Vitamin D status and recommendations to improve vitamin D status in Canadian youth

Sean Mark

Doctor of Philosophy

Department of Dietetics and Human Nutrition

McGill University
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ABSTRACT

Little is known regarding the vitamin D status of Canadian youth. Our objectives were: (i) to describe the vitamin D status of Québec youth using a representative sample; (ii) examine the relative contributions of diet, physical activity and fat mass to the variance in plasma 25-hydroxyvitamin D{25(OH)D}, the best biomarker of vitamin D status; and (iii) examine the influence of household income and food insecurity on the intakes of dietary vitamin D, calcium and dairy foods.

To describe vitamin D status, we used data from a cross-sectional survey representative of Québec youth aged 9, 13 and 16, the **Q**uébec **C**hild and **A**dolescent **H**ealth and **S**ocial Survey (QCAHS). For the second objective, 159 youth, aged 8-11 whose parents (at least one) were obese or had the metabolic syndrome were used for cross-sectional analysis in the **Q**uébec **A**dipose and **L**ifestyle **I**nves**T**igation in **Y**outh (QUALITY). Fat mass was measured using Dual X-ray Absorptiometry (DXA) and physical activity was assessed by accelerometer. Finally, we analyzed data from the **C**anadian **C**ommunity **H**ealth **S**urvey (CCHS), a sample of 8960, 9-18-year-olds representative of Canadian youth for whom a single 24 hour dietary recall, measured height and weight, sociodemographic and information on food insecurity were available.

Greater than 90% of youth had sub-optimal vitamin D levels {plasma 25(OH)D < 75 nmol} at the end of winter and beginning of spring in both the QUALITY and QCAHS study. In the QCAHS study, older youth had a higher prevalence of vitamin D deficiency {25(OH)D < 27.5 nmol} (> 10%) than younger youth and girls from low income households had lower plasma 25(OH)D concentrations. In the QUALITY study, milk consumption and physical activity had modest associations with plasma 25(OH)D corresponding to 2.9 nmol/L and 2.1 nmol/L higher plasma 25(OH)D per standard deviation increase in these exposures,

respectively. In the CCHS study, we found evidence that milk intake was being displaced by sweetened beverages amongst low income boys and food insecure girls.

Population wide measures to increase dietary vitamin D intake should be examined in Canadian youth.

RÉSUMÉ

Il y a peu de connaisances concernant le statut vitamin D des jeunes Canadiens. Nos objectifs étaient de: (i) décrire le statut vitamin D des jeunes Québécois en utilisant un échantillon représentatif; (ii) examiner la contribution de la diète, l'activité physique et l'adiposité a expliquer la variance du 25-hydroxyvitamin D, {25(OH)D.}, le meilleur biomarqueur du statut vitamine D; et (iii) examiner l'influence du statut socio-économique et l'insécurité alimentaire sur le consommation des produits laitiers, du calcium et de la vitamine D alimentaire.

Pour décrire le statut vitamine D on a utilisé les données transversales d'un échantillon représentatif des jeunes Québecois agés de 9, 13 et 16 ans. Pour le deuxième objectif, 159 jeunes, âgés 8-11 ans avec des parents (au moins un) qui étaient obèses ou avaient le syndrome métabolique etaient utilisés pour une analyse transversale dans l'étude **Q**uébec **A**dipose and **L**ifestyle **I**nves**T**igation in Youth (QUALITY). Le tissu adipeux a été mesuré avec le dual X-ray absorptiometry (DXA) et l'activité physique était mésurer par accéléromètre. Finalement, on a utilisé des données du **C**anadian **C**ommunity **H**ealth **S**urvey (CCHS), un échantillon de 8960 jeunes, agés de 9-18 ans qui avaient un rappel alimentaire de 24 heures, le poids et la taille mesuré, l'information sociodémograhique et le statut de sécurité alimentaire.

Dans l'étude QUALITY et le QCAHS plus de 90% des jeunes avaient un statut de vitamine D sub-optimal {plasma 25(OH)D < 75 nmol} à la fin de l'hiver et au début du printemps. Dans l'étude QCAHS, les adolescents avaient une prévalence de déficience de vitamine D élevé {25(OH)D < 27.5 nmol} (> 10%) et les filles venant des foyers défavorisés avait des niveaux de vitamine D plus bas. Dans l'étude QUALITY, un augmentation d'un écart-type de la consommation du lait et l'activité physique était associée avec une augmentation du niveau de vitamin D de 2.9 nmol/L and 2.1 nmol/L respectivement. Dans l'étude CCHS nous avons

remarqué que les garçons de milieux défavorisés et les filles avec une insécurité alimentaire consommaient moins de lait et le lait étaitremplacé par les breuvages sucrés.

Des mesures pour augmenter la consommation de vitamine D parmi les jeunes Canadiens devraient être examinées.

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CONTRIBUTIONS OF COAUTHORS

The manuscripts in this thesis have been prepared by a group of authors; I (Sean Mark) however, have been the principal contributor to all of them. I was involved in multi-disciplinary bilingual research team in order to procure data sources for manuscript 1 and manuscript 2. This involved attendance of bi-monthly meetings and presenting research findings periodically to the group. I wrote this thesis and the first drafts of the manuscripts. I extracted the data from the three data sources detailed in this thesis. I had the original research ideas for all papers. The contribution of co-authors for each manuscript is described below.

Manuscript 1

LOW VITAMIN D STATUS IN A REPRESENTATIVE SAMPLE OF YOUTH FROM QUEBEC, CANADA

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Sean Mark, PhD Candidate: Was the primary author, conceived and performed statistical analysis, wrote the 1st draft of the manuscript and contributed to the interpretation of the data.

Katherine Gray-Donald, PhD: Contributed to the interpretation of data and to the writing of the manuscript.

Marie Lambert, MD: Assisted in the funding of the study, contributed to the interpretation of data and to the writing of the manuscript

Edgard Delvin, PhD: Contributed to the interpretation of data and the writing of the manuscript

Jennifer O'Loughlin, PhD: Contributed to the interpretation of data and the writing of the manuscript.

Gilles Paradis, MD, MSC: Contributed to the interpretation of data and the writing of the manuscript.

Dr. Emile Levy, MD, PhD: Contributed to the interpretation of data and the writing of the manuscript.

Manuscript 2

HIGHER LEVELS OF VITAMIN D INTAKE ARE NEEDED TO ACHIEVE OPTIMAL LEVELS OF VITAMIN D IN QUÉBEC YOUTH

Sean Mark, PhD Candidate: Was the primary author, performed statistical analysis, wrote the 1st draft of the manuscript and contributed to the interpretation of the data.

Marie Lambert, MD: Contributed to the funding, collection and interpretation of data and to the writing of the manuscript

Edgard Delvin, PhD: Contributed to the writing of the manuscript.

Jennifer O'Loughlin, PhD: Contributed to the interpretation of data and the writing of the manuscript.

Katherine Gray-Donald, PhD: Contributed to the funding and interpretation of data and to the writing of the manuscript.

Manuscript 3

HOUSEHOLD INCOME, FOOD INSECURITY AND NUTRITION IN CANADIAN YOUTH

Sean Mark, PhD Candidate: Was granted access to a Research Data Center of Statistics Canada through a series of security clearance checks. I received training in Ottawa on the dietary methods used in the Canadian Community

Health Survey (Cycle 2.2). I performed all statistical analysis, was the primary author, wrote the 1st draft of the manuscript and contributed to the interpretation of the data.

Marie Lambert, MD: Contributed to the interpretation of data and to the writing of the manuscript

Jennifer O'Loughlin, PhD: Contributed to the interpretation of data and the writing of the manuscript.

Katherine Gray-Donald, PhD: Contributed to the interpretation of data and to the writing of the manuscript.

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LIST OF ABBREVIATIONS

25(OH)D: 25-hydroxyvitamin D

1,25(OH)2D: 1,25-dihydroxyvitamin D

AI: Adequate Intake

ALDH: Aldehyde dehydrogenase

Ca2+: Calcium

BMI: Body mass index

CCHS: Canadian Community Health Survey (Cycle 2.2)

CDC: Center for Disease Control

DIN: Drug Identification Number

DRI: Dietary Reference Intake

DXA: Dual X-ray absorptiometry

EAR: Estimated Average Requirement

ECF: Extracellular fluid

EQAHS: External Quality Assessment Scheme

FGF-23: Fibroblast growth factor 23

HDL: High-density lipoprotein

IGF-1: Insulin growth factor-1

LDL: Low-density lipoprotein

NHANES: National Health and Nutrition Examination Survey

NS: Non-significant

QCAHS: Québec Child and Adolescent Health and Social Survey

QUALITY: Québec Adipose and Lifestyle InvesTigation in Youth

PA: Physical activity

PTH: Parathyroid hormone

UVB: Ultraviolet B radiation

VDR: Vitamin D Receptor

WHI: Women's Health Initiative

CHAPTER 1: INTRODUCTION

Vitamin D has been implicated in the attainment of peak bone mass [1, 2], glucose homeostasis [3] and a number of common malignancies [4]. Moreover, low vitamin D status has been described as a public health problem in many regions of the globe [5]. Even in sunny climates, concealing clothing has been associated with low vitamin D status [6]. In Canada, for about half the year, ultraviolet radiation is not of sufficient intensity to catalyze cutaneous vitamin D production [7]. Cutaneous production accounts for the vast majority of bodily vitamin D stores [8]. In youth, long-term exposure to low vitamin D levels may be a risk for lower bone density [9] in addition to the aforementioned chronic diseases. Little, however, is known regarding the vitamin D status of Canadian youth [10].

One of the unifying themes of this thesis is the use of a population health approach to describe vitamin D status and examine its correlates in Canadian youth. Population health is defined by John Last's Dictionary of Epidemiology as 'the disciplines involved in studying the determinants and dynamics of a population's health [11]. Some definitions of population health embrace public health actions based on sound evidence, other definitions do not. Implicit in this definition is the use of a number of disciplines. This thesis concerns itself with the discipline of nutrition and epidemiology. Using these tools we will build a case for why the findings of this thesis should be incorporated into evidence based public health action for Canadian youth.

One of the challenges in this thesis was the use of three different databases to answer distinct questions relating to vitamin D status and means to improve vitamin D status in Canadian youth. The research questions dictated the use of one or the other databases. The **Q**uébec **C**hild and **A**dolescent **H**ealth and Social **S**urvey (QCAHS) was representative of the Québec youths aged 9, 13 and 16 while the **C**anadian **C**ommunity **H**ealth **S**urvey (CCHS) Cycle 2.2 was representative of Canadian youth. These studies offered great strengths with respect to statistical power owing to the large samples sizes and the generalizability of study findings. The trade-off however, was in the constraints

placed on the measurement of complex exposures in large numbers of individuals. There is thus generally a trade off between the number of participants in a study and the feasibility to measure exposures accurately [12]. For example, the QCAHS was representative of Caucasian youth of Québec, had a questionnaire measure of physical activity (PA) but lacked dietary measures. The accuracy and availability of measures have an impact on the ability to detect associations and to control for important confounders in analyses.

Population health is also germane to population wide measures to improve health, such as food fortification. The fortification of flour with folic acid in Canada and the US was an important means to reduce the incidence of neural tube defects. [13, 14]. In keeping with the work of Dr. Geoffrey Rose, measures to improve health for a population should endeavor to shift the entire distribution of a population [15]. For example, amongst 15 000 Canadian women there was a 64% increase in serum folate and a decrease in folate deficiency from 6.3 % to 0.88% after the mandatory fortification of flour with folate [14]. This example may be germane to the vitamin D nutritional status of the Canadian population as a number of smaller, non-representative, studies have suggested that vitamin D deficiency may be prevalent at the end of winter despite vitamin D intakes above recommended levels [16-18].

CHAPTER 2: LITERATURE REVIEW

Few studies have examined the vitamin D status of Canadian youth although residence at high latitude places them at risk for low vitamin D levels [10]. What few data are available suggest that low vitamin D status is prevalent amongst Canadian adults and children [16, 19]. Many phenomena may be at work to explain this. Lack of exposure to UVB from low amounts of outdoor activity, sunscreen use, skin covering and dark skin pigmentation, are all factors limiting the cutaneous synthesis of vitamin D [20]. Foods which naturally contain vitamin D include: fatty fish, liver and fat from fish-eating mammals. The major dietary sources of vitamin D in Canada, however, are fortified foods, including milk (fluid milk, evaporated milk, and goat's milk), margarine and some yogurts [21]. The consumption of milk is declining in some youth settings [22]. For example, data from Canada suggest that there has been a secular decline in milk consumption [23]. Consistent with this, in Canadian youth the mean intake of milk and dairy products consumed does not meet the Canadian Food Guide for Healthy Eating recommendations [24]. Lower vitamin D status combined with lower than recommended calcium intake in youth could have implications for the attainment of peak bone mass [2] and a number of other health outcomes such as cardiovascular disease, metabolic regulation and a number of common malignancies [25]. The majority of studies relating to non-calcemic functions of vitamin D, however, have been conducted in adults [26].

2.1 Vitamin D and the metabolism of phosphorus and calcium

The spectrum of functions associated with vitamin D and calcium are thought to be overlapping [27] due to vitamin D dependent gene activation which facilitates dietary calcium absorption [28]. Vitamin D regulates the absorption and metabolism of phosphorus, this mineral, however, is not limited in the food supply of industrialized countries [29], thus it will only be briefly discussed.

Phosphorus

Phosphorus accounts for 0.7 to 1.1 percent of body weight such that a 70 kg adult body will contain about 700 grams. Approximately 80% of this is stored in the skeleton in the form of crystalline hydroxyapatite (Ca10(PO4)6(OH)2). The remaining phosphorus includes 9% in skeletal muscle, approximately 10% in the viscera and the final 0.1 % in extracellular fluid. Phosphorus is important as a building block for a large number of biomolecules, including: adenosine-tri-phosphate, deoxyribose nucleic acid (DNA) and deoxyribose nucleic acid (RNA). As these are basic building blocks of both plants and animals, they are widely distributed in the diets of industrialized countries. Thus, phosphorus deficiency occurs only in settings of extreme malnutrition [29]. Nonetheless, vitamin D plays an essential role in maintaining phosphorus reserves in the body since the actions of vitamin D are thought to account for 40 % of the absorbed phosphorus [5].

Calcium

Calcium is the most abundant mineral in the body, accounting for 1-2 % of body weight. Calcium has a large nutrient reserve in the body, the bone which has assumed a structural role throughout the course of evolution [27]. In the circulation, 40-45 % of calcium is bound to serum proteins, 8-10 % is complexed to citrate and 45-50% is found as free ions. These free ions are highly regulated by the parathyroid glands, which release parathyroid hormone when ionized calcium concentrations fall. The skeleton maintains calcium concentration in the body by balancing accretion (calcium deposition) with resorption (release of calcium) [29]. Calcium cannot be extracted directly from the skeleton; bone tissue is broken down leading to the release of calcium. It is for this reason that calcium is described as a threshold nutrient such that when consumption is below bodily needs, bone tissue is broken down and calcium is released into the serum. Conversely when absorption is in excess of needs, the remaining calcium gets deposited within bone [27].

Functions of calcium

Calcium has a number of functions, these include: being a structural component of bones and teeth, facilitating muscle contraction, blood clotting, maintenance of cell membranes and nerve cell transmission [29]. The need to maintain constant levels of calcium in the serum are underscored by the ubiquity of ionized calcium as a second messenger in a wide variety of cells and organisms [30]. As part of a signaling pathway, calcium is released locally from the endoplasmic reticulum, an interconnected network of tubules and vesicles within the cell. Calcium signaling manifests intracellularly through Ca 2+ binding to and changing the conformation of specific adaptor proteins. As an example, I will highlight one such protein. Calmodulin is a small protein acting as a cellular sensor and adaptor protein. Its amino acid sequence has remained conserved over 1.5 billion years of evolution and it is transcribed from three different chromosomes in the human genome underscoring its ubiquity throughout the cellular environment. Calmodulin undergoes a conformational change upon binding to calcium, which enables it to bind to proteins for a specific response. Hundreds of cellular proteins bind calmodulin facilitating its role as a second messenger in propagating signals as diverse from cellular activation to programmed cell death [30].

Calcium absorption

Calcium absorption in the human intestine occurs at 15-35% efficiency depending on the dietary calcium intake, age and an individual's vitamin D status [31]. There are two mechanisms by which calcium is absorbed. First, there is a receptor mediated process dependent on Calbindin, a 9 kilodalton protein located in the duodenum and upper jejunum, which is activated by 1,25(OH)2Vitamin D [32]. The other means of absorption occurs paracellularly whereby calcium is passively absorbed when a sufficient concentration gradient is established between the lumen of the gut and the serosal side of the intestine [33]. The relative proportions of each of these means of calcium absorption depend on the concentration of luminal calcium. For example, when gut luminal calcium concentrations are high, the paracellular route accounts for the greatest amount of calcium absorption.

Alternatively, when gut luminal calcium concentrations are low, the vitamin D dependent means of calcium absorption predominates [34]. It has been noted, however, that, in the absence of vitamin D receptors in both humans [35] and mice, passive diffusion is unable to provide sufficient calcium absorption to prevent rickets even at high calcium intakes [36]. In adults the passive diffusion of calcium is thought to account for 8-23% of calcium absorption [37].

Dietary factors influencing calcium absorption and metabolism

A number of dietary factors influence calcium absorption [38]. For example, lactose has been found to facilitate the absorption of calcium [39], thus making dairy products effective vehicles for calcium absorption [40]. Certain plant metabolites such as oxalates and phytates have been shown to inhibit calcium absorption. Meal spacing of calcium rich foods has also shown to play an important role in calcium absorption where greater spacing of meals results in higher calcium absorption [38]. Absorption of calcium of commonly consumed vegetables is thought to be quite close to that of milk, between 71% and 86% [41]. Higher protein intake is thought to cause increases in urinary calcium; in addition, high sodium intake is thought to cause higher calcium losses [42].

Toxicity of calcium

While a number of scientists have argued for higher than recommended calcium intakes [43, 44], there is some evidence suggesting that this could be detrimental to health. A recent report from a randomized trial in post-menopausal women found that those randomized to 1 gram of calcium for 5 years, in addition to a calcium intake of approximately 850 mg / day, had a higher incidence of myocardial infarction. Here, the relative risk for confirmed cases of myocardial infarction was 2.12 (95% CI: 1.01-4.47) [45]. This increased risk of vascular events in the context of calcium supplementation has been observed in a number of different trials. For example, in a trial of 36 282 postmenopausal women, participants were randomized to 1000 mg of calcium carbonate and 400 IU of vitamin D / day or placebo, using a composite end point of myocardial infarction, coronary heart disease death, coronary artery bypass graft and percutaneous coronary

intervention found a hazard ratio of 1.08 (95% CI: 0.99 to 1.19) [46]. The mechanisms by which calcium increased the risk of vascular complications are not known but could be due to the acute elevation of calcium levels through calcium supplementation [47]. Acutely elevated calcium levels are thought to increase vascular calcification, which in turn is associated with increased number of vascular events [48]. Paradoxically calcium supplementation at this level raises HDL and lowers LDL, thus it is unlikely that the deleterious effect of calcium supplementation on vascular events operates through lipid based risk factors [45].

Calcium economy

Calcium metabolism is tightly regulated and plays a non-redundant role in the physiology of a wide diversity of organisms. There are approximately 200 mg of obligatory losses of calcium per day in an adult [49]. Calcium losses also occur through sweat and in athletes under heavy exertion losses can be up to 200-400 mg/day. Thus, exertion in athletes over a prolonged period of time can result in measurable decreases in bone mineral density [50], underscoring the need for adequate dietary intake.

Ionized calcium is an important component of extracellular fluid (ECF) which is an intermediate between the bone, the reserve of calcium, and dietary intakes. ECF calcium is highly regulated; bone mass, the calcium nutrient reserve, is regulated not so much by maintenance of mineral homeostasis in the bone matrix but by structural constraints. For example, when absorption from dietary sources falls, parathyroid hormone (PTH) rises, activating 1-alpha-hydroxylase enzyme which catalyzes the second hydroxylation of 25(OH)D, the activated form of vitamin D. This, in turn, initiates cellular processes to degrade mineralized bone matrix, thus liberating calcium. The regulation of the 1-alpha-hydroxylase enzyme is complex. For example, Ca2+, PTH, growth hormone and insulin growth factor-1 are positive regulators while phosphate, fibroblast growth factor (FGF-23) and 1,25(OH)2 vitamin D are negative regulators [51].

Parathyroid hormone

PTH is produced by the parathyroid glands located behind the thyroid glands. PTH stimulates osteoblasts and stimulates the differentiation of pro-osteoclast into osteoclasts and transfers osteoclasts onto bone surfaces. In the kidney, PTH enhances the active reabsorption of calcium and magnesium from the distal tubules. In the intestine, PTH stimulates the absorption of calcium through the action of activated vitamin D. PTH secretion is determined by the aggregate calcium output of all organ systems involved with calcium metabolism [27].

Modulating the biologic responses of PTH target organs

The PTH target organs have evolved some degree of regulatory autonomy while maintaining calcium homeostasis. For example, the bones of individuals of African descent are relatively resistant to the effects of PTH. This results in higher PTH and calcitriol (the activated form of vitamin D) but less bone remodeling leading to higher bone mass than individuals of Caucasian or Asian descent. In women, estrogen attenuates the effect of PTH on bone. During menopause, however, the levels of estrogen decrease thus increasing the effect of PTH on bone remodeling. Calcium absorption in post-menopausal women is also thought to decrease owing to lower levels of estrogen [27, 52].

Interactions between calcium and vitamin D

Calcium and vitamin D interact in a number of different ways. The first mechanism has been well characterized in adult populations as the increased calcium absorption fraction owing to a vitamin D dependent absorption process [31]. The correlation between 25(OH)D and calcium absorption in adults suggests that the vitamin D receptor may be responsive to 25(OH)D and not just the active form of vitamin D, 1,25(OH)2D [53]. In youth, calcium absorption is thought to be regulated by processes associated with growth like levels of insulin like growth factor 1 (IGF-1) levels as opposed to vitamin D levels [54]. The second interaction between calcium and vitamin D is the more rapid conversion of 25(OH)D to 1,25(OH)2D caused by a low dietary calcium intake and the activation of 1-alpha-hydroxylase enzyme in the kidney [55]. This finding has been shown in animal

models but has limited evidence in humans. The final interaction between vitamin D and calcium relates to the purported additional health benefits of high dietary calcium and optimal levels of vitamin D. Health benefits, such as cancer prevention, were shown in a small randomized trial of post-menopausal women with optimal vitamin D levels and higher than recommended calcium [43]. The higher than recommended doses of calcium, however, must be weighed against the higher probability of vascular events associated with calcium supplementation reported amongst older women in a randomized trial [45].

Metabolism of vitamin D

Vitamin D enters the circulation through either dietary intake or through sunlight exposure. It is metabolized by the liver to 25(OH)D in a largely unregulated step [26] . 25(OH)D, a fat soluble vitamin, is stored in adipose tissue [56]. The 1-alpha-hydroxylase plays an important role in the final activation of vitamin D. It had long been thought that the 1-alpha hydroxylase enzyme was only expressed in the kidney [26]. Interestingly, a number of tissues including monocytes, beta cells of the pancreas and parathyroid glands also express the 1 alpha-hydroxylase, thereby allowing tissues to regulate synthesis of 1,25(OH)2D locally [57]. These findings highlight the need to reconceptualize active vitamin D not as a hormone centrally regulated in the kidney but also as a paracrine and autocrine effector with physiological effects tightly regulated at the cellular level. Optimal substrate concentration for extra-renal 1-alpha-hyroxylase is thought to be about 75 nmol/L [25]. Studies are eagerly awaited that examine the genetic elements which govern 1-alpha-hydroxylase expression in a number of different tissues.

Assessing vitamin D status

25(OH)D has a half-life in the circulation of about 3 weeks. It is generally considered the best indicator of vitamin D status [58]. The active form of vitamin D, 1,25(OH)2D has a short half life and is available at low concentrations in the bloodstream. It is thus not a good indicator of vitamin D status. Measurement of 25(OH)D is not standardized between laboratories with as much as 30% variation in the measurement of the same sample by different laboratories [59]. Thus the

diagnosis of vitamin D deficiency using serum levels of 25(OH)D and defining accurate cut-offs consistent with functional outcomes has been made difficult [60]. In response to this, the Vitamin D External Quality Assessment Scheme was established in 1989. In 2004, there were 100 participating laboratories in 18 countries [61]. Participating laboratories are sent 5 samples of human sera every 3 months; for each sample an all laboratory mean 25(OH)D concentration is calculated and the % deviation from the mean is computed for each laboratory. In 1997, a small validation study compared the all laboratory mean obtained primarily through radio-immunoassays with a gas chromatography-mass spectrometry measurement of 25(OH)D, the latter being the gold standard, and found the all laboratory mean to reflect the true value of 25(OH)D [62]. Measurements are considered acceptable if 80% of results are within 30% of the all laboratory mean [61].

2.2 Vitamin D requirements in youth

When the vitamin D requirements were set in 1997, there was inadequate evidence to establish an Estimated Average Requirement (EAR). An EAR is 'the nutrient intake value that is estimated to meet the requirement defined by a specified indicator of inadequacy in 50 percent of the individuals in a life stage and gender group' [29]. Instead, an adequate intake (AI) was set, which was based on observed estimates of nutrient intake in a group of healthy people. Assessing nutrient intake in the case of vitamin D is difficult as the majority of vitamin D comes from cutaneous production through exposure to UVB [20]. UVB exposure is difficult to measure, particularly by questionnaires [63]. Thus the AI for vitamin D is defined by IOM (Institute of Medicine) as 'the intake that is considered likely to maintain adequate serum 25(OH)D for individuals in the population group who have limited and uncertain sun exposure and stores, multiplied by a safety factor of 100 percent for those unable to obtain sunlight'[29]. The AI is thus meant to over-estimate the true biological need. Defining adequate serum 25(OH)D is dependent on life stage and is largely based on indicators of bone health. For example, in infants, rickets and parathyroid hormone are useful indicators of

vitamin D status while in older youth and adults, bone mineral density, bone mineral content and PTH are criteria with which to define adequate serum 25(OH)D [29]. The use of bone health as an indicator of vitamin D status is complicated by the fact that indicators of bone metabolism are dependent not only on vitamin D levels but on calcium intakes, physical activity and genetic factors [64].

Defining cut-offs for vitamin D

There are a number of cut-offs used in the literature to describe vitamin D deficiency and hypovitaminosis. These cut-offs differ for pediatric and adult populations. This situation is complicated further by inter-laboratory variability in 25 (OH) D measurements. There is, however, widespread consensus in the pediatric literature that vitamin D deficiency can be defined as serum 25(OH)D below 25-30 nmol/L [65]. The Institute of Medicine defines vitamin D deficiency as 25(OH)D $\leq$ 27.5 nmol/L from infancy to youth 18 year olds and $\leq$ 37.5 nmol/L in adults [29]. In pediatric populations hypovitaminosis D is defined as $\leq$ 37.5 nmol/L [66]. Optimal cut-offs have been defined largely in adults and are thought to be levels of 25(OH) $\geq$ 75 nmol/L [67]. Much of the work, however, defining the optimal cut-off of vitamin D has been done using observational studies. We will discuss cut-offs for optimal and vitamin D deficiency as both of these cut-points have been the topic of considerable investigation.

Vitamin D deficiency, rickets and osteomalacia

The manifestations of vitamin D deficiency depend on the stage of (linear) growth. For example, when the epiphyseal plates are closed in adulthood, deficiency manifests as osteomalacia, a condition associated with the improper mineralization of bone [5]. While the epiphyseal plates are open, vitamin D deficiency results in a flaring of the growth plates, incurvation of long bones and hypotonia, a condition known as rickets. While most rickets cases occur in infants, rickets has also been reported in adolescents [68]. The clinical signs and symptoms of rickets include: bone pain and tenderness, dental problems, hypocalcemia, muscle weakness, increased tendency of fractures. Rickets is also

well known for its skeletal deformities including cranial, spinal and pelvic deformities in toddlers this also includes bowed legs and knocked-knees [5]. Significant variability in 25(OH)D assays, the importance of dietary calcium and the variable clinical presentation of rickets make it difficult to ascribe a definitive level of plasma 25(OH)D to rickets outside of the clinical presentation of rickets and the exclusion of a genetic basis for the condition [29].

Calcium intake and vitamin D deficiency interact to produce rickets. For example, in tropical countries, inadequate dietary quality associated with low dietary calcium can precipitate rickets [69]. Youth in these studies do not present with vitamin D deficiency despite clinically confirmed rickets [65] and this condition resolves with the administration of dietary calcium [69].

A number of genetic mutations in vitamin D, calcium or phosphorus metabolism in humans are associated with the clinical presentation of rickets. For example, a number of mutations have been described to explain X-linked hypophosphatemic rickets. One such case is the loss of function of dentin matrix protein 1, a non-collagenous matrix protein that results in the clinical presentation of rickets [70]. Similarly, a mutation in a protein called FGF-23, which has an important role in the absorption of phosphorus, is associated with autosomal dominant hypophosphatemic rickets [71].

Optimal plasma concentration of 25(OH)D

A number of vitamin D experts have suggested that, for adults, optimal levels of 25(OH)D status are between 70-80 nmol/L [72] This estimate is based on multiple lines of evidence including, a low plateau of parathyroid hormone, optimal calcium absorption [31], bone health, lower extremity function, colorectal cancer among other health outcomes. For example, amongst 1569 French adults located in 20 French cities (43^{o}-51^{o} N) intact parathyroid hormone reached a low plateau at 78 nmol/L of 25(OH)D. Parathyroid hormone levels increased in concentrations of 25(OH)D below 78 nmol/L [72]. Similarly, a review of calcium absorption [31] studies amongst postmenopausal women [28, 73] and men with a mean age of 26 [74] found that the calcium absorption fraction was maximized at a

25(OH)D level of 80 nmol/L. A number of vitamin D experts concur with an optimal range of 25(OH)D levels between 70 and 80 nmol/L based on evidence for fracture prevention [67]. Similarly another publication of vitamin D experts have supported this range of 25(OH)D levels using observational data in adults for bone mineral density, lower-extremity function, dental health, risk of falls, fractures and colorectal cancer. While the evidence for an optimal cut-off of 25(OH)D levels between 70-80 nmol / L is supported by intermediate endpoints such as optimal calcium absorption and the plateau of PTH, much of the evidence to define optimal vitamin D levels is derived from observational studies with disease based outcomes [75].

Limitations of optimal cut-offs in youth

The use of an optimal cut-off to define vitamin D status in youth is problematic as it is derived from clinical end-points which are of limited relevance to youth [75]. Instead, intermediate functional indices are used such as parathyroid hormone, bone mineral density and bone turn-over markers. The long-term implications of unfavorable intermediate indicators in youth are unclear [76] as youth are undergoing rapid periods of bone accretion. This is particularly evident in the context of calcium absorption. In adults calcium absorption is associated with 25(OH)D levels [77]. In youth, however, IGF-1 an anabolic hormone [54], and 1,25(OH)2D [78] are associated with calcium absorption whereas 25(OH)D is not.

2.3 Vitamin D status in youth and adults

Levels of Vitamin D in Canadian youth have not been systematically studied. The one study identified used a convenience sample from pediatric emergency departments in Edmonton, Alberta (52^0 N). The study reported serum 25(OH)D on 68 youth aged 2 to 16 at a time corresponding to the end of winter (April). The mean 25(OH)D from this sample was 47.2 nmol/L; 34 % of the participants in the study had levels of 25(OH)D lower than 40 nmol/L. From this sample, 6% of youth were reported deficient, where deficiency is defined as a serum level of 25(OH)D less than 25nmol/L. The strongest positive correlate of sufficient vitamin D levels ($\geq$ 40 nmol/L) was vitamin D intake from diet measured using a

semi-quantitative food frequency questionnaire. Male sex and increasing age were related to lower levels of 25(OH)D [16].

A number of studies have examined the vitamin D status of Canadian adults. For example, a study of 796 Canadian women (43^0 N) aged 18-35 found that self-reported vitamin D intake from milk and/or multivitamins did not prevent vitamin D insufficiency in the winter months. This was consistent with a lack of association between dietary vitamin D intake and vitamin D status during the winter months in these participants [18]. A study of mothers and newborns conducted in Winnipeg (49^0 N) found that 46% of mothers were considered vitamin D deficient {25(OH)D < 37.5 nmol / L}. Of the mothers that were vitamin D deficient, 70% gave birth to infants who were also vitamin D deficient {25(OH)D < 27.5 nmol / L}. This sample, however, consisted of 44% non-white participants and darker skin pigmentation is known to decrease the cutaneous production of vitamin D [20]. Thus for aboriginal populations living at high latitudes long winters and darker skin pigmentation increase the risk of vitamin D deficiency. Consistent with this, a number of studies have documented a high prevalence of vitamin D deficiency in this population [79, 80].

While much of the work on vitamin D levels in Canadians was conducted in convenience, clinic based samples, a small sample of adults randomly selected from telephone listings were recruited in Calgary (51^0 N). Participants were aged 27 to 89 years and the sample consisted of 128 women and 60 men. Vitamin D levels were assessed in each season. In this sample, winter 25 (OH)D was 57.3 nmol/L while summer levels were 71.6 nmol / L [17].

2.4 Canadian Pediatric Surveillance System

Surveillance is the routine accumulation of data for the analysis and the timely dissemination of information to those who need to know so that action can be taken [11]. The Canadian Pediatric Surveillance System was established to improve the health of children by gathering data on disorders that are high in disability, morbidity and economic cost to society despite their low frequency. A number of disorders are the subject of surveillance including: neonatal hyperbilirubinemia,

necrotizing fasciitis, neonatal Herpes Simplex Virus infection and vitamin D deficiency rickets [81]. As vitamin D deficiency rickets is preventable, it is an amenable target for surveillance. With respect to rickets, a total of 2325 pediatricians were sent a monthly questionnaire asking whether new cases of vitamin D deficiency rickets cases had been identified. The case definition for rickets in the Canadian Pediatric Surveillance System is clinically confirmed rickets in conjunction with low vitamin D levels to exclude genetic causes of rickets [81]. If a new case is identified a detailed form is sent to obtain demographic data and medical profiles of the confirmed cases. The case definition for the surveillance system includes being < 18 years of age and with a serum 25(OH)D < 27.5 nmol / L. Participants were excluded if rickets were caused by a heritable disorder of vitamin D metabolism, including 1-alpha-hydroxylase deficiency, vitamin D receptor defects or hypophosphatemic rickets.

Recently the Canadian Pediatric Surveillance Network reported 104 cases of vitamin D deficient rickets between July 2002 and June 2004 (incidence rate = 2.9/100 000) [82]. Affected children had a mean age of 1.4 years and 89% of youth had intermediate or dark skin. There was also a higher incidence of rickets amongst youth residing in Northern Canada. Both skin color and living in the North have been associated with decreased cutaneous synthesis of vitamin D [7, 20]. In the Pediatric surveillance system, 94% of infants who had rickets had been breastfed. Low vitamin D levels in breastmilk have been well documented [83]. This surveillance system likely under-reports the incidence of rickets as only pediatricians participate in the surveillance system. It is possible that the clinically confirmed cases of rickets in this surveillance system represent an iceberg effect of vitamin D deficiency. That is, there may be a much higher prevalence of sub-clinical vitamin D deficiency which may be clinically silent for a number of years but contribute to an increased risk of osteoporosis in later life. Further, one can expect that access to a pediatrician is not frequent in Northern Aboriginal communities.

2.5 Vitamin D_2 versus Vitamin D_3

Vitamin D_2, and vitamin D_3 have slightly different chemical structures, the former is derived from fungus and plants upon UV radiation whereas the latter is synthesized in the skin after UVB irradiation. There is conflicting literature on the ability of vitamin D_2 to increase levels of serum 25(OH)D. This controversy, however, is of less public health significance in Canada as staple foods are fortified with vitamin D_3 and vitamin D supplements are D_3. In Canada some soy based beverages are fortified with vitamin D_2 to avoid animal derived vitamin D_3 [84]. Vitamin D_2 is derived from fungal cell membranes while vitamin D_3 is derived from the lanolin of sheep's wool. In the US, foods and supplements consist of vitamin D_2. The controversy relates to the ability of vitamin D_2 versus vitamin D_3 to increase 25(OH)D levels. A number of reports showed that vitamin D_2 was less efficacious than vitamin D_3 at increasing 25(OH)D levels [85]. A recent report, however, shows that vitamin D_2 is as efficacious as vitamin D_3 at increasing serum 25(OH)D [86]. More studies are eagerly awaited to resolve this controversy.

2.6 Safety and toxicity of vitamin D

Currently the upper limit of dietary vitamin D intake is 2000 IU per day [29]. It has been shown in clinical trials that up to 10 000 IU/day of vitamin D is non-toxic in adults [87]. Hypervitaminosis D is defined as plasma 25(OH)D concentrations between 400 to 1250 nmol/L. The most proximal effect of hypervitaminosis D is the increased intestinal absorption of calcium leading to hypercalcemia, which is thought to mediate the toxic effects of vitamin D [29]. Moreover in adolescents, supplementation with 2000 IU/day for one year resulted in no side effects such as hypercalcemia and hypercalciuria [88]. Given that the amount of dietary vitamin D required to achieve optimal vitamin D levels is in the order of 1700 IU for adults [89], the UL for dietary vitamin D will likely be needed to be revised upward.

A small number of studies, in youth, have examined doses of oral vitamin D at or above the upper limit of vitamin D, set at 2000 IU by the Institute of Medicine. A study in 10-17 year old youth found that doses of vitamin D_3 up to 2000 IU / day resulted in no hypercalcemia and achieved vitamin D levels > 75

nmol / L [88]. Another study of youth in Argentina found that 18 healthy youth with a mean age of 7 years supplemented with 100 000 IU of vitamin D_2 at the beginning of winter and the same dose 3 months later reported no hypercalcemia [90] . A study amongst 30 healthy infants aged 15 day given a single dose of vitamin D_3 up to 600 000 IU reported no hypercalcemia [91].

2.7 The vitamin D receptor

The receptor for vitamin D is a complex and multifaceted protein expressed in most tissues of the body [92]. It is found predominantly in the nucleus where it binds its preferred ligand, 1,25(OH)2D, also known as calcitriol, but it also localizes to so called 'lipid rafts' on plasma membranes. With respect to its nuclear action, once calcitriol has bound the vitamin D receptor, it dimerizes with the RXR vitamin A receptor and subsequently binds vitamin D response elements. Vitamin A and vitamin D have distinct physiologic roles but both are implicated in the regulation of the cell cycle [93]. Approximately 3% of genes of the human and the mouse genome respond, directly or indirectly, to active vitamin D [94]. After the active receptor complex has bound DNA it stimulates gene transcription. Vitamin D responsive genes are primarily involved in mineral metabolism but also include genes involved in cell cycle progression [94]. The rapid (minutes and hours), non genomic actions of vitamin D were first described in 2001 [95]. As these cellular responses were too rapid to be dependent on gene transcription and protein translation, they are thought to be non-genomic. The non-genomic responses of vitamin D receptors include the rapid intestinal absorption of calcium, secretion of insulin by β-cells of the pancreas and the opening of voltage-gated $Ca2^+$ and Cl^- channels in osteoblasts. There remains speculation as to whether the vitamin D receptor is able to bind 25(OH)D in addition to 1,25(OH)2D as in adults calcium absorption from the intestine correlates better with 25(OH)D than 1,25(OH)2D [55].

2.8 Genetic ablation of the vitamin D receptor gene in vivo

The use of mice with genetic ablation of genes is a powerful tool to examine the function of those genes 'knocked-out' of the genome. In 1997 mice lacking

vitamin D receptors (VDR) were created. These mice survive gestation but exhibited abnormal mineral homeostasis, osteomalacia like bone formation and growth retardation after weaning [96]. Mice with the genetic ablation of VDRs can be rescued with diets high in calcium, phosphorus and lactose. Thus the skeletal structure of VDR null mice fed a rescue diet, resulting in normal mineral ion homeostasis, is the same as wild-type mice [97]. When not on the 'rescue diet' vitamin D receptor deficient mice have grossly normal immune systems but have a predisposition to autoimmune diseases such as type I diabetes and experimentally induced inflammatory bowel disease. Further, these mice do not have spontaneously higher levels of cancer but are predisposed to chemically induced tumours [51]. These mice also develop high renin hypertension, cardiac hypertrophy and increased thrombogenicity [51]. Mouse gene 'knock-outs' must be interpreted carefully as mouse knockout experiments do not model human physiology in the majority of rickets cases. For example, there is a very low prevalence of genetic forms of rickets in the general population [98].

2.9 Emerging role of vitamin D in human health

Recent findings allude to an expansion of vitamin D's function into a number of non-calcemic physiological systems; these include: cellular differentiation [94], immunological regulation [99] and, recently, insulin secretion in normo-glycemic [100] individuals. The responsiveness of a diverse number of tissues to vitamin D is supported by the presence of the vitamin D receptor (VDR) in a wide array of tissues [57]. We will provide a brief overview of the literature on vitamin D as it relates to a variety of physiologic systems and its implications for health.

Role of vitamin D in bone biology

It is widely accepted that the functions of calcium and vitamin D are tightly linked with respect to bone homeostasis. The synergistic effect of vitamin D and calcium is also reflected in a meta-analysis of 23 trials, with 41 419 participants aged 50 or older, with measured bone density which showed a decreased rate of bone loss in participants given calcium and vitamin D supplements [101]. With respect to youth, a 3-year prospective study of 171 peri-pubertal Finnish girls reported that baseline

vitamin D deficiency {25(OH)D ≤ 20 nmol/L} in a context of high dietary calcium intake (1575 mg/day) was associated with a decrease in bone mineral density of the lumbar spine 3 years later [9]. In addition to interactions between vitamin D and calcium, these nutrients interact with load-bearing physical activity to produce optimal bone mass in youth [64, 102].

Vitamin D and cancer prevention

While a role for sunlight in promoting mutagenesis has been established for skin malignancies, there is a hypothesis that vitamin D has anti-cancer effects at a number of tissue sites [4, 43]. Biologically, this can be explained by vitamin D's role in promoting cellular differentiation [94]. Evidence for an anti-cancer effect of vitamin D is strongest for common malignancies including: cancers of the digestive tract, breast cancer and prostate cancer. Recently, a randomized control trial of vitamin D (1100 IU) and a calcium (1400mg) supplementation study of 1179 post-menopausal women found that participants with both vitamin D and calcium supplementation had 60% reduced cancer incidence over a 4 year follow-up period [43]. These findings stand in contrast to the findings of the Women's Health Initiative (WHI) which recruited 18 176 women who were randomized to receive both 1000mg supplemental calcium and 400 IU supplemental vitamin D_3 or placebo for an average follow-up of 7 years which found no difference between those on treatment and those on placebo in the incidence of colorectal cancer [103]. Similar negative findings have been reported for invasive breast cancer incidence in the WHI [104]. It has been suggested that the negative findings of the WHI could also be explained by a lack of appropriate placebo group which had a dietary calcium intake of 1100-1200 mg/day. The negative findings with respect to cancer incidence could also be explained by lower doses of supplemental vitamin D than those used in the smaller trial of post-menopausal women D [52]. Alternatively, it is possible that vitamin D might inhibit cancer progression as opposed to cancer incidence [105]. Further adequately powered randomized control trials with sufficient doses of vitamin D and calcium are required to settle the controversy in this area.

Vitamin D and the immune system

Immune cells have been shown to carry vitamin D receptors suggesting that vitamin D may play a role in modulating immune function [106]. Consistent with this, a study of a birth cohort in Finland found that infants (n=12 055) who were provided vitamin D supplements had a lower risk of type I diabetes, a pancreatic auto-immune condition. The risk ratio was 0.12 (95% CI 0.03-0.51) comparing those who regularly took vitamin D supplements versus no supplementation, though participants were not randomized [107]. The lack of randomization opens the possibility that administration of supplements was socially patterned suggesting that a number of health promoting behaviors may be associated with supplementation in infants. Multiple sclerosis, an autoimmune condition of the nervous system, has a clear geographical distribution, with a higher prevalence at higher latitudes [108]. A role for vitamin D in multiple sclerosis is supported by work in mice which show a benefit of vitamin D on a mouse model of multiple sclerosis [108] and case-control studies in humans. In a mouse model of experimental inflammatory bowel disease vitamin D deficiency exacerbates inflammation induced by a genetic ablation of Interleukin-10, an important anti-inflammatory cytokine. Dietary calcium has an independent protective effect on experimental inflammatory bowel disease [99].

Vitamin D and metabolic homeostasis

Beginning in the early 1980s, work in isolated rat pancreatic cells demonstrated that vitamin D deficiency inhibits insulin secretion from the beta cells [109, 110]. Further in vivo work supported by observational epidemiologic studies have corroborated this finding [3, 100]. For example, a number of cross-sectional studies have shown an association between decreased concentration of 25-(OH)D and beta cell dysfunction [3], insulin resistance [111], glucose tolerance [112], the metabolic syndrome and type 2 diabetes [113]. Further, in cross-sectional studies a high prevalence of vitamin D deficiency was found in individuals with type II diabetes [114]. Adequately powered randomized trials are required to support or refute the role of vitamin D in various aspects of the metabolic syndrome.

A role for vitamin D in metabolic regulation is supported by the presence of a vitamin D response element, the binding site for the nuclear vitamin D receptor, on the transcriptional regulatory region of the insulin gene. Insulin, a central regulator of metabolism, induces the cellular uptake of glucose. Insulin resistance occurs when the actions of insulin fail to produce the entry of glucose into fat, muscle and liver tissues. This results in elevated levels of plasma glucose, triglycerides and free fatty acids. Insulin resistance is central in the pathogenesis of metabolic syndrome and greatly increases the risk of type II diabetes and cardiovascular disease [115].

Vitamin D levels have also been associated with a myriad of metabolic abnormalities. For example, a study reported negative correlations between vitamin D, triacylglycerol and LDL in individuals free of metabolic syndrome [116]. With respect to blood pressure, animal studies [117] and some limited work in humans [118], has shown that vitamin D may be implicated in the regulation of blood pressure. For example, mice genetically engineered to be vitamin D receptor deficient had a 7-fold increase in renin expression and developed hypertension and cardiac hypertrophy [119].

Much of the work on vitamin D and metabolic homeostasis has been conducted in animals or in observational studies making it difficult to establish a causal relationship between vitamin D and metabolic parameters in humans [120]. An intervention study conducted in 2008, however, found that high doses of vitamin D did not improve blood glucose levels in 33 adult men with vitamin D insufficiency {25(OH)D < 50 nmol / L}[121]. One of the potential reasons for these negative findings is that vitamin D levels must be frankly deficient before disrupting metabolic homeostasis. For example, adults in the intervention study began with a mean 25(OH)D level of 39.9 nmol / L. It is also possible that the relationship between vitamin D and metabolic outcomes is confounded by physical activity. For example, physical activity (PA) has been shown to be associated with components of the metabolic syndrome, including higher HDL, reduced blood glucose, blood pressure and lower triglycerides [122]. Another PA intervention showed that one year of moderate resistance training decreased

inflammatory markers in overweight women [123]. Inflammation is believed to play an important role in pathogenesis of cardiovascular disease and type II diabetes [124]. As PA is difficult to measure [125], and thus control for in large epidemiologic studies the possibility of residual confounding in studies examining vitamin D levels and metabolic variables is real [126].

2.10 Correlates of vitamin D status

Correlates of vitamin D levels are important to understand for two reasons. First, from an analytic perspective an understanding of the correlates is relevant to understanding possible confounding in the relationship between vitamin D levels and a myriad of health outcomes such as cardiovascular disease, type II diabetes and various common malignancies. Secondly, certain correlates of vitamin D levels like age, socio-economic status and adiposity are not as amenable to public health intervention as are correlates like dietary vitamin D intake. Thus an understanding of the correlates of vitamin D status would inform efforts to remediate vitamin D deficiency in the Canadian population.

Sunlight

There are three types of UV radiation, UVA (400-320-nm), UVB (320-280nm) and UVC (280-100nm). Vitamin D is produced in large part (90-95%) by cutaneous exposure to UV rays between 290-315 nm, the UVB range. In many northern regions the urban environment (tall buildings, air pollution) may reduce UVB exposure [20]. During summer months sunscreen use reduces 90-95% of cutaneous vitamin D synthesis [20]. Though a recent report of adults in the UK found that sunscreen use was associated with higher levels of vitamin D [127] suggesting that sunscreen use was correlated with sun exposure and that possibly coverage was not always complete. The efficacy of UV exposure in increasing vitamin D status is illustrated by individuals who use tanning beds once a week. These individuals had optimal vitamin D levels while control participants had sub-optimal levels of vitamin D [128]. Use of tanning beds, however, is associated with frequent burns [129]. Burning due to excessive UV light has been associated with skin cancer and in particular malignant melanoma [130].

The measurement of sunlight exposure

The ability to accurately quantify the sunlight exposure has been limited despite widespread interest in measuring this exposure [131]. Ambient UV levels can vary up to 30 fold depending on latitude, season, time of day, ground surface, and tree cover. Overall ambient exposure can be measured by dosimetry which measures UV and can specifically measure UVB [132]. Alternatively sunlight exposure can be assessed by using questionnaires though these can take up to 30 minutes for participants to complete and may have limited validity. For example, in an Australian study sunlight exposure assessed by questionnaire had a low correlation with vitamin D levels [63]. Questionnaires can be validated against observed exposure and UV dosimetry though neither are considered gold standards [63]. A dosimeter is a device used to measure an individual's exposure to an environmental exposure, such as UVB, over a long period of time.

Dietary vitamin D intake

Very few foods in the Canadian food supply naturally contain vitamin D. In Canada milk and margarine are fortified such that each 250 ml serving of milk contains 100 IU of vitamin D_3 [29]. There is expert consensus, however, that current levels of dietary vitamin D dietary intake are insufficient, in of themselves, to support optimal vitamin D status [89] or prevent vitamin D insufficiency [18]. For example, the vitamin D supplementation study in Finnish girls showed that participants taking 400 IU of vitamin D_2 during the winter months were not protected from vitamin D deficiency [133]. In youth, it is not known if current recommendations are adequate to prevent vitamin D deficiency in Canadians. Complicating matters is the difficulty in accurately assessing the dietary intake of vitamin D. For example, 62 % of milk samples had less than 80% of the vitamin D on the label. No vitamin D was found in 3 of the 14 skim milk samples and 70% of infant formula contained 200 percent of the vitamin D written on the label[134].

There are two major limitations inherent in the assessment of vitamin D intake of Canadian youth. The first limitation relates to the absence of vitamin D

values in the Canadian Nutrient File for certain commonly consumed foods. For example, many meat products contain small amounts of vitamin D which are not included in earlier versions of the Canadian Nutrient File [135]. Some brands of orange juice and yogurt are fortified with vitamin D and these values are also not included in the database [136]. The fortification of certain food products is a way of adding value and thus increasing the cost to the consumer [137]. Thus a national regulatory framework which allows the discretionary fortification of foods by the food industry has the potential to exacerbate existing income inequalities in nutrient and food intake in Canada [138]. Moreover, it is probable that the proposed amendments to existing food regulatory framework allowing increased food industry control over the discretionary fortification of foods would be inconsistent with public health efforts to ameliorate inadequate nutrient intakes in the Canadian population [139]. The second limitation of the Canadian Nutrient File relates to the high variability in vitamin D content of foods and the difficulty in obtaining representatives sample of foods across Canada [140]. For example, there is considerable variability in the vitamin D content of fish, even within the same species. For example, farmed salmon was shown to have 4 times less vitamin D than Alaskan wild caught salmon [141]. Efforts are ongoing in the US to acquire representative foods samples and then assay for vitamin D in these foods to update the US Department of Agriculture (USDA) Database [136]. Canada is likely to benefit from these initiatives as many values in the Canada Nutrient File are obtained from the USDA database.

Vitamin D and adiposity

Vitamin D is a fat soluble vitamin though the relationship between vitamin D levels and adiposity is unclear. For example, serum 25(OH)D has been reported to be negatively associated with adipose mass [142, 143] in some studies but not others [144]. Oral dosing studies of vitamin D also yield conflicting results. For example, a study conducted in 2001 demonstrated that upon supplementation greater adiposity in study participants was associated with lower increases in vitamin D levels [145]. Another oral dosing study of vitamin D found adipose tissue had no impact on the change in level of serum 25(OH)D [146]. There are at least two

explanations for these inconsistent findings. First as vitamin D is fat soluble [56] higher adiposity could result in greater sequestration of vitamin D from cutaneous synthesis and dietary sources in adipose tissue [145]. But there may be a larger behavioral effect where excess adiposity is associated with less (outdoor) physical activity. Consistent with this, excess adiposity has been associated with lower physical activity as assessed by an objective measure of movement, the accelerometer [147].

Vitamin D and age

Young people are able to synthesize four times as much cutaneous vitamin D from the ultraviolet radiation compared to elderly individuals (>65 years) [20]. This was determined by using skin samples taken from Caucasians of different ages. Samples were standardized in size, irradiated with UV and the synthesized vitamin D measured by high performance liquid chromatography [148]. The reasons for this age associated decrease in cutaneous synthesis of vitamin D are not known. In elderly individuals there is also a resistance to the actions of vitamin D [149]. As a result, the Food and Nutrition Board proposed a higher AI for vitamin D being 10 μg / day for 51-70 year olds and 15 μg / day for individuals >70 years old [29]. In addition a number of studies have reported that adolescence is associated with lower serum levels of this vitamin [16, 150]. This decrease in vitamin D levels during adolescence might be related to well characterized declines in physical activity, and hence outdoor activity, across adolescents [151].

Vitamin D, latitude and season

At latitudes higher than 35^0, cutaneous synthesis of vitamin D is limited to April through October as UVB is of insufficient intensity to catalyze UVB production. Further, the refractory period for cutaneous vitamin D synthesis is longer at higher latitudes [7]. Canadians reside north of the 35^0 placing them at risk for hypovitaminosis D during the winter months, reinforcing the need for latitude specific data for vitamin D status. Low vitamin D levels are also reported in sunnier climates of lower latitudes. For example, Turkish youth who wore concealing clothing reported vitamin D deficiency, despite a sunny climate [152].

Vitamin D and calcium intake

There is some evidence to suggest that low dietary calcium can result in decreased vitamin D status. This is thought to act through an increased conversion rate of 25(OH)D into 1,25(OH)D. Much of this evidence comes from animal models and the evidence for this, in humans, is limited [55].

Vitamin D and socio-economic status

Low socio-economic status has been associated with low vitamin D status in a number of studies in both youth [150, 153] and adults [154]. This could occur through decreased sun exposure and/or decreased consumption of vitamin D containing food or supplements. Consistent with this, a study of White British adults supplement use was higher in individuals of high socio-economic position [154]. Sunscreen use was also more frequently used in individuals of higher socio-economic position. Interpreting time spent outdoors is difficult in this population as sunscreen blocks the vast majority of the vitamin D generating UVB [155]. A study conducted amongst youth in Philadelphia (40^0N) found that parental education and income were associated with vitamin D status in univariate analysis. These variables, however, were not included in the final model thus they could have been confounded by season or some other exposures [150]. A number of studies showed that, in youth, vitamin D and dietary calcium intake are patterned by socio-economic position though this effect varies by study population [156, 157].

Vitamin D, physical activity and sedentary behavior

Associations between vitamin D status and physical activity, seen in a number of studies in adults [158-161] suggest that (outdoor) physical activity is an important contributor to vitamin D levels. This association is likely mediated through UVB exposure on the cutaneous production of vitamin D. In NHANES III outdoor physical activity, measured by the frequency of leisure activity in the last month, was associated with higher vitamin D status in 23 258 participants between the ages of 20 to greater than 60 years of age. Moreover, higher levels of moderate, but not vigorous, physical activity was associated with higher vitamin D status. As most individuals spend little time in vigorous activity the association with

moderate activity may reflect greater time spent outdoors. Intensity of physical activity was assessed by assigning metabolic equivalents to each different physical activity and then summing activities [158].

Physical activity is difficult to measure in large epidemiologic studies. Moreover, the measurement of leisure physical activity does not integrate information on activities that may be embedded within daily routines. For example it was shown that leisure physical activity is more prevalent in higher socio-economic groups while non-leisure activity more prevalent in low socio-economic groups [162]. Measuring physical activity by questionnaire is difficult, resulting in either over or under-estimation of energy expenditure by physical activity depending on the physical activity questionnaire [163]. The cost of using objective methods of measuring physical activity, such as doubly labeled water and accelerometers in large scale epidemiologic studies can be prohibitive [164]. Accelerometers, however, are becoming increasingly affordable and being used in larger studies.

There are few reports examining the relationship between vitamin D status and sedentary behavior. A report from a population based sample of UK adults found that time spent watching TV or using a computer was associated with lower vitamin D status [154]. This association, however, is likely mediated by a lack of outdoor sun exposure [155]. There are competing hypothesis about the relationship between physical activity and sedentary behavior. Some authors suggest that sedentary behavior displaces physical activity [165, 166], while other authors point to a lack of correlation between physical activity and sedentary behavior [167, 168]. Thus it is probable that sedentary behavior will be inconsistently associated with vitamin D levels.

2.11 Limitations of epidemiologic studies

Observational and experimental epidemiologic studies have a number of important limitations. For example, one of the principal limitations of observational studies is the difficulty in measuring confounders between a nutrient and a health outcome of interest. Another limitation relates to the

exclusion of confounders in regression, such as socio-economic status, which can be associated with the nutritional exposure and outcome [169]. These limitations have produced associations in observational studies which were not repeated when these nutrients and minerals, administered in the form of supplements, were the subject of well conducted randomized trials. This has been the case for a number of nutrients including: β-Carotene and vitamin A in lung cancer [170], vitamin C and cardiovascular disease [171], selenium and cancer [172], to name a few. Randomized trials are difficult in nutrition as they are expensive, require sufficient follow-up time and participants may not be blinded to their treatment. Moreover, food is a complex mixture of chemicals and food consumption patterns are embedded in complex social structures, all of which makes it difficult to ascribe a biologic effect to a single nutrient. Single nutrients are often the subject of randomized trials [173]. The problem of reducing a complex biologic exposure into a single nutrient is analogous to sunlight which is also a complex exposure. For example, lack of UV exposure is thought to cause seasonal affective disorder [174]. Further, UVB exposure is associated with serotonin production in the skin [175, 176] and may also have cutaneous immunomodulatory effects [177]. Finally, fairer weather is associated with higher levels of outdoor physical activity [178]. It is thus difficult to isolate the effect of vitamin D from that of sunlight in observational studies as the greatest source of vitamin D is UVB from sunlight and sunlight is notoriously difficult to measure [63].

2.12 Are vitamin D levels a marker of healthy living choices?

Vitamin D levels may be a marker of healthy living choices such as participation in physical activity and healthy dietary choices. These exposures are difficult to measure in epidemiologic studies. For example, as previously mentioned, vitamin D levels have been associated with physical activity [158] and higher socio-economic status [150, 154, 179]. Both of these exposures are also associated with a myriad of health benefits [180, 181]. Physical activity, for example, has been associated with decreased blood pressure in normotensive and hypertensive persons independent of adiposity [182]. Physical activity has also been shown to

have protective effects with respect to colorectal cancer, breast cancer, insulin resistance and type II diabetes [181]. Physical activity is difficult to measure in larger epidemiologic studies [183] and self-reported questionnaires, the most commonly used method to assess PA, have limited validity [183]. Thus studies between vitamin D and health outcomes such as type II diabetes [114], colorectal and breast cancer [4] may be confounded if PA is not measured accurately. As previously stated, vitamin D levels are associated with socio-economic status in a number of different study populations. It has long been recognized that lower socio-economic status is associated with a higher prevalence of obesity, a higher incidence of cardiovascular disease and shorter life expectancy [184]. Higher chronic disease morbidity and mortality amongst those with lower socio-economic position are believed to be explained, in part, by a higher prevalence of smoking, binge drinking, lower dietary quality and a higher prevalence of obesity in some settings [180, 185]. In a number of studies lower vitamin D levels have been associated with socio-economic position [150, 154]. This opens the possibility for confounding when observational studies examining vitamin D levels in relation to a number of health outcomes do not control for socio-economic status thus producing spurious results [169, 186]. Novel methods such as Mendelian randomization and genome wide association studies are needed to produce non-confounded estimates of the effect of low vitamin D levels on numerous health outcomes.

2.13 Randomized trials and future research avenues

The health effects of vitamin D have been studied in a number of randomized controlled trials. Some of the findings of these trials have provoked controversy [187] and/or criticism of the trial design [55]. A randomized controlled trial (RCT) is defined by John Last's Dictionary of Epidemiology as: 'An epidemiologic experiment in which subjects in a population are randomly allocated into groups to receive or not receive an experimental intervention' [11]. I will examine three such examples of randomized trials in greater detail to illustrate the complexities of studying vitamin D in randomized trials.

A double blind placebo trial of 1179 community-dwelling postmenopausal women (> 55 years of age) tested the hypothesis that calcium and vitamin D, alone and together, could reduce the incidence of cancer. The trial had three arms a placebo arm, a calcium arm (1400-1500 mg of calcium) and a calcium and vitamin D arm (27.5 μg vitamin D_3). Participants were followed up for 4 years. The unadjusted relative risk of incident cancer was 0.40 (p= 0.01) and 0.53 (p = 0.06) for the calcium and vitamin D and calcium only arm, respectively [43]. While the randomized trial showed impressive decreases in cancer incidence the number of incident cancer cases was small, 20 in the placebo group and 13 in the vitamin D and calcium group. Moreover, the most common cancer in all 3 groups was breast cancer, showing reductions with calcium and vitamin D treatment which were not consistent with the findings of the Women's Health Initiative (WHI) which had a much larger sample size [104].

The WHI was the largest randomized trial in history, costing the US government an estimated 700 million dollars. The trial was designed to provide definitive answers regarding the health benefits of calcium and vitamin D supplementation in women [188]. The WHI randomized 36 282 postmenopausal women aged 18 176 women aged 50-79 years of age to 1100 mg/day of supplemental calcium as calcium carbonate and 10 μg /day of supplemental vitamin D_3 or placebo. Participants were followed up an average of 7 years and the primary outcome was risk of fracture. This large study also examined outcomes related to vitamin D and health. For example no association was found between calcium, vitamin D supplementation and breast cancer incidence [104]. Similarly no significant associations were observed between calcium, vitamin D supplementation and incidence of colorectal cancer [103] and risk of hip fractures [189]. A number of explanations have been advanced to account for these negative findings which stand in contrast to the observational studies of these outcomes [4]. Reasons for this possible discrepancy include an inadequate dose of vitamin D and calcium and the long latency associated with most chronic disease relative to the length of the trial [55].

A meta-analysis of 57 311 participants examined the effect of vitamin D_2 or vitamin D_3 supplementation in randomized trials on all cause mortality. Daily dose of vitamin D supplements varied from 7.5 μg to 50 μg. The mean age of participants in this study was not provided but the majority of these trials were conducted in the elderly. This study found that the intake of vitamin D supplementation was associated with a relative risk of 0.93 (95% CI 0.87-0.99) for all cause mortality [190]. The baseline vitamin D status of these participants was not known but it is possible that there was a high prevalence of vitamin D deficiency which is commonly reported in the elderly [191]. Thus supplementation with vitamin D may correct vitamin D deficiency which has been associated with osteomalacia and osteoporosis in adults [5] which may increase the probability of falls, hip fracture and the ensuing health sequelae.

Despite the clear advantages of RCTs, they face a number of limitations. Well conducted randomized controlled trials are expensive and require many years of follow-up to accrue sufficient health events to adequately power a study. The question of interest is thus, whether or not a lifetime of greater exposure to, for example vitamin D, results in improved health outcomes. It is impractical to conduct an RCT over the entire lifecourse. RCTs may also have limited generalizability as inference from findings may not extend to groups beyond those included in the study. For example, it is uncertain if the findings of the WHI would apply to men. Novel methods are needed to produce non-confounded estimates of the effect of low vitamin D levels on health. An example of one of these methods is a disciplinary mélange of functional genetics, population genetics and epidemiology.

Mendelian randomization

Mendelian randomization is defined as the use of genetic variants in observational studies, which either alter the level of, or mirror the biologic effects of, a modifiable exposure which in itself alters disease risk [192]. Genetic variants can be chosen which are associated with the metabolism of an environmental exposure. For example, alcohol is oxidized to acetaldehyde which in turn, is oxidized by

aldehyde dehydrogenase (ALDH) to acetate. Acetaldehyde is toxic. For example half of the Japanese population is heterozygote or homozygote for a null variant of the aldehyde dehydrogenase gene (ALDH2*2) and blood acetaldehyde concentrations are 5 and 18 times higher than those with functional aldehyde dehydrogenase genes after the consumption of alcohol, respectively. The higher acetaldehyde concentrations cause facial flushing, palpitations, drowsiness and other symptoms which make drinking unpleasant. Thus heterozygote or homozygotes drink less alcohol than individuals with two functional variants of the aldehyde dehydrogenase gene [192].

In observational studies alcohol consumption is confounded by lifestyle factors such as cigarette smoking, poor diet and other lifestyle factors. These covariates are difficult to measure and thus difficult to control for in observational studies. This makes it difficult to discern the independent effect of alcohol on intermediate health indicators like high density lipoprotein (HDL) [193]. In the case of the ALDH, there is a dose-response relationship between the functionality of ALDH variants and HDL such that heterozygotes have intermediate levels of alcohol consumption and HDL and those with two functional alleles, mimicking higher alcohol consumption, have higher HDL [194]. The distribution of these genetic variants in populations is uncorrelated with lifestyle factors which are difficult to control for in epidemiologic studies.

Other genetics variants suitable for nutrition epidemiology have been found and used in Mendelian randomization studies. For example the GSTM1 and the GSTTT1 are two genes associated with the metabolism of isothiocyanates a phytochemical found in cruciferous vegetables [195]. Another example is the gene encoding the cytochrome P450 1A2 gene in the liver which has two variants which metabolizes caffeine slowly or rapidly [196]. With respect to vitamin D, no clear functional variants of the vitamin D metabolizing genes or the vitamin D receptor have yet been found [197]. There are, however, three adjacent restriction fragment length polymorphisms near the 3′ end of the vitamin D receptor (VDR) gene which are most likely non-functional but maybe in linkage disequilibrium with functional variants elsewhere in the VDR gene [197]. It has been difficult to

find genes involved in vitamin D metabolism which could mimic low vitamin D levels to be used for Mendelian randomization studies [192]. Genome wide association studies may be needed to identify novel genes associated with vitamin D metabolism [198]. These genes could then be used in Mendelian randomization studies to establish the unconfounded effects of vitamin D levels on health outcomes varying from heart disease to common malignancies.

Limitations of Mendelian randomization

There are a number of limitations associated with the use of Mendelian randomization. First, as the effect of gene instruments is small, large sample sizes are required to discern the effects of the genetic instruments. Another limitation relates to the difficulty in finding an appropriate genetic variant. These genetic variants need to have well described functional effects and must not be in linkage disequilibrium with genes which will influence the health outcome of interest.

2.14 Conclusion

The preceding introduction provided a critical overview of the literature on vitamin D and health and reviewed what few studies were available on the vitamin D status in Canadian youth. We showed that the most well established role of vitamin D was in the absorption of dietary calcium. As a result of the functional inter-relationship between these two nutrients, the health consequences of low calcium intake or vitamin D levels are linked. For example, vitamin D deficiency has been implicated in osteomalacia and rickets amongst infants. Cases of rickets also occur in tropical countries and these cases can be treated with the administration of dietary calcium [65, 69]. More recently, there has been the recognition that vitamin D may be involved in a broad number of non-calcemic physiologic systems. These were reviewed and include immunologic function with implications for autoimmune disease such as type I diabetes and multiple sclerosis [99]. There also been some observational studies [199] and some limited evidence from randomized trials [43] suggesting that optimal vitamin D levels may be implicated in the prevention of common malignancies such as: breast, colon and prostate.

Canada‘s high latitude, and thus long winters, together with vitamin D’s involvement in a wide range of physiologic systems, highlights the need to describe the vitamin D status of Canadian youth. Prior to this thesis, there were no population based samples of Canadian youth from which to assess vitamin D status. The only study that examined the vitamin D status of Canadian youth showed considerable levels of vitamin D deficiency, though the study was a clinic based convenience sample [16].

Finally, in this literature review we discussed methodologies used in studies of vitamin D and health and the limitations of findings and the implications of residual confounding in observational studies. Finally the use of a novel method, Mendelian randomization, was discussed as was the need to find genetic variants which mimic life-long exposure to lower vitamin D status using genome wide association studies.

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CHAPTER 3: OBJECTIVES AND METHODS

This section outlines the objectives and methods used in this thesis.

The specific objectives are:

1) To document the vitamin D status of Québec youth using a population representative sample of Québec children aged 9, 13 and 16.

2) To examine the respective contributions of diet, physical activity and fat mass to explain the variance in serum vitamin D status in youth at high risk of obesity.

3) To examine dietary vitamin D, calcium and dairy foods intake in relation to household income and food security status in a representative sample of Canadian youth.

In order to address these three objectives three different databases were used. The databases are: the Québec Child and Adolescent Health and Social Survey (QCAHS), The Québec Adipose and Lifestyle InvesTigation in Youth (QUALITY) study and finally the Canadian Community Health Survey (CCHS) cycle 2.2.

3.1 Québec Child and Adolescent Health and Social Survey

Objective 1: Document the plasma vitamin D status of Québec youth using a representative sample of Québec children aged 9, 13 and 16.

Study population

This study population comprised a probability sample of Québec 9, 13 and 16 year olds who participated in the Québec Child and Adolescent Health and Social Survey, conducted in schools between January and May 1999. Three (one per age group), independent, stratified, cluster random samples were drawn from the Québec Ministry of Education list of children attending public or private schools. Schools in the most remote regions of Québec, federal government and aboriginal schools, those in which >50% of students had severe handicaps, and those with

<12 eligible students were excluded. This sampling frame represented 97% of youth of the targeted ages. The survey comprised a youth questionnaire, clinical measurements, a parent questionnaire and a questionnaire for school principals. Two to three weeks before data collection, an explanatory letter, a consent form and a parent questionnaire were mailed to subjects' homes with letters of support from the principal and parents' committee. All other survey procedures were conducted on school premises in a room designated for this purpose.

Biochemical measurements

After an overnight fast, venous blood was collected between 0800h and 1000h in a 1 g/L EDTA collection tube, and placed on ice. Samples were centrifuged on site within 45 minutes of collection, transported on dry ice and stored at –80oC. 25(OH)D mostly bound to vitamin D binding protein and albumin is stable under those conditions. 25-hydroxyvitamin D was measured in duplicate by a radio-immunoassay following extraction in dichloromethane (IDS Laboratories, UK). The inter assay coefficient of variation was 5.9% nmol / L at 30.6 nmol / L and 6.0 % at 115.1 nmol / L. The laboratory participates in the international External Quality Assessment Scheme (EQAS) on vitamin D metabolites and meets the performance target set by the Advisory Panel for Data Analysis. Two assays of 25(OH)D, for each participant, were averaged and this value was considered the true serum 25(OH)D value. When the two results were more discrepant than 3 nmol /L the sample was repeated.

Statistical analysis

Objective 1: Age and sex specific Z-scores for BMI were computed using a SAS program developed by the CDC based on the 2000 US CDC growth charts [1]. To take the complex study design into account, sampling weights and clustering effects by school were estimated and incorporated into computations of prevalence, percentile values and 95% confidence intervals. We used the non-parametric method developed by Hutson [2] to estimate percentiles and their confidence intervals. Because of the low observed frequencies we calculated exact binomial 95% confidence intervals [3] for the prevalence of the three cut-offs

for vitamin D. Differences in prevalence between sex and age groups were tested using a likelihood ratio test in a generalized linear logistic model to take the clustering effect within schools into account. Generalized linear models are a flexible generalization of ordinary least squares regression. Generalized linear models incorporate other statistical models including linear, logistic and Poisson regression into a single framework [4]. The associations between mean 25(OH)D, sex and age were tested in univariate regression analyses. We used hierarchical regression models to estimate regression coefficients for univariate and multivariate associations. Hierarchal regression (multi-level analysis) allows variance to be analyzed at various levels. For example, we found significant clustering of 25(OH)D variance at the school level that was taken into account with the hierarchal model. Explanatory variables were treated as fixed effects and clustering between subjects in the same school was treated as a random effect. Analyses were stratified by sex because of a significant age-sex interaction ($p < 0.0001$) in a model predicting vitamin D status which included sex, age, BMI Z score, month of blood draw, parental income and location of residence. Statistical analyses were performed with SAS version 9.1 (SAS Institute, Inc., Cary, NC).

3.2 The Québec Adipose and Lifestyle InvesTigation in Youth (QUALITY)

Objective 2: To examine the respective contributions of diet, physical activity and % of total mass as fat mass to explaining the variance in serum vitamin D status in youth.

Study sample

A recruitment goal of 600 youth at risk of obesity was set. We examined the first 159 youth for which measurement of vitamin D was analysed for this purpose. To be eligible, the child had to be between 8 and 10 years of age at inclusion, and both biological parents had to be available for the study. Further, at least one of the biological parents had to be obese, which was defined using a standard definition of having either a body mass index of ≥ 30 kg/m^2 (according to reported height and weight) or having a reported waist circumference of greater than 102 cm in men or greater than 88 cm in women. Finally, families had to be

Caucasians of Western-European ancestry. Families were not eligible to participate if one of the parents had a type of diabetes other than type-2, the mother was pregnant or lactating at the baseline evaluation, or if the family had plans to leave the province of Québec in the near future. Exclusion criteria for the child under study included having been diagnosed with diabetes (types 1 or 2), having a serious illness that prevents participation at some or all of the tests, taking anti-hypertensives or steroids (except if administered topically or through inhalation), being on a very restrictive diet (less than 600 kcal/day), or having serious psychological or cognitive problems that would limit participation in the study.

Families were recruited through flyers sent to primary schools situated within 75 km of Montréal, Québec City and Sherbrooke. The flyers informed parents of children in grades 3 to 5 about the study, its objectives and the eligibility criteria. Families interested in participating, and who thought that they might be eligible to participate, were invited to contact the research coordinator for more information and to confirm eligibility. Once eligibility was confirmed, and consent obtained, families were given an appointment for baseline data collection and are subsequently followed for follow-up two years later. Measurements were undertaken at the clinical research units of CHU Sainte Justine (Montréal) and Hôpital Laval (Québec City). Families at high risk of obesity were chosen to ensure a sufficient number of children would present with or develop overweight / obesity during the period beginning with the ending of childhood and the follow-up period concluding at the end of adolescence. Recruitment into the study was limited to Caucasian families to reduce genetic admixture of populations.

Anthropometry and DXA

Height, weight, waist and hip circumferences were measured according to standardized protocols. BMI was computed as weight (kg) / height (m^2). Sexual maturity stage of children was scored, by visual inspection, according to Tanner [5]. Determination of percentage of body fat was done using dual energy x-ray

absorptiometry (DXA) (Prodigy Bone Densitometer System, DF+14664, GE Lunar Corporation).

Measurement of physical activity in epidemiologic studies

We will briefly review the different methods used to measure physical activity to contextualize the significance of the objectively measured PA in the QUALITY study. One of the principal limitations of epidemiologic studies is the difficulty in accurately measuring both independent and dependent variables.

Physical activity is defined as muscular movement which results in energy expenditure. It has 3 principle dimensions: frequency, intensity and duration. PA is a complex exposure and is difficult to measure, particularly in youth [6]. This is complicated further as there is no gold standard to measure physical activity. For example, doubly labelled water can provide activity based energy expenditure over a 2 to 3 week period in free-living individuals. It is, however, expensive thus limiting its use in larger studies making it more difficult to perform validation studies for PA questionnaires and accelerometers. Measurement of PA is made more difficult in youth due to the unstructured nature of PA in youth and cognitive difficulties associated with the recall of complex behaviors [7]. I will briefly describe three methods to measure physical activity, this, however, is not a comprehensive review of measurement methods for physical activity.

Doubly Labeled Water: Doubly labeled water is the closest technique to a gold standard to measure physical activity in free-living individuals. It measures energy expenditure over a two three- week period. Individuals are administered isotopes of oxygen and hydrogen which equilibrate in the body fluids and CO_2 is collected 2-3 weeks later through inhalation. Measured CO2 can be converted to energy expenditure, using equations and assumptions about hydration level. The measurement of energy expenditure is conceptually different to the measurement of physical activity which is defined as the muscle movement which causes energy expenditure. Activity based energy expenditure, however can be calculated from doubly-labeled water by calculating the resting metabolic rate and the thermogenic effect of food, the energy associated with food digestion. The

limitations of this approach to measure physical activity are that it is very expensive. It is thus not a suitable approach to measure PA in large scale epidemiologic studies.

Accelerometer: An accelerometer is a device which measures acceleration. Accelerometers to measure PA are small devices which can be worn at the hip in free-living individuals. Some accelerometers measure acceleration in one plane, others can measure acceleration in three planes, though this does not result in greatly enhanced validity [8]. Accelerometers do not capture the context of physical activity, for example it does not indicate what activities are being performed to generate the accelerometer data. Further, they are more valid when measuring walking and running than activities such as house and yard work and golfing. These devices do not capture upper body movement, load bearing and movement up an incline [8].

Questionnaire: Physical activity questionnaires are the most frequently used method for measuring PA, particularly in epidemiologic studies. Most PA questionnaires are self-administered. PA questionnaires are able to capture structured PA and the context within which activities occur. This is problematic when measuring the PA of youth, where much of the activity is unstructured [9]. PA questionnaires have been used to generate information regarding the intensity of PA by assigning a metabolic equivalent to a particular activity and then summing the duration of activities conducted with different intensities. The use of metabolic equivalent, however, assumes that youth are conducting activities at the assigned level of intensity and that the duration of the activity has been accurately measured [10, 11].

Physical activity assessment in the QUALITY study

The QUALITY study measured physical activity using accelerometers and questionnaires. For objectively assessed PA, children wore a uniaxial Actigraph LS 7164 activity monitor (Actigraph LLC, Pensacola, Florida) for a 7-day period following the clinic visit. Data were downloaded in one-minute intervals. Days were excluded when the accelerometer was worn for less than 80% of the average

time worn on the other days. No modifications were made to the data to account for children who indicated they had gone swimming or cycling on any of the seven days. A total of 140 children (58.5% boys, aged 8-11 years) provided accelerometer data. Overall, 89% of youth had greater than 4 days of accelerometer reporting, a number of days shown to ensure adequate reliability [12]. The mean daily accelerometer time worn was 13.6 hours for both sexes. 74 % of the sample reported no cycling or swimming during the evaluation week, activities which are not accurately represented by the accelerometer.

Dietary assessment

Children's dietary assessment was based on three non-consecutive 24-hour dietary recalls completed on different days of the week including one weekend day. A dietician obtained recalls within six weeks of the clinic visit. In order to minimize the burden to families, recalls were conducted by telephone. Each subject received a small disposable kit of food portion models prior to the interview. At the clinic visit the child was given a short training session on the use of the graduated cup, bowl etc. Telephone interviews for the 24-hour dietary recalls have been validated against dietician administered 24 hour recalls administered in person with good results in youth [13]. Cooking details for meals cooked at home were obtained from parents. Food groups were created for dairy, fruit and vegetable servings using the Canadian Food Guide [14]. A food group was created for sweetened drinks and the portion size used was based on a 250 mL serving size. We also collected information on the use of all dietary supplements including the drug identification number. The nutrient analysis of the food and supplement data was completed using the CANDAT software (Godin London Incorporated, London, Ontario, Canada).

Lifestyle and Health Questionnaires

A questionnaire administered to the child, by a research assistant, collected information on child lifestyle behaviors and general health. A self-administered questionnaire for parents collected information on socio-demographic

characteristics, child medical history, parental lifestyle behaviors, parental health and health of other family members.

Biochemical measurements

After an overnight fast, venous blood was collected between 0800h and 0900h in a 1 g/L EDTA collection tube, and placed on ice. Samples were centrifuged within 15 minutes of collection, and stored at –80oC until use. 25-OH vitamin D was measured as described in methods for section 1.

Statistical analysis

25(OH)D were not normally distributed in the QUALITY study and was natural log transformed in a sensitivity analysis to test the robustness of the findings of the regression. Generalized linear regression was used with 25(OH)D as the dependent variable. Independent variables included: % body fat, physical activity measured by accelerometers, dietary intake of vitamin D including supplements, sex and age. Vitamin D containing foods and supplements were tested for their contributions to serum 25(OH)D status in a second model.

3.3 The Canadian Community Health Survey Cycle 2.2

Objective 3: To examine dietary vitamin D, calcium and dairy product intake in relation to income and household food insecurity.

Study Sample

The survey targets respondents from all age groups living in private occupied dwellings in the ten provinces. Excluded from the sampling frame were residents of the three territories, persons living on Indian reserves or Crown lands, persons living in institutions, full-time members of the Canadian Forces and residents of some remote regions. The sample of 35,000 individuals was selected from four different frames: the Labour Force Survey (LFS) area frame, a list of CCHS 2.1 dwellings, the PEI and Manitoba Healthcare registries. The use of more than one frame was necessary to ensure the minimum number of 80 individuals required for each Dietary Reference Intake (DRI) group per province. We examined youth

who were 9-18 years old and excluded girls who were pregnant or breastfeeding bringing our final sample to 8938.

Interviewers received 3.5 days of training before going out into the field. Data collection began in 2004, spanning the entire year in order to eliminate possible seasonal effects and to spread out the interviewer workload in the field. The average length of interview was 60 minutes including the 24-hour dietary recall module (30 minutes). The majority of interviews were conducted in person and the 24 hour recall was done using a computer-assisted interviewing method. Measured height and weight in all respondents aged 2 and older were collected at the end of the interview.

The validity of dietary intake in youth

Under-reporting of energy intake is common in dietary surveys and has been associated with obesity and socio-economic position [15]. Basal metabolic rate was calculated for youth participants using equations provided by the Institute of Medicine [16]. Using a measure of leisure physical activity in the past 3 months we classified individuals into active, moderate or inactive. Estimated energy expenditure was calculated using the basal metabolic rate and the leisure physical activity score [16]. This, in turn, was compared to the total energy intake. We conducted a sensitivity analysis to examine if individuals with implausible energy intakes modified trends in the data [17].

Statistical analysis

As the 24 recall methodology is only appropriate for assessing the intake of foods or nutrients for groups owing to the day-to-day variability within individuals, data were analyzed by groups which were quartiles of income taking into account household size. For example the third quartile of income was defined as a household income of 40 – 80 thousand dollars / year with 3 or 4 / per members in the household. Income groups, food insecurity, age and sex were used as stratification variables. In order to examine the effect of low income and food insecurity together as a measure of what is more likely persistent food insecurity, we excluded youth who came from households in the upper two quartiles of

income in the food insecure group. We chose to analyze dairy servings, fruits and vegetables, sweetened drinks, dietary fiber, calcium, vitamin D and saturated fat. These foods and nutrients were chosen for their established role in preventing or promoting disease [18, 19]. Descriptive statistics and comparisons of means and proportions were undertaken to examine pre-defined risk groups (food insecure/secure, quartile of income by age-sex group and analysis of variance was done to compare means). The role of supplements in improving nutrient adequacy was examined in addition to food sources; however the methodologies to accurately adjust for within subject variability in supplement intake have not been developed.

Quality Assurance / Quality Control

The QCAHS, QUALITY and the CCHS studies used standardized, high quality data acquisition methods. These methods include: 1) develop detailed protocols that clearly describes all data collection procedures; 2) training study personnel centrally; 3) regularly monitoring of performance of the personnel using meaningful criteria; and 4) determine standards for unacceptable levels of measurement quality and implement corrective action as appropriate.

3.4 Ethics

The QCAHS study

At the planning phase of the QCAHS studies permission was obtained to store residual samples from the Direction de Sante Québec and CHU Sainte-Justine Ethics Review Boards. Two to three weeks before data collection, an explanatory letter, a consent form and a parent questionnaire were mailed to subjects' homes with letters of support from the principal and parents' committee. Specific and explicit written consent was obtained from parents (or guardians) and assent was obtained from each youth. All data were stored anonymously. Individuals cannot be traced because the list of names has already been destroyed as specified by the Commission d'accès à l'information in the case of QCAHS.

The QUALITY study

The QUALITY study was designed to assess a broad array of health indicators in youth at high risk of obesity. Study participants were given an information package and given a number of days to reflect on the information before agreeing to participate in the study. Specific and explicit written consent was obtained from parents (or guardians) and assent was obtained from youth.

All data were stored anonymously. A toll free number was made available to families for questions pertaining to their participation in the study. All information on participants was stored in locked filing cabinets. The QUALTY study has been reviewed and approved by the Ethics Review Board of CHU Sainte-Justine approved this sub-study.

CCHS study

Statistics Canada followed federal government and Statistics Canada policies to ensure the privacy and confidentiality of respondents of the CCHS. The CCHS was also guided by the Policy on Informing Survey Respondents by telling respondents, before and at the time of data collection, that their participation was voluntary. Data was stored anonymously. Access to the Statistics Canada data center required a number of personal security checks. Prior to the disclosure of data for use in publication, a Statistics Canada employee verified the data to ensure that no less than 5 respondents were in each cell to protect the confidentiality of participant information.

3.5 References

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CHAPTER 4: LOW VITAMIN D STATUS IN A REPRESENTATIVE SAMPLE OF QUÉBEC YOUTH

Sean Mark [1], Katherine Gray-Donald [1 2], Edgard E Delvin [3], Jennifer O'Loughlin [4], Gilles Paradis [2], Emile Levy [5], Marie Lambert [6]

Department of Dietetics and Human Nutrition [1] and Department of Epidemiology, Biostatistics and Occupational Health [2], McGill University, Montréal, Qc, Canada.

Departments of Clinical Biochemistry [3], Nutrition [5] and Pediatrics [6], CHU Sainte.-Justine and Université de Montréal and Department of Social and Preventive Medicine [4], Université de Montréal, Montréal, Qc, Canada

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Keywords: Vitamin D status, 25-hydroxyvitamin D, deficiency, children, adolescents.

List of abbreviations: 25(OH)D, 25-hydroxyvitamin D; PTH, parathyroid hormone; BMI, body mass index; ns, not significant.

Corresponding author and reprint request: Marie Lambert, MD, Medical Genetics Service, CHU Sainte-Justine, 3175 Côte-Sainte-Catherine, Montréal, Québec, Canada, H3T 1C5. Telephone: 514-345-4736, Fax: 514-345-4766, E-Mail: marie.lambert@umontreal.ca

4.1 Abstract

Background: Adequate vitamin D status is important for bone growth and mineralization and has been implicated in the regulation of autoimmunity, metabolic function and cancer prevention. There are no reports of population-based studies on the vitamin D status of Canadian youth, a population where vitamin D is fortified in staple foods.

Methods: We measured plasma 25-hydroxyvitamin D, the best indicator of vitamin D status, in a school-based cross-sectional sample representative of Québec French Canadian youth (n=1753) aged 9, 13 and 16 years. Blood samples were collected from January to May 1999. We defined the threshold for vitamin D deficiency as ≤ 27.5 nmol/L, hypovitaminosis as ≤ 37.5 nmol/L and optimal as > 75.0 nmol/L.

Results: More than 93% of youth in each age and sex group had sub-optimal vitamin D concentrations. The prevalence of vitamin D deficiency increased with age in both sexes ($p < 0.0001$). It was 2%, 3% and 13% in 9, 13 and 16-year-old boys and 2%, 8% and 10% in 9, 13 and 16-year-old girls. Girls with higher body mass index and girls from households with lower income had lower vitamin D concentrations. These effects were not observed in boys.

Conclusions: Inadequate vitamin D status is a potentially serious public health problem among Québec youth. Youth living at high latitudes in countries with no fortification of vitamin D are also, likely, at heightened risk of vitamin D deficiency. These results call for renewed efforts to assure adequate vitamin D concentrations among growing children and adolescents.

4.2 Introduction

Vitamin D facilitates the intestinal absorption of calcium and phosphorus and plays an important role in bone mineralization [1]. It follows that maintaining optimal levels of vitamin D concentrations is particularly important during the growth period in children and adolescents, when much of adult bone mass is established [2]. Vitamin D is believed to exert physiological effects beyond the skeletal system [3]. Indeed, the identification of vitamin D-specific nuclear receptors which modulate the expression of a variety of genes [4] in a number of tissues indicates that this hormone plays a role in several physiological processes including cancer prevention [5], immune regulation [6] and glucose homeostasis [7].

Ultraviolet activation of 7-dehydrocholesterol in the epidermis is the predominant source of cholecalciferol or vitamin D_3 [8]. This secosteroid is transported to the liver where it is hydroxylated to yield 25-hydroxyvitamin D_3 {25(OH)D}. When dietary calcium intake is low, the parathyroid glands respond to minute decreases in ionized serum calcium by releasing parathyroid hormone (PTH). PTH in turn, regulates the final hydroxylation of vitamin D in the renal mitochondria yielding the biologically active hormone, 1-alpha, 25-dihydroxycalciferol [9]. Active vitamin D has three target tissues relevant to the calcium metabolism. First, active vitamin D initiates the break down of bone tissue, releasing calcium into the serum, second, it increases the absorption of dietary calcium in the gut, finally, active vitamin D increases the reabsorption of calcium in the distal tubule of the kidney [3].

At high latitudes cutaneous vitamin D synthesis is season-dependent and from approximately October to March supplements or fortified foods are the only widely available sources [10]. The 1997 Institute of Medicine guidelines for dietary intake of vitamin D were meant to prevent the seasonal increase in PTH, associated with lower vitamin D status, assuming little or no cutaneous production of vitamin D [11]. Elevated plasma concentrations of PTH are a marker of bone remodeling; where consistently elevated levels are associated with an increase in risk for osteoporosis in later life [12] .

A number of studies have documented vitamin D deficiency and hypovitaminosis from populations inhabiting a wide range of latitudes, including: male adolescents in Paris [13], adolescents in Maine [14], inner city adolescent girls in Manchester UK [15], school-aged youth from Lebanon [16] and adolescent girls wearing concealing clothing in Turkey [17]. Despite this, there are very few countries with fortification of vitamin D in staple foods. Mandatory fortification does exist, however, in the US, Canada and Finland, where staple foods, such as fluid milk, are fortified and have been shown to increase vitamin D intake consistent with dietary intake guidelines proposed by the Institute of Medicine [18]. Since fortification, however, an expert consensus amongst vitamin D researchers have stated that current levels of food fortification are inadequate to support optimal vitamin D status in adults [19]. It is not known, in older youth, the effectiveness of fortification in the mitigation of vitamin D deficiency or supporting optimal vitamin D status. Further, there are no reports of large population-based studies on the vitamin D status of youth at high latitude in a country with mandatory fortification of staple foods with vitamin D.

As 25(OH)D is widely recognized as the best indicator of vitamin D status [8], we have assessed the vitamin D status of a representative sample of French Canadian children and adolescents in Québec (450– 480 N) by measuring its concentration in stored plasma samples collected from January to May, the nadir for skin vitamin D synthesis. We also tested the influence of age, sex, adiposity and socio-demographic characteristics on vitamin D status.

4.3 Methods

Study population

The study population comprised children and adolescents who participated in the Québec Child and Adolescent Health and Social Survey (QCAHS), a school-based survey conducted between January and May 1999. Details on the survey design and methods have been reported previously [20]. Briefly, the QCAHS used a cluster sampling design to draw three independent, provincially representative samples of youth aged 9, 13 and 16 years. Questionnaire and anthropometric data

were collected for 83% (1267 of 1520), 79% (1186 of 1498) and 81% (1212 of 1495) of 9, 13, and 16 year-olds, respectively. This current analysis was restricted to French Canadians who comprised 80% (1019 of 1267), 79% (931 of 1186) and 78% (942 of 1212) of the 9, 13 and 16-year-old samples, respectively. Sixty three percent (638 of 1019), 69% (640 of 931) and 75% (709 of 942) of 9, 13 and 16 year-olds provided a fasting blood sample. Of 1987 blood specimens available for analysis, 234 (12%) were excluded because parents refused consent for analyses other than glucose and lipids, the samples were thawed on arrival at the laboratory or they were of insufficient quantity for the vitamin D assay. There were no differences in sex, body mass index (BMI) Z score, or parental income among youth for whom blood samples were studied versus those not studied. The Ethics Review Board of CHU Sainte-Justine approved this study. Written informed assent and consent were obtained from participants and their legal guardians, respectively.

Variables

Height was measured to the nearest 0.1cm at maximal inspiration using a measuring tape and a triangular level. Weight was measured in light indoor clothing with shoes removed. Body mass index (BMI) was calculated by dividing weight by the square of height (kg /m^2). Youth were categorized as overweight or obese if their BMI was ≥ 85th and < 95th or ≥ 95th percentile values, respectively, for their sex and age according to the 2000 US-CDC growth charts [21]. Household income was categorized as superior, upper middle, lower middle or lowest based on total income and number of persons living in the household [22] Household income was coded as missing if parents did not respond to the income question. We used the location of each school as a proxy for the location of the participants' residences. Schools were classified as rural or urban based on the census classification used by Statistics Canada.

After an overnight fast, venous blood was collected between 0800 and 1000h in 1 g/L EDTA collection tubes and placed on ice. Samples were centrifuged on site within 45 minutes of collection, transported on dry ice and

stored at -80^0 C. We used a radioimmunoassay for the quantitative determination of plasma 25(OH)D (Immunodiagnostic Systems Limited, Boldon, UK). Our laboratory participates in the International Vitamin D External Quality Assessment Scheme on vitamin D metabolites and meets the performance target set by the Advisory Panel for Data Analysis. The interassay coefficient of variation was 5.9% at 30.6 nmol/L and 6.0 % at 109.4 nmol/L.

Defining cut-offs for vitamin D

We used three cut-offs to describe vitamin D status: deficiency, hypovitaminosis and optimal. In the absence of rickets or osteomalacia, there are no outcome-based criteria to define vitamin D deficiency, although there is widespread consensus in the paediatric literature that vitamin D deficiency can be defined as plasma 25(OH)D below 25-30 nmol/L [23]. The Institute of Medicine defines vitamin D deficiency as 25(OH)D ≤ 27.5 nmol/L [24]. For hypovitaminosis D, we used a cut-off of ≤ 37.5 nmol/L, a value commonly reported in the literature [25]. The value for optimal vitamin D status was > 75 nmol/L, a value thought to be consistent with both improved bone health [26] and other health outcomes [27] in adults.

Statistical Analysis

Age and sex specific Z-scores for BMI were computed using a SAS program developed by the CDC based on the 2000 US CDC growth charts [28]. To take the complex study design into account, sampling weights and clustering effects by school were estimated and incorporated into computations of prevalence, percentile values and 95% confidence intervals. We used the non-parametric method developed by Hutson [29] to estimate percentiles and their confidence intervals. Because the low observed frequencies we calculated exact binomial 95% confidence intervals [30] for the prevalence of the three levels of vitamin D status. Differences in the prevalence between sexes and across ages were tested using a likelihood ratio test in a generalized linear logistic model to take the clustering effect within school into account. The association between mean 25(OH)D, sex and age was tested in univariate regression analysis. We used

hierarchical maximum likelihood regression to estimate regression coefficients for univariate and multivariate associations. Explanatory variables were treated as fixed effects and clustering between subjects in the same school was treated as a random effect. Analyses were stratified by sex because of a significant age*sex interaction ($p < 0.0001$) in a model including sex, age, BMI Z score, month of blood draw, parental income and location of residence. Statistical analyses were performed with SAS version 9.1 (SAS Institute, Inc., Cary, NC).

4.4 Results

Selected characteristics of participants are shown in Table 4.1. The majority of blood samples (78%) were drawn between January and March. Plasma 25(OH)D concentrations ranged from 11.4 nmol/L to 115.9 nmol/L. Mean age and sex specific plasma 25(OH)D concentrations and selected percentiles values are presented in Table 4.2. With the exception of 16-year old girls, the value of the 95th percentile in each age and sex group was below the optimal concentration of 75 nmol/L. We observed no significant differences in mean 25(OH)D between sexes ($p = 0.9$). In both sexes combined, 13- and 16-year-olds had significantly lower mean 25(OH)D than 9-year-olds ($p < 0.0001$).

More than 10% of 16-year-olds were vitamin D deficient (Table 4.3). The prevalence of deficiency increased significantly with age in both sexes. As many as 38% of 16-year-old boys and 30% of the same-age girls had vitamin D concentrations consistent with hypovitaminosis. The prevalence of hypovitaminosis increased significantly with age in both sexes. Vitamin D concentrations were not optimal in the vast majority of children and adolescents.

Girls in the lowest category of household income had significantly lower plasma vitamin D than those in the superior income category after adjustment for age, month of blood draw, area of residence and BMI Z score (Table 4.4). Male and female participants with missing income had the lowest concentrations of vitamin D. In contrast to boys, BMI Z score was significantly associated with vitamin D concentrations in girls: a 1 SD increase in BMI was associated with a 1.7 nmol/L decrease in vitamin D. There was no association between plasma

25(OH)D and month of blood draw or living in rural or urban setting in both sexes.

4.5 Discussion

Our study, the first to examine vitamin D status during winter and spring months in a representative, population-based sample of French Canadian youth in the province of Québec, revealed a relatively high prevalence of vitamin D deficiency, in particular among 16-year old boys and girls. We did not detect any differences in vitamin D concentrations between samples collected in January-March and April-May suggesting that the period of deficiency or hypovitaminosis extended beyond the winter months. As Canada, the US and Finland are the only countries with mandatory fortification of vitamin D in staple foods, our results likely represent a 'best case scenario' for vitamin D status of Caucasian youth at high latitudes.

Our results concur with those of others to suggest that vitamin D deficiency and hypovitaminosis D are widespread among children and adolescents at high latitudes. For example, white girls from Manchester, UK reported a mean serum 25(OH)D concentration of 37.3 nmol / L at the end of May, though this sample size was small [15]. A population based sample of adolescent girls from Denmark, Finland, Ireland, and Poland taken during February and March reported a median serum 25(OH)D of 29.4 nmol / L [31]. Similar findings were reported among youth in Philadelphia [32] and in New Zealand [33]. In Canadian children, rickets is an ongoing concern. Ward et al. [34] reported 104 cases of vitamin D-deficient rickets between July 2002 and June 2004 (incidence rate = 2.9/100 000). Affected children had a mean age of 1.4 years and many were dark skinned or residing in Northern Canada. Both skin color and living in the North have been associated with decreased cutaneous synthesis of vitamin D [3].

In Québec youth, a high prevalence of vitamin D deficiency and hypovitaminosis associated with lower than recommended dietary calcium intake, as revealed in a 1999 province-wide survey [20], may prevent the attainment of an optimal bone mass, which may in turn impact on the development of osteoporosis in later life. A 3-year prospective study of 171 peri-pubertal Finnish youth reported that baseline vitamin D deficiency {defined as 25(OH)D ≤ 20 nmol/L} in a context of high dietary calcium intake (1575 mg/day) was associated with a decrease in bone mineral content of the lumbar spine 3 years later in older girls (9). Furthermore, recent findings from observational and randomized trials suggest that optimal vitamin D levels and adequate dietary calcium intakes are important for the prevention of cancer [5], of type I diabetes [35], of type II diabetes [36] and of other health outcome [3].

Similar to a study of New Zealand youth [33], our analyses showed an inverse association between vitamin D concentrations and body weight in girls. The sequestration of vitamin D into adipose tissue is thought to explain this association [37]. Our finding that girls of low-socio-economic position had lower levels of 25(OH)D was not consistent with a report from Philadelphia in which caregiver education and annual income were used as measures of socio-economic position [32]. Youth whose parents did not respond to the question on income had the lowest concentrations of 25(OH)D. Non-response is generally thought to be most common among individuals in highest and lowest socio-economic position [38]. Further research is needed to explore the association between vitamin D concentrations and socio-economic position.

Because we restricted the sample to French Canadians, the findings may not be generalizable to other groups in which there may be a higher risk of deficiency due to darker skin color [3]. Our blood specimens were collected in 1999. However it is unlikely that vitamin D status has changed substantially since 1999. Our plasma vitamin D samples have been stored since 1999 raising concerns over the stability of 25(OH)D. It has, however, been shown that 25(OH)D is stable under repeated freeze/thaw cycles [39]. Finally we did not collect

information on the sources of vitamin D, namely dietary or supplement intake and sun exposure.

In conclusion, vitamin D deficiency and hypovitaminosis were highly prevalent in Winter-Spring 1999 in Québec children and adolescents, a population where staple foods are fortified with vitamin D. No recent representative surveys of vitamin D status in youth are available in Québec and none are available elsewhere in Canada. This lack calls for an urgent monitoring of indicators of the vitamin D status in Canada and elsewhere. In that respect, Vieth et al.[19], in their editorial, have recently called for international agencies to reassess the dietary recommendations for vitamin D to ensure optimal concentrations of 25(OH)D. Our study militates in favour of such a plea.

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Tables

Table 4.1: Selected characteristics of study participants in the QCAHS study (n= 1753)

Characteristic	Boys, % (n = 882)	Girls, % (n = 871)
Age, years		
9	30.9	30.1
13	31.7	29.3
16	37.4	40.6
Household income		
Superior	14.2	11.6
Upper middle	31.3	34.9
Lower middle	27.4	27.8
Lowest	12.6	12.4
Missing	14.5	13.3
Area of residence		
Urban	54.4	54.9
Rural	45.6	45.1
Month of blood draw		
January – March	78.1	78.2
April – May	21.9	21.8
BMI category [a]		
Normal weight	77.5	78.5
Overweight	12.8	13.3
Obese	9.7	8.52

[a], normal weight is defined as ≤85th percentile, overweight is the 85th to 95th percentile and obese is ≥ 95th percentile of the 2000 US-CDC growth charts.

Table 4.2: Selected percentile values and mean plasma 25(OH)D (nmol/L) by sex and age

Percentile (95% CI):	Boys			Girls		
	9 years (n = 284)	13 years (n = 293)	16 years (n = 305)	9 years (n = 275)	13 years (n = 249)	16 years (n = 347)
5th	35.3 (30.9–37.8)	30.1 (27.3–31.5)	23.5 (20.0–24.9)	30.3 (29.0–34.8)	25.3 (22.4–27.7)	24.6 (22.4–25.9)
25th	44.7 (42.9–45.9)	37.2 (35.0–38.3)	33.0 (30.2–35.2)	42.6 (41.2–43.4)	34.9 (33.2–36.2)	34.4 (32.8–37.8)
50th	51.1 (49.7–52.7)	44.0 (42.2–45.4)	41.1 (39.7– 43.6)	48.6 (46.7–50.2)	40.2 (38.8–42.6)	44.7 (42.4–47.0)
75th	57.2 (56.5–58.7)	50.2 (49.2–51.1)	51.6 (49.3–53.2)	55.2 (53.7–57.0)	47.1 (45.7–49.4)	56.7 (52.9–60.0)
95th	70.3 (67.3–74.2)	60.9 (55.9–65.3)	66.6 (62.3–70.8)	65.8 (62.5–68.9)	57.9 (55.3–63.9)	78.8 (73.1–89.7)
Mean (95% CI)	51.5 (50.2–52.7)	43.9 (42.8–45.1)	42.7 (41.2–44.3)	48.6 (47.3–49.9)	41.3 (40.0–42.6)	47.3 (45.4–49.1)

[a] Data are percentile (95% CI) and mean (95% CI).

Table 4.3: Percent of participants according to three cut-off values of plasma 25-hydroxyvitamin D by age and sex

Plasma 25-hydroxyvitamin D cut-off values (95% CIs)	9 years	13 years	16 years	P[a]
Deficient:≤ 27.5 nmol/L				
Boys	1.5 (0.3 – 4.3)	3.3 (1.3 – 6.7)	12.6 (9.0 – 17.3)	<0.0001
Girls	1.5 (0.2 – 4.5)	7.9 (4.4 – 12.7)	10.1 (6.7 – 14.4)	<0.0001
P value [b]	Ns	0.036	Ns	
Hypovitaminosis:≤ 37.5 nmol/L				
Boys	7.8 (4.6 – 12.2)	25.5 (19.9 – 31.7)	37.8 (31.9 – 43.9)	<0.0001
Girls	13.0 (8.6 – 18.6)	34.5 (27.8 – 41.8)	29.9 (24.4 – 35.8)	<0.0001
P value [b]	Ns	0.020	0.016	
Sub-optimal:≤ 75 nmol/L				
Boys	97.3 (94.2 – 99.1)	99.7 (97.8 – 100)	98.8 (96.7 – 99.8)	Ns
Girls	98.7 (95.8 – 99.8)	99.5 (96.9 – 100)	93.2 (89.4 – 95.9)	0.0003
P value [b]	Ns	Ns	0.002	

[a], p values for differences across ages.
[b], p values for differences between sexes.
Ns, not significant ($p \geq 0.05$).

Table 4.4: Associations between 25-hydroxyvitamin D concentration (nmol/L) and selected socio-demographic and anthropometric variables

Explanatory variable	Boys (n= 878)			Girls (n= 867)		
	B [a]	SE	p value	β [a]	SE	*P*
Age, years						
9	Ref.	-	-	Ref.	-	-
13	-6.9	1.0	<0.0001	-6.7	1.3	<0.0001
16	-8.6	1.1	<0.0001	-1.2	1.2	ns
Month of blood draw						
April – May	Ref.	-	-	Ref.	-	-
January – March	0.13	1.0	ns	-1.5	1.2	ns
Household income						
Superior	Ref.	-	-	Ref.	-	-
Upper middle	-1.4	1.3	ns	-1.2	1.6	ns
Lower middle	-2.1	1.3	ns	-1.9	1.6	ns
Lowest	-2.4	1.6	ns	-4.8	1.9	0.0100
Missing	-3.5	1.5	0.022	-6.8	1.9	0.0003
Area of residence						
Urban	Ref.	-	-	Ref.	-	-
Rural	-0.30	0.88	ns	-0.65	1.1	ns
BMI Z score (1 SD)	-0.58	0.38	ns	-1.7	0.42	0.0001

β: regression coefficient; SE: standard error; *P*: probability value for the regression coefficient; ns: not significant ($p \geq 0.05$); BMI: body mass index.
[a], β refers to the difference, in nmol/L, of plasma 25(OH)D per unit increment of the explanatory variable.

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BRIDGE STATEMENT

Prior to this study, little was known with respect to the vitamin D status of Canadian youth. Moreover, there are few population based samples of youth at high latitudes. The QCAHS study was thus a unique opportunity in Canada to examine the vitamin D levels of a representative sample of Caucasian youth at the end of winter and beginning of spring. We showed that vitamin D deficiency is observed in Québec youth and was more prevalent in older youth and amongst youth from lower socio-economic status. While a role for vitamin D in the resolution of rickets has long been recognized, more recent work has found associations between lower vitamin D levels and an elevated risk of a number of chronic diseases{Holick, 2005 #24}. Thus exposure to low vitamin D status in youth may contribute to an increased risk of chronic disease in adulthood.

One of the limitations of the QCAHS study was the absence of dietary and physical activity measurements to assess the lifestyle correlates of vitamin D status. The inability to collect in depth information on study participants is an implicit limitation of epidemiologic studies. For example, in the QCAHS study we found that girls from lower income households had lower vitamin D status but we had not measured exposures to discern the reasons for this. Further, in not measuring dietary vitamin D intake we were unable to determine if inadequate dietary vitamin D and/or inadequate sunlight exposure was also associated with low socio-economic position. Consequently we are unable to assess if the vitamin D deficiency seen in Canadian youth could be remediated by higher vitamin D intake through supplementation or perhaps higher levels of fortification. The QUALITY study represents an opportunity to examine the correlates of vitamin D levels, including diet and accelerometer measured physical activity in a sample of youth who are at high risk of obesity. Examination of lifestyle correlates of vitamin D levels could help inform public health measures to increase vitamin D levels.

CHAPTER 5: HIGHER LEVELS OF VITAMIN D INTAKE ARE NEEDED TO ACHIEVE OPTIMAL LEVELS OF VITAMIN D IN QUÉBEC YOUTH

Sean Mark [1], Marie Lambert [2], Edgard Delvin [3], Jennifer O'Loughlin [4], * Katherine Gray-Donald [1 5]

[1] Department of Dietetics and Human Nutrition, McGill University, Montréal, Qc, Canada [2] Department of Pediatrics, CHU Sainte.-Justine and Université de Montréal. [3] Department of Clinical Biochemistry, CHU Sainte.-Justine and Université de Montréal. [4]Department of Social and Preventive Medicine, Université de Montréal, Montréal. [5] Department of Epidemiology, Biostatistics and Occupational Health, McGill University, Montréal.

Short Title: Correlates of Vitamin D Status in Youth

Keywords: Vitamin D status, 25-hydroxyvitamin D, deficiency, children, adolescents.

List of abbreviations: 25(OH)D, 25-hydroxyvitamin D; PA, physical activity; BMI, body mass index; DXA.

* To whom correspondence should be addressed: katherine.gray-donald@mcgill.ca

5.1 Abstract

Introduction: Sub-optimal vitamin D levels are observed in Canadian youth despite mandatory food fortification. We examined the modifiable correlates of plasma vitamin D concentrations to inform public health efforts to remediate low vitamin D status.

Methods: We recruited 159 children aged 8-11 sampled once throughout the year. Vitamin D status was assessed by measuring plasma 25-hydroxyvitamin D {25(OH)D} using a radioimmunoassay. Dietary intake was measured using three dietitian-administered 24 hour diet recalls. Fat mass was measured by dual energy X-ray absorptiometry. Computer Science and Applications accelerometers were worn for 7 days to estimate physical activity (PA).

Results: Correlates of plasma 25(OH)D concentrations were examined using multiple regression in an analysis controlling for season of measurement. Seven percent of youth had hypovitaminosis D {25(OH)D < 37.5 nmol / L} during winter and spring. Only 3.8% of participants had optimal vitamin D status {25(OH)D > 75nmol / L} in all seasons. The mean dietary vitamin D intake was above recommendations. A serving increase in milk consumption and a standard deviation increase in physical activity was associated with a 2.9 nmol/L and 2.1 nmol/L increase in plasma 25(OH)D, respectively. There was no association between 25(OH)D and adiposity.

Conclusion: Our results suggest currently recommended intake of dietary vitamin D will not support optimal vitamin D status in youth. Increased levels of vitamin D intake should be examined in Canada.

5.2 Introduction

Vitamin D status has been associated with the attainment of peak bone mass [1, 2], glucose homeostasis, [3] and is potentially protective of colorectal and breast cancer [4]. Although important for health, vitamin D deficiency and sub-optimal vitamin D levels are prevalent among Canadian youth [5, 6]. Plasma vitamin D concentrations are lower in older youth thus increasing the risk of vitamin D deficiency and insufficiency in this group. In fact, more than 10% of 16-year old adolescents in a representative sample of Québec youth had vitamin D deficiency at the end of winter [6].

In adults, the modifiable correlates of vitamin D status are intake of food and supplements containing vitamin D, sunlight exposure and adiposity. Little, however, is known about the correlates of vitamin D status in youth. Latitude of residence, which is associated with length of winter, is an important determinant of vitamin D status. During winter, at latitudes higher than 37^0, ultraviolet B radiation (UVB) is not of sufficient intensity to catalyze the cutaneous synthesis of vitamin D [7], thus necessitating dietary intake of vitamin D to maintain adequate levels. The level of intake of dietary vitamin D needed to obtain optimal vitamin D status in youth (plasma 25(OH)D > 75 nmol /L) [8], is not well characterized and may be greater than the currently recommended 200 IU per day at high latitudes [9]. As vitamin D is fat soluble, excess adiposity sequesters vitamin D and is thought to be a risk factor for low vitamin D status [10]. The association between excess body fat and lower plasma 25(OH)D concentrations, however, may be confounded by the negative association between weight status and physical activity [11, 12]. In adults, physical activity has been associated with higher vitamin D status in NHANES III [13] as well as in other studies in adults [14-16], but this has not been examined in youth.

Our aim is to describe the modifiable correlates of vitamin D status in youth; potential correlates investigated included intake of vitamin D from foods and supplements, physical activity and adiposity. Identification of the independent correlates could inform public health actions by measuring the degree to which differences in these modifiable factors explain vitamin D status.

5.3 Materials and methods

Study population

The Ethics Review Board of Ste-Justine Hospital approved the study, and written informed assent and consent were obtained from the participants and their legal guardians. We studied the first 159 children aged 8-11 years recruited in the Québec Adipose and Lifestyle InvesTigation in Youth (QUALITY) study. These children were at risk of obesity because the recruitment criteria required that at least one biological parent had overall (BMI $\geq$30 kg/m^2) or central obesity (waist circumference >88 cm for women and >102 cm for men). Participants were recruited between 2005 and 2007 through public or private primary schools using pamphlets distributed to children in grades 2-5 in schools located within 75 km of Montréal or Québec City, Québec, Canada (45^0 N). The pamphlet invited parents to contact the study center if they met the inclusion criteria. Both parents had to be available for the study. For families that expressed interest, screening for eligibility was conducted in a structured telephone interview using pre-selection criteria. Criteria for children included: an absence of diabetes, no dieting, no regular medications and no psychological ailments. Recruitment into the study was limited to Caucasian families due to genetic analyses undertaken in the QUALITY study. Parental education was categorized by completion of university degree (yes/no). For analysis, study participants were grouped according to the season during which they had blood drawn. As there was no difference in 25(OH)D concentrations between youth who had their blood drawn during the summer or fall (p= 0.60), they were placed in the same category.

Assessment of vitamin D status

Plasma 25(OH)D concentration is widely recognized as the best indicator of vitamin D status [17]. We measured 25(OH)D concentration using a radio-immunoassay (IDS Laboratories, UK). This assay measures vitamin D_3 with 100% specificity and vitamin D_2 with 75% specificity [18]. The lower specificity of the assay for vitamin D_2 was not a concern as in Canada, milk is fortified with vitamin D_3 and supplements also contain vitamin D_3 [19]. Samples were assayed in duplicate. Our laboratory participates in the International Vitamin D External

Quality Assessment Scheme on vitamin D metabolites and meets the performance target set by the Advisory Panel for Data Analysis. The interassay coefficient of variation was 5.9% at 30.6 nmol/L and 6.0 % at 109.4 nmol/L.

Defining cut-offs for vitamin D status

We used two different cut-offs to describe vitamin D status, hypovitaminosis and optimal. In the absence of rickets or osteomalacia, there are no outcome based criteria to define vitamin D deficiency although the Institute of Medicine defines vitamin D deficiency as 25(OH)D $\leq$ 27.5 nmol/L [20]. None of the youth had this level of deficiency in this study. We used $\leq$ 37.5 nmol/L to define hypovitaminosis a value above which the IOM states that 25(OH)D levels are consistent with bone health [20]. More recently, it was observed in a study of adolescents that 25(OH)D levels below $\leq$ 37.5 nmol were associated with hypertension, fasting hyperglycemia, low high-density lipoprotein cholesterol and metabolic syndrome [21]. Although there is no widely agreed upon cut-off for optimal levels of vitamin D levels, 25(OH)D levels $\geq$ 75 nmol/L are thought to be consistent with overall health and disease prevention in adults.

Anthropometry and DXA

Height was measured with a stadiometer with participants standing against a wall with their heads in the Frankfort horizontal plane and looking ahead. Height was recorded to the nearest millimeter during maximal inspiration. Weight was measured to the nearest 0.1 kg, with an electronic scale, with participants wearing light indoor clothing and no shoes. BMI was computed as weight (kg)/height (m^2). Age and sex percentiles for BMI were computed using a SAS program developed by the CDC based on the 2000 US CDC growth charts [22]. Dual energy X-ray absorptiometry (DXA) (Prodigy Bone Densitometer System, DF+14664, GE Lunar Corporation, USA) was used to assess fat mass which was converted into a fat mass index by dividing fat mass by the square of height (m) [23].

Physical activity assessment

To obtain objective measures of physical activity, children wore a uniaxial Actigraph LS 7164 activity monitor (Actigraph LLC, Pensacola, Florida) for a 7-day period following the clinic visit. These accelerometers have been validated in 9 year old children with a correlation (r=0.54) between mean counts / minute and activity based energy expenditure assessed by doubly-labeled water [24]. One-minute intervals of data were downloaded and categorized as light (≥ 800 and < 3199 counts•min-1), moderate (3200 to 8199 counts•min-1) or vigorous (> 8200 counts•min-1) activity. A total of 140 children provided accelerometer data. Days were excluded when the accelerometer was worn for less than 80% of the average time worn on the other days. Of the 140 youth with accelerometer data, 97% had more than 4 days of data, which has been shown to ensure adequate reliability [25]. In both sexes, the mean number of hours that the accelerometer was worn daily was 13.6 hours. Since 74 % of the sample reported no cycling or swimming activity during the evaluation week, no modifications were made to the data to account for children who indicated they had gone swimming or cycling on any of the seven days.

Dietary assessment

Children's dietary assessment was based on three 24-hour dietary recalls completed on non-consecutive, different days of the week including one weekend day. A dietitian obtained recalls following the clinic visit. In order to minimize the burden to families, recalls were conducted by telephone. At the clinic visit the child was given a short training session on the use of a graduated cup, bowl and other portion size models. Each participant received a small disposable kit of food portion size models prior to the interview. Telephone interviews for the 24-hour dietary recalls have been validated in youth with good results [26]. Cooking details for meals cooked at home were obtained from parents. Food groups were created for dairy, fruit and vegetable servings following the Canadian Food Guide for Healthy Eating [27]. A food group was created for sweetened drinks and units of a 250 mL serving size were reported. We also collected information on the use of dietary supplements, using the Drug Identification Number (DIN) on each day of

dietary recalls. The nutrient analysis of the food and supplement data were completed using the CANDAT software (Godin London Inc., London, Ontario, Canada), using food composition data obtained from the 2007b Canadian Nutrient File. In order to measure the potential for attenuation in the relation of dietary intake and vitamin D status, we assessed the number of days needed to obtain an estimate of observed dietary intake within 20% of the true intake using the method outlined by Willett [28]. We assessed attenuation in milk servings since it had the strongest association with vitamin D levels, and it also the most important source of dietary vitamin D and calcium.

Statistical analysis

T statistics and non-parametric statistics were used to test for differences between sexes in non-normally distributed variables. Since plasma 25(OH)D was not normally distributed we conducted a sensitivity analysis to test the robustness of the findings from Table 2 by natural log transforming plasma 25(OH)D values. To compare aspects of diet quality according to the plasma concentration of 25(OH)D, we stratified participants according to the lowest and upper three quartiles of 25(OH)D specific for each season. T statistics were used to test for differences between these two categories of 25(OH)D. Since there was a non-significant trend of higher energy intake in the upper 3 quartiles we conducted a sensitivity analysis to assess whether energy intake confounded the relationship between the quartile of vitamin D levels and dietary quality. The sensitivity analysis was done by including energy intake, as an independent variable with the season adjusted quartile of vitamin D status, in a multiple regression model with dietary variables as dependent variables. We used logistic regression to assess whether youth were meeting adiposity and physical activity guidelines using season specific quartiles of 25(OH)D as the independent variable. All statistical analyses were conducted using SAS version 9.1 (SAS Institute, Inc., Cary, NC).

5.4 Results

Characteristics of the study participants are presented in Table 5.1. Girls had more educated parents, lower energy intakes, a higher mean fat mass index and lower

mean accelerometer counts than boys. Participants sampled in winter had, on average, 10.4 nmol/L lower plasma 25(OH)D levels than participants sampled in the summer/fall. The mean 25(OH)D concentrations, all seasons included, were 52.5 nmol/L (95% CI: 49.9-55.1) and 54.0 nmol/L (50.9-57.1) in boys and girls, respectively. Only 8% of participants had optimal levels of vitamin D {25(OH)D > 75 nmol/L} in the summer and fall when vitamin D levels are at their zenith. The range of dietary vitamin D intakes, including supplements, for both sexes was 0.5 to 25 µg and the means and medians of total dietary vitamin D intake were above 5 µg per day as recommended by the Institute of Medicine (IOM). The mean daily vitamin D intake as supplements in users (N = 32) was 5.1 µg. After adjusting for season there was no difference in plasma 25(OH)D between supplement users and non-users (p= 0.374).

In Table 5.2, we present correlates of plasma 25(OH)D status. Season was the strongest correlate of plasma 25(OH)D concentrations, therefore all associations between 25(OH)D and the other potential correlates were examined after adjustment for the season of blood draw. In bivariable models, after controlling for season, physical activity, fat mass, milk intake, and dietary calcium intake were all associated with 25(OH)D concentrations. In a multivariable analysis including season, physical activity, sex, fat mass and milk intake a serving increase in milk intake was associated with a 2.9 nmol/L mean increase in plasma 25(OH)D. Similarly, a 1 SD increase in physical activity was associated with 2.1 nmol/L mean increase in plasma 25(OH)D. Physical activity accounted for 3% of the variance in 25(OH)D after accounting for sex and season. The results of the sensitivity analysis using log transformed 25(OH)D status were analogous to regression results presented in Table 2. We included only milk intake (and not dietary vitamin D and calcium intakes) in the multivariable model, since milk was strongly correlated with dietary vitamin D {r = 0.62 (p <.0001)} and calcium {(r = 0.83 (p <.0001)} intakes. Since we assessed usual diet with three days of intake, the β coefficient for milk intake in the regression was attenuated due to the imprecision of the measurement [28]. To assess the degree of attenuation we found that 12 days of dietary reports are required to obtain an

estimate of observed milk intake within 20% of the usual intake [28]. We thus applied a correction factor to the β coefficient for milk intake. [28] Using this factor, one serving of milk was associated with a mean increase of 3.5 nmol/L of plasma 25(OH)D.

We compared the diet quality between participants in the season-specific lowest quartile of 25(OH)D and those in the upper 3 quartiles to see if any lifestyle habits were linked to low vitamin D status (Table 5.3). Those in the lowest quartile of 25(OH)D concentrations had lower dietary fibre intake and a trend towards less milk and calcium intake. There was a higher proportion, though a non-significant trend, of youth meeting guidelines for steps / day [29] and MVPA [30] in the 3 highest season specific quartiles of 25(OH)D.

5.5 Discussion

Our study of the correlates of vitamin D status showed that milk consumption and levels of physical activity, assessed by accelerometer were associated with vitamin D status. Moreover, despite mean vitamin D intakes above recommended levels, the vast majority of youth had sub-optimal vitamin D levels. Thus the sizes of the measured effects are most likely insufficient to achieve optimal vitamin D status in Québec youth.

The mean levels of 25(OH)D reported in this study {49.5 nmol / L (95% CI 47.6 – 51.5)}were similar to those reported in 9 year olds from a representative sample of Québec youth evaluated in winter and spring 1999 {50.0 nmol/L (49.1-50.8)} [6]. While there was no vitamin D deficiency {25(OH)D ≤ 27.5 nmol / L} among QUALITY participants, 1.5% of 9 year olds from the representative sample of Québec youth had vitamin D deficiency [6]. As these youth age, however, they may be at heightened risk of vitamin D deficiency. The level of deficiency is higher in older youth as 10% of 16 year olds in Québec had vitamin D deficiency at the end of winter or beginning of spring [6]. Consistent with other studies of Canadian youth, greater than 90% of QUALITY participants had sub-optimal vitamin D status. Sub-optimal vitamin D status has been associated with the development of common malignancies in studies of adults [4].

Similar to other studies, we found that physical activity, some of it likely occurring outdoors, was associated with vitamin D status. The magnitude of change in physical activity required to meaningfully increase vitamin D levels, however, is large. For example, in QUALITY, one standard deviation increase in accelerometer measured physical activity was associated with a 2.1 nmol / L increase in mean 25(OH)D, while a 20 nmol/L increase was required to achieve optimal concentrations. In a study of adults from NHANES III participants who undertook daily outdoor activity ≥ 31 times per month had approximately 10 nmol / L higher levels of vitamin D compared to those who reported no outdoor activity in the last month [13]. In addition, at latitudes greater than 37^{o} UVB rays are not of sufficient intensity to generate vitamin D production during winter [7]. Hence, sun exposure has no effect on vitamin D status for half of the year. In youth, time spent outside has been associated with increased physical activity [31]. Thus a public health message calling for more time spent outside during summer months could potentially increase vitamin D levels and physical activity as well. However, sunlight exposure without adequate sun protection is a risk factor for skin malignancies, particularly malignant melanoma. The annual incidence of malignant melanoma amongst Canadians is 12 / 100 000 [32] so there is reluctance to suggest high levels of UVB exposure. To further inform this debate about risks and benefits of sun exposure, the broad spectrum of possible health consequences of low vitamin D status requires further investigation.

Adiposity was not related to vitamin D levels in our study even though we had a wide range of adiposity to examine this issue. In a representative sample of Québec youth, BMI was negatively associated with vitamin D levels in girls, but not boys, such that a standard deviation increase in BMI Z score was associated with a 1.7 nmol/L lower level of plasma 25(OH)D [6]. Similarly, in a population based sample from New Zealand, obese youth had 6 nmol / L lower levels of vitamin D than healthy weight and overweight youth [33]. In a study of youth from Northeastern U.S., however, adiposity measured by DXA was not associated with vitamin D levels [34]. Higher levels of adiposity are associated with lower levels of physical activity [11], potentially confounding the adiposity and 25(OH)D

relationship. Consistent with this, in our study adiposity was significantly related to 25(OH)D in bivariable analysis which included season, but when physical activity, assessed by accelerometer, was entered into the regression, adiposity was no longer associated with 25(OH)D levels.

In multivariable regression a serving of vitamin D fortified milk was associated with 2.9 nmol / L higher levels of vitamin D. In Canada, a 250 ml serving of milk is legislated to contain 2.5 μg of vitamin D [19]. Increasing milk consumption amongst Canadian youth as a means of increasing vitamin D levels would require major shifts in the consumption of milk. Levels of vitamin D in fortified milk would have to be considerably higher for milk consumption to remediate sub-optimal levels of vitamin D. Amongst Finnish girls (60^0N) supplementation of 20 μg of vitamin D per day was required to prevent hypovitaminosis {25(OH)D < 37.5nmol / L} during winter months [35]. Twenty percent of youth in our study used vitamin D supplements. Participants who took vitamin D supplements on the days of the dietary recall had the same vitamin D levels as participants who did not take vitamin D supplements. Moreover, encouraging supplementation in all youth creates challenges as this is unlikely to be adhered to for a variety of reasons, including family income. Evidence from the Dutch public health campaigns to increase the consumption of folic acid found that poorly educated women had a lower consumption of folate supplements than better educated women [36]. In a representative sample of Québec youth, girls from low income households had lower vitamin D levels [6]. Thus a public health campaign aiming to increase levels of vitamin D supplementation amongst youth may exacerbate inequities in vitamin D status. Ensuring that the whole population increases their vitamin D intake levels argues towards further fortification of a variety of commonly consumed foods.

Youth in the lowest season-specific quartile of vitamin D levels had similar diets as youth in the upper quartiles with the exception of dietary fibre. We compared diet quality using group means, since accurate quantification of diet at the individual level is difficult due to the high intra-individual variation in nutrient and food intakes [37]. Group means, however, can be used as an accurate

representation of dietary intake [38]. Despite the difference in dietary fibre intake, fruit and vegetable intake was not different between the two groups and was below the 6 servings recommended for this age group by Canada's Food Guide for Healthy Eating [27]. Similarly dairy consumption was below the 3-4 servings recommended by the Canada's Food Guide for Healthy Eating [27] in all groups.

This study has a number of limitations. Youth in the QUALITY study were not representative of Québec youth since they had a higher prevalence of overweight and obesity. They did, however, have very similar levels of plasma 25(OH)D as compared to a representative sample of 9 year olds from Québec. Our sample allowed for the measurement of adipose tissue with DXA, objective measurement of physical activity and good quantitative dietary assessment. We did not measure UV exposure directly even though cutaneous production of vitamin D is the largest determinant of vitamin D levels [39]. Sunlight exposure, however, is difficult to measure [40] and when measured by questionnaires, correlates weakly with vitamin D levels [41]. Physical activity was not likely a strong proxy for sun exposure since much of the physical activity would have taken place indoors or in winter [31, 42]. Consistent with this, physical activity explained only 3% of the variance in 25(OH)D levels after accounting for season of blood draw and sex.

Vitamin D status is sub-optimal in many Canadian youth, even in the summer months [43]. We found that even though our population had vitamin D intakes above recommended levels, vitamin D status remained sub-optimal. Dietary intakes above the currently recommended adequate intake value will be needed to achieve optimal levels of vitamin D status; we believe that it is unlikely that increasing milk consumption or increasing (outdoor) physical activity would increase vitamin D status sufficiently to achieve optimal levels. Improving levels of vitamin D in the Canadian population will likely require re-evaluation of current dietary needs for vitamin D and examination of the best means to increase dietary vitamin D intake.

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Tables

Table 5.1: Characteristics of QUALITY study participants (n=159)

Characteristic	Both sexes Mean (SD)	Boys (59%) Mean (SD)	Girls (41%) Mean (SD)	Difference between sexes (P)
Age (years)	9.2 (0.83)	9.3 (0.84)	9.1 (0.83)	0.220
Parent's university educated (%)	47.8	40.4	57.8	0.030
Plasma 25(OH)D (nmol / L)				
Winter (n= 32)	47.8 (8.8)	46.9 (8.0)	49.5(10.5)	
Spring (n= 61)	50.4 (9.8)	49.6 (9.2)	51.5 (10.9)	0.570
Summer / Fall (n=66)	58.2 14.5)	58.4 (15.2)	57.8 (13.8)	
Difference by seasons (P)	< 0.0001	< 0.0001	< 0.0001	
Vitamin D status				
Optimal (%)	3.8	4.3	3.1	
Suboptimal (%)	96.2	95.7	96.9	
Hypovitaminosis (%)	3.8	4.2	3.1	
Energy intake (kcal)	1767 (359)	1844(374)	1652 (306)	0.001
Milk intake (servings)	1.30 (0.96)	1.41 (1.06)	1.16 (0.78)	0.089
Vitamin D in foods (μg)	5.6 (3.5)	6.3 (3.9)	4.7 (2.6)	<0.001
Vitamin D in supplements in 32 users (μg)	5.1 (3.9)	5.4 (4.1)	5.1 (3.7)	0.854
Total vitamin D in foods and supplements	6.6 (4.3)	7.3 (4.7)	5.8 (3.6)	0.043
Dietary calcium intake (mg)	908 (341)	959(366)	836 (285)	0.019
BMI ≥ 85th percentile (%)	42.1	45.7	37.5	0.304
% body fat	25.7 (11.3)	24.2 (11.0)	27.8 (11.6)	0.050
Mean accelerometer counts/min	561 (161)	608 (173)	497 (117)	<0.001

Table 5.2: Associations between plasma 25(OH)D and selected covariates in the QUALITY study (n= 159)

Explanatory variable	Bivariable model including season			Multivariable model with lifestyle covariates		
	β	SE	P	β	SE	P
Season *						
Winter	-10.36	2.54	< 0.001	-9.76	2.48	<0.001
Spring	-7.77	2.11	< 0.001	- 7.53	2.11	<0.001
Summer / Fall	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
Physical activity (1 SD)†	1.95	0.97	0.044	2.14	1.02	0.036
Sex						
Girls	0.94	1.92	0.62	3.83	2.03	0.045
Boys	Ref	Ref.	Ref.	Ref	Ref.	Ref.
Fat mass (1 SD)	-2.88	0.92	0.002	-1.45	1.00	0.145
Milk (serving)	2.97	0.96	0.002	2.90	0.96	0.003
Dietary calcium (1 SD)	2.42	0.93	0.009	-	-	-
Dietary vitamin D (1 SD)	1.60	0.94	0.089	-	-	-
Parents university educated (yes / no)	2.73	1.87	0.145	-	-	-
Age (years)	-0.19	1.12	0.866	-	-	-

* Season is examined in univariable analysis

† Activity is available for 140 participants and is based on counts/minute assessed by accelerometer.

Table 5.3: Comparison of diet between the season-specific lowest and top 3 quartiles of 25(OH)D in the QUALITY study participants (n=159)

Variable	Lowest 25(OH)D quartile mean (95% CI) (n=40)	3 highest 25(OH)D quartiles mean (95% CI) (n=119)	P
Total energy intake (kcal)	1716 (1609-1823)	1784 (1718-1851)	0.247
Dietary carbohydrate (% of kcal)	54.0 (51.9-55.9)	53.1 (52.0-54.1)	0.623
Dietary fat (% of kcal)	32.2 (30.5-34.0)	32.6 (31.7-33.4)	0.704
Dietary protein (% of kcal)	15.5 (14.9-16.0)	15.0 (14.2-15.9)	0.652
Dietary fiber (g)	11.7 (10.7-12.7)	14.0 (13.2-14.7)	0.002
Fruit and vegetable (servings)	3.9 (3.3-4.5)	4.2 (3.8-4.6)	0.412
Milk (servings)	1.1 (0.8- 1.4)	1.4 (1.2-1.6)	0.064
Dairy products (servings)	1.8 (1.5-2.1)	2.1 (1.8-2.3)	0.149
Sweetened drinks (servings)	0.5 (0.4-0.7)	0.5 (0.4-0.6)	0.575
Calcium from foods (mg)	827 (721- 933)	935 (873- 997)	0.086
Vitamin D from foods (μg)	5.5 (4.0-7.0)	5.7 (5.1- 6.2)	0.340
Vitamin D from supplements (μg)	1.2 (0.3 - 2.1)	1.0 (0.5- 1.5)	0.449
Adherence to adiposity and physical activity guidelines			
Percent > 85th percentile of BMI	42.0 (33.0-51.0)	42.5 (26.5-58.5)	0.958
Meets guidelines for steps / day *	25.0 (10.9-39.0)	36.1 (27.4-44.9)	0.199
Meets guidelines for MVPA†	75.0 (61.0-89.0)	78.2 (70.6-85.7)	0.683

* Guidelines are 12000 steps / day for girls and 13000 steps /day for boys

† MVPA guidelines are greater than 60 minutes per day for youth of both sexes

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BRIDGE STATEMENT

In chapter 4, the QCAHS study, we described a high prevalence of vitamin D deficiency in a representative sample of Québec youth. We also found that vitamin D levels were patterned by socio-economic position. For example, girls and boys that came from the lowest income households had 4.8 nmol / L and 2.4 nmol / L less vitamin D than households with the highest income, respectively. Amongst vitamin D researchers there has been a recognition that currently recommended levels of vitamin D intake will not be sufficient to support optimal vitamin D levels.

In chapter 5, the QUALITY study, we found that the lifestyle correlates of vitamin D level had a modest association with vitamin D levels. For example, a serving increase in milk intake is associated with a 2.9 nmol / L higher levels of vitamin D. Our results suggest that a public health message to increase vitamin D levels by increasing milk consumption is likely to have limited impact on the prevalence of vitamin D deficiency in Canada. Consistent with the need for higher vitamin D intake, the Institute of Medicine has initiated a review of the evidence concerning vitamin D and health with the aim of revising guidelines for vitamin D dietary intake. This review will likely result in the upward revision of currently recommended vitamin D intake. If dietary vitamin D guidelines are updated, population wide interventions to improve vitamin D levels, such as a review of the current fortification policy in Canada, will likely be needed. In chapter 6, using the CCHS database, we will examine the diet quality of a representative sample of Canadian youth by income and food insecurity. To assess diet quality we will examine those nutrients related to bone health which include dietary vitamin D, calcium and vitamin D fortified dairy products. Understanding how income influences and food insecurity these nutrients could help inform efforts to remediate vitamin D deficiency possibly through revision of current fortification policies. The success of fortification policies in effecting public health change is shown by the addition of folic acid to flour resulting in a lower incidence of neural tube defects amongst offspring of women of periconceptional age.

CHAPTER 6: HOUSEHOLD INCOME, FOOD INSECURITY AND NUTRITION IN CANADIAN YOUTH

Sean Mark [1], Marie Lambert [2], Katherine Gray-Donald [1,3]

School of Dietetics and Human Nutrition 1, McGill University, Montréal, Qc, Canada, Department Pediatrics 2, CHU Sainte.-Justine and Université de Montréal, Department of Epidemiology, Biostatistics and Occupational Health 3, McGill University, Montréal, Qc

Short Title: INCOME, FOOD INSECURITY AND DIET IN CANADIAN YOUTH

Keywords: children, adolescent, diet, household income, food insecurity

List of abbreviations: BMI, body mass index; ns, not significant.

Corresponding author and reprint request: Katherine Gray-Donald, PhD, School of Dietetics and Human Nutrition, Room MS2-039 Macdonald-Stewart Building, McGill University, 21 111 Lakeshore Road. Ste Anne de Bellevue, Québec H9X 3V9 Telephone: 514-398-7677, Fax: 514-398-7739,

E-Mail: katherine.gray-donald@mcgill.ca

6.1 Abstract

Introduction: Low socio-economic position is associated with poorer health. This association is evident at all ages and in adults is associated with a higher prevalence of chronic disease. The contribution of diet to health inequalities is poorly understood.

Methods: We used data from the Canadian Community Health Survey (CCHS) Cycle 2.2, a nationally representative sample, to examine the diets of 8938 youth aged 9-18. A single 24 hour recall was used to collect dietary information. Interviews were conducted in person with anthropometric measurements provided in 71 % of the sample. Estimates of variance for means were calculated using bootvar with weights specific to the CCHS survey. Generalized linear models were used to examine the associations between the independent variables income, low income-food insecurity and the dependent variables of anthropometric measures, food and nutrient intake.

Results: Low income girls had a higher prevalence of BMI≥ 85th percentile and mean height percentiles were higher amongst high income boys and girls. Vitamin D fortified milk consumption was lower in low income boys, while sweetened beverage intake was higher in this group. Calcium and vitamin D intakes were lower in low income households. Among low income-food insecure households there was evidence of a higher prevalence of BMI ≥ 85 percentile in boys and low income-food insecure girls had lower milk intakes and a greater intake of sweetened beverages. Finally, the intake of fruits and vegetables was lower amongst low income-food insecure boys.

Conclusion: Public health messages aiming to increase diet quality amongst low income youth may be unsuccessful due to budgetary constraints amongst low income and particularly the subset of low income-food households which are food insecure.

6.2 Introduction

There are important health disparities by income in both Canada and the US [1, 2]. Among disadvantaged children, there is higher infant mortality, a higher incidence of deaths from infectious diseases and accidental deaths [3]. Older youth living in disadvantaged settings may be more adversely exposed to smoking, obesity, poor diet and physical inactivity, contributing to chronic disease in adulthood [4, 5]. Moreover the contribution of these exposures to increased morbidity and mortality appears to be additive [6] and clustering of risk behaviors appears to be common in Canadian youth, particularly amongst those from low income households [7]. The contribution of lower diet quality to poor health and higher risk of chronic disease in youth is not well understood [8].

The high cost of nutrient dense foods, such as milk, fruits and vegetables, relative to inexpensive foods rich in added sugar and fat are thought to constrain dietary choices in low income households, and may contribute to long term nutritional deprivation [9, 10]. In NHANES (1999-2004) sweetened beverage intake was higher in youth from households living below the poverty line [11]. And there is concern that sweetened beverage intake, which is associated with weight gain [12], will displace milk intake in youth [13]. Low income is associated with greater adiposity and lower height in industrialized countries [14, 15]. The association between low income and adiposity has been documented in youth from the US [16] and a number of European countries [17]. A higher prevalence of overweight and obesity has been documented in low income Canadian youth, although this study was limited by use of self-reported height and weight [7].

Food insecurity is defined as the ability to acquire or consume an adequate diet quality or sufficient quantity of food in socially acceptable ways, or the certainty that one will be able to do so [18]. A recent report from the Canadian Community Health Survey (Cycle 2.2), found that teenage girls that came from food insecure households consumed less milk products, and fruits and vegetables when compared to food secure youth [19]. The relationship between food insecurity

and height and weight has not examined in a representative sample of Canadian youth.

We set out to examine the influence of low income and food insecurity on diet quality in a representative sample of Canadian youth aged 9-18 years. We also examined longer term indicators of diet quality, height and weight in this sample. As the experience of food insecurity is not confined to the lowest income groups and food insecurity and low income have both been shown to compromise diet quality, we sought to isolate the effect of food insecurity while controlling for low income by dichotomizing youth from the two lowest income groups into a food secure and food insecure group.

6.3 Methods

The Canadian Community Health Cycle 2.2 targeted respondents from all age groups living in private occupied dwellings in Canada's ten provinces. Residents of the three Canadian territories, persons living on First Nations reserves or Crown lands, persons living in institutions and residents of some remote regions were excluded from the sampling frame. Our study population included 8938 youth aged 9-18 y; those who were pregnant or breastfeeding were excluded.

Data were collected in all months of 2004. Interviewers received 3 days of training and interviews were conducted in person using a computer-assisted interviewing method. Twenty-four hour dietary recalls of participants between the ages of 6 to 11 were conducted with the guardian, dietary recalls with youth 12 years and older were conducted alone. We chose to analyze milk, calcium and vitamin D intakes, as they are implicated in bone health in youth and there is considerable vitamin D deficiency amongst Canadian youth [20, 21]. Sweetened drink consumption has been associated with a greater risk of obesity [12]. We also examined fruits and vegetables which have been associated with a myriad of health benefits [22]. We used Canada's Food Guide to Healthy Eating [23] to define fruit, vegetables (excluding potatoes) and milk servings which included soy milk. A sweetened drink category was created which included pop and sweetened fruit drinks. To examine the quality of dietary intake data, basal metabolic rate was

calculated for youth participants using equations provided by the Institute of Medicine [24]. Energy expended during physical activity was assessed by presenting participants with a list of physical activities and asked the frequency and duration of the activities conducted in the last 3 months. As participants were not asked about the intensity of physical activity, the lowest metabolic equivalent of each physical was assumed. Energy expenditure was calculated using the frequency, duration and metabolic equivalents of each task (MET) value of the activity[25]. Participants were classified as inactive if their total energy expenditure (kcal/kg/day) was < 1.5 to ≥ 0, moderately active was classified as < 3.0 to ≥ 1.5 and active was classified as < 96 to ≥ 3.0 [25]. The ratio of energy intake divided by the estimated energy requirements (EI / EER) was compared across income and food insecure groups to identify potential differences in reporting of energy intake. Sedentary activity included the number of hours in a typical week in the last 3 months. Sedentary activities included time spent viewing television, using a computer, playing video games and reading. The number of hours of sedentary activity per week was ranked such that youth who were sedentary for 5-9 hours / week were assigned a value of 2 for the sedentary activity variable, 10-14 hours corresponded to a value 3, and 15-19 hours corresponded to a value of 4.

A parent or guardian was asked questions relating to household income and food security if the participant was ≤ 17 years old. A variable integrating information on total household income and household size was stratified into 4 income groups where low income was defined as $<$ \$ 20 000 if 3 or 4 people lived in the house and the highest income category was defined as $\geq$ \$ 80 000 for 3+ individuals in the household. Household food security was measured using a set of 18 questions adapted from the U.S. Food Security Survey Module [26]. The module consists of 10 adult referenced questions and 8 child-referenced questions designed to categorize households into food secure, insecure without hunger, insecure with hunger at any time in the last 12 months. The relationship between low income and household food insecurity is complex as both of these states can be fluid for some households but not others. In the Canadian National Longitudinal Survey of Children and Youth of those who reported hunger in

1994, only 23 % reported hunger in 1996 [27]. Further, while the proximal cause of food insecurity is low-income, less than 50% of Canadians who reported food insecurity in the last 12 months were from the lowest and lower middle income groups [19]. Thus, in Canada, the experience of food insecurity is not restricted to the lowest income households [28]. To examine the effect of food insecurity amongst low income households, we dichotomized youth from the two lowest income groups into a food secure and food insecure group. For participants' ≥ 12 years old, a dichotomous variable was created differentiating ever smokers from never-smokers. Height and weight were measured for 71.1 % of our sample. There were no differences in household income or age in youth who had height and weight measured vs. those who did not. Height was measured to the nearest 0.1cm at maximal inspiration using a measuring tape and a triangular level. Weight was measured in light indoor clothing with shoes removed. Body mass index (BMI) was calculated by dividing weight by height squared (kg/m^2). Youth were categorized as being overweight if they were $\geq 85\%$ percentile of BMI for their sex and age using CDC norms [29].

All analyses used a weighting scheme specific to the Canadian Community Health Survey Cycle 2.2 to take into account unequal probabilities of selection resulting from the sample design, non-response, and planned over-sampling of selected subgroups. Bootvar was used to estimate the variance for the generation of confidence intervals. Multiple regression using variance estimates from bootvar was used to generate p-values for estimates and in the case of food and nutrients, the confounding influence of age and height were controlled by including energy intake as an independent variable. To control for the confounding influence of age on physical activity, sedentary behavior and the BMI percentiles, we adjusted for age in the regression as it was higher amongst higher income and food secure groups. All analyses were conducted using SAS version 9.1 (SAS Institute, Inc., Cary, NC).

6.4 Results

A comparison of youth (9-18 y) characteristics by family income level indicated that boys from higher income households were older than low income boys whereas this was not so for girls (Table 6.1). There were more youth from single parent households in the low income groups and a higher prevalence of youth with BMI values ≥ 85th percentile amongst lower income girls and a similar non-significant trend in boys. Youth from lower income households had lower age and sex-specific height percentiles than youth from higher income households. We also found that 9-11 old youth had income associated height disparities similar to those 12-18. There were no differences in prevalence of ≤ 5th percentile of BMI across income levels. Smoking, reported by those over 12 years of age, was more prevalent amongst low income girls. Sedentary activities were significantly higher amongst higher income boys. There were no differences in self-reported leisure physical activity in either sex (data not shown).

As shown in Table 6.2, higher income boys were older and thus had higher mean energy intake. All nutrient and food analyses for both boys and girls were adjusted for energy intake. Both boys and girls from high income households had higher calcium intake than those from low income households after adjusting for energy intake. Only boys from the highest income group had a mean calcium intake above the recommended 1300 mg / day, the recommendation for this age group. Dietary vitamin D intakes were lower in low income boys but the means of the different income groups were above 5 µg, the daily dietary vitamin D intake recommended by the Institute of Medicine [30]. Vitamin D fortified milk intake was lower in low income boys while the intake of sweetened beverage was higher in this group. The mean servings of fruit and vegetable consumption was below 6-8 servings recommended by the Canadian Food Guide for Healthy Eating in all groups [23]. Girls from the highest income households had a lower ratio of energy intake to estimated energy expenditure, suggesting dietary under-reporting in this group. There was a lower intake of dietary supplements amongst low income groups (data not shown).

In Table 6.3, we examine the impact of food insecurity in the two lowest income groups and compare these food insecure youth to youth in similarly low income groups who were not food insecure. There was a higher prevalence of youth from single parent households in the food insecure group and youth were, on average, younger than those in the food secure group. Boys from food insecure households had a higher prevalence of BMI ≥ 85th percentile. There was no difference in height percentiles between the two groups. Boys from food insecure households had lower mean hours of sedentary behavior than boys from food secure households. There was no difference in smoking prevalence or amount of leisure physical activity (data not shown) between different food security groups.

As food insecurity is sometimes observed in middle income households [19], we sought to examine the influence of food insecurity amongst the two lowest income groups on nutrient intake and anthropometric measures. The comparison between low income-food secure and low income-food insecure groups (Table 6.4) largely supported the findings from the comparison of the low income groups. Dietary vitamin D intakes were lower in food insecure girls and were below the 5 µg/day recommended by the Institute of Medicine. Compared to girls in the lowest income group, girls who came from food insecure households had lower milk consumption. Amongst the low income-food insecure girls there was a higher intake of sweetened drink consumption than the low income-food secure girls. Fruit and vegetable consumption was higher in food insecure boys though the mean intakes for all groups, was the mean intake was below 6-8 servings recommended by the Canadian Food Guide for Healthy Eating [23].

6.5 Interpretation

Our analysis of nutritional health indicators from the CCHS indicated a clear socio-economic gradient in calcium intake. Consistent with this, milk consumption was low compared to higher income youth and sweetened beverages intake was higher. Long term indicators of nutritional status supported the nutrient intake findings. For example, height was lower in low income boys and girls. Low

income girls and low income-food insecure boys had a higher prevalence of ≥ 85th percentile of BMI. Smoking was more prevalent amongst the low income girls. These results suggest that, amongst Canadian youth, exposures associated with a higher risk of chronic disease tend to be more prevalent in disadvantaged households.

Amongst low income and low income households which reported food insecurity in the previous 12 months we found that purchasing patterns may be constrained with respect to expensive, nutrient dense foods such as milk and fruit and vegetables. For example, milk intake was lower in low income-food insecure girls but sweetened drink consumption was higher. This was consistent with lower milk consumption amongst 14-18 year old girls from food insecure households compared to those from households which were food secure in the CCHS 2.2 [19]. This result was also supported by a study in NHANES (1999-2004) which showed that sweetened beverage intake was higher in youth who were living below the poverty line [11]. As milk intake is highly correlated with calcium and vitamin D intake [31], we found that these nutrients were lower in low income and food insecure households. Consistent with this, studies from the U.S and Canada have shown that serum vitamin D levels were lower amongst low income youth [20, 32] and vitamin D deficiency is relatively common in Canadian youth, particularly during the end of winter and beginning of spring [20, 21]. Although much of our vitamin D comes from sunlight, Canadians rely on vitamin D from food and supplements to maintain vitamin D levels in winter. These were both lower in less advantaged youth. Amongst low income-food insecure boys, fruit and vegetable consumption was lower than low income households which were food secure. Amongst 9-18 year old boys in the CCHS 2.2 food insecurity alone was not associated with a decrease in fruit and vegetable consumption [19]. This suggests that food insecurity and low income may have additive effect on nutritional deprivation. The largely community-based response to food-insecurity and income subsidies to low income households may have shielded low-income and food insecure youth from greater nutritional deprivation [33].

We found evidence that low income was associated with lower height in youth from both sexes. Youth from households belonging to the two lowest income groups who had reported being food insecure in the previous 12 months did not have lower height percentiles compared to their food secure counterparts. Similar results have been reported in youth recruited from a study in Northern Ireland where 15 year olds had a 3cm height difference comparing those whose parents had manual occupations versus youth whose parents had non-manual occupations [34]. We also found that 9-11 old youth had income associated height disparities similar to 12-18 year olds suggesting that these height differences are established before puberty. Approximately 80% of income associated height differences are established early, during the period of rapid postnatal growth. Thus the height differences reported here are likely due to nutritional and/or psychosocial exposures in the first 2 years of life [35].

As shown by our data and others [36], the association between low income and obesity is complex. We found low income was associated with a higher prevalence of obesity in girls. As low income women are widely reported to be at increased risk of being overweight [37] our results suggest that the relationship between low income and overweight in women may be established early. The relationship between low income and overweight in men is less consistent. In our data, there was a non-significant trend of increased prevalence of $\geq$ 85th percentile of BMI in boys from lower income households. The reasons for the inconsistency in the relationship between overweight and low socio-economic status across sexes is not well understood. Low income-food insecure boys had a higher prevalence of obesity than low income-food secure boys. Amongst the food insecure families there was a tendency for there to be more boys, the boys were significantly more overweight and the boys tended to be taller. As these data are cross-sectional, it may be that the greater caloric intake of larger boys may prove difficult for low-income households to maintain the dietary adequacy. This finding is supported by a qualitative study which showed that even with two incomes the large appetite of growing children makes it difficult to maintain a balanced budget [38].

Among low income and low income-food insecure households there was a higher prevalence of single parent households than higher income households and low income-food secure households, respectively. Lone parent households, and in particular single mothers, experience heightened economic vulnerability [27]. In Canadian youth from lone parent households there was a higher prevalence of multiple chronic disease risk factors such as excess adiposity, smoking and sedentary behavior [7]. Our results are consistent with this report as there was a higher prevalence of smoking and greater adiposity amongst low income girls though we did not isolate the contribution of lone parent households in our analysis.

This study has a number of limitations. For example, the sampling frame does not capture hard to reach populations, such as street youth, who experience higher levels of socio-economic [39] and nutritional vulnerability [40]. There is a high degree of intra-individual variability in a single dietary 24 hour recall [41]. A single 24 hour recall, however, can be used to accurately assess the mean dietary intake of a group of individuals but there is likely some attenuation of the effects seen due to misclassification. There was likely dietary under-reporting in the highest income girls. Dietary under-reporting is common in youth, particularly youth who may have body image issues [42]. Consistent with this, girls from the highest income households had the lowest prevalence of $\geq$ 85th percentile of BMI. Thus, while these youth may be under-reporting unhealthy food choices, like sweetened beverages, the effect of these food choices on the anthropometric measures was not evident. Finally, as this study is cross-sectional we cannot establish the causality between low income, food insecurity and anthropometric measures such as height and obesity.

To conclude, using a representative sample of Canadian youth, we found evidence that girls from low income households and boys from food insecure household had higher levels of $\geq$ 85th percentile of BMI. Both boys and girls from low income households had lower height percentiles than those of high income households. We also found evidence that milk was and may be partially replaced by less costly sweetened beverages. Consistent with this, calcium and

vitamin D intakes were lower in low income and there was some evidence of this amongst low income-food insecure households. Public health campaigns to increase diet quality amongst low income and food insecure youth may be unsuccessful due to the budgetary constraints on low income households [43]. Alternative means of increasing diet quality amongst low income and food insecure youth may be needed [44].

Acknowledgments

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Tables

Table 6.1: Demographic, height, weight and activity measured by income in youth of the CCHS study aged 9-18 (n=7378)

(%)	Sex	Lowest income (10.4)	Low Middle (20.8)	Upper middle (35.9)	Highest income (33.0)	P
% Female		51.8 (45.7-57.9)	49.1 (45.1- 53.0)	49.2 (46.5 – 51.9)	46.7 (44.1- 49.3)	0.151
Age	Boys	13.0 (12.5– 13.5)	13.1 (12.8 –13.3)	13.3 (13.1 – 3.5)	13.4 (13.2 – 13.6)	0.020
	Girls	13.0 (12.5– 13.5)	13.3 (13.0 – 13.6)	13.0 (12.8 – 3.3)	13.4 (13.2 – 13.7)	0.160
% of households with post-sec. degree	Boys	49.4 (41.0-57.7)	57.0 (51.4-62.6)	72.5 (69.0-76.0)	86.7 (83.4-90.0)	<0.001
	Girls	47.4 (39.7-55.1)	62.3 (57.3-67.4)	72.8 (69.0-76.6)	85.8 (82.8-88.8)	<0.001
% Food insecure	Boys	34.7 (25.6 - 43.8)	14.3 (9.9 -18.6)	4.1 (2.5 – 5.7)	2.2 (0.50-3.9)	<0.001
	Girls	30.0 (23.2–38.7)	9.9 (6.6-13.2)	3.7 (2.2 – 5.1)	0.5 (0.1 – 0.9)	<0.001
% Single parent households	Boys	43.8 (35.4-52.1)	32.9 (27.4-38.3)	20.0 (16.6-23.3)	6.7 (4.4-9.0)	<0.001
	Girls	47.6 (39.9-55.2)	27.8 (23.2-32.3)	15.4 (12.6-18.3)	6.2 (4.1-8.2)	<0.001
% Immigrant households	Boys	13.3 (8.2-18.4)	11.1 (7.5-14.5)	7.7 (5.0-11.0)	4.3 (3.9-6.0)	<0.001
	Girls	16.1 (9.9 – 22.3)	10.5 (7.0-14.1)	5.8 (3.9 – 7.8)	3.4 (1.9-4.9)	<0.001
% $\geq 85^{th}$ percentile †	Boys	30.9 (20.5-41.3)	31.0 (24.9-37.2)	33.7 (28.6-38.8)	26.6 (21.7-31.6)	0.313
	Girls	31.9 (22.9-40.9)	27.8 (21.2-34.4)	29.5 (25.1-33.9)	21.5 (17.0-26.0)	0.031
Height percentile	Boys	51.4 (45.0-57.9)	57.9 (54.4-61.3)	56.7 (53.7-59.7)	59.9 (56.7-63.1)	0.047
	Girls	52.3 (47.0-57.6)	47.8 (44.0-51.7)	55.6 (52.3-58.9)	58.0 (54.8-61.3)	0.001
Sedentary behaviour (hours) †	Boys	2.7 (2.2-3.2)	3.1 (2.8-3.5)	3.5 (3.2-3.8)	3.3 (3.0-3.6)	0.012
	Girls	2.5 (2.0-2.9)	2.7 (2.3-3.0)	2.6 (2.4-2.8)	2.9 (2.7-3.5)	0.099
% Ever smoked *	Boys	14.5 (10.2-18.8)	12.7 (9.8-15.6)	13.0 (10.9-15.1)	10.5 (8.6-12.5)	0.258
	Girls	21.3 (16.3-26.3)	15.5 (12.4-18.6)	14.0 (11.7-16.3)	11.3 (9.1-13.5)	0.039

Values in cells are means (95% confidence interval); P-values represent a trend in income

† Adjusted for age in regression; * Smoking is not measured in participants < 12 years of age

Table 6.2: Foods and nutrients of public health importance by household income in 9-18 year olds in the CCHS 2.2 (n= 7378)

(%)	Sex	Lowest income (10.4)	Low Middle (20.8)	Upper middle (35.9)	Highest income (33.0)	P
Total calories	Boys	2490 (2333-2647)	2660 (2526-2794)	2592 (2500–2685)	2778 (2662–2893)	0.013
	Girls	2115 (1983-2247)	2087 (1979-2195)	2088 (2015-2162)	2013 (1955-2071)	0.124
Fruit / vegetable servings	Boys	2.3 (1.8-2.8)	2.6 (2.2-3.0)	2.5 (2.2-2.8)	2.4 (2.2-2.6)	0.410
	Girls	2.3 (1.9-2.7)	2.7 (2.1-3.2)	2.6 (2.3-2.8)	2.7 (2.4-2.9)	0.150
Sweetened drink servings	Boys	1.9 (1.5-2.3)	1.6 (1.4-1.9)	1.8 (1.7-2.0)	1.8 (1.6-2.0)	0.006
	Girls	1.3 (1.0-1.6)	1.4 (1.2-1.5)	1.3 (1.2-1.4)	1.1 (1.0-1.2)	0.410
Milk servings	Boys	1.3 (1.1 – 1.5)	1.4 (1.3 – 1.6)	1.5 (1.4 – 1.7)	1.8 (1.6 – 1.9)	0.001
	Girls	1.1 (0.9 – 1.3)	1.1 (0.9-1.3)	1.1 (1.0 – 1.2)	1.2 (1.1 – 1.3)	0.111
Vitamin D (μg)	Boys	6.4 (5.6-7.2)	7.3 (6.6-8.0)	6.9 (6.5-7.3)	8.0 (7.5-8.5)	0.026
	Girls	5.3 (4.6-6.0)	5.5 (4.7-6.3)	5.3 (5.0-5.7)	5.7 (5.2-6.2)	0.210
Calcium (mg)	Boys	1145 (1010-1281)	1200 (1119-1282)	1203 (1143-1263)	1374 (1306-1443)	0.005
	Girls	914 (828-1001)	920 (838-1002)	967 (919-1015)	1014 (951-1077)	< 0.001
Fiber (grams)	Boys	17.1 (15.7-18.5)	17.0 (15.8-18.2)	16.9 (16.1-17.8)	17.7 (16.9- 18.5)	0.490
	Girls	14.4 (13.0-15.8)	14.4 (13.5-15.3)	14.4 (13.7-15.1)	14.7 (14.0-15.3)	0.130
Saturated fat (% of energy) †	Boys	10.1 (9.6-10.7)	11.1 (10.6-11.7)	10.6 (10.3-10.9)	11.1 (10.7-11.4)	0.131
	Girls	10.1 (9.5-10.8)	10.6 (10.1-11.0)	10.4 (10.0-10.8)	10.6 (10.3-11.0)	0.270
Energy intake / estimated energy expenditure †	Boys	0.93 (0.83-1.04)	0.97 (0.91-1.03)	0.91 (0.87-0.96)	0.98 (0.93-1.02)	0.563
	Girls	1.01 (0.93-1.09)	1.03 (0.95-1.1)	1.03 (0.98-1.07)	0.95 (0.90-0.99)	0.042

Values in cells are means (95% confidence interval); P-values represent a trend in income after adjusting for energy intake
† Not adjusted for energy intake; * Smoking is not measured in participants < 12 years of age

Table 6.3: Demographic, height, weight and activity stratified by food security status amongst the two lowest income groups in youth of the CCHS study aged 9-18 (n=2280)

Characteristic (%)	Sex	Food secure and low income (79.7)	Food insecure and low income (20.3)	P
Female (%)		50.9 (47.5-54.4)	45.0 (37.4-52.7)	0.181
Age (years)	Boy	13.3 (13.0-13.5)	12.1 (11.6-12.6)	< 0.001
	Girl	13.2 (13.0-13.5)	12.9 (12.3-13.5)	0.318
% of households with post-secondary degree	Boy	56.3 (51.3-61.2)	59.1 (54.3-64.0)	0.280
	Girl	48.6 (38.1-59.2)	50.6 (41.2- 60.0)	0.094
% Single parent households	Boy	32.5 (27.6-37.5)	51.1 (40.2-61.9)	0.002
	Girl	31.2 (26.6-35.9)	49.9 (40.2-61.9)	< 0.001
% Immigrant household	Boys	13.6 (10.3 – 16.9)	4.5 (1.2 - 7.7)	0.003
	Girl	12.5 (9.0- 16.0)	12.4 (4.7- 20.2)	0.995
$\geq 85^{th}$ percentile of BMI	Boy	27.1 (21.8-32.4)	45.0 (31.1-58.9)	0.034
	Girl	30.0 (24.0-36.1)	26.8 (17.0-36.7)	0.525
Height percentile	Boy	54.8 (51.4-58.2)	60.0 (52.3-67.7)	0.209
	Girl	49.6 (46.1-53.2)	49.1 (42.7-55.5)	0.876
Sedentary behaviour † (hours / day)	Boy	3.0 (2.7-3.3)	2.8 (2.2-3.5)	0.038
	Girl	2.6 (2.3-2.9)	2.4 (1.9-2.9)	0.471
Physical activity †	Boy	2.5 (2.2-2.8)	2.1 (1.4-2.7)	0.920
	Girl	1.6 (1.4-1.9)	1.3 (1.0-1.5)	0.082
% Ever smoked *	Boy	9.1 (6.2-11.9)	6.6 (2.9 - 10.3)	0.849
	Girl	12.0 (8.4-15.6)	6.8 (2.9 – 10.6)	0.140

Values in cells are means (95% confidence interval); P-values represent the effect of food insecurity amongst individuals in the two lowest income groups

† Adjusted for age in regression

* Smoking not measured in participants < 12 years old

Table 6.4: Nutrients and foods of public health importance by strata of food security for CCHS participants aged 9-18 years (n= 2280)

(%)		Low income and Food Secure (79.7)	Lower Income and Food Insecure (20.3)	P
Total calories	Boy	2611 (2494-2728)	2581 (2332-2830)	0.833
	Girl	2091 (1996-2185)	2143 (1990-2297)	0.573
Fruits and vegetable servings	Boy	2.7 (2.3-3.0)	2.0 (1.4-2.7)	<0.001
	Girl	2.6 (2.1-3.0)	2.2 (1.6-2.8)	0.535
Sweetened drink servings	Boy	1.8 (1.6-2.0)	1.5 (1.1-1.9)	0.204
	Girl	1.3 (1.1-1.4)	1.7 (1.3-2.0)	0.032
Milk servings	Boy	1.4 (1.3-1.6)	1.3 (1.0-1.5)	0.302
	Girl	1.2 (1.0 – 1.3)	0.8 (0.6-0.9)	<0.001
Vitamin D from food (μg)	Boy	7.1 (6.5-7.8)	6.5 (5.4-7.6)	0.262
	Girl	5.6 (4.9-6.3)	4.7 (4.0-5.3)	0.008
Calcium	Boy	1181 (1107-1256)	1187 (1003-1370)	0.718
	Girl	932 (859-1004)	874 (774-973)	0.377
% energy from fat saturated fat †	Boy	10.8 (10.4-11.2)	11.1 (9.8-12.3)	0.656
	Girl	10.4 (10.0-10.9)	10.4 (9.7-11.1)	0.963
Fiber (grams)	Boy	17.1 (16.0-18.2)	16.6 (14.5-18.7)	0.730
	Girl	14.6 (13.8-15.4)	13.9 (12.4-15.5)	0.202
Energy intake / estimated energy expenditure †	Boy	0.95 (0.89-1.00)	1.0 (0.86-1.13)	0.547
	Girl	1.00 (0.95-1.08)	1.06 (0.96-1.16)	0.503

Values in cells are means (95% confidence interval); P-values represent the effect of food insecurity amongst individuals in the two lowest income groups after adjusting for energy intake † Not adjusted for energy intake in regression analysis

6.6 References

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CHAPTER 7: PUBLIC HEALTH POSSIBILITIES TO IMPROVE VITAMIN D LEVELS

Chapter 4 of this thesis established that vitamin D deficiency is observed amongst Québec youth at the end of winter and beginning of spring. Data from the 2007-2009 Canadian Health Measures Survey (CHMS), representative of the Canadian population, has been released allowing us to comment on the generalizability of our findings. The mean 25(OH)D levels of the 6-19 year old youths of the CHMS, comparable in age to the youth sampled in this thesis, had near optimal levels of vitamin D [1]. Even in winter, the mean of 6-11 year olds was close to optimal levels of vitamin D (> 75 nmol/L). In the QCAHS study there was a mean 25(OH)D level in youth of about 50 nmol/L during winter, about 15 nmol/L lower than youth of comparable age and season in the CHMS.

Despite these differences in 25(OH)D levels our results, reflecting the vitamin D status of Québec youth, are still likely consistent with the findings of the CHMS. In the QCAHS the samples were collected in 1999 but 25(OH)D assays were done in 2006 raising the possibility that our samples had degraded. We thus assessed if there had been 25(OH)D degradation over time by testing a small sub-set of 25(OH)D samples 18 months apart. We found no evidence of sample degradation. The apparent differences between the QCAHS and the CHMS results also cannot likely be explained by differences in latitude. For example, the highest latitude sampled in the QCAHS was 48.3 ^{0} N which is a more southerly latitude than Vancouver, British Columbia (49.2 ^{0} N), the 3rd largest city in Canada. There are no data yet available to examine differences in 25(OH)D levels by province in the CHMS, thus we are unable to ascertain if there is heterogeneity, by province, in 25(OH)D levels as there is for other chronic disease risk factors [2]. In the CHMS, however, the 10th percentile of 25(OH)D levels for 12-19 year olds was 32.0 nmol/L, a value which reflects youth sampled both in the summer and winter [3]. Thus the CHMS indicates, similar to the QCAHS that there is significant vitamin D deficiency in the winter. Further in both the CHMS and the QCAHS there was an age associated decline in vitamin D levels suggesting that adolescents are at risk of vitamin D deficiency, particularly at the end of winter.

Canadian Public Health Authorities must decide if vitamin D levels observed in Québec and Canadian youth are incompatible with bone health and and/or will place youth at greater risk of cardiovascular disease [4] and common malignancies in adulthood [5]. If vitamin D levels are considered to be too low then population wide measures will be needed to increase vitamin D levels. Chapters 5 and 6 of this thesis provide important information to guide public health actions to increase vitamin D levels in youth. In the QUALITY study we showed that a serving increase in milk consumption was associated with a 2.9 nmol/L increase in vitamin D levels and that physical activity conducted outdoors is not likely a viable public health option to increase vitamin D levels. Finally using a representative sample of Canadian youth, the CCHS study, we found evidence that milk intake may be being displaced by sweetened beverages in low socio-economic position households. Thus as we showed that both milk intake and vitamin D levels were patterned by household income in representative samples, care must be taken not to exacerbate socio-economic disparities in vitamin D levels through public health actions. Some of the possibilities to increase vitamin D levels at the population level are outlined below.

7.1 Safe sunlight exposure and malignant melanoma

Safe sunlight exposure is defined as exposure to sunlight which brings benefits such as cutaneous vitamin D production without the detrimental effects of skin damage and its ensuing risk of skin malignancies. The amount of safe sun exposure depends on the amount of pigmentation (melanin) in the skin. Dermatologists have classified skin type into 5 types. The darkest, class 5, is resistant to UV damage, the lightest skin is from individuals who, upon exposure to UV light, accrue immediate DNA damage to epidermal cells [6].

Malignant melanoma is a potentially lethal skin malignancy caused by a malignant transformation of melanocytes. The age standardized incidence rate of malignant melanoma in Canadians is 12 / 100 000 [7]. The estimated five-year survival ratio for malignant melanoma is 90%. The incidence of malignant melanoma is increasing in Canada at approximately 1.5% / year. It is not clear, however, if the increase in malignant melanoma incidence is an artifact of improved clinical detection [7].

The primary etiologic exposure causing malignant melanoma is believed to be ultraviolet radiation, though the relationship between exposure and malignancy is not linear [8]. For example, outdoor workers have a lower incidence of malignant melanoma than indoor workers, suggesting that chronic sun exposure is protective. Further, malignant melanoma develops most frequently on the backs of men and the legs of women, areas of the body which are less exposed to sunlight. Evidence in support of UV light as an etiological exposure includes a higher incidence of this malignancy in Caucasians. Moreover individuals with the genetic disorder xeroderma pigmentosum, who lack DNA repair enzymes which repair thymidine dimers caused by UV exposure to the skin, have a 1000 fold greater risk of malignant melanoma than those without this condition. Malignant melanoma has also been associated with intermittent sun exposure. For example, a meta-analysis of 23 studies found that intermittent sun exposure was associated with an odds ratio of 1.71 while chronic sun exposure had an odds ratio of 0.76, suggesting a protective effect [9]. There has been some suggestion that UVA exposure also poses a risk for the development of malignant melanoma [10]. The association with UVA is germane as most tanning salons use UVA bulbs to produce cosmetic tans [11]. Consistent with a role for UVA in malignant melanoma, a number of reports have found an association between tanning bed use, sunlamps and malignant melanoma [12].

Public health concerns over malignant melanoma have been primarily responsible for 'Sun Safe' public health messages [13]. As UVB exposure is the largest determinant of vitamin D status a conflict exists between scientific and corporate interests, such as the artificial tanning industry and the sun-screen manufacturers, who advocate for more sunlight exposure for vitamin D synthesis or less sunlight exposure to protect against skin malignancies, respectively [14]. For example malignant melanoma researchers insist that vitamin D can be obtained using dietary supplements or through fortification. Some vitamin D researchers, however, have argued for 'safe' sunlight exposure as a solution to a high prevalence of low vitamin D status [15].

7.2 Dietary vitamin D

Dietary vitamin D plays a small but important role in maintaining vitamin D levels, particularly at high latitudes during winter months. Milk, however, often does not contain the legislated amount of vitamin D [16]. For example, 62% of milk samples, which included 13 different brands, had less than 80% of the vitamin D stated on the label [17]. Other dietary sources of vitamin D are equally variable in their vitamin D content. For example, farmed salmon has substantially less vitamin D than wild caught salmon [18]. It is becoming increasingly clear that doses of vitamin D required to prevent hypovitaminosis ($\leq$ 37.5 nmol /L) may have to be higher than the currently recommended 5 μg/day in youth. For example, 20 μg were required to prevent hypovitaminosis in Finnish (60^0 N) youth [19]. Thus increased amounts of vitamin D intake are unlikely to be achieved through dietary means as most foods, except for oily fish do not contain sufficient vitamin D.

7.3 Fortification

Fortification of foods is defined as a public health policy of adding nutrients to foods to ensure that minimum dietary requirements are met in the population. The scientific justification for fortification should be a public health need, such as a level of nutrient deficiency documented in a representative sample of a population. There are two different means by which nutrients can be added to the food supply, through either mandatory or through discretionary fortification. Mandatory fortification is a policy implemented by a central governing agency when a public health need is identified. All individuals are expected to benefit from fortification through the consumption of staple foods [20]. Discretionary fortification, on the other hand, is an unregulated process left to food manufacturers who often use the addition of extra nutrients as a means to market food products [21].

7.4 Discretionary versus mandatory fortification

Discretionary fortification is not likely to achieve higher levels of nutrient intake in the whole population as the cost of fortified foods tends to be higher than their non-fortified counterparts. In the US, discretionary fortification of vitamin D is found in a range of food products including: cereal flours, such as rice and macaroni, yogurt, fluid milk and

dry whole milk [20]. Discretionary fortification is further complicated by the difficulty of manufacturers in adhering to fortification guidelines resulting in too little or too much vitamin D than stated on the label [16]. This underscores the need for the active regulation of the addition of vitamin D in foods by conducting regular surveys of the vitamin D content of staple foods.

7.5 Vitamin D fortification in Canada

As vitamin D is fat soluble, accumulating in fatty tissues [22], the excessive intake of vitamin D through fortification has resulted in a number of cases of vitamin D toxicity owing to the consumption of excessively supplemented milk [23, 24]. The clinical symptoms of vitamin D toxicity include: hypercalcemia, hypercalciuria, anorexia, nausea and vomiting. Health Canada, under the authority of the Food and Drugs Act, regulates the safety and nutritional quality of all foods, this includes the mandatory fortification of fluid milk and margarine with vitamin D (16). Under the current fortification scheme each 250 ml serving of milk is mandated to contain 6.25 μg of vitamin D [25]. Thus for youth and adults less than 50 years of age, consumption of 2 glasses of milk provide more than the recommended daily intake of vitamin D.

It is widely recognized that current levels of dietary vitamin D in the North American food supply are inadequate to support optimal vitamin D levels [26]. In response, the IOM has commenced a review of the Dietary Reference Intakes for calcium and vitamin D as they relate to various health outcomes including those of bone health and for a number of chronic disease as well [27]. This committee includes experts from Canada and the US. The results from this enquiry are expected to be published in 2010. If the IOM should increase the recommended amount of dietary vitamin D this would open the possibility of increasing the amount of dietary vitamin D in the food supply through higher levels of fortification.

7.6 Challenges associated with fortification

One of the rationales for the modification of a fortification policy is to meet the nutritional needs of a population. The IOM recognizes four distinct life stages during which individuals' nutrient needs are physiologically different. These stages are: infancy,

toddlers aged 1 to 3 years, pregnancy and lactation (25). With respect to infancy, the Canadian Pediatric Surveillance System has reported an annual incidence of 2.9 / 100 000 of clinically confirmed cases of vitamin D deficient rickets [28]. Vitamin D deficiency has also been observed amongst pregnant and lactating women in Canada [29]. Our work has shown that youth are also at risk of vitamin D deficiency, particularly at the end of winter and beginning of spring [30]. Finally, increased age is associated with a decreased ability to synthesize cutaneous vitamin D placing the elderly at risk of low vitamin D levels [18]. Thus the target populations for vitamin D fortification are broader than the fortification of flour with folic acid which reduced the incidence of neural tube defects in Canada and the US [31].

Fortifying staple foods without increasing the risk of adverse physiologic effects is complex. For example, long-term consumption of higher than recommended folic acid increases the risk of colorectal malignancy in individuals at high risk of colorectal neoplasia [32]. This finding is supported by work in animal models which showed that folate supplementation may have a pro-mutagenic effect on neoplastic foci once they have become established [33]. Thus while the fortification of flour with folic acid met its objective of reducing the incidence of neural tube defects, a greater exposure to folic acid may be detrimental to other physiologic states and increase the risk of disease in an unforeseen way. Moreover as energy intake is highly correlated with the intake of most nutrients, individuals who have the greatest food intake, such as growing adolescents, have the greatest exposure to a nutrient fortified in a staple food [34].

7.7 Knowledge translation steps

Knowledge translation, defined by the Canadian Institute of Health Research (CIHR), is the dynamic and iterative process that includes synthesis, dissemination, exchange and ethically-sound application of knowledge to improve health, provide more effective health services [35]. In this thesis we showed that levels of vitamin D deficiency in the first representative sample of Canadian youth may constitute of a public health problem. Unfortunately, the modifiable correlates of vitamin D levels are not likely effective public health targets to obtain optimal levels of vitamin D [36]. This suggests that an increase in vitamin D levels in youth will require a change in Canada's fortification

policy. This is a complex multi-step process. We will propose a number of steps by which to achieve this end.

1) Establish a multidisciplinary committee to examine the long-term consequences of vitamin D deficiency or sub-optimal vitamin D in Canadian youth versus the risks of increasing the amount of vitamin D in a staple food through fortification. This committee would consist of clinicians, epidemiologists and experts on vitamin D toxicology. These efforts would coincide with the initiative of the Institute of Medicine, to revise, likely upward, the recommended intake level of vitamin D. Similarly, the upper level (UL) of vitamin D consumed without adverse effects is also likely going to be revised upwards in adults and in youth [23].

2) Examine different fortification strategies with which to increase the amount of vitamin D in the Canadian food supply. Our results from the CCHS study showed an income related gradient in milk intake in Canadian youth. Thus increasing the amount of vitamin D in milk would likely exacerbate income related disparities in vitamin D levels that we observed in the QCAHS study. The fortification of white flour with vitamin D should be considered. A study in adults showed that bread supplemented with (50 μg) of vitamin D per day was associated with optimal vitamin D levels.

CHAPTER 8: CONCLUSIONS AND SUMMARY

We established, using the first representative sample of youth in Canada, that vitamin D deficiency was observed amongst Québec youth. Data from the Canadian Health Measures Survey [1] suggest our results are generalizable to Canadian youth. We found that milk intake resulted in only a modest association with vitamin D level and that milk consumption was being displaced by sweetened beverages amongst low socio-economic position youth. Thus a public health message to increase milk consumption as a means to increase vitamin D levels in youth might not be the most efficacious means to increase vitamin D levels.

This thesis has a number of limitations. First, all three data sources were cross-sectional thus we cannot infer causality from our results. It is, however, unlikely that vitamin D levels could influence the season with which vitamin D was sampled or that vitamin D levels could influence physical activity. Second, we did not measure sun exposure in the QUALITY study and this is the greatest source of vitamin D [37]. Sunlight exposure, however, is difficult to measure particularly using questionnaires [38]. Our sample was restricted to Caucasian youth thus our results represent a best case scenario as darker skin pigmentation is associated with lower vitamin D levels [18]. Finally, though we found low vitamin D levels in Québec youth, we did not assess the physiologic implications of this. For example, low vitamin D levels have been associated with lower bone density in youth [19] and have been associated with a number of common malignancies in adults [5].

As most dietary vitamin D in the Canadian food supply comes from fortified foods, efforts to increase vitamin D levels will require changing levels of fortification in staple foods. To help inform this public health action, the associations between low vitamin D levels and a number of chronic diseases will have to be validated in well designed and powered randomized trials. Our results, using the first representative sample of youth in Canada, suggest that vitamin D deficiency is observed in Canada and public health actions are needed to increase vitamin D levels.

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APPENDIX