met this recommendation, and 20% of the participants had trace amounts of DHA in the diet (<2 mg/d).

2.2 Calcium

2.21 Calcium Transferred During Pregnancy and Lactation

Pregnancy and lactation are times of high calcium demand [36, 37]. Over the course of human pregnancy the fetus acquires between 20 to 30 g of calcium

from its mother [37-39]. Maximal calcium accretion occurs during the third trimester with an average accretion rate of 200-250 mg/d [37, 38]. This demand for calcium is met primarily through an increase in maternal absorption from the diet in addition to a slight increased resorption from the skeleton [39]. Therefore, the DRI for calcium intake does not increase during pregnancy.

With the onset of lactation, the demands on maternal calcium metabolism accelerate [39]. Calcium transfer between mother and infant ranges from 280-400 mg/day [37-40]. For mothers who breastfeed for more than 3-6 months, the total calcium transfer via breast milk in one lactation period is greater than that transferred across the placenta during the whole of pregnancy [36-38].

The total amount of calcium that a breastfeeding infant ingests depends on the calcium concentration of the milk and on the quantity of milk produced, with no association between the two [37, 38, 40]. Calcium concentrations in breast milk are not nutrient-intake dependent but are rather dependent on the type of milk itself. For example, in a group of lactating Indian mothers calcium concentrations in colostrum (48 hours to 5 days) were an average of 256 mg/l but decreased as milk reached transitional stage (6 to 10 days) to 228 mg/l and decreased even more as milk became mature (11 to 30 days) to 223 mg/l [41]. Diet does not affect breast milk calcium concentrations. It has been reported that women with usual low intakes of calcium, such as in parts of Africa and Asia, have breast milk calcium concentrations (200 mg/liter of milk) comparable to those of women with adequate intake of calcium in areas such as United States

and Europe (300 mg/liter of milk) [40]. The fact that diet or supplementation has no effect on breast milk calcium concentrations has been further confirmed by 2 randomized control trials [42, 43]. Lactating Gambian women with a customary intake of 400 mg/day of calcium were supplemented with an additional 1000 mg/day of the mineral. Supplementation had no effect on breast milk calcium concentration [43]. These results were agreeable with those seen in Kalkwarf et al [42] whereby supplementation with 1000 mg/day failed to influence breast milk calcium concentration in a sample of women with low to moderate usual intake of calcium (≤ 800 mg/d).

In light of the above, considering that the maternal skeleton contains about 900 g of calcium, the loss of calcium during pregnancy and six months of lactation are equivalent to 3-5%, and sometimes more, of a mother's total skeletal calcium content [40]. In light of this augmented demand for calcium, the mechanisms of maternal calcium metabolism during lactation are quite different from those observed in pregnancy [39].

2.22 Calcium Metabolism During Lactation

The physiological adaptations that occur during lactation in order to secure the calcium necessary for breast milk differ markedly from those stimulated during pregnancy [40]. During pregnancy, parathyroid hormone (PTH) levels decrease into the low normal range and calcitonin levels increase partly because of production of parathyroid related peptide hormone (PTHrP) by the breast and placenta [39]. In turn, 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) concentrations

double which in turn is most likely responsible for the doubling in efficiency of calcium absorption by the gastrointestinal (GI) tract [39]. This increase in calcium absorption and lowering of PTH levels as well as increased renal filtered load of calcium, and the increased glomerular filtration rate (GFR) of pregnancy elicit increased calcium excretion in the urine [39]. In contrast with pregnancy, there is no elevation in postpartum serum concentrations of $1,25(OH)_2D$ [44, 45] or intestinal calcium absorption in lactating as compared with non lactating women [40, 46, 47]. In fact, maternal free and bound $1,25(OH)_2D$ levels decrease to normal within days of labour and remain constant throughout lactation [38]. In addition, serum PTH, as established using a two-site immunoradiometric (IRMA) assay has been found to decrease by as much as 50% in lactating women when compared with nonlactating women in the first 3 months postpartum [38, 40, 44, 45].

Lactating women meet calcium requirements during through temporary demineralization of the skeleton; the mechanism of which is not fully understood [38]. The act of breastfeeding elicits prolonged postpartum amenorrhea and hypoestrogenemia as a consequence of suppression of the hypothalamic-pituitary-gonadal axis [40]. It is an established fact that estrogen withdrawal accelerates bone resorption as is the case in postmenopausal women who suffer from osteoporosis [39]. However, the rate of bone loss during lactation exceeds that which happens after menopause, therefore, there seems to be another factor contributing to lactation associated bone loss [39]. This other factor is PTHrP, made in the mammary gland. It stimulates resorption of calcium from the

maternal skeleton, renal tubular reabsorption of calcium, and indirect suppression of PTH [38]. Animal and human studies have found that the concentration of serum calcium is more highly correlated with PTHrP levels and that higher PTHrP levels stimulate greater bone resorption and subsequently more loss of bone mineral density (BMD) [38, 40, 48]. Sowers et al [49] showed that PTHrP was strongly and negatively associated with change in BMD over time, at both the lumbar spine ($P<0.01$) and the femoral neck ($P<0.001$). This physiological response starts at pregnancy with concentrations of PTHrP increasing gradually from the 1st trimester to term [50]. This is perhaps what elicits resorption of calcium from the bone during the third trimester [51]. Most bone resorption occurs at trabecular sites with the trochanter and femoral head being the most affected [51]. The femoral neck, being predominantly cortical when compared to the trochanter and femoral head and being the main weight bearing region of the proximal femur [51], is not prone to significant resorption during pregnancy. However, there have been reports of pregnant women experiencing transient osteoporosis in that region that starts off as a dull pain during the third trimester and continues post partum [40-43]. These women may have started their pregnancy being osteoporotic or may have experienced severe bone loss [39]. These women are advised to discontinue lactation and are supplemented with calcium and vitamin D [52-55].

Alleviating hypoestrogenemia and its effect on bone resorption via treatment with estrogen may only mitigate bone loss but not prevent it [56].

Moreover, review studies have cited that in humans, GFR decreases during lactation and the renal excretion of calcium is reduced to very low levels, suggesting increased tubular reabsorption of calcium [37, 38, 40]. However, primary studies have very inconsistent results on the matter ranging from studies that found no difference in urinary calcium excretion between lactating and non lactating mothers [44, 45, 57], to others which reported a 20 – 50% decrease in urinary calcium [40, 43, 46, 58, 59]. However, the diets of these women were not examined and hence information on comparability of calcium intake of women among these studies is lacking.

2.23 Changes in Bone Mass and Density during Lactation

Studies on bone resorption during lactation reflect unanimous results pointing towards decreases within the range of 2 to 10% in bone mass and density of trabecular sites (lumbar spine, hip, and distal radius) [37, 38, 40, 42, 45, 57, 58, 60-62]. This takes place during the first 3-6 months postpartum and is transient [37, 38, 40, 57, 60, 62].

Recovery of bone loss associated with lactation takes place shortly after weaning with recovery of bone mass occurring earlier for the spine than for the femoral neck [42, 45, 57, 61, 63-65]. An explanation for this difference is that trabecular bone (predominantly in the vertebral bodies) has a higher remodelling rate due to higher surface/volume ratio compared with cortical bone (predominantly in the total hip), hence a recovery after weaning is detected earlier in the lumbar spine than at the femoral neck [63]. In addition, during pregnancy

there may be maintenance of bone at sites that are highly stressed, such as the femoral neck (due to the extra weight of the fetus), which is the main weight bearing region of the proximal femur [51]. With some of the trabecular bone density (spine site) having been lost during pregnancy and perhaps having reached a BMD loss threshold, the maternal body may draw on calcium stores from the femoral neck rather than the spine. Hence, the recovery of femoral neck BMD may be prolonged significantly beyond cessation of lactation while lumbar spine BMD recovery starts at an earlier period.

The time it takes for lactating women to recover all their BMD may differ from one population to another due to reasons such as diet, lifestyle, genetics, and multiple pregnancies [66]. Hence longer studies have to be conducted (2 year follow up) in different populations in order to look at such factors and whether they have any impact on BMD recovery. Longer follow up periods may also be beneficial in the sense of looking at women who may become pregnant with a second child while breastfeeding their first offspring. Matsushita et al [66] found that bone loss was not greater in women who became pregnant while lactating, when the decrease in bone mineral is still evident, as compared to women who became pregnant again after having allowed for the lapse of sufficient time following their previous pregnancy and lactation. In addition, it was found that following a subsequent (second) pregnancy, BMD of the lumbar spine was higher with a change of 1.4% as compared to lumbar spine BMD after a primary pregnancy with a change of about 3%. Comparable results were seen in animal

studies whereby goats and sheep exhibited less prominent bone loss in the second pregnancy and lactation as compared to the first lactation [67].

The time course of recovery of lactation associated bone loss as well as the amount of bone lost during lactation is said to be very closely linked to cessation of breastfeeding (length of lactation) or to the return of ovarian function and menstruation [37, 40, 58, 61, 62, 64, 68, 69]. The fact that the former and latter are strongly interrelated makes it very hard to examine the influence of each independently on bone mineral status or change in bone density [37, 62]. Many studies have looked at both aspects with respect to changes in bone density however, the subjects in these studies had different length of lactation and varied resumption of menses [58, 61, 63, 64]. The pattern of change in BMD was wide ranging due to the variously defined timings of the final measurements relative to termination of lactation or resumption of menstruation [37]. However, a recent Italian study encompassed a design that required women to exclusively breast feed for 6 months and wean their babies at 7 months, at which point lactation was suppressed pharmacologically [65]. The authors found less of a deficit in spine BMD in lactating women who resumed menses by 5 months postpartum than in those that remained amenorrheaic for more than 5 months (-3.0% vs. -5.8%). However, those with an early return of menses had lower subsequent gain in BMD, so that by 18 months there was no difference relative to women with a later return of menses [65]. On another note, the ethical aspect of this study may be questionable in the sense that women had to cease breastfeeding their infants at

six months. However, each woman gave informed consent at enrolment. Additionally, 24 women started bottle feeding between 2 and 6 months postpartum and hence had to withdraw. Although not stated in the article, it may be assumed that perhaps women who wanted to breastfeed for longer than 6 months may have done so after withdrawing from the study. However, desire to breastfeed past 6 months was not mentioned as a reason for withdrawal.

2.24 Effect of Calcium Supplementation on Bone Turnover during Lactation

The physiologic adaptations that occur during lactation, mainly bone loss and recovery after weaning, have been shown to be relatively independent of calcium intake [37, 40, 57, 60, 62]. Observational studies failed to report any correlation between changes in bone density during lactation and maternal calcium intake [57, 59, 60, 62, 64]. Lactation-induced bone loss at the femoral neck and spine has been reported for women with high calcium intake regardless of whether it is dietary or supplemental [57, 60, 62]. Randomized calcium-supplementation trials have also revealed that calcium supplementation has little effect on lactation-induced bone loss [42-44, 60, 65]. It is important to mention that serum 25(OH)D was not measured in these studies. Lactation may deplete 25(OH)D and thus a higher $1,25(OH)_2D$ response cannot be mounted, rendering the women studied unable to properly use the calcium given to them. A study coming from Italy [65] randomly assigned 274 lactating women to two groups. The first group was lactating women without calcium supplementation and the second group was lactating women with 1 g calcium supplementation. At the end

of the 6 month intervention period there was no difference between the supplemented group and the placebo group in the amount of bone loss at the lumbar spine (-4.0% vs. -4.4%) (p=0.162). A similar trend was seen with the ultra-distal radius (-2.0% vs. -2.2%) (p=0.162). It was hypothesized that the period of weaning rather than the period of lactation may be the stage at which supplemental calcium makes a difference with regard to recovery of lactation induced bone loss. However, recovery of lost bone density occurs after weaning despite low or high maternal calcium intake during the period of weaning [40, 42, 65].

Thomas and Weisman [70] refuted the ideology that lactation-induced bone loss is not associated with calcium intake. The authors used data from several studies to argue that dietary calcium counteracts maternal bone loss during lactation. Thomas and Weisman stated that Kwalkarf et al [42] reported a "significant positive effect of calcium supplementation on the lumbar spine region BMD" in the lactation subgroup. However, in reviewing the original study, results indicate less bone loss or slightly increased lumbar spine density in postpartum women in general (both lactating and nonlactating). This effect was more significant for nonlactating women ($P<0.01$), whereas among lactating women it was not significant ($P=0.38$). Furthermore, there was no statistical interaction between the lactation group and the calcium group ($P=0.23$), suggestive of calcium supplementation being no more beneficial in the lactating group than in the nonlactating group. Additionally, Thomas and Weisman drew upon the work

of Krebs et al [45]. This study comprised of 26 lactating women and 8 non-lactating women followed from 0.5 to 7 months postpartum. Once again the claim by the authors was “high calcium intake positively associated with lumbar BMD”. Looking at the original study by Krebs et al, it is stated that “stepwise regression indicated that calcium intake and L2-L4 BMD were significantly associated (P=0.03) in lactating subjects”. However, nowhere in the study was there a table or graph showing L2-L4 BMD results at any point during the 7 months follow up. In addition, the authors do not show associations between lumbar spine BMD and calcium intake for any time points apart from that one generalised statement of association mentioned above. We do not know where this association is from due to lack of data reporting in the primary study, hence it does not seem fit to claim that the study by Krebs et al is proof of a link between calcium intake and lumbar spine BMD. A third piece of evidence used by Thomas and Weisman, to show that dietary calcium counteracts maternal bone loss during lactation is a study by Chan et al [71] in which 21 calcium supplemented lactating adolescents (Ca > 1600 mg/d), 15 control lactating adolescents (Ca = 900 mg/day) and 12 adult lactating women were followed from 2 weeks postpartum until 16 weeks postpartum. Thomas and Weisman quoted “In the control group, BMC decreased by 10% but there was no statistical decrease in the high-calcium group”. This statement was correctly taken from the original primary article by Chan et al and there indeed was a decrease in BMC in the control group. However, it is important to note that the primary population of interest in this study was adolescents and it may very well be that lactating adolescents with a low intake of

dietary calcium are at very high risk for decreased BMD and BMC due to their own skeletal demands (which is not the case in adult women who have already reached peak bone mass) as well as those of lactation. In a comparison between pregnant, lactating and never pregnant never lactating adolescents (NPNL) (Ca = 500mg/d) with pregnant, lactating and NPNL adults (Ca = 500mg/day), substantial differences were found [72]. Pregnancy seemed to impair bone formation in adolescents rendering lactation a period during which they may be partially catching up on skeletal growth that ceased or slowed down during pregnancy [72]. The minimal calcium attained from the diet will be channelled towards the adolescent's skeletal growth; however calcium for lactational demands will be resorbed from bone. Hence, if bone formation is lower than bone resorption it is normal that a 10% decrease in BMC is observed in adolescents. Adolescents with low intakes of calcium recover lactation associated bone loss in the post weaning period but there may be deficits with respect to recovery of lumbar spine BMD [73]. Adolescents with habitually low calcium intake and who had lactated may experience a less than optimal rate of bone recovery. Consequently these adolescents may not attain the same level of BMD as adolescents who were never pregnant [73]. Future studies should aim to investigate whether supplementation during lactation and the post weaning phase will prompt a faster rate of bone accretion in lactating adolescents.

2.25 Calcium and Maternal Health during Lactation

The increased calcium demand experienced by lactating mothers is supplied via resorption from the maternal skeleton, which in turn has raised concern whether this is a potential risk of osteoporosis in later life, especially if customary calcium intake is low [37]. Both early evidence and more recent studies are inconsistent and inconclusive; some studies suggest that lactation history and duration of breastfeeding are associated with increased BMD while others reflect decreased BMD or no effect at all [74-77]. It is doubtful that supplemental calcium has any effect on decreasing risk of osteoporosis or hip fracture in women who had breast fed due to the ability of the maternal skeleton to restore its calcium content post weaning. However, studies that have looked at calcium restoration, encompassed women who breast fed for a maximum of 1 year. It is unclear whether lactation past 1 year and perhaps 2 years has a different effect on maternal BMD restoration and whether calcium supplementation may have any influence in these situations. Typical durations of exclusive breastfeeding in Canadian women reach a maximum of 6 months [78]. In fact, only 60% and 30% of Canadian women exclusively breast feed at 3 and 6 months respectively [78]. Hence, such long durations (1 year and 2 years) are not as critical to examining adequacy of dietary intake for this specific population.

On the other hand, a Tufts evidence-based systematic review suggests that women with calcium intake levels of 1000-1250 mg/d have lower risk of breast cancer (RR 0.75, 95% CI 0.55, 0.99) compared to those with intake levels less

than 500 mg/d [79]. In addition, the review stated associations between higher calcium intake and lower colorectal cancer but further studies need to be conducted in order to draw conclusions. The Women's Health Study (WHS) which comprised of 29, 000 women illustrated a significantly higher rate of hypertension in women in who had low intakes of calcium (189-557 mg/day) compared to women with intakes above 679 mg/day [79]. Whether decreased calcium intake during lactation could possibly place women at higher risk for the previously mentioned complications is unknown. There are no studies looking at calcium intake during a metabolically stressful period such as lactation and future risk of hypertension, colorectal cancer or breast cancer. However, in order to prevent the development of such incidences lactating women are urged to sustain an adequate intake of 1000 mg/day.

2.26 Recommended Calcium Intakes for Pregnant and Lactating Women over Time

Previously, it had long been assumed that pregnant and lactating women need to consume greater amounts of calcium to compensate for the loss of calcium from the mother to the fetus and infant. The inception of the Recommended Dietary Allowances (RDA's) in the early 1940's, reflected this belief. The recommended calcium intakes were higher for pregnant and lactating women as compared with those for nonpregnant, nonlactating women. Recommended calcium intakes have been as high as 1500 mg/d for pregnant women and 2000 mg/d for lactating women [40]. In 1964, recommended calcium

intake for lactating women was decreased to meet the 1500 mg/d threshold of pregnant women. The release of the 1997 Dietary Reference Intake (DRI) brought about a change to this trend. It was the first time that recommended calcium intakes for pregnancy and lactation were the same as those for nonpregnant, nonlactating women-1000 mg/d [80]. Although the 1997 DRI for calcium was an adequate intake, which differs conceptually from an RDA, the similarity in requirement for dietary calcium by pregnant, lactating and nonpregnant women remains prominent due to all the literature that points towards the fact that calcium needs during lactation are delivered from the bone regardless of increases in dietary intake or supplementation [40].

2.3 Vitamin D

The importance of vitamin D is widely recognized and in parallel with this recognition comes the need for a definition of healthy vitamin D status. The definition of vitamin D status has been modified due to research into the relationship between vitamin D, PTH, serum calcium and bone resorption [81]. In adults, optimal plasma 25(OH)D levels have been defined as levels at which parathyroid hormone production and calcium resorption from bone are minimized, and intestinal calcium absorption is stabilized [81]. This definition however may not be appropriate for pregnant women due to already decreased PTH levels as a result of pregnancy. Different criteria perhaps are needed but are unavailable.

Table 2**:** Cut off values for serum 25(OH)D status categories[1]

25(OH)D	**ng/ml**	**nmol/L**
Deficient	<10	<25
Insufficient	10-30	25-75
Optimal	30-90	75-225
Pharmacological	>90	>225
Potentially toxic	>200	>500

[1]Canadian Paediatric Society [112]

With all the knowledge pertaining to the significance of vitamin D and the ability to measure serum concentrations of 25(OH)D, it is surprising that vitamin D deficiency is widespread [82]. Modern lifestyle has been deemed a mediator of vitamin D deficiency. Urbanization, indoor activities, and use of sunscreen [82] decrease sun exposure. Furthermore, persons with dark skin pigmentation and those who cover themselves for cultural or religious reasons as well as those at higher latitudes, particularly in winter are at higher risk of developing vitamin D deficiency [82].

A threshold of 18-20 mJ/cm^2 of ultraviolet B (UVB) light is needed in order for the skin to produce vitamin D. With the increased concern over skin cancer, the recent trend is to apply sun screen when going outdoors. Sunscreen of SPF 8 or higher, blocks vitamin D production [82]. Persons of dark skinned races who have elevated melanin content do not produce vitamin D in the same quantities as people with white skin. In addition, the threshold of 18-20 mJ/cm2 is not usually attained during the winter in areas above latitude 40° making skin pigmentation less of an issue since there is not enough UVB to make vitamin D in the first place [82].

2.31 Vitamin D in Lactation

2.311 Vitamin D Content of Human Milk

The current recommended intake of 200 IU vitamin D per day for lactating women results in vitamin D activity of 20-70 IU/L in milk [82]. This amount is

far below the recommended dose of 400 IU/day of vitamin D for infants (although the scientific community does not know the amount actually needed by infants).

The important sources of vitamin D activity in human milk are the parent compounds; vitamin D_3 and vitamin D_2. Vitamin D_3 [83] and the metabolites $25(OH)D_3$ and $25(OH)D_2$ are of particular importance. Other metabolites such as $1,25(OH)_2D_3$ and $1,25(OH)_2D_2$ are at insufficient concentrations to measurably increase activity [82]. In fact, to rephrase, vitamin D (vitamin D_2 and vitamin D_3) passes readily into breast milk, 25(OH)D passes very poorly and $1,25(OH)_2D$ does not appear to pass at all [84].

The concentration of 25(OH)D in human milk, which represents approximately 1% of the maternal circulating 25(OH)D provides a steady supply of antirachitic activity that is resistant to daily changes in short-term vitamin D supply. Alternatively, 20-30% of maternal circulating vitamin D is expressed in milk [82]. “This expression allows maternal variation in vitamin D metabolite due to UVB exposure or fluctuation in intake to be transferred to the milk” [82]. In lactation maintaining both circulating 25(OH)D and vitamin D are important because D_3 in particular is what is quantitatively transferred into mother’s milk as opposed to circulating 25(OH)D, which is transferred on a very limited basis into human milk [83]. Hence for human milk to achieve sufficient vitamin D status, the parent compound vitamin D (mostly D_3) is the required form [82].

The concentration of vitamin D in human milk relies entirely on maternal vitamin D status which in turn is influenced either by UV exposure or oral

supplementation [82]. Such findings have been reflected in studies as early as 1984 [82]. Lactating white women receiving total body UVB exposure equal to 30 minutes of sunshine at midday at temperate latitudes significantly increased the vitamin D content of their milk with a peak at 48 hours and return to baseline at 7 days. Maternal circulating 25(OH)D concentrations also increased from 13.9 to 20.5 ng/ml (34.75 to 51.25 nmol/L) and remained elevated for 14 days, however there was no significant change in the milk 25(OH)D concentrations [82]. This highlighted the importance of the parent compound vitamin D and its relatively short half life which calls for consistent dosing. However, due to reasons mentioned above, UVB exposure is not a viable option for many lactating women to achieve vitamin D sufficient breast milk. A better alternative is to increase consumption of vitamin D perhaps from food or supplementation.

2.312 Maternal Vitamin D Status

With breast milk vitamin D content depending on the vitamin D status of the lactating mother, the first step toward achieving vitamin D sufficient breast milk must address the vitamin D intake (dietary or supplementation) required to achieve vitamin D sufficiency in the mother [82]. Since vitamin D content of food is not always sufficient enough to maintain adequate levels of circulating vitamin D in lactating women, supplementation is vital. Nesby-Odell et al [85] found that 42.4% of African American women and 4.2% of white women of child bearing years (15-49) in the United States exhibited circulating 25(OH)D below 15 ng/ml (37.5 nmol/L).

Currently most obstetricians recommend 400 IU/day vitamin D_3 supplementation for pregnant and lactating women in the United States [69]. However, the scientific community is rethinking these values due to studies that suggest 400 IU/day is inadequate [82, 86]. Researchers are lobbying for an increase in the dose of supplementation to amounts exceeding 1,000 IU/day. This stems from the argument that 400 IU/day is not enough for women who are initially vitamin D deficient. Heaney et al [87] reported that for every µg intake of vitamin D, serum 25(OH)D_3 is elevated by 0.7 nmol/L. Therefore an intake of 400 IU/day (10 ug) would increase 25(OH)D by 7.0 nmol/L. This may be a sufficient amount of supplementation if the mother's dietary intake of vitamin D and solar exposure are optimal however, this is usually not the case. Usually, prenatal vitamins are prescribed into lactation in order to provide extra vitamin D to support the mother's and infant's needs. If the lactating mother is vitamin D deficient, 400 IU/day provides no extra vitamin D during lactation [82]. This point is important because maternal vitamin D deficiency during the period of lactation can affect the vitamin D status of the infant. Dawodu et al [88] looked at 90 breastfeeding mother-infant dyads in the United Arab Emirates during the summer. The authors found that the median serum 25(OH)D concentrations in mothers and infants was 8.6 ng/ml (21.5 nmol/L) and 4.6 ng/ml (11.5 nmol/L) respectively. Sixty percent of the mothers stated that they had either avoided commercial milk or had consumed unfortified milk, while only 40% reported consuming fortified milk with a calculated daily average intake of 88 IU of vitamin D. Furthermore, despite the abundance of UVB in this region, vitamin D

status of these women was poor due to clothing that covers the majority of the body and minimises exposure to UVB [82, 88]. In Eastern Turkey, 39 children aged between 0-3 years were diagnosed with vitamin D deficiency rickets (VDDR). The mothers of these infants had mean serum 25(OH)D of 15 ng/ml (37.5 nmol/L) while the infants' mean serum 25(OH)D was 5.8 ng/ml (14.5 nmol/L). These low maternal values were attributed to veiling and lack of vitamin supplementation during pregnancy and lactation [89]. In sunny Greece, there was no difference in the mean serum values of 25(OH)D between mothers who had given birth in winter-spring and those who had delivered in the summer-autumn season (10.8 ng/ml vs. 12.9 ng/ml) (27 nmol/L vs. 32.3 nmol/L) [90]. Although, not directly pertaining to intake of vitamin D, nevertheless this last study from Greece shows that maternal vitamin D deficiency is relatively wide spread. In Canada, 34 infants below the age of one year were diagnosed with vitamin D-deficiency rickets from July 1, 2002, to June 30, 2004 [91]. Breastfeeding without supplementation was stated as the most frequent factor [91]. In light of the fact that human milk has very low vitamin D content, this finding comes as no surprise. Furthermore, 12.5% of the mothers (104 mothers in total) said they had received vitamin D supplements during pregnancy and only 5% received supplements following delivery [91]. Seventy six percent of the mothers stated that they did not drink milk pre- or postnatally [91].

If some lactating mothers are not receiving supplements of vitamin D then they should be obtaining it from their diet. Fish oil, egg yolk or liver, although

very rich in vitamin D may not be frequently consumed by many women [92]; nonetheless a readily available source of vitamin D is milk and dairy products. In Canada, milk including soy beverage and many other dairy products such as some yogurts, some cheeses and margarine (530 IU/100 g) are fortified with vitamin D [92]. Canada currently has mandatory fortification of foods, through the Canadian Food and Drug regulation [92]. "*Eating well with Canada's food guide"* recommends all Canadians including pregnant and lactating women consume 500 ml (2cups) of milk per day" [93]. Two cups of milk would supply the DRI of 200 IU/day of vitamin D for lactating women [93]. However, lactating women are not abiding by this recommendation [6, 88, 91]. Mannion et al [6] observed that 23% of 175 exclusively breastfeeding women restricted their intake of milk (< 250 ml of milk). These women's intake of vitamin D was below the recommended 5 µg (mean of 2.2 µg) and only reached that number and beyond with the help of supplements.

Whether a lactating mother attains the recommended intake via dietary sources or supplementation, the persisting question in the literature is whether 200-400 IU/day from food or supplements is adequate for a lactating woman in order to cater to her needs as well as the requirements of her growing breastfeeding child. This stems from the fact that vitamin D status of breastfeeding child parallels that of the lactating mother's milk, which in turn is influenced by the mother's vitamin D status. Therefore, is it possible to

supplement mothers with a dose that would provide sufficient circulating serum vitamin D in both mother and child?

2.32 Supplementing Mothers in Order to Meet her Needs and her Infant's Needs

It is believed that vitamin D supplementation of lactating mothers has a dual purpose: (1) to increase the nutritional vitamin D status of the mother and (2) to improve the vitamin D nutrition of her breastfeeding infant [83]. Two studies looking at supplementing lactating women with vitamin D at doses above 2000 IU/day show an increase in maternal total circulating 25(OH)D concentrations from baseline [94, 95]. The same trend was seen for changes in milk anti-rachitic activity and circulating 25(OH)D in infants. Results reflected the notion that higher supplementation of the mother benefits both mother and child. One major difference between the study by Hollis et al [94] and the study by Wagner [95] was that milk antirachitic activity did not increase the same amount although maternal supplementation in both studies was comparable. This discrepancy was attributed to the fact that the first study [94] used vitamin D_2 for supplementation while the second study used vitamin D_3 [95]. Research on men found that vitamin D_2 is less potent than vitamin D_3 and also has a shorter duration of action relative to vitamin D_3 [83, 96]. In an attempt to measure the efficacy of daily or monthly supplementation of vitamin D_2 in nulliparous and lactating women, Saadi et al [97] found that "increments in serum 25(OH)D concentrations were substantially lower than those reported for equimolar doses of vitamin D_3" [97]. In this study, both oral supplementation with 2000 IU/d or 60,000 IU/month for 3 months was

safe and increased serum 25(OH)D substantially; however only 20-36% of subjects achieved serum concentrations of greater than 50 nmol/L. This was highly attributable to the fact that vitamin D_2 was used to supplement the women rather than vitamin D_3 [97]. It is established that supplementation with vitamin D is of benefit to both mother and child with regard to bettering serum status of the vitamin in both parties. However, if the mother was deficient and not obtaining the DRI or more of vitamin D, would that adversely affect her or her ability to provide adequate nutrition for the infant? The bulk of literature available on the topic leans towards the fact that vitamin D deficiency does not negatively affect the mother or her nursing infant if given supplements. Nevertheless, we do not know whether this deficiency, if not corrected, may impact her future health.

2.33 Maternal Outcomes from Lactation and Vitamin D

Vitamin D deficient rats and vitamin D receptor-null mice lactate normally and experience similar skeletal losses to controls [84]. Intestinal calcium absorption in lactating vitamin D-deficient rats is upregulated to the same level as in vitamin D-sufficient rats [84]. In humans, ingested calcium during lactation whether low or high has no effect on skeletal demineralization [37, 40, 57, 59, 60, 62, 64]. Hence it would be logical to suggest that supplementation with vitamin D would probably have no effect on skeletal resorption in lactating women [84], especially since $1,25(OH)_2D$ levels decrease to non pregnancy concentrations postpartum. Furthermore, Basile et al found that maternal vitamin D status or vitamin D supplementation did not affect breast milk calcium content [98].

Therefore, vitamin D does not seem to affect the mother's skeletal health at the time of lactation or the content of calcium in her breast milk however, recent studies have found associations between vitamin D deficiency and other adverse health consequences such as insulin insensitivity, rheumatoid arthritis (RA) , multiple sclerosis and osteoporosis. These do not necessarily occur in vitamin D deficient lactating mothers; however the chance that they may manifest themselves in the future in this at risk population must not be underestimated.

Chiu et al [99] found that increased circulating 25(OH)D concentrations improved insulin sensitivity. In fact, the authors stated that 25(OH)D concentration "was a highly significant and independent predictor for insulin sensitivity". When tested with the covariates sex, body mass index (BMI), diastolic blood pressure, age and ethnicity, 25(OH)D concentrations accounted for 42% of the variation in insulin sensitivity in a sample of 126 subjects. Vitamin D deficiency impairs insulin secretion of pancreatic β cells and increases insulin resistance which are two major factors in the pathogenesis of type 2 diabetes [100]. Accordingly, Knekt et al [100] showed significant findings for men, whereby a relative odds of 0.28 (95% CI 0.10-0.81) for developing diabetes was seen between the highest (75.2 nmol/L) and lowest quartiles (26.0 nmol/L) of serum vitamin D concentration.

In addition, vitamin D seems to have positive defensive effects against autoimmune diseases such as RA [101]. There is robust evidence for an inverse relationship between vitamin D and RA [101]; greater intake of total vitamin D is

inversely associated with development of rheumatoid arthritis (RR 0.67, 95% CI 0.44-1.00) [101]. Very similar results were obtained when vitamin D intake was categorized as dietary (RR 0.72, 95% CI 0.46-1.14) or supplemental (RR 0.66, 95% CI 0.43-1.00) [101]. Beyond insulin sensitivity and RA, there is indication of serious outcomes due to chronic vitamin D deprivation, such as increased risk of multiple sclerosis [95, 102], periodontal disease, decreased muscle function, asthma, pathogenesis of specific types of cancer, and depression [95]. The more commonly known effects of vitamin D include its role in calcemic functions thus, vitamin D deficiency has been linked with osteopenia and osteoporosis [95, 103]. $1,25(OH)_2D$ can promote bone mineralization indirectly by stimulating calcium and phosphate supply, mainly by absorption from the gut [103]. Without vitamin D, only 10-15% of dietary calcium and about 60% of phosphorus is absorbed [103]. Additionally, vitamin D deficiency is positively correlated with loss of BMD at different skeletal sites [103]. Furthermore, when people are vitamin D deficient, the decrease of intestinal calcium absorption will increase serum PTH, which in turn activates osteoblasts and stimulates preosteoclasts to differentiate into mature osteoclasts. Osteoclasts dissolve the mineralized collagen matrix in bone causing loss of bone mass and low BMD if mineral is not replaced [103]. On the other hand maternal free and bound $1,25(OH)_2D$ levels decrease to normal within days of labour and remain constant throughout lactation and PTH decreases significantly as part of a lactation-induced adaptation process. This mechanism makes it unclear whether vitamin D deficiency may actually elicit an increase in serum PTH in lactating women.

The female population in general will benefit from an adequate intake of vitamin D. However, addressing pregnant and lactating women is crucial because their bodies are stressed and have to cater to the increased needs of the fetus and subsequently infant. If they start off with optimal concentrations of vitamin D but end up with suboptimal concentrations, they may become at risk for the above mentioned health complications. On the other end of the spectrum is the breastfeeding infant's vitamin D levels. What are the consequences of inadequate maternal intake of vitamin D on the infant?

2.34 Infant Outcomes from Mother's Vitamin D Deficiency

2.341 Pregnancy

In utero, the only vitamin D that a fetus obtains is provided by the mother hence, maternal vitamin D deficiency during pregnancy can affect an infant's calcium homeostasis, causing hypocalcaemia, osteomalacia and craniotabes (softening of the skull bones) [104]. Furthermore, positive associations exist between maternal vitamin D status in pregnancy and birthweight, birth length and bone mineral accretion at 9 years [105, 106]. Conversely, Javaid et al did not find any association between maternal vitamin D status in late pregnancy with birth weight or birth length [107]. However, the authors found that low maternal vitamin D status during the last trimester of pregnancy caused a decrease in bone size and bone mineral content in their children which extended to their nineth birthday [107].

2.342 Lactation

The infant born to a vitamin D replete mother is protected from vitamin D deficiency for the first few months of life as 25(OH)D readily crosses the placenta and neonatal levels approximate two thirds of maternal serum concentrations [108]. Since serum 25(OH)D has a half life of approximately 3 weeks, an infant is protected against vitamin D deficiency for a couple of months even if the young infant does not receive vitamin D. This is provided that the mother's vitamin D status during pregnancy was adequate and had allowed for ample transfer of the vitamin to the fetus [108]. On the other hand, an infant who is exclusively breast fed and gets minimal sunlight exposure runs the risk of developing vitamin D deficiency rickets by 4-6 months of age.

In Turkey [109], 42 infants were diagnosed with vitamin D deficiency and/or nutritional rickets. The predominant cause of the above was exclusive breastfeeding (83% of the infants). None of the infants were supplemented with vitamin D and furthermore none of the 42 mothers were supplemented with vitamin D during pregnancy. Moreover, sunlight exposure was limited for 23 of the infants for whom a history of sun exposure was available. Thirty three mothers wore clothes that covered their entire body and hence with no sunlight exposure or adequate intake these women were vitamin D deficient. Therefore, these infants were diagnosed with vitamin D deficiency rickets as result of a combination of reasons (lack of sun exposure, vitamin D deplete mothers, lack of

supplementation) rather than exclusive breastfeeding alone. Cardiomyopathy as well as myelofibrosis are complications of vitamin D deficiency (rickets) [110].

The peak age at which rickets is most prevalent is 3-18 months which is the age [111] when the child normally starts walking hence, any aberration from normal skeletal development (such as rickets) may delay the process of walking [111]. In India, 25 patients (children with mean age of presentation of 1.6 years) which had presented with none or delayed walking as chief complaint, were diagnosed with nutritional rickets. Upon treatment with vitamin D and calcium, 90% of the children started walking [111]. Delayed walking has been previously reported as a presentation of rickets. In Canada 10% of a total of 56 patients in the age group of 1-2 years had delayed walking as a result of vitamin D-deficiency rickets [91].

Therefore, many health organizations call for supplementing newborn infants with vitamin D [112]. However, supplementing infants without supplementing their mothers means that lactating women remain deficient or become at risk of deficiency. Saadi et al [113] reported on 90 lactating vitamin D supplemented women (2000 IU/day or 60,000 IU/mo for 3 months). Both groups of women exhibited positive results with follow up mean serum 25(OH)D reaching 38.7 nmol/L from a baseline of 25.3 nmol/L [113].

Optimal supplementation in order to achieve sufficient vitamin D status in lactating women is still very controversial. Although recommendations are in

place, recent evidence suggests that these recommendations do nothing to sustain the woman's needs or her infant's.

2.35 Vitamin D Recommendations in Lactating Women

Currently Health Canada recommends an AI of 200 IU/d of vitamin D for both pregnant and lactating women in the 19-30 and 31-50 age groups [20]. The Canadian Pediatric Society on the other hand states that "Consideration should be given to administering 2000 IU of vitamin D daily to pregnant and lactating women, especially during the winter months, to maintain vitamin D sufficiency. The effectiveness of this regimen and possible side effects should be checked with periodic assays for 25(OH)D and calcium" [112]. This statement comes in face of the above mentioned research (previous sections) that highlights the fact that doses below 2000 IU/d do not accomplish much with regard to maintaining lactating women's levels of vitamin D. Furthermore, the Institute of Medicine (DRI committee), like Health Canada, recommends 200 IU/d of vitamin D "in the absence of adequate sunlight exposure" for lactating women [20]. Hence, studies that have supported the need to revaluate the DRI's for vitamin D, has resulted in wide spread criticism of the 1997 recommendations for vitamin D. This is not to say that these recommendations are wrong and misleading but simply to show that sound and correct evidence was lacking at that point. The conservative tolerable upper intake level (UL) of 2000 IU/day for vitamin D was based on uncertain reports of hypercalcemia and hypercalciuria [87]. Currently the DRI values are under revision; whether higher intakes will be suggested remains to be seen [114].

2.4 Essential Fatty Acids

2.41 LCPUFA in Pregnancy and Lactation

Brain accretion of DHA starts *in utero* and increases markedly in the second half of gestation with continual accumulation after birth [12]. Total DHA accretion reaches about 4 g between the ages of two and four years [12]. Furthermore, DHA is also a significant structural component of retina lipids, encompassing as much as 50% of total fatty acids of rod and cone outer segments [12]. There is high accumulation of AA in addition to DHA during pre but mostly postnatal development [100].

2.411 Pregnancy

The enzymes needed for PUFA conversion to LCPUFA are present in the fetal liver early on in gestation, but it has been found that this activity seems to be low before birth [12]. Therefore, the much needed n-6 and n-3 LCPUFA that the fetus accretes during gestation is predominantly provided by the mother through placental transfer, with the quantity in cord blood influenced by maternal diet [12]. Maternal dietary intake of LCPUFA and lifestyle influence the LCPUFA amount available for transfer to the fetus [12]. The physiological importance of DHA is further mediated by the support of "active and preferential maternal fetal placental transfer" [12, 34, 115]. This transfer is arbitrated by particular fatty acid transfer proteins and membrane binding proteins that favour placental transport of DHA over other fatty acids such as LA [12]. It is important to mention that pregnancy is a period of high estrogen secretion, and this enhances elongations

and desaturation of ALA to EPA and DHA [116]. However, maternal dietary intake of these fatty acids remains the most important source of DHA for maternal fetal transfer.

2.412 Lactation

Preterm and term infants both have the capacity to synthesize DHA and AA, however the rate of conversion of these LCPUFA's from their C18 precursors is deemed insufficient in infants to maintain stable plasma and red blood cell LCPUFA levels [12, 117, 118]. Hence, human milk provides LA, ALA, DHA, AA and other LCPUFA to breastfed infants [12]. The lipid fraction is the greatest macronutrient in human milk, supplying almost 50% of energy as fat to the breastfeeding infant [119].

The main compounds of milk fat are fatty acids [119]. These are esterified mainly in the form of triacylglycerols (TG's) which account for 98% of milk fat, or in the form of phospholipids (PL) [119]. In mature human milk, approximately 85% of LCPUFA is in the form of TG's and 15% is in the form of PL's [119], AA and DHA being within these percentages.

The concentration of AA in breast milk is more or less constant on a worldwide basis; however the level of DHA is more variable depending on maternal diet [12]. A few studies have looked at the variability in concentration of DHA and AA in human milk amongst different populations [117, 120]. Population means of AA in breast milk ranges from 0.35-0.7 weight % of total

fatty acids while means of DHA range from 0.17% to 1.4% of total fatty acids [12, 117, 120].

Brenna et al [117] identified the Canadian Arctic, Japan, Dominican Republic, Philippines, and Congo as being locales with the greatest breast milk DHA concentration (1.4-0.6%). All these areas (apart from Congo) are coastal or island populations that may have high marine food consumption. Congolese women may have a high fish intake due to imported fish from the neighbouring Gabon which has a long coast along the Atlantic Ocean. On the other end of the spectrum, the lowest breast milk DHA levels were found in Pakistan, rural South Africa, urban Canada, the Netherlands and France (0.06-0.14%). These populations are either inland or are developed countries, both of which have been associated with low intake of marine food [117]. Pakistani mothers' milk has the lowest concentration LCPUFA which is consistent with their suboptimal essential fatty acid status, particularly n-3 fatty acids due to the minimal intake of vegetable oil and marine food [121]. The milk of Caribbean and Chinese mothers contained the highest LCPUFA which may be in conjunction with the high consumption of fish [121, 122]. These variations in the LCPUFA concentrations of breast milk may be further proof that diet may have a significant role in dictating levels of LCPUFA, particularly DHA in human milk. The scientific literature reports that women receiving DHA supplementation exhibit an increase in milk DHA concentration. Gibson et al [123] and Jensen et al [124] stated that a dose dependent relationship exists between maternal DHA consumption and DHA

concentration in milk. Gibson et al also showed that breast milk DHA levels above 0.8% of total fatty acids had little effect on plasma or red blood cell (RBC) DHA content of the study infants. Maternal fatty acid intake and milk lipid content are not the only two indices that have been shown to correlate [124]. Significant correlations between the DHA content of maternal plasma phospholipids and those of milk lipids have been reported [124].

As mentioned previously, AA does not seem to be as sensitive to dietary intake as DHA is; nevertheless studies that have looked at the effect of supplementation of AA have observed that milk AA increases subsequent to supplementation [125, 126]. This has been attributed to the longer observation time of these studies implying that influence of dietary AA becomes noticeable after prolonged supplementation and even then milk content of AA still does not increase as readily as DHA does after supplementation.

The biggest concern is the tendency of supplemental DHA to reduce milk AA or vice versa. DHA and AA compete with one another for many metabolic processes and therefore one may theoretically inhibit the synthesis and mammary secretion of the other. Van Goor et al [125] stated that supplementing with fish oil or DHA had no effect on milk AA levels. Weseler et al [126] tested the effect of DHA supplementation only on milk AA levels in addition to assessing whether coadministration of low and high doses of AA along with the DHA would attenuate increases in milk DHA. DHA supplementation did not alter the level of AA in milk and further coconsumption of low dose of AA (200 mg/day) or a high

dose (400 mg/day) had no influence on the increase in milk DHA [126]. Alternatively, a randomized control trial found that supplementation with DHA tended to reduce milk AA [125]. Breast milk in both studies was collected at similar times; 2 weeks and then 8-12 weeks postpartum. The key to suitable incorporation of all LCPUFA's into milk lipids maybe in finding the appropriate ratio of n-6 to n-3 fatty acid whereby absorption of one fatty acid does not affect the other. It is important to add that finding the right ratio of n-6 to n-3 is not a new topic of study or debate. It is well established that modern diets, particularly western diets, have evolved from containing roughly equal amounts of n-6 and n-3 to containing very high n-6 fatty acids to n-3 fatty acid ratio [127]. High intakes of n-6 fatty acids shift the physiologic state to a more prothrombotic and proaggregatory one with increase in blood viscosity, vasospasm, vasoconstriction and decrease in bleeding time as the main characteristics of this state [127]. Omega-3 fatty acids on the other hand, have anti-inflammatory, antithrombotic, antiarrhythmic and vasodilatory effects. Hence perhaps an approporiate ratio or equal amounts of the two types of fatty acids is recommended in order to achieve a balance.

2.42 Maternal LCPUFA and its Link to Infant Outcome Pertaining to Visual and Neural Development

An increase in DHA intake among lactating women through supplementation with fish, fish oils, single-cell PUFA, DHA-enriched eggs or other sources of DHA increases the secretion of DHA in milk and subsequently

the plasma and erythrocyte levels of DHA in the breastfeeding infant [128]. This is of particular importance because DHA is present in high concentrations in phosphatidylserine and the ethanolamine phosphoacylglycerols of brain grey matter and the outer segments of rod and cone photoreceptors in the retina [128]. DHA comprises up to 80% of the PUFA in the retina outer segment disks, and phospholipids exist in which both fatty acids are DHA [128]. DHA has an imperative role in photoreceptor signal transduction by enhancing the ability of photons to transform rhodopsin to the activated meta-rhodposin 11 state. In the brain DHA has diverse roles in growth, function and protects against oxidative stress [128]. Reduced levels of DHA in the brain impair neurogenensis and neurite outgrowth. Although the exact molecular mechanisms underlying such an effect are not fully understood, it has been suggested that DHA elevated neuromodulin (GAP43) mRNA which was a substrate of protein kinase C and involved in neurite formation of different neuronal cells [129]. Calderon and Kim found that DHA increased the number of hippocampal neurons with longer neurites and a higher number of branches [130]. Moreover decreased levels of DHA change the metabolism of several neurotransmitters, including dopamine serotonin and acetylcholine. Thus DHA is important in the central nervous system and in visual acuity [128].

Various studies have been conducted on whether the variability in human milk DHA is likely to be related to visual and neural development among breast fed infants [32, 33, 115, 118, 131, 132]. Studies of visual acuity and cognitive

function and their relation to DHA in term infants during lactation have very mixed results. A Cochrane review did not find a significant relationship between supplementation and vision or general development with the exception of a slight bettering of information processing [133].

2.43 Other Benefits of LCPUFA

Of great interest is a recent study assessing the correlation between LCPUFA and bone mass in human infants. Weiler et al [11] conducted correlation and regression analysis of cord RBC LCPUFA with infant BMC obtained from 30 maternal-child pairs. Results concluded that cord RBC AA was associated with infant whole-body BMC in both correlation and regression analysis [11] while cord AA: EPA was associated with infant lumbar-spine BMC in both correlation and regression analysis [11]. Other results were that cord RBC AA: EPA was correlated with femur BMC and that lumbar spine 1-4 and femur BMC were negatively influenced by maternal DHA status after accounting for infant weight as predicted by regression equations. The scientific community's insight into the influence of dietary intake of PUFA on bone health is very limited. Nevertheless, human and animal studies both point in the direction that fatty acids do have a role in bone mineral accrual [11, 134]. Perhaps the most marked of these studies is by Hogstrom et al [134] whereby the authors found significant associations between n-3 fatty acids, namely DHA, with both BMD at 22 years of age and changes in BMD of the spine between 16 and 22 in a cohort of 78 healthy young men. No studies have been conducted on the effect of PUFA in breast milk on

bone mineral accrual in breastfeeding infants. However if BMC in newborn infants is proven to be affected by cord or maternal PUFA status, then it is logical to postulate that breast milk PUFA may have an important influence on bone mineral accumulation in infants postnatally. If that is the case, further studies will need to be conducted in order to arrive at the combination of LCPUFA needed to achieve this benefit and whether one type of omega fatty acid is more beneficial to bone mineral accrual than the other.

2.44 Maternal LCPUFA Status and its Effect on the Mother Herself

During lactation, the mother's body transfers about 70-80 mg DHA/d to breast milk in addition to the amount lost to oxidation or used to fulfill the mother's own requirements [135]. Furthermore, in women who have resumed menstruation, DHA may also be lost in menstrual blood [135]. On the positive side of DHA balance, attention needs to be paid to dietary intake (which is highly varied across populations), synthesis by the mother, DHA that may be available from maternal stores accumulated during pregnancy, and DHA saved as a result of lactational amenorrhea [135]. Hence it is evident that determining maternal fatty acid balance during lactation, as well as the risk of LCPUFA depletion is very complicated. Nevertheless, although understanding the components of the balance equation may be complicated, taking plasma samples is probably not. In doing so, Makrides and Gibson [135] observed a 30% decrease in maternal plasma phospholipid DHA concentration between day 5 and week 6 postpartum. They then compared maternal plasma phospholipid DHA concentration between

mothers who were exclusively breastfeeding their infants and those that chose to formula feed; a small yet significant difference was found ($P<0.01$). Otto et al [34] reported that while percentages of plasma LA, AA, and EPA, increased over time, the percentage of DHA in plasma and erythrocyte phospholipid fatty acids decreased significantly in both lactating and non lactating women. The decrease in plasma DHA was more evident in lactating women and was augmented when the lactation period was extended [34]. This decrease in certain LCPUFA's may be a result of normalization after an initial increase of LCPUFA's throughout pregnancy or may actually be related to lactation; perhaps even the combination of both processes along with enhancement by some hormonal effect and increased utilization of maternal stores [34]. Especially that increased levels of estrogen in pregnancy (3-8 times higher compared to normal levels) may stimulate DHA synthesis and promote its storage in adipose tissue for use in lactation.

Hibbeln [13] claims that lower concentrations of plasma DHA and lower national rates of seafood consumption are significantly associated with higher rates of major postpartum depressive symptoms. In his opinion supplementation with n-3 and n-6 fatty acids, particularly n-3 fatty acids will dramatically alleviate this problem [13]. Another benefit of dietary lipids in postpartum lactating women may be in the form of positive influence on bone modeling and remodelling [14]. The primary assumption in this field is that dietary fat (particularly omega fatty acids) may influence bone metabolism by altering prostaglandin (PG) biosynthesis [14]. The PGs, locally produced from 20-carbon essential fatty acid

precursors (20:4 n-6 and 20:5 n-3) in osteogenic cells regulate both bone formation and bone resorption [14]. In fact the effect of PGE_2on bone formation is described as a biphasic, dose-dependent effect; stimulatory at low concentration but inhibitory at high concentrations [136]. PGE_2 at 3 mg/Kg body weight per day increased tibial shaft cortical bone formation rate by enhancing modeling activity in aged male rats [14] but PGE_2 at high concentration depressed osteogenesis in fetal rat calvariae [14].

The two essential fatty acid families (n-3 and n-6) are converted into two distinct families of eicosanoids [136] each with unique properties. PUFA of the n-6 family lead to production of PGE_2 while the n-3 family tends to increase PGE_3 and other prostacyclins. Thus diets rich in n-3 modulate eicosanoids production away from PGE_2 formation [136]. It is established that osteoporosis associated with a decline in estrogen production in postmenopausal women results in increased production of osteoclastogenic cytokines such as IL-1, IL-6 and TNF-α. These cytokines induce the expression of COX-II in osteoblastic and stromal cells, resulting in the production of PGE_2 [15]. PGE_2 along with TNF-α through specific pathways enhance the differentiation of osteoclast progenitors into mature osteoclasts. This process leads to osteoporosis. Since the period of lactation is one of low *in vivo* estrogen production (similar to menopause) due to inhibited ovarian function perhaps adequate n-3 fatty acid intake in this population should be recommended. Especially that research has illustrated inhibition of pro-inflammatory cytokine (IL-6, IL-1 and TNF- α) mRNA expression by n-3 fatty

acids in immune cells as well as kidney tissue from animal models of autoimmune disease [15]. Aside from n-3 fatty acids being able to decrease PGE_2 production, it could perhaps downregulate COX-II activity. Nitric oxide is postulated to play an important role in bone metabolism and it is known that DHA and EPA enhance NO formation. Hence by decreasing the n-6/n-3 ratio in food and increasing DHA intake, bone resorption may be decreased in lactating women therefore decreasing lactation- induced BMD loss.

2.45 Recommendation for Dietary Fat Intake for Lactating Women

Currently the DRI as recommended by the Institute of Medicine stands at 13 g/day of n-6 for lactating women in both the 19-30 and 31-50 age categories. Of the n-3 fatty acids, ALA has an AI of 1.3 g/day in the same age categories of lactating women [19].

In 2005, a consensus recommendation on dietary intakes for pregnant and lactating women was issued on behalf of the European Commission Research projects Perinatal Lipid Metabolism and Early Nutrition Programming [133]. Upon reviewing all the available scientific evidence the committee drew conclusions and recommendations for intake of n-3 and n-6 during lactation. Some of the recommendations include but are not limited to "Pregnant and lactating women should aim to achieve a dietary intake of n-3 LCPUFA that supplies intake of at least 200 mg/d" [133]. In addition, they stated that "women of child bearing age whose dietary intake of LA is adequate do not need additional intake of AA". With regard to intake of fish and risk of dietary

exposure to contaminants such as methylmercury, the committee stated that it is possible to achieve the recommended intake of DHA by ingesting one to two portions of sea fish per week including oily fish (which is rich in n-3 fatty acids) without exceeding the tolerable intake of environmental contaminants [133]. Close attention should be paid to the type of fish chosen for consumption with evasion of undue preference for large predatory fish such as marlin, pike, swordfish and shark since these fish are the highest in methylmercury content [133].

In light of the importance of calcium, vitamin D and n-3 and n-6 fatty acids during lactation, this thesis aims to examine intakes of these nutrients from diet and in combination with supplements amongst Canadian lactating women.

2.5 Summary and Objectives

Lactation is a metabolically stressful period during which mothers have to maintain adequate nutrient intake in order to sustain optimal nutritional status of both themselves and their infants. Studies on nutrient intakes of calcium, vitamin D and n-3 and n-6 fatty acids in Canadian lactating mothers suggests that these nutrients may be insufficiently consumed. This may place both lactating women and their breastfeeding infants at risk for adverse health effects. In view of this, objectives of this thesis include quantifying the number of lactating women in Montreal who are consuming at or above the AI for the above nutrients from diet alone and in combination with supplements. In addition, determine predictors of intakes (such as age, pre-pregnancy weight, weight gain during pregnancy, weight one month postpartum) of the above mentioned nutrients amongst lactating women using three 24-hour recalls. Lastly, determine the mean group intake of the above nutrients without supplements and with supplements. These objectives were compiled as a result of the hypothesis that a significant percentage of lactating women in Montreal do not attain the AI for calcium, vitamin D and n-3 and n-6 fatty acids.

3.0 Manuscript

Nutrient intake of lactating women in Montreal with emphasis on calcium, vitamin D and omega fatty acids

Samira Bou Raad[1], Sina Gallo[1], Catherine Vanstone[1], Celia Rodd[1,2] , Hope Weiler[1]

[1]McGill University, School of Dietetics and Human Nutrition, 21 111 Lakeshore, Ste. Anne de Bellevue Québec, Canada H9X 3V9

[2]Montréal Children's Hospital, 2300 Tupper St.
Montréal Québec, Canada H3H 1P3

3.1 Abstract

Data regarding consumption of calcium, vitamin D and n-3 and n-6 fatty acids by lactating women in Canada are scarce, yet these nutrients are of great significance to maternal health and may be insufficiently consumed. The primary objective of this study was to examine whether calcium, vitamin D and n-3 and n-6 fatty acid consumption in lactating women living in Montreal meet DRI values. A sample of 70 predominantly lactating (>80% of infant feeds from breast milk) women were recruited and assessed using a 24-hour recall, administered at 1(baseline), 3 and 6 months postpartum and analyzed using the Canadian Nutrient File. Supplement use and demographic information were collected. Women who attained the AI for calcium, vitamin D, linoleic acid (LA), alpha-linolenic acid (ALA) and docosahexaenoic acid (DHA) via diet mounted to 52%, 46%, 15%, 31% and 11% respectively. Supplement intake increased the percentage of women achieving the AI for calcium, vitamin D and DHA to 82%, 90% and 13% respectively. However supplements had no impact on LA and ALA intake. There were no significant predictors of reaching the AI for any of the nutrients except for ALA. Body mass index at 1 month postpartum, parity and family income above 75,000 Canadian dollars were predictors of LA intake. These data support that many lactating women in Montreal are not meeting AI for calcium and vitamin D unless supplements are added to their diet. Omega fatty acids are insufficiently consumed by lactating women in Montreal even upon addition of supplements.

3.2 Introduction

Exclusive breastfeeding for six months has become universally recommended by many organizations and scientific bodies [1, 2] including Health Canada. Despite the presence of international and national recommended nutrient intakes specific for lactation [17-20], research on evaluating dietary practices and nutrient adequacy of lactating women is limited. In Canada, data on nutrient intake of lactating women is more limited than other populations despite the fact that breastfeeding initiation is successful in Canada. Eighty five percent of Canadian females initiate breastfeeding with geographic variations ranging from 63% in Newfoundland to 87% in Ontario and 76% in Quebec [23, 24].

While attainment of optimal nutrition is important, some data suggests that calcium, vitamin D and n-3 and n-6 fatty acids are of concern in the diet of lactating women. For example in Western Canada (Calgary), lactating women restricted intake of certain foods such as milk, due to reasons including prevention of infant colic, and perceived maternal lactose intolerance among other reasons. Therefore, these women put themselves at risk for calcium and vitamin D deficiency [6]. Furthermore, Vatanparast [7] illustrated that generally Canadian women of childbearing age (19-45) do not meet the recommendation for calcium and vitamin D even when supplements are added to their diet. This information along with a recent study reflecting low vitamin D intake during pregnancy have created concern over the possibility that Canadian lactating mothers may not be meeting current nutrient recommendations for calcium and vitamin D [21].

Vitamin D intake is of particular importance in the Canadian population due to minimal UVB solar radiation exposure as a result of long winters in addition to higher latitudes [6].

Furthermore, post partum women often feel the pressing need to lose weight accumulated throughout their pregnancy and attempt to do so by decreasing their intake of fat and dairy products at the expense of reduced consumption of calcium and vitamin D, as well as polyunsaturated and monounsaturated fatty acids [6, 8]. On that note a survey of 1544 Canadians using 24-hour dietary recalls reflected intakes of 10.6% and 5.0% energy from monounsaturated and polyunsaturated fat respectively among women 18-34 years [9, 10]. These figures are comparable to a group of 55 Canadian pregnant women studied by Innis et al [10]. In this study 1 in 6 women consumed <67 mg DHA per day during the latter part of gestation, 60% had an intake of <150 mg DHA/d and 16% consumed >300 mg DHA/d. Mean intakes of LA, alpha-linolenic acid ALA, AA, EPA and DHA were 11.2 g, 1.6 g, 121 mg, 78 mg and 160 mg per day, respectively. There is concern that lactating women may be following similar dietary patterns in view of the fact that low DHA intake may result in postpartum depression [13]. Additionally, low maternal DHA intake may have an adverse effect on infant visual and neurodevelopment [114].

Such dietary practices raise the question of whether supplementation during lactation is a necessary solution to compensate for inadequate intake [6, 137]. Although data regarding nutrient adequacy of foods plus supplements in

lactating women is meagre, studies have reflected widespread use of vitamin/mineral supplements during pregnancy and into lactation [3, 6, 137]. Therefore the purpose of this study is to determine whether calcium, vitamin D and n-3 and n-6 fatty acid intake from food alone and then in combination with supplements in lactating women meet the DRI values. To do so, women will be quantified as percentage of sample who reach the AI and percentage of sample who do not reach the AI for the above nutrients from diet alone and with supplements. In addition, specific objectives include identification of determinants of achieving recommendations of these nutrients in view of pregnancy weight gain and other maternal factors.

3.3 Materials and Methods

Recruitment

This research is a part of a larger vitamin D dose response study (clinicaltrials.gov; #NCT00381914), which is a randomized clinical trial of different vitamin D doses supplemented to breast fed infants. Women were recruited from 7 paediatric clinics and one primary care facility from the Western region of the island of Montreal, Quebec (latitude 47.5° N). Approval for the research, including this sub analysis was obtained from the Research Ethics Board of McGill University in Montreal, Quebec. Informed written consent was obtained from all mothers.

Description of Subjects and Measurements

Healthy lactating women were referred to the study by their paediatricians and contacted within 2 weeks of delivery by the research team. Eligibility criteria

included singleton births between 37-43 weeks gestation, between the 5th and 95th percentile for weight and gender using the Centers for Disease Control growth charts [138] and predominantly breastfeeding at time of recruitment. Predominantly breastfeeding is defined as >80% of infant feeds from breast milk. Women with a medical condition that may have affected pregnancy outcome, nutrient absorption, or delivery of nutrients to the infant were excluded. Specific diseases screened for were gestational diabetes, gestational hypertension, Crohn's disease, celiac disease, diabetes, liver disease and kidney disease. Additionally, women on medications which may have affected absorption or utilization of nutrients were also excluded (for example bile acid sequestrants and anticonvulsants). For this analysis, maternal data were collected at 1 (baseline), 3 and 6 months postpartum at the Mary Emily Clinical Nutrition Research Unit, Montreal, Quebec. At baseline, women's height was measured to the nearest 0.1 cm using a stadiometer (model 242, Seca, Hanover, USA). Weight at baseline, pre-pregnancy weight and pregnancy weight gain were self-reported. Pre-pregnancy and baseline body mass index (BMI) were calculated using the standard formula: weight (Kg) / height^2 (meters). Education level, family income, parity and gravida were also recorded at baseline using a sociodemographic questionnaire.

Lactation continuance was assessed via a health assessment questionnaire at each contact point. Women were asked if they were still lactating and if so whether their infants were still predominantly breastfeeding (>80% of infant feeds

from breast milk). Infant total number of feeds per day was collected as well as amount of breast milk or formula per feed using 24-hour test weighing (Tanita BLB-12) and food records. Women were instructed to weigh their infants before each breastfeeding and again post breastfeeding in order to note the volume of breast milk that was ingested (24-hour test weighing).

Dietary Assessment

One 24-hour recall was conducted with a registered dietitian at each visit (1, 3, 6 months postpartum). A four stage, multiple pass interviewing technique was used in prompting for food item, quantity, time of day, cooking method and brand name as well as other details [139]. Three-dimensional models of foods (Spectrum Nasco Nutrition Teaching aids, Newmarket, ON) as well as measuring cups and spoons were used as memory aids and in quantifying amounts of food consumed. Each subject provided a mix of week days and weekend days of dietary intake.

Twenty four recalls were only conducted if the mother was still lactating. If the mother had weaned her infant, no 24-hour recalls were obtained. However, the family was not dropped from the larger vitamin D dose response study. All food and beverage items collected were entered into the program Nutritionist PRO version 4.1.0 (Axxya Systems LLC., Stafford, TX) which contains the Canadian Nutrient File 2007b. Moreover, 4 food items (apple juice fortified with calcium, n-3 eggs, calcium fortified punch, margarine fortified with n-3 fatty acid) were

imported into the program using nutrition information provided by the food manufacturers.

Supplement Use

All vitamins, mineral, omega fatty supplements and any other medications were recorded in a health assessment questionnaire that was completed at each contact point. The women were prompted to report if they had changed their supplement brand since their last visit, the dosage of their supplements and how many times per week they were taking their supplements. The supplements of interest for this study were calcium, vitamin D and n-3 and n-6 fatty acids in single or multi-nutrient formulations. Supplement composition was determined using standard references from the Health Canada Drug Product Database (DPD) [140] and verified from manufacturers' data. Supplement intake per day for calcium, vitamin D and omega fatty acids for each contact point (1, 3 and 6 months postpartum) was calculated as total ingested dosage reported by the women per week, divided by 7 days. The averages of these intakes were calculated in order to obtain the mean supplemental intake across the 3 contact points.

Statistical Analysis

Although 106 women consented to the study, only 70 women were included in the analysis based on lactation until 6 months postpartum. This sample size is adequate for exploring intake of both calcium and vitamin D at a

confidence level of 80% [141]. The desired precision for calcium was ±100 mg/day and the standard deviation as calculated from previous studies was 582 mg/day. The desired precision for vitamin D was ±0.5 µg/day with a standard deviation (calculated from previous studies) of 2.8 µg/day.

Descriptive statistics were used to summarize sample characteristics. In addition to the nutrients of interest, other nutrients that were examined included fat, protein, carbohydrate, fibre, cholesterol, total long chain polyunsaturated fatty acids, iron, and magnesium. This gave us a more comprehensive understanding of the general nutrient intake of these women in addition to the intake of calcium, vitamin D and n-3 and n-6 fatty acids.

Results obtained were tested for normality quantitatively using q-q plots and Shapiro Wilks test, respectively. Postpartum BMI and pre-pregnancy BMI were divided in to 2 categories; women at and above BMI of 25 and women below 25. Chi-square tests compared differences in intake across BMI categories of women above and below the AI for the nutrients of interest. Student's t-tests were conducted to test for differences in total fat intake between the group of women who reached the AI and those who did not for calcium, vitamin D, linoleic acid and linolenic acid as well as test for differences in BMI at 1 month postpartum between women who reached the AI and who did not reach AI for linoleic and linolenic acid. Furthermore, Student's t test and Chi square analysis was used to examine any differences between the group of women who lactated

until 6 months and the women who opted to drop out or wean their infants before 6 months.

Repeated measures analysis of variance using general linear model was conducted in order to assess whether intakes of the above nutrients differed between contact points. It was deemed that if there were no differences in group mean intakes across time, then the intakes were reflective of usual maternal consumption across 6 months of lactation. No differences were found (Table 3); hence the intakes from each visit were collapsed to form one averaged intake across the three study visits. These nutrient intakes are reported as means and standard deviations. Carbohydrate, total fat and protein were also reported in comparison to the accepted macronutrient distribution range as well as values for the respective Recommended Dietary Allowance (RDA). Fat categories such as cholesterol, polyunsaturated fatty acids, and monounsaturated fatty acids amongst others were reported as medians and ranges due to the skewed nature of the data. Iron and magnesium were compared with values from the RDA as well. Vitamin D, calcium and n-3 and n-6 fatty acid intake can only be reported as percent of women attaining 100% of the DRI or more since only AI have been set.

Multiple logistic regressions were performed to assess for any significant predictors of reaching recommended intakes for calcium, vitamin D and n-3 and n-6 fatty acids. Mother's age, postpartum BMI, pregnancy weight gain, pre-pregnancy BMI, parity, mother's education and family income were all entered into the regression model. These variables were chosen because they have been

identified in the literature as possible predictors of intake [142,143]. Odds ratios were calculated and reported. Statistical analyses were performed using Statistical Analysis System (SAS version 9.2, SAS Institute, Cary, NC). A p-value of less than 0.05 was considered statistically significant.

3.4 Results

Subject Characteristics

Of the 106 women who enrolled in the study, 70 women completed the study (Figure 1). Characteristics of the all subjects are shown in Table 4. There was a significant difference in use of prenatal vitamin between women who lactated until 6 month and those who weaned their babies earlier on or dropped out shortly after their baseline or second visit. Additionally, a higher number of black women weaned their babies before 6 months when compared with women of the same race who continued to lactate until 6 months. All women in the study were from two-parent families (married or living with a spouse) and 96% of mothers had completed an education greater than high school (college or university). Ninety-two percent of mothers were of white race and 64% of mothers had a combined family income above 75,000 Canadian dollars per year before taxes. A hundred percent of the women (n=70) were predominantly breastfeeding at baseline with minor decreases to 98% (1 woman) at 3 months and 96% (3 women) at 6 months due to the women with declining breastfeeds. However, these women were still included in the study because their infants were ingesting between 60% and 80% of feeds from breast milk.

Ninety three percent of women continued to take their prenatal multivitamin/mineral supplements throughout the period of lactation (6 months postpartum). Only 10 % of the women took any form of omega fatty acid supplements. The most commonly used fatty acid supplements were omega-3 (8.6% of women) supplements followed by omega 3-6-9 (1.4% of women). The average DHA content of omega-3 supplements, used by the women in this study, is 150 mg while average linoleic, α-linolenic and DHA content of omega 3-6-9 is 350 mg, 400 mg and 150 mg respectively [140].

Dietary Analysis

Based on food intakes alone (Table 5), about half the women met AI for calcium and vitamin D however only a minority of women met the AI for linoleic acid, alpha-linolenic acid and DHA. When comparing the total intake from food and supplements, a higher percentage of women met the AI for calcium and vitamin D respectively. However, for the n-3 and n-6 fatty acids, supplement intake did not increase the percentage of women who attained the AI (Table 5). The most important dietary sources of calcium for this group of women in rank order of providing calcium were milk, cheddar cheese, feta cheese, ice cream and yogourt. Forty three percent of the women attained the recommended number of food guide servings per day of 2 milk and alternatives set by Health Canada. Only 10 % of them were consuming full fat milk (3,25% fat). With regard to vitamin D intake, top five sources in descending order were milk, margarine, salmon, vitamin D fortified juices and eggs. As for the n-3 and n-6 fatty acids, most important sources of linoleic acid were peanut butter, mayonnaise, French fries,

and trail mix. More than 90% of dietary DHA came from salmon and the rest was contributed by omega-3 eggs. Top linolenic acid sources were store bought pancake mixes, margarine, mayonnaise, canola and soy bean oil.

Table 6 shows the dietary analysis of the sample of women using a total of three 24-hour recalls. Mean carbohydrate, protein, and total fat intake were within the acceptable macronutrient distribution range (AMDR). As for the micronutrient analysis, mean magnesium and iron intake were 304 mg and 16 mg respectively. These values are higher than the EAR of 265 mg and 6.5 mg for magnesium and iron respectively. Fat components such as cholesterol and unsaturated fatty acids, amongst others are reported as medians and ranges (Table 6). Of specific interest in these results were the low medians yet high ranges (difference between minimum and maximum value) of these nutrients. In addition, there was a significant difference in fat intake between women who reached AI for calcium (92 g) and those who did not (80 g) ($p=0.0254$). As for vitamin D, difference in fat intake approached significance ($p=0.0881$) with women who reached the AI and did not reach the AI ingesting 92 g and 82 g, respectively. There was a significant difference in total fat intake between women who reached the AI for linoleic acid (114 g) and those who did not (81 g) ($p=0.0006$). However, there was no significant difference in BMI 1 month postpartum between the two groups. On the other hand there was significant difference in total fat intake between women who attained the AI for linolenic acid (98 g) and women who did not (81 g) ($p=0.0033$), as well as a small but significant difference in BMI at 1 month post

partum between the women who achieved the AI for linolenic acid (25 kg/m^2) and those who did not (27 kg/m^2) (p=0.0127).

There were no significant explanatory variables for reaching adequate intake for calcium, vitamin D or the omega fatty acids except for alpha linolenic acid. Each unit increase in post partum BMI was associated with a decrease in the chance of reaching the AI for alpha linolenic acid (OR: 0.75, p= 0.003, 95%CI: 0.62-0.90) when controlling for other variables (Table 10). Additionally, family income below 74,999 compared to equal or above 75,000 Canadian dollars also decreased the chance of reaching AI for linolenic acid (OR: 0.17 p=0.026, 95%CI: 0.03-0.80). Chi-square analyses comparing differences in intake across BMI categories of women above and below the AI for the nutrients of interest did not show any significant results (data not shown).

Correlation coefficients showed that pre-pregnancy BMI and BMI at 1 month postpartum were highly correlated (90% p<0.0001) and similarly there was significant but small correlation between pregnancy weight gain and BMI at 1 month postpartum (28% p=0.017). Both pre-pregnancy BMI and pregnancy weight gain were removed from the regression model in order to avoid collinear variables.

3.5 Discussion

This study, examining whether lactating women consume the DRI values for calcium, vitamin D and n-3 and n-6 omega fatty acids, indicated that percentage of women who reached the DRI levels for these nutrients via diet

alone is less than optimal. Within our sample, 52% and 46% of women attained the AI for calcium and vitamin D, and only 15%, 31% and 11% attained the AI for linoleic acid, alpha-linolenic acid and DHA respectively. Although supplementation did not raise the percentage of women who attained the AI for calcium and vitamin D to 100% of the sample, it significantly increased percentage of women reaching AI for calcium and vitamin D from 52% to 82% and from 46% to 90% respectively. Supplementation did not raise the percentage of women who reached AI for the omega fatty acids. This finding, in addition to being a result of lack of daily intake, was also attributed to the low number of women ingesting supplements as well as the low amounts of linoleic and linolenic acid present in frequently used omega fatty acid supplements (350 mg of linoleic acid, 400 mg of linolenic acid). On the basis of these results we conclude that although the percentage of women who reached the AI for calcium and vitamin D via diet alone was low, supplementation significantly raised this percentage to cover more than 80% of the sample. On the other hand percentage of women who reached the AI for n-3 and n-6 fatty acids was low and remained low even with supplementation. No significant differences were found between women who participated in the study and those who did not except for prenatal vitamin use and race. Perhaps women who weaned their infants or dropped out before the 6 month mark knew that they were not going to lactate and hence opted to discontinue use of prenatal supplements. A higher number of black women weaned their infants before the 6 months mark when compared with women of the same race who continued to lactate. Similar results were reported in the United

States whereby mothers of black children were less likely to initiate and maintain breastfeeding than mothers of white children (51.5% vs 72.1% for ever lactating,19.7% vs 36.6% for continuing at 6 months) [144].

When looking at food sources of calcium and vitamin D, milk is the most important one. This is significant in view of the fact that milk may be considered as a high fat food and studies have shown that some women tend to restrict milk as a means of cutting down on fat intake if dieting, thereby decreasing their intake of calcium and vitamin D [6]. However, 40% of the women in this study attained the recommended milk servings or alternatives and only 10% of these women consumed full fat milk. The rest of the women consumed 1% or 2% fat milk. Hence, with the availability of low fat milk, women may still ingest calcium and vitamin D but may be forgoing other fat dense foods such as cheeses, ice cream and margarine in order to decrease their fat intake, consequently decreasing their intake of calcium and vitamin D. In this sample of women, those who reached the AI for calcium and vitamin D had a higher total fat intake than the women who did not. At this point it is important to mention that 40% attaining recommended milk or alternatives is still low. There may be other reasons that lactating women may restrict milk and dairy products if it is not based on fat content. Mannion et al [6] reported that lactating women restricted milk and dairy intake for reasons such as prevention of infant colic, perceived maternal lactose intolerance and perceived infant allergy to milk.

With respect to intake of linolenic acid, an increasing postpartum BMI and a family income below 74,999 Canadian dollars decreased the chances of attaining AI for linolenic acid. Other studies have documented nutritional inadequacies among population groups characterized by low income [27, 145]. Although income below 74,999 Canadian dollars is not necessarily considered low, it is associated with linolenic acid intake in these women. Reasons as to why linolenic acid in particular was the only nutrient with predictors of intake are unclear. It may be that the distribution of intakes specific to this nutrient made the regression analysis more successful. If comparing to linoleic acid, a higher percentage of women attained the AI for linolenic acid as compared to linoleic acid. Foods that are sources of either or both fatty acids are fat dense, therefore it is no surprise that women who attained the AI for both of these fatty acids had a higher fat intake as compared to those who did not. Nonetheless, the recommendation for linolenic acid is only 1.3 grams which makes it more achievable than linoleic acid. For example, 29 grams of mayonnaise provides 4.7 grams of linoleic acid (36% of recommendation) and 0.6 grams of linolenic acid (46% of recommendation) since key sources of linoleic acid and linolenic acid were soybean and canola. The ratios of linoleic acid to linolenic are 8:1 for soybean oil and 2:1 for canola oil. The fact that a higher number of women had achieved the AI for linolenic acid perhaps made the regression model more sensitive to the predictor variables, particularly to the variable of BMI at one month postpartum.

The issue at hand remains the fact that a certain percentage of lactating women did not attain the AI for calcium, vitamin D and n-3 and n-6 fatty acids (through food and supplementation combined). This raises the question of whether this specific subgroup is at risk of any adverse effects. With respect to calcium, diet does not affect breast milk calcium concentration as reported by two randomized control trials even with intakes as low as 400 mg/day [42, 43]. In fact lactating women meet calcium requirements during lactation through temporary demineralization of the skeleton elicited by hypoestrogenemia, decreased PTHrP and decreased urinary calcium excretion. The resulting physiologic adaptations, mainly bone loss and recovery after weaning have been shown to be relatively independent of dietary calcium intake or calcium supplementation [37, 40, 42, 44, 57, 59, 62, 65]. On the other hand a Tufts evidence-based systematic review suggests that women with calcium intake levels of 1000-1250 mg/d have lower risk of breast cancer and colorectal cancer compared to those with intake levels less than 500 mg/d [79]. The Women's Health Study (WHS) reported an association between low calcium intake and increased risk of hypertension [79]. However, there are no studies looking at calcium intake during a metabolically stressful period such as lactation and future risk of hypertension, colorectal cancer or breast cancer. Hence we cannot assume that the 18% of women who did not attain the AI for calcium are at risk for the above adverse effects. Nevertheless, lactating women are urged to sustain an adequate intake of 1000 mg/day.

Several studies examined women's dietary vitamin D intakes and 25(OH)D status. In Weiler et al [146] women who were classified as vitamin D deficient (< 37.5 nmol/L) had average plasma 25(OH)D of 28.6 nmol/L and a mean intake of 149 IU/day (3.7 µg/day). On the other hand women who were of adequate status had an average plasma 25(OH)D of 61.6 nmol/L and a mean intake of 242 IU/day (6 µg/day). In another study by Sowers et al [147], women's plasma 25(OH)D ranged between 22.0 and 30.0 ng/ml (55 nmol/L and 75nmol/L) with mean vitamin D intakes varying between 119 IU/day (2.9 µg/day) and 316 IU/day (7.9 µg/day). The women in this study were also exposed to UVB. In our study, mean vitamin D intake was 5.2 µg/day and 13.5 µg/day from food alone and food plus supplements respectively. Moreover, upon addition of vitamin D supplements 90% of women attained the AI implying that risk of vitamin D deficiency may be low in this group of women. This is not to say that the remaining 10% of women could be vitamin D deficient. However, the concern is if they are vitamin D deficient due to inadequate intake and insufficient UVB exposure, their breast fed infants (if not supplemented) may develop nutritional rickets, a phenomenon which was once thought to have been eradicated. Lebrun et al [29] investigated the vitamin D status of 80 mother-child pairs in a Manitoba community with a high incidence of rickets. The authors observed that 70% of the mothers said that they drank no milk while another 24% declared that they are milk intolerant. Moreover, 24% of the mothers did not take any supplements and those that did, admitted to taking them only occasionally. There is indication of serious outcomes due to chronic vitamin D deprivation such as increased risk of

multiple sclerosis [95, 102] and rheumatoid arthritis [101], impaired insulin secretion [99, 100] and decreased utilization of dietary calcium which in turn can lead to enhanced bone resorption resulting in osteopenia and osteoporosis [95, 103]. There is no data to suggest that vitamin D deficient lactating mothers are at particular risk for these outcomes.

Of the n-3 and n-6 fatty acids discussed in this paper, DHA is by far the most important during lactation due its effect on both mother and infant. In infants DHA is important in the central nervous system and visual acuity [128]. Studies [115, 118, 132] that have been conducted on whether visual and neural development among breast fed infants is likely to be related to the variability in human milk DHA have mixed results. Hence we are not sure whether the inadequate intake of DHA in this group of mothers has any effect on the neural and visual development of their breastfeeding infants. Additionally, Hibbeln [13] claims that lower concentrations of plasma DHA (through inadequate consumption) and lower national rates of sea food consumption are significantly associated with higher rates of maternal postpartum depressive symptoms. Moreover, emerging indications from human and animal research support the hypothesis that dietary lipids influence bone modeling and remodelling [14] which may be important in lactating women at a time when they are experiencing heightened bone resorption. In light of these facts, there is concern over the low percentage of women in this sample that had achieved linoleic, linolenic and DHA recommendations.

Since this study was based on a total of three 24-hour recalls, usual intake of calcium, vitamin D and n-3 and n-6 fatty acids of lactating women spanning 6 months of lactation may be slightly underestimated. More specifically the intakes of n-3 and n-6 fatty acids, since these nutrients would have been better assessed using a FFQ. This is because 24-hour recalls may have missed the days on which seafood was consumed. This nutrient underestimation alongside the small sample size may have affected the outcome of our regression analysis. Nevertheless, this sample of women, albeit small, gave a clearer picture about some of the eating habits of lactating women in Montreal with respect to calcium, vitamin D and n-3 and n-6 fatty acids. These trends however, cannot be generalised to the rest of the Canadian women due to the narrow margin of diversity within the sample.

Another issue is that information is lacking on these women's dietary knowledge and whether they had received any education on diet during lactation and simply taking part in the study may have unintentionally affected eating habits. Furthermore, data on maternal serum vitamin D and DHA status was not available. Both vitamin D and DHA can be endogenously produced and hence can be influenced by factors other than diet.

In summary, the percentage of lactating women who attained the AI for calcium and vitamin D through diet alone proved less than optimal. However, supplement use significantly increased the percentage of women who attained the AI for calcium and vitamin D implying that supplement use should be endorsed when adequacy from diet is not achieved. The percentage of women who attained

the AI for omega fatty acids was very low and supplementation did not have an impact on the percentage of women who had attained the AI for n-3 and n-6 fatty acids. These results in addition to the reported mean group intakes of macronutrients suggest that apart from n-3 and n-6 fatty acid intake, this sample of women seem to have an adequate diet. Nevertheless, large random population studies regarding nutrition of lactating women are now justified towards sustaining and improving maternal health. Larger studies will reflect intakes of nutrients amongst lactating women in different regions (not only one part of Montreal) thereby allowing the scientific community to gain a more insightful understanding of what impacts maternal health with regard to nutrition.

3.6 Tables

Table 3: Difference between mean dietary intakes of lactating women among 3 contact points (n= 70)

	Baseline	**3 months postpartum**	**6 months postpartum**	**P-value[a]**
Calcium (mg)	1180	1067	1079	0.239
Vitamin D (µg)	5.5	5.6	4.6	0.255
Linoleic acid (g)	9.6	8.9	9.6	0.8046
Alpha linolenic acid (g)	1.2	1.3	1.2	0.9206
DHA (mg)	72.6	146.1	80.1	0.4543

[a]Repeated Measures ANOVA between continuous variables of dietary intake as assessed by one 24-hour recall at each contact point

Table 4: Maternal characteristics of participating and non-participating women

	Women participating in study (n=70)	**Non-participating women**[e] (n=36)	**P-value**[a]
Presented as Mean ± standard deviation			
Age (years)	32.9±4.1	32.6±3.0	0.6118
Weight 1 month post partum (kg)	73.2±13.4	73.6±15.4	0.8936
BMI 1 month post partum (kg/m^2)	26.7±4.6	27.8±6.4	0.3746
Parity (#)	1.8±0.8	2.0±0.9	0.3764
Gravida (#)	1.8±0.8	2.0±0.9	0.4635
Pre-pregnancy weight (kg)[c]	65.1±11.5	66.5±14.2	0.6079
Pre-pregnancy BMI (kg/m^2)	23.8±4.1	25.2±6.1	0.2332
Pregnancy weight gain (kg)[c]	15.9±5.1	15.6±6.4	0.8378
Presented as n(%)			
Using prenatal vitamins	69(98.6)	32(88.9)	0.0444[b]
Non smoking	65(92.9)	33(94.3)	1.0000
Mother's education > high school	67(95.7)	32(88.9)	0.2248
Income > 75,000	42(63.6)	22(62.9)	0.9383
White race Other[d]	57(91.9) 5(8.1)	21(72.4) 8(27.6)	0.0222[b]

[a]Student-test between continuous variables and X^2 between categorical variables
[b]Fisher's exact test due to small sample size between groups
[c]Self reported measurements
[d]Includes Black, Asian, Indian, and Hispanic
[e]Enrolled in study but dropped out

Table 5: Relative distribution for lactating women meeting dietary standards for selected nutrients from food and food plus supplements (n=70)

Nutrient	Food only	Food and supplement	*AI/UL**
Calcium (mg/day)			1000/2500
Mean±SD[a]	1111±405	1358±477	
intake > 100% AI(%)	52	82	
intake ≥ UL(%)	1.4	2.8	
			AI/UL
Vitamin D (µg/day)			5/50
Mean±SD[a]	5.2±3.8	13.4±7.4	
intake > 100% AI (%)	46	90	
intake ≥ UL(%)	0	0	
			AI
Linoleic acid (n-6) (g/day)			13
Median, min-max[a]	8.6, 2.5-24	8.6, 2.5-24	
intake > 100% AI(%)	15	15	
			AI
a-linolenic (n-3) (g/day)			1.3
Median, min-max[a]	1.0, 0.3-4.1	1.1, 0.3-4.1	
intake > 100% AI(%)	31	31	
			ISSFAL
DHA (mg/day)			300
Median, min-max[a]	27, 0-1355	30, 0-1355	
intake ≥ recommendation(%)	11	13	

*Adequate intake as stated by Institute of Medicine

**Upper tolerable intake level as stated by Institute of Medicine

[a]Means and Medians calculated using a total of three 24-hour recalls

Table 6: Dietary mean energy and macronutrient intakes and fat intakes of lactating women using three 24-hour recalls (n=70)

Macronutrient	**Mean±SD (%AMDR)***	**AMDR**
Energy (kcal)	2364±488	-
Protein (g)	97±24 (16)	10-35
Carbohydrate (g)	302±72 (52)	45-65
Dietary fibre (g)	23±7	-
Fat (g)	86±24 (33)	20-35
Fat Categories	**Median, min-max**	
Cholesterol (mg)	269, 4.1-934	
Saturated fatty acid (g)	30, 11-60	
Polyunsaturated fat (g)	11, 3.4-30	
Oleic fatty acid (g)	22, 5.6-52	
*Energy from macronutrient as a percentage of total energy intake		

Table 7: Odds Ratio for reaching AI for calcium (n=36)

Variable	**Odds Ratio**	**p**	**95% CI**
BMI 1 month postpartum (kg/m^2)	0.93	0.702	0.62-1.37
Parity(#)	1.18	0.657	0.57-2.47
Mother age (years)	0.97	0.638	0.82-1.12
Mother education ≤ high school vs. education > high school	0.31	0.421	0.68-1.31
Family income ≤ 74,999 vs. income > 75,000	1.14	0.809	0.38-1.44

Table 8: Odds Ratio for reaching AI for vitamin D (n=32)

Variable	**Odds Ratio**	**p**	**95% CI**
BMI 1 month postpartum (kg/m^2)	0.97	0.867	0.65-1.43
Parity(#)	1.51	0.270	0.72-3.16
Mother age (years)	1.00	0.970	0.87-1.16
Mother education ≤ high school vs. education > high school	0.40	0.522	0..3-5.63
Family income ≤ 74,999 vs. income > 75,000	1.28	0.661	0.43-3.80

Table 9: Odds Ratio for reaching AI for linoleic acid (n=11)

Variable	**Odds Ratio**	**p**	**95% CI**
BMI 1 month postpartum (kg/m^2)	1.03	0.921	0.54-1.57
Parity(#)	3.00	0.098	0.81-11.07
Mother age (years)	0.67	0.069	0.45-1.11
Mother education ≤ high school vs. education > high school	0.05	0.094	0.001-1.64
Family income ≤ 74,999 vs. income > 75,000	0.05	0.069	0.003-1.02

Table 10: Odds Ratio for reaching AI for alpha-linolenic acid (n=22)

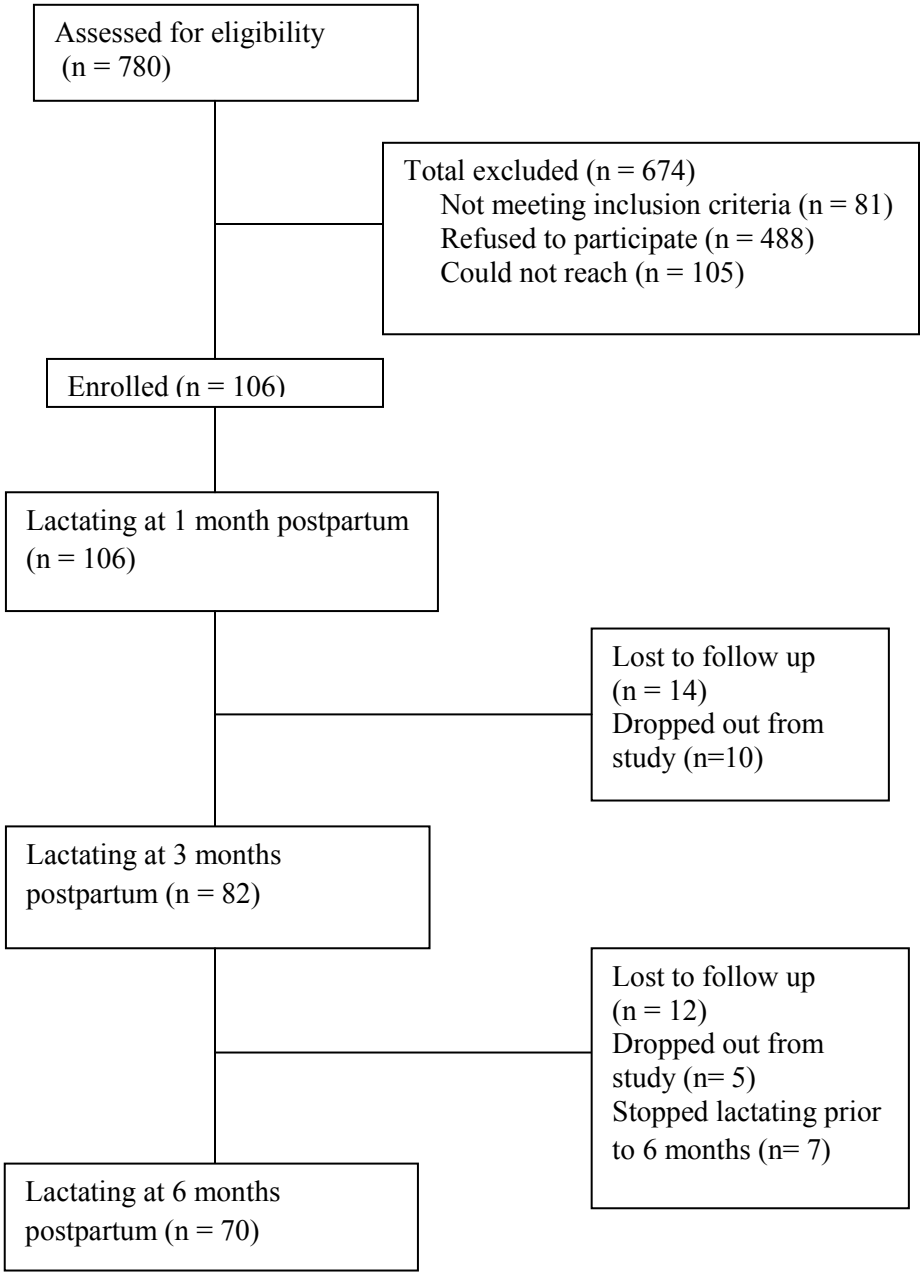
Variable	**Odds Ratio**	**p**	**95% CI**
BMI 1 month postpartum (kg/m^2)	0.75	0.003	0.62-0.90
Parity(#)	3.13	0.058	1.00-9.73
Mother age (years)	0.90	0.310	0.75-1.09
Mother education ≤ high school vs. education > high school	0.200	0.257	0.01-3.22
Family income ≤ 74,999 vs. income > 75,000	0.17	0.026	0.03-0.80

Table 11: Odds Ratio for reaching AI for DHA (n=8)

Variable	**Odds Ratio**	**p**	**95% CI**
BMI 1 month postpartum (kg/m^2)	1.68	0.237	0.71-4.00
Parity(#)	3.45	0.130	0.69-17.0
Mother age (years)	1.01	0.910	0.78-1.31
Mother education ≤ high school vs. education > high school	0.02	0.056	0.00-1.03
Family income ≤ 74,999 vs. income > 75,000	0.27	0.303	0.02-3.28

3.7 Figures

Figure 1: Diagram of lactating women enrolled in study



4.0 Extended Discussion

4.1 Summary of Findings

The results of this study show that only half the women in this sample attained the AI for calcium and vitamin D and a small percentage of women attained the AI for n-3 and n-6 fatty acids from diet. Supplementation significantly increased the percentage of women who achieved the AI for calcium and vitamin D but had no impact on the percentage of women who attained the AI for omega fatty acids. An assessment of inadequacy however, cannot be made due to the fact that AI values are limiting in comparison to more exact requirements such as the EAR or RDA [148].

Supplement use in this group of women is very common (98.6%) and is comparable to other populations [3, 6]. However, supplement use did not increase the percentage of women attaining the AI for calcium, vitamin D and n-3 and n-6 fatty acids as much as desired (100%). Ideally, lactating women should be achieving their dietary requirements from food however supplementation is endorsed when adequacy from diet is not achieved.

There were no predictors of intake for any of the nutrients except for linolenic acid. Perhaps the variables chosen as predictors of intake do not apply to this specific population of lactating women. Guendelman and Abrams [143] described age, BMI, education and parity as predictors of low dietary intake amongst white non-Hispanic women in the USA. The concept of BMI was thus adopted and expanded to include postpartum BMI and prepregnancy BMI.

Moreover, the Canadian Community Health Survey [142] examined family income when assessing distributions of obesity within the population (men and women). Although these studies do not pertain to lactating women per se, they do allow for an understanding of what factors may influence women's dietary habits. There are no studies that look at predictors of dietary intake specifically in lactating women.

4.2 Limitations

Data are lacking on whether this group of women had received any form of nutrition education or alternative advice, prior to joining the study, regarding certain aspects of their diet. For example, in Mannion's work it was found that dairy product restriction was supported and deemed appropriate by physicians and other health care providers as a means to decrease infant colic, alleviate perceived maternal lactose intolerance, and improve baby's behaviour [6]. The presence of such data could shed some light on some of the dietary choices women make while lactating. Most importantly it could point towards the source for these decisions. Lactating women should be advised where to obtain correct nutrition information. Again, in Mannion's study lactating women were receiving dietary advice from internet sites, magazines and homeopaths [6]. On the other hand, health care professionals should inquire about the reasons women have for any sort of dietary restrictions and dismiss "commonly held myths" [6].

While the 24-hour recall is a good method to capture dietary intake in populations, the present study may have benefited from an increase in the number

of administered 24-hour recalls in order to better relay dietary intake of calcium, vitamin D and n-3 and n-6 fatty acids. There are no data describing the number of days needed to capture calcium and vitamin D intake of lactating women. Therefore, our next best alternative is to use the equation described by Willett [149] in order to estimate the number of days needed to assess calcium and vitamin D intake. Using the equation $n= (Z_\alpha CV_w/D_0)^2$ where n is number of 24-hour recall days needed per person, Z_α is normal deviate for the per cent of times the measured value should be within a specified limit, CV_w is within person coefficient of variation obtained from analysis of repeated days of dietary intake provided by Willett and D_0 is the specified limit (as a percentage of long term true intake) [149], the number of days needed to capture intakes of calcium and vitamin D can be calculated.

Table 12: Number of days needed to estimate intake of calcium and vitamin D

Nutrient	Z_α: 1.96 (95%) D_0: 20%	Z_α: 1.96 D_o: 40%	Z_α :1.645 (90%) D_0: 40%
Calcium	17	4	3
Vitamin D*	106	26	19

*There is no available CV for vitamin D, hence the CV for vitamin A was used since it is also a fat soluble vitamin and found sparsely in food

Hence three 24-hour recalls as administered in this study is sufficient to estimate a woman's calcium intake to within 40% of her mean intake 90% of the time. As for vitamin D the values in the above table may be exaggerated due to the use of the coefficient of variation pertaining to vitamin A. Vitamin D is more readily available in food than vitamin A hence less days are probably needed to estimate intakes. Nevertheless, administering a large number of 24-hour recalls is "beyond practical possibilities in epidemiological studies" [149]. Omega-3 and omega-6

fatty acids particularly DHA are abundant in the infrequently consumed seafood. Future studies looking at lactating women's intakes of n-3 and n-6 fatty acid may achieve better results if a food frequency questionnaire (FFQ) is used as compared to 24-hour recall. Moreover, dietary values for certain nutrients like vitamin D and n-3 and n-6 fatty acids are not up to date in nutrition databases like the Canadian Nutrient File. Thus, these results may have underestimated the actual intakes.

The sample size and sample characteristics both constitute limitations. The sample size may be too small to detect predictors of intake as well as capture true intake of n-3 and n-6 fatty acids especially in view of few women achieving the AI. The equation $N= 4z^2_{\alpha}s^2/ w^2$ was used to calculate the sample size using an averaged standard deviation for calcium and vitamin D obtained from previous studies. In order to achieve a 90% CI for dietary vitamin D and calcium measurements at a precision of ±0.5 μg/day and ±100 mg/day respectively, a sample size of 100 would have been necessary. However, our sample size constituted 70 women which is a CI of 80%. Using the same equation and data obtained from this study, it appears that a twofold increase in participants is necessary to obtain a CI of 90% when accounting for both vitamin D and calcium.

Table 13: Sample size calculation to measure vitamin D and calcium at different confidence intervals

Nutrient	CI: 99%	CI: 95%	CI: 90%	
Calcium	109	63	44	W= 200 S=405 mg/day
Vitamin D	384	221	156	W=1.0 S=3.8 µg/day
W:desired total width of the confidence interval (precision)				S:Standard deviation

Sample characteristics unfortunately are not representative of the Canadian population due to very limited diversity within the sample. Attaining a larger more diverse sample proved quite difficult for two main reasons. Firstly, only women who had lactated for 6 months and completed three 24-hour recalls were included in the study. Secondly, recruitment for the vitamin D dose response study occurred in one area of the island of Montreal rendering the sample demographically homogenous.

4.3 Future Directions

The global aim of this study is to better understand the dietary intakes of lactating women in Canada, as it pertains to calcium, vitamin D and n-3 and n-6 fatty acids. In looking at the results from this study, lactating women do not appear to be at risk of inadequate intake of calcium and vitamin D so long as they are consuming supplements. However, there are two areas of concern. Firstly, although many women continue to take their prenatal supplements during lactation, others may opt to discontinue taking them. At this point if these women are not reaching the AI for calcium and vitamin D through their diet they may become at risk of adverse effects. Secondly, the percentage of women who

reached the AI for n-3 and n-6 fatty acids from diet is and remained low even with addition of supplements. Recurrent studies looking at dietary and supplemental intake of the mentioned nutrients will help inform the scientific community on the predictors and causes of inadequate diets particularly of n-3 and n-6 fatty acids. This in turn will enable health professionals to take subsequent action and pay more attention to this population's dietary needs.

The limitations previously mentioned, if addressed and corrected for in future studies, can pave the way for more rounded and informative research. Principally, a bigger more diverse sample of women can be examined. Research on dietary intakes of lactating women across Canada should be conducted paying specific attention to different family incomes and education levels. This comes in light of the fact that if a "best case scenario" group of women such as the ones in this study are not attaining the AI for certain nutrients then there may be women who are worse off with regard to their intake simply because they do not have the financial means or the knowledge on healthy diets. This being said, "best case scenario" has to be interpreted with care. High income and high education does not automatically imply a healthy and adequate diet. However, it does mean that women may have the means of purchasing healthy foods and attaining recommendations. Data from the Canadian Community Health Survey [142] reported that while women who attained post secondary education had the lowest levels of obesity, women from upper-middle class families had a high level of obesity. Nevertheless, examining lactating women's dietary intake would warrant

random sampling across Canada using census data or Statistics Canada as guidelines. The challenge lies in obtaining a sample that covers all socioeconomic and demographic characteristics.

Moreover, women's perceptions of their diets, nutrition education if any, dietary beliefs and sources of dietary information should all be inspected. Furthermore, future dietary research should also involve the breastfeeding mother's serum vitamin D and DHA status. This should be done for two reasons; firstly because vitamin D can be endogenously produced and low intake by the mother does not mean inadequate status and secondly, to evaluate the method of assessment of intake. For example, DHA concentrations in red blood cells can be used to evaluate the accuracy of assessment methods (24-hour recalls, FFQ) in measuring dietary DHA. DHA is also endogenously produced however in low quantities. Hence, a low RBC DHA would still reflect low DHA consumption. This method was previously used in validating an FFQ for evaluation of EPA and DHA intake [150].

As mentioned previously, the 24-hour recall may not be the best method to capture n-3 and n-6 fatty acid intake because the days where fish may have been consumed can be overlooked. A more comprehensive assessment method such as a FFQ may be more precise as compared to 24-hour recall. Additionally this method would allow in examination of whether lactating mothers are achieving the recommended servings of seafood per week (150 g of fish per week). Furthermore, a more precise estimate of calcium and vitamin D intake would warrant increasing the number of days for 24-hour recalls as shown in Table 12.

Future studies should not only examine usual intakes of calcium and vitamin D of lactating women but should also assess whether the quality of women's diets change over the period of lactation. This could be achieved by administering repeat 24-hour recalls at every contact point. Using our study design, an example would be to collect dietary information via 2 or more 24-hour recalls at baseline and then at 3 and 6 months postpartum. This would allow for more accurate dietary estimates as well as an opportunity to monitor dietary changes.

5.0 Conclusion

There is little information as to the adequacy of lactating women's diet with respect to calcium, vitamin D and n-3 and n-6 fatty acids. The results of this study indicated that almost half of the women included in this research appear to be at elevated risk for lower than recommended dietary intakes of calcium, vitamin D and n-3 and n-6 fatty acids. However, supplementation with calcium and vitamin D significantly increased the percentage of women who reached the AI for these nutrients implying that supplementation should be endorsed in this population. On the other hand supplementation did not affect percentage of women who reached AI for n-3 and n-6 fatty acids. The majority of the women in this study were well-educated and were all predominantly breastfeeding. Future studies should aim at exploring why women are not attaining recommendations for these nutrients through diet. Additionally, future studies should address the problem of inadequate intake of n-3 and n-6 fatty acids in lactating women.

6.0 References

1. Canadian Paediatric Society home page, 2009. *Pregnancy and Babies; Breastfeeding*. Retrieved July 27, 2010, from www.caringforkids.cps.ca/pregnancybabies/breastfeeding.htm
2. World Health Organization home page, 2010. *Exclusive Breastfeeding*. Retrieved July 27, 2010 from www.who.int/nutrition/topics/exclusive_breastfeeding/en/
3. Mackey, A.D., et al., *Self-selected diets of lactating women often fail to meet dietary recommendations.* Journal of the American Dietetic Association, 1998. **98**(3): p. 297-302.
4. Caire-Juvera, G., et al., *Food components and dietary patterns of two different groups of Mexican lactating women.* Journal of the American College of Nutrition, 2007. **26**(2): p. 156-62.
5. Giammarioli, S., *Nutrient intake of Italian women during lactation.* International Journal for Vitamin and Nutrition Research, 2002. **72**(5): p. 329-35.
6. Mannion, C.A., et al., *Lactating women restricting milk are low on select nutrients.* Journal of the American College of Nutrition, 2007. **26**(2): p. 149-155.

7. Vatanparast, H., *Many adult Canadians are not meeting current calcium recommendations from food and supplement intake.* Applied Physiology, Nutrition, and Metabolism, 2009. **34**(2): p. 191.

8. Todd, J.M. and W.R. Parnell, *Nutrient intakes of women who are breastfeeding.* European Journal of Clinical Nutrition, 1994. **48**(8): p. 567.

9. Gray-Donald, K., L. Jacobs-Starkey, and L. Johnson-Down, *Food habits of Canadians: Reduction in fat intake over a generation.* Canadian Journal of Public Health-Revue Canadienne De Sante Publique, 2000. **91**(5): p. 381-385.

10. Innis, S.M. and S.L. Elias, *Intakes of essential n-6 and n-3 polyunsaturated fatty acids among pregnant Canadian women.* American Journal of Clinical Nutrition, 2003. **77**(2): p. 473-478.

11. Weiler, H., et al., *Maternal and cord blood long-chain polyunsaturated fatty acids are predictive of bone mass at birth in healthy term-born infants.* Pediatric Research, 2005. **58**(6): p. 1254-1258.

12. Koletzko, B., et al., *The roles of long-chain polyunsaturated fatty acids in pregnancy, lactation and infancy: review of current knowledge and consensus recommendations.* Journal of Perinatal Medicine, 2008. **36**(1): p. 5-14.

13. Hibbeln, J.R., *Seafood consumption, the DHA content of mothers' milk and prevalence rates of postpartum depression: a cross-national, ecological analysis.* Journal of Affective Disorders, 2002. **69**(1-3): p. 15-29.

14. Watkins, B.A., et al., *Omega-3 polyunsaturated fatty acids and skeletal health.* Experimental Biology & Medicine, 2001. **226**(6): p. 485-97.

15. Sun, D., et al., *Dietary n-3 fatty acids decrease osteoclastogenesis and loss of bone mass in ovariectomized mice.* Journal of Bone & Mineral Research, 2003. **18**(7): p. 1206-16.

16. National Health and Medical Research Council home page. 2006. *Nutrient Reference Values for Australia and New Zealand including Recommended Dietary Intakes*. Retrieved June 10, 2009, from http:// www.nhmrc.gov.au/publications/synopses/_files/n35.pdf

17. Committee on Medical Aspects of Food Policy home page. 2002. *Scientific Advisory Committee on Nutrition; paper for information: key dietary recommendations*. Retrieved June 10, 2009, from http://www.sacn.gov.uk/pdfs/sacn_02_26.pdf

18. AFSSA, Agence Francaise do securite sanitaire des aliments home page. Retrieved June 10, 2009, from http://www.afssa.fr/index.htm

19. Institute of Medicine home page. 2010. *Dietary reference values for vitamin D, calcium and fatty acids*. Retrieved June 10, 2009, from http://iom.edu/Activities/Nutrition/SummaryDRIs/~/media/Files/Activity %20Files/Nutrition/DRIs/DRISummaryListing3.ashx

20. Health Canada home page. 2004. *Food and Nutrition; Dietary Reference Intakes*. Retrieved June 10, 2009, from http://www.hc-sc.gc.ca/fn-an/nutrition/reference/index-eng.php

21. Ward, L.M. and L.M. Ward, *Vitamin D deficiency in the 21st century: a persistent problem among Canadian infants and mothers.* Canadian Medical Association Journal, 2005. **172**(6): p. 769-70.

22. Schofield, C., E. Wheeler, and J. Stewart, *The diets of pregnant and post-pregnant women in different social groups in London and Edinburgh: energy, protein, fat and fibre.* British Journal of Nutrition, 1987. **58**(3): p. 369-81.

23. Dennis, C.L., *Breastfeeding peer support: Maternal and volunteer perceptions from a randomized controlled trial.* Birth-Issues in Perinatal Care, 2002. **29**(3): p. 169-176.

24. Millar, W.J., *Breastfeeding practices.* Rapports sur la Santé, 2005. **16**(2): p. 23.

25. Sanchez, C.L., et al., *Calcium intake nutritional status in breastfeeding women.* Archivos Latinoamericanos de Nutricion, 2008. **58**(4): p. 371-6.

26. Berti, P.R., R. Soueida, and H.V. Kuhnlein, *Dietary Assessment of Indigenous Canadian Arctic Women with a focus on Pregnancy and Lactation.* International Journal of Circumpolar Health, 2008. **67**(4): p. 349-362.

27. Doran, L. and S. Evers, *Energy and nutrient inadequacies in the diets of low-income women who breast-feed.* Journal of the American Dietetic Association, 1997. **97**(11): p. 1283-1287.

28. Black, A.E., S.J. Wiles, and A.A. Paul, *The nutrient intakes of pregnant and lactating mothers of good socio-economic status in Cambridge, UK:*

some implications for recommended daily allowances of minor nutrients. British Journal of Nutrition, 1986. **56**(1): p. 59-72.

29. Lebrun, J.B., et al.,*Vitamin-D deficiency in a manitoba community.* Canadian Journal of Public Health-Revue Canadienne De Sante Publique, 1993. **84**(6): p. 394-396.

30. Torres, A.G. and N.M.F. Trugo, *Evidence of inadequate docosahexaenoic acid status in Brazilian pregnant and lactating women.* Revista De Saude Publica, 2009. **43**(2): p. 359-368.

31. Silva, M.H.L., et al., *Fatty acid composition of mature breast milk in Brazilian women.* Food Chemistry, 2005. **93**(2): p. 297-303.

32. Jensen, C.L., et al., *Effects of maternal docosahexaenoic acid intake on visual function and neurodevelopment in breastfed term infants.* American Journal of Clinical Nutrition, 2005. **82**(1): p. 125-132.

33. Jorgensen, M.H., et al., *Is there a relation between docosahexaenoic acid concentration in mothers' milk and visual development in term infants?* Journal of Pediatric Gastroenterology and Nutrition, 2001. **32**(3): p. 293-296.

34. Otto, S.J., et al., *Comparison of the peripartum and postpartum phospholipid polyunsaturated fatty acid profiles of lactating and nonlactating women.* American Journal of Clinical Nutrition, 2001. **73**(6): p. 1074-1079.

35. Denomme, J., K.D. Stark, and B.J. Holub, *Directly quantitated dietary (n-3) fatty acid intakes of pregnant Canadian women are lower than current dietary recommendations.* Journal of Nutrition, 2005. **135**(2): p. 206-211.

36. Kalkwarf, H.J., *Calcium in pregnancy and lactation*.

37. Prentice, A., *Calcium in pregnancy and lactation.* Annual Review of Nutrition, 2000. **20**: p. 249-272.

38. Kovacs, C.S. and G.E. Fuleihan, *Calcium and bone disorders during pregnancy and lactation.* Endocrinology and Metabolism Clinics of North America, 2006. **35**(1): p. 21-+.

39. Wysolmerski, J.J., *The evolutionary origins of maternal calcium and bone metabolism during lactation.* Journal of Mammary Gland Biology and Neoplasia, 2002. **7**(3): p. 267-276.

40. Weaver, C. and R.P. Heaney., *Calcium in human health*. 2006, Totowa, N.J.: Humana Press.

41. Gupta, A., et al., *Mineral content of breast milk from North Indian mothers giving birth preterm and at term. Implication for mineral nutrition of preterm infants.* Journal of Tropical Pediatrics, 1984. 30(5): p. 286-288.

42. Kalkwarf, H.J., et al., *The effect of calcium supplementation on bone density during lactation and after weaning.* New England Journal of Medicine, 1997. **337**(8): p. 523-528.

43. Prentice, A., et al., *Calcium requirements of lactating Gambian mothers - effects of a calcium supplement on breast-milk calcium-concentration,*

maternal bone-mineral content, and urinary calcium excretion. American Journal of Clinical Nutrition, 1995. **62**(1): p. 58-67.

44. Kalkwarf, H.J., B.L. Specker, and M. Ho, *Effects of calcium supplementation on calcium homeostasis and bone turnover in lactating women.* Journal of Clinical Endocrinology and Metabolism, 1999. **84**(2): p. 464-470.

45. Krebs, N.F., et al., *Bone mineral density changes during lactation: Maternal, dietary, and biochemical correlates.* American Journal of Clinical Nutrition, 1997. **65**(6): p. 1738-1746.

46. Fairweather-Tait, S., et al., *Effect of calcium supplements and stage of lactation on the calcium absorption efficiency of lactating women accustomed to low calcium intakes.* The American Journal of Clinical Nutrition, 1995. **62**(6): p. 1188.

47. Moser-Veillon, P.B., et al., *Calcium fractional absorption and metabolism assessed using stable isotopes differ between postpartum and never pregnant women.* Journal of Nutrition, 2001. **131**(9): p. 2295-2299.

48. VanHouten, J.N., et al., *Mammary-specific deletion of parathyroid hormone-related protein preserves bone mass during lactation.* Journal of Clinical Investigation, 2003. **112**(9): p. 1429-1436.

49. Sowers, M.F., et al., *Elevated parathyroid hormone-related peptide associated with lactation and bone density loss.* JAMA Journal of the American Medical Association, 1996. **276**(7): p. 549-54.

50. Ardawi, M.S., H.A. Nasrat, and B.A.A. HS, *Calcium-regulating hormones and parathyroid hormone-related peptide in normal human pregnancy and postpartum: a longitudinal study.* European Journal of Endocrinology / European Federation of Endocrine Societies, 1997. **137**(4): p. 402-9.

51. Kaur, M., et al., *Longitudinal changes in bone mineral density during normal pregnancy.* Bone, 2003. **32**(4): p. 449-54.

52. Aynaci, O., et al., *Bilateral non-traumatic acetabular and femoral neck fractures due to pregnancy-associated osteoporosis.* Archives of Orthopaedic & Trauma Surgery, 2008. **128**(3): p. 313-6.

53. O'Sullivan, S., et al., *Bisphosphonates in pregnancy and lactation-associated osteoporosis.* Osteoporosis International, 2006. **17**(7): p. 1008-1012.

54. Sweeney, A.T., M. Blake, and M.F. Holick, *Transient osteoporosis of hip in pregnancy.* Journal of Clinical Densitometry : the Official Journal of the International Society for Clinical Densitometry, 2000. **3**(3): p. 291-7.

55. Xyda, A., et al., *Postpartum bilateral transient osteoporosis of the hip: MR imaging findings in three cases.* Radiologia Medica, 2008. **113**(5): p. 689-94.

56. Vanhouten, J.N. and J.J. Wysolmerski, *Low estrogen and high parathyroid hormone-related peptide levels contribute to accelerated bone resorption and bone loss in lactating mice.* Endocrinology, 2003. **144**(12): p. 5521-5529.

57. Affinito, P., et al., *Changes in bone mineral density and calcium metabolism in breastfeeding women: A one year follow-up study.* Journal of Clinical Endocrinology and Metabolism, 1996. **81**(6): p. 2314-2318.

58. Ritchie, L.D., et al., *A longitudinal study of calcium homeostasis during human pregnancy and lactation and after resumption of menses.* American Journal of Clinical Nutrition, 1998. **67**(4): p. 693-701.

59. Specker, B.L., et al., *Calcium kinetics in lactating women with low and high-calcium intakes.* American Journal of Clinical Nutrition, 1994. **59**(3): p. 593-599.

60. Cross, N.A., *Changes in bone mineral density and markers of bone remodeling during lactation and postweaning in women consuming high amounts of calcium.* Journal of Bone and Mineral Research, 1995. **10**(9): p. 1312.

61. Kalkwarf, H.J. and B.L. Specker, *Bone-mineral loss during lactation and recovery after weaning.* Obstetrics and Gynecology, 1995. **86**(1): p. 26-32.

62. Kolthoff, N., et al., *Bone mineral changes during pregnancy and lactation: a longitudinal cohort study.* Clinical Science, 1998. **94**(4): p. 405-12.

63. Karlsson, C., K.J. Obrant, and M. Karlsson, *Pregnancy and lactation confer reversible bone loss in humans.* Osteoporosis International, 2001. **12**(10): p. 828-834.

64. Laskey, M.A. and A. Prentice, *Bone mineral changes during and after lactation.* Obstetrics & Gynecology, 1999. **94**(4): p. 608-15.

65. Polatti, F., et al., *Bone mineral changes during and after lactation.* Obstetrics and Gynecology, 1999. **94**(1): p. 52-56.

66. Matsushita, H., et al., *The effect of multiple pregnancies on lumbar bone mineral density in Japanese women.* Calcified Tissue International, 2002. **71**(1): p. 10-3.

67. Liesegang, A., J. Risteli, and M. Wanner, *Bone metabolism of milk goats and sheep during second pregnancy and lactation in comparison to first lactation.* Journal of Animal Physiology & Animal Nutrition-Zeitschrift fur Tierphysiologie Tierernahrung und Futtermittelkunde, 2007. **91**(5-6): p. 217-25.

68. Holmberg-Marttila, D., H. Sievanen, and R. Tuimala, *Changes in bone mineral density during pregnancy and postpartum: prospective data on five women.* Osteoporosis International, 1999. **10**(1): p. 41-6.

69. Yasumizu, T., et al., *Bone metabolism after human parturition and the effect of lactation: Longitudinal analysis of serum bone-related proteins and bone mineral content of the lumbar spine.* Endocrine Journal, 1998. **45**(5): p. 679-686.

70. Thomas, M., et al., *Calcium supplementation during pregnancy and lactation: effects on the mother and the fetus.* American Journal of Obstetrics & Gynecology, 2006. **194**(4): p. 937-45.

71. Chan, G.M., et al., *Effects of increased dietary calcium intake upon the calcium and bone-mineral status of lactating adolescent and adult women.* American Journal of Clinical Nutrition, 1987. **46**(2): p. 319-323.

72. Bezerra, F.F., et al., *Pregnancy and lactation affect markers of calcium and bone metabolism differently in adolescent and adult women with low calcium intakes.* Journal of Nutrition, 2002. **132**(8): p. 2183-2187.

73. Bezerra, F., et al., *Bone mass is recovered from lactation to postweaning in adolescent mothers with low calcium intakes(1-3).* American Journal of Clinical Nutrition, 2004. **80**(5): p. 1322-1326.

74. Aksakal, O., et al. *Effect of lactation on bone mineral density later in life in Turkish women*. 2008: Tubitak Scientific & Technical Research Council Turkey.

75. Dursun, N., et al., *Influence of duration of total breast-feeding on bone mineral density in a Turkish population: does the priority of risk factors differ from society to society?* Osteoporosis International, 2006. **17**(5): p. 651-655.

76. Hoffman, S., et al., *parity, lactation and hip fracture.* Osteoporosis International, 1993. **3**(4): p. 171-176.

77. Michaelsson, K., et al., *Influence of parity and lactation on hip fracture risk.* American Journal of Epidemiology, 2001. **153**(12): p. 1166-1172.

78. Millar, W.J. and H. Maclean, *Breastfeeding practices.* Health Reports-Statistics Canada, 2005. **16**(2): p. 23-34.

79. Chung, M., E. M. Balk, et al. *Vitamin D and Calcium: A Systematic Review of Health*. Evidence Report No. 183, 2009. (Prepared by the Tufts Evidence-based Practice Center under Contract No. HHSA 290-2007-10055-I.)

80. Picciano, M.F., *Pregnancy and lactation: physiological adjustments, nutritional requirements and the role of dietary supplements.* The Journal of Nutrition, 2003. **133**(6).

81. Greer, F.R., *Issues in establishing vitamin D recommendations for infants and children.* The American Journal of Clinical Nutrition, 2004. **80**(6).

82. Taylor, S.N., C.L. Wagner, and B.W. Hollis, *Vitamin D supplementation during lactation to support infant and mother.* Journal of the American College of Nutrition, 2008. **27**(6): p. 690-701.

83. Hollis, B.W., *Vitamin D requirement during pregnancy and lactation.* Journal of Bone and Mineral Research : the Official Journal of the American Society for Bone and Mineral Research, 2007. **22**: p. 39-44.

84. Kovacs, C., *Vitamin D in pregnancy and lactation: maternal, fetal, and neonatal outcomes from human and animal studies.* The American Journal of Clinical Nutrition, 2008. **88**(2): p. 520S.

85. Nesby-O'Dell, S., et al., *Hypovitaminosis D prevalence and determinants among African American and white women of reproductive age: third National Health and Nutrition Examination Survey, 1988-1994.* The American Journal of Clinical Nutrition, 2002. **76**(1): p. 187-92.

86. Hollis, B.W. and C.L. Wagner, *Review Articles - Assessment of dietary vitamin D requirements during pregnancy and lactation.* The American Journal of Clinical Nutrition., 2004. **79**(5): p. 717.

87. Heaney, R.P., et al., *Human serum 25-hydroxycholecalciferol response to extended oral dosing with cholecalciferol.* American Journal of Clinical Nutrition, 2003. **77**(1): p. 204-210.

88. Dawodu, A., et al., *Hypovitaminosis D and vitamin D deficiency in exclusively breast-feeding infants and their mothers in summer: A justification for vitamin D supplementation of breast-feeding infants.* Journal of Pediatrics, 2003. **142**(2): p. 169-173.

89. Ozkan, B., et al., *Prevalence of vitamin D deficiency rickets in the eastern part of Turkey.* European Journal of Pediatrics, 2009. **168**(1): p. 95-100.

90. Challa, A., et al., *Breastfeeding and vitamin D status in Greece during the first 6 months of life.* European Journal of Pediatrics, 2005. **164**(12): p. 724-729.

91. Ward, L.M., et al., *Vitamin D-deficiency rickets among children in Canada.* Canadian Medical Association Journal, 2007. **177**(2): p. 161-166.

92. Chung, M., E. M. Balk, et al. *Vitamin D and Calcium: A Systematic Review of Health*. Evidence Report No. 183, 2009. (Prepared by the Tufts Evidence-based Practice Center under Contract No. HHSA 290-2007-10055-I.)

93. Health Canada home page. 2004. *Eating Well With Canada's Food Guide.* Retrieved July 10, 2010, from http://www.hc-sc.gc.ca/fn-an/food-guide-aliment/order-commander/eating_well_bien_manger-eng.php

94. Hollis, B.W. and C.L. Wagner. *Vitamin D requirements during lactation: high-dose maternal supplementation as therapy to prevent*

hypovitaminosis D for both the mother and the nursing infant. 2004: Amer Soc Clinical Nutrition.

95. Wagner, C.L., *High-dose vitamin D3 supplementation in a cohort of breastfeeding mothers and their infants: a 6-month follow-up pilot study.* Breastfeeding Medicine, 2006. **1**(2): p. 59.

96. Armas, L.A.G., *Vitamin D_2 is much less effective than vitamin D_3 in humans.* The Journal of Clinical Endocrinology and Metabolism, 2004. **89**(11): p. 5387.

97. Saadi, H.F., *Efficacy of daily and monthly high-dose calciferol in vitamin D-deficient nulliparous and lactating women.* The American Journal of Clinical Nutrition, 2007. **85**(6): p. 1565.

98. Basile, L.A., *The effect of high-dose vitamin D supplementation on serum vitamin D levels and milk calcium concentration in lactating women and their infants.* Breastfeeding Medicine, 2006. **1**(1): p. 27.

99. Chiu, K.C., et al., *Hypovitaminosis D is associated with insulin resistance and beta cell dysfunction.* American Journal of Clinical Nutrition, 2004. **79**(5): p. 820-825.

100. Knekt, P., et al., *Serum vitamin D and subsequent occurrence of type 2 diabetes.* Epidemiology, 2008. **19**(5): p. 666-671.

101. Merlino, L.A., et al., *Vitamin D intake is inversely associated with rheumatoid arthritis - Results from the Iowa Women's Health Study.* Arthritis and Rheumatism, 2004. **50**(1): p. 72-77.

102. Hayes, C.E. *Vitamin D: a natural inhibitor of multiple sclerosis*. Proceedings of the Nutrition Society, 2000. **59**(4): p. 531-535

103. Xia, W.B., L. Xu, and H. Su, *Vitamin D deficiency and osteoporosis.* International Journal of Rheumatic Diseases, 2008. **11**(4): p. 366-373.

104. Yu, C.K.H., *Vitamin D deficiency and supplementation during pregnancy.* Clinical Endocrinology, 2008. **70**(5): p. 685.

105. Mannion, C.A., K. Gray-Donald, and K.G. Koski, *Association of low intake of milk and vitamin D during pregnancy with decreased birth weight.* Canadian Medical Association Journal, 2006. **174**(9): p. 1273-1277.

106. Morley, R., et al., *Maternal 25-hydroxyvitamin D and parathyroid hormone concentrations and offspring birth size.* Journal of Clinical Endocrinology and Metabolism, 2006. **91**(3): p. 906-912.

107. Javaid, M.K., et al., *Maternal vitamin D status during pregnancy and childhood bone mass at age 9 years: a longitudinal study.* Lancet, 2006. **367**(9504): p. 36-43.

108. Balasubramanian, S. and R. Ganesh, *Vitamin D deficiency in exclusively breast-fed infants.* Indian Journal of Medical Research, 2008. **127**(3): p. 250-255.

109. Hatun, S., et al. *Vitamin D deficiency in early infancy*. The Journal of Nutrition, 2005. 135: p. 279-282.

110. Balasubramanian, S., S. Shivbalan, and P.S. Kumar, *Hypocalcemia due to vitamin D deficiency in exclusively breastfed infants.* Indian Pediatrics, 2006. **43**(3): p. 247-251.

111. Agarwal, A., et al., *Rickets: A Cause of Delayed Walking in Toddlers.* Indian Journal of Pediatrics, 2009. **76**(3): p. 269-272.

112. Vitamin D supplementation: Recommendations for Canadian mothers and infants. First Nations, Inuit and Métis Health Committee. Canadian Paediatric Society (CPS). Paediatr Child Health 2007;12(7)583-9 Reference No. FNIM07-01.

113. Saadi, H.F., et al., *Effect of combined maternal and infant vitamin D supplementation on vitamin D status of exclusively breastfed infants.* Maternal and Child Nutrition, 2009. **5**(1): p. 25-32.

114. Yetley, E.A., *Dietary Reference Intakes for vitamin D: justification for a review of the 1997 values.* The American Journal of Clinical Nutrition, 2009. **89**(3): p. 719.

115. Jacobson, J.L., et al., *Beneficial effects of a polyunsaturated fatty acid on infant development: evidence from the inuit of arctic Quebec.* Journal of Pediatrics, 2008. **152**(3): p. 356-64.

116. Doria, A., *Estrogens in pregnancy and systemic lupus erythematosus.* Annals of the New York Academy of Sciences, 2006. **1069** (Basic and Clinical Aspects of Neuroendocrine Immunology in Rheumatic Diseases): p. 247.

117. Brenna, J.T., et al., *Docosahexaenoic and arachidonic acid concentrations in human breast milk worldwide.[see comment]*. American Journal of Clinical Nutrition, 2007. **85**(6): p. 1457-64.

118. Helland, I.B., et al., *Maternal supplementation with very-long-chain n-3 fatty acids during pregnancy and lactation augments children's IQ at 4 years of age.* Pediatrics, 2003. **111**(1): p. 6.

119. Sala-Vila, A., et al., *Lipid composition in human breast milk from Granada (Spain): Changes during lactation.* Nutrition, 2005. **21**(4): p. 467-473.

120. Yuhas, R., K. Pramuk, and E.L. Lien, *Human milk fatty acid composition from nine countries varies most in DHA.* Lipids, 2006. **41**(9): p. 851-858.

121. Smit, E.N., et al., *Estimated biological variation of the mature human milk fatty acid composition.* Prostaglandins Leukotrienes and Essential Fatty Acids, 2002. **66**(5-6): p. 549-555.

122. Ruan, C., et al., *Milk composition in women from five different regions of China: the great diversity of milk fatty acids.* Journal of Nutrition, 1995. **125**(12): p. 2993-8.

123. Gibson, R.A., M.A. Neumann, and M. Makrides, *Effect of increasing breast milk docosahexaenoic acid on plasma and erythrocyte phospholipid fatty acids and neural indices of exclusively breast fed infants.* European Journal of Clinical Nutrition, 1997. **51**(9): p. 578-584.

124. Jensen, C.L., et al. *Effect of docosahexaenoic acid supplementation of lactating women on the fatty acid composition of breast milk lipids and*

maternal and infant plasma phospholipids. American Journal of Clinical Nutrition, 2000. **71**(1): p. 292S-299S.

125. Van Goor, S.A., et al., *Human milk arachidonic acid and docosahexaenoic acid contents increase following supplementation during pregnancy and lactation.* Prostaglandins Leukotrienes & Essential Fatty Acids, 2009. **80**(1): p. 65-9.

126. Weseler, A.R., et al., *Dietary arachidonic acid dose-dependently increases the arachidonic acid concentration in human milk.* Journal of Nutrition, 2008. **138**(11): p. 2190-7.

127. Simopoulos, A.P. *Essential fatty acids in health and chronic disease*. American Journal of Clinical Nutrition, 1999. **70**(3): p. 560S-569S

128. Innis, S.M. and S.M. Innis, *Human milk: maternal dietary lipids and infant development.* Proceedings of the Nutrition Society, 2007. **66**(3): p. 397-404.

129. Ikemoto, A., et al., *Membrane fatty acid modifications of PC12 cells by arachidonate or docosahexaenoate affect neurite outgrowth but not norepinephrine release*. Neurochemical Research, 1997. 22(6): p. 671-678.

130. Calderon, F. and H. Kim, *Docosahexaenoic acid promotes neurite growth in hippocampal neurons*. Journal of Neurochemistry, 2004. 90(4): p. 979-988.

131. Innis, S.M., J. Gilley, and J. Werker, *Are human milk long-chain polyunsaturated fatty acids related to visual and neural development in breast-fed term infants?* Journal of Pediatrics, 2001. **139**(4): p. 532-538.

132. Lauritzen, L., et al., *Maternal fish oil supplementation in lactation: Effect on visual acuity and n-3 fatty acid content of infant erythrocytes.* Lipids, 2004. **39**(3): p. 195-206.

133. Koletzko, B., et al., *Dietary fat intakes for pregnant and lactating women.* British Journal of Nutrition, 2007. **98**(5): p. 873-877.

134. Hogstrom, M., P. Nordstrom, and A. Nordstrom, *n-3 fatty acids are positively associated with peak bone mineral density and bone accrual in healthy men: the NO2 Study.* American Journal of Clinical Nutrition, 2007. **85**(3): p. 803-807.

135. Makrides, M. and R.A. Gibson, *Long-chain polyunsaturated fatty acid requirements during pregnancy and lactation.* American Journal of Clinical Nutrition, 2000. **71**(1 Suppl): p. 307S-11S.

136. Albertazzi, P. and K. Coupland, *Polyunsaturated fatty acids. Is there a role in postmenopausal osteoporosis prevention?* Maturitas, 2002. **42**(1): p. 13-22.

137. Ladipo, O., *Nutrition in pregnancy: mineral and vitamin supplements.* American Journal of Clinical Nutrition, 2000. 72(1): p. 280S

138. Centres for Disease Control. *Growth Curves*, 2000.

139. Gibson, R. S. (Ed.). *Principles of Nutritional Assessment*. 2005, New York (NY): Oxford University Press

140. Health Canada home page. 2003. *Drug Product Database (DPD).* Retrieved July 12, 2010 from www.hc-sc.gc.ca/hpb/drugs-dpd/searching.html.

141. Hulley, S.B., Cummings, S.R., Browner, W.S., Grady, D.G. & Newman, T.B. (Ed). *Designing Clinical Research*. 2007, Philadelphia (PA): Lippincott, Williams and Wilkins

142. Tjepkema, M., *Adult obesity in Canada: measured height and weight*. Catalogue, 2005. Ottawa: Statistics Canada.

143. Guendelman, S. and B. Abrams, *Dietary intake among Mexican-American women: generational differences and a comparison with white non-Hispanic women.* American Journal of Public Health, 1995. 85(1): p. 20

144. Li, R., et al., *Breastfeeding rates in the United States by characteristics of the child, mother, or family: the 2002 National Immunization Survey*. Pediatrics, 2005. 115(1): p. e31.

145. Tarasuk, V., et al., *Nutrition inequities in Canada.* Applied Physiology, Nutrition, & Metabolism = Physiologie Appliquee, Nutrition et Metabolisme. **35**(2): p. 172-9

146. Weiler, H., et al., *Vitamin D deficiency and whole-body and femur bone mass relative to weight in healthy newborns*. Canadian Medical Association Journal, 2005. **172**(6): p. 757-61

147. Sowers, M.F.R., et al., *Parameters related to 25-OH-D levels in a population-based study of women*. American Journal of Clinical Nutrition, 1986. **43**: p. 621-628.

148. Institute of Medicine. *Dietary Reference Intakes: Applications in Dietary Assessment*. 2000, Constitution Avenue N.W, D.C.: National Academy Press

149. Walter Willett. (Ed). *Nutritional Epidemiology*. 1998, New York (NY): Oxford University Press.

150. Lucas, M., et al., *Validation of an FFQ for evaluation of EPA and DHA intake.* Public Health Nutrition, 2008. **12**(10): p. 1783-1790.