MILK FAT CONTENT, VITAMIN D, AND ADIPOSITY IN EARLY CHILDHOOD

by

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A thesis submitted in conformity with the requirements for the degree of Master of Science

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2016

ABSTRACT

Background: Vitamin D is fat soluble and cow’s milk is the main dietary source of vitamin D in North America. I hypothesized higher fat milk would be associated with higher 25-hydroxyvitamin D and decreased BMI in children.

Methods: Multivariable linear regression and bivariate regression models were used to test the associations between percent fat content of milk and 25(OH)D, and both 25(OH)D and BMI z-scores (zBMI) in children 12-72 months.

Results: Higher fat milk had a positive effect on 25(OH)D, but a negative effect on zBMI. Children who drank whole milk had 25(OH)D 5.4 nmol/L (95% CI 4.3 to 6.5) higher than children who drank 1% milk, but a 0.72 (95% CI 0.68 to 0.76) lower zBMI. Volume of milk modified the relationship between percent fat content of milk and 25(OH)D (p=0.003), but not zBMI (p=0.77).

Conclusion: Consumption of lowfat milk may compromise both 25(OH)D and adiposity in children.
ACKNOWLEDGMENTS

My master’s degree has been an incredible learning experience. It would seem obvious that I have predominantly gained academically, but I have grown in so many other ways. I have learned discernment, gained confidence, and grown in creativity as a result of the mentorship of Jonathon Maguire, among other leaders. I’d like to thank him for being approachable, inspiring and flexible throughout my degree. Being a part of the TARGetKids team has been both a challenging and encouraging experience – I am so lucky to be able to collaborate with and learn from such accomplished and intelligent people. I feel that my experience as a graduate student is unrivaled to any student who doesn’t have the opportunity to be a part of a team such as this.

I am appreciative of everyone who has worked hard to make TARGetKids the team that it is, and those who believe in advancing it even further. The innovation, creativity and passion within the group is inspiring and has enabled me to see how caring and driven each member is. I’d like to recognize each family who took time to participate in TARGetKids, and the practitioners who were willing to incorporate our research into their practice. To principal investigators Catherine Birken and Patricia Parkin, as well as the research manager, Dalah, coordinator, Kanthi, as well as assistants Tarandeep, Kathleen, Antonietta, Megan, Laurie and Dharma, thank you for your hard work to make my project possible. Thank you to my committee members, Deborah O’Connor and Gerald Lebovic, for your encouragement and constructive feedback. Gerald, I have so much gratitude for your incessant patience with me, making time for my endless questions and analysis glitches.

Thank you to Grace Lee and Jessica Omand for being the hard working and bright role models you are. Grace, you always made time for my questions, but most of all are a great friend and colleague; therefore making my degree far less stressful and more fun than it could have been. Jess, your skills and passion for research are so admirable. Thanks for always being available and resourceful. Jill, thanks for being a supportive friend and eagerly doing your best to understand my research. To my family, I am grateful for your ongoing support, encouragement, and attempts to figure out what it is I do.

To everyone who has helped me through this process, I am appreciative of your support.
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**LIST OF ABBREVIATIONS**

25(OH)D  25-hydroxyvitamin D  
AAP    American Academy of Pediatrics  
BMI    Body Mass Index  
CCHS   Canadian Community Health Survey  
CI     Confidence Interval  
CPS    Canadian Pediatric Society  
DBP    Vitamin D Binding Protein  
DRI    Dietary Reference Intake  
EAR    Estimated Average Requirement  
EWCFG  Eating Well with Canada’s Food Guide  
IOM    Institute of Medicine  
IU     International Units  
NHANES National Health and Nutritional Examination Survey  
OR     Odds Ratio  
RDA    Recommended Dietary Allowance  
UL     Tolerable Upper Level  
WHO    World Health Organization  
zBMI   z-score for Body Mass Index
STUDENT CONTRIBUTIONS

- Conceptualized research questions and designed analyses
- Performed statistical analysis using R version 3.1.1
- Presented research findings at several research symposia:
  - Sick Kids Research Retreat (2015)
  - University of Toronto Department of Paediatrics Research Day (2015)
- Wrote thesis
- Submitted 2 research articles for publication to Applied Physiology, Nutrition and Metabolism (Study 1) and the Canadian Medical Association Journal (Study 2)
CHAPTER 1: INTRODUCTION

Childhood nutrition is important for growth and development and disease prevention later in life (1). Adequate nutrition includes maintaining a healthy micronutrient and macronutrient balance (2). Optimal nutrition in early life is important to minimize risk of adverse health outcomes throughout the life course (3) and an important field of study.

Vitamin D is known to play a critical role in child bone development and health, being a key nutrient in the prevention of rickets (4, 5). The American Academy of Pediatrics (AAP) recommends children’s serum 25-hydroxyvitamin D, the main indicator of circulating vitamin D levels in the blood, to be above 50 nmol/L (6). The Canadian Pediatric Society (CPS) advises the level to be above 75 nmol/L (7). Emerging research suggests links between vitamin D and various health conditions during childhood, including upper respiratory tract infections (8), asthma (9) and obesity (10, 11).

Children typically receive vitamin D from ultraviolet B rays; specifically in Toronto at a latitude of 43°N, during summer months (May-September) (12). The main dietary source of vitamin D for Canadian children is fortified cow’s milk (13-15). However, cow’s milk consumption is often inadequate to satisfy the daily requirement of vitamin D in children, and in winter months when ultraviolet rays are also insufficient (7, 12). According to the National Health and Nutrition Examination Survey (NHANES) in the United States 14% of children aged 1-5 years have serum 25-hydroxyvitamin D <50 nmol/L, and 62% have serum 25-hydroxyvitamin D <75 nmol/L (16). Survey data on Canadian children’s serum 25-hydroxyvitamin D levels were collected in 2011 by the Canadian Health Measures Survey, and show that 11% of three to five year olds were insufficient, which was defined as <50 nmol/L (17).
Cow’s milk vitamin D fortification is standardized in Canada at 100 IU per cup (250mL) (18). Guidelines given by the CPS and AAP state that children between the ages of one and two years should consume 2 cups per day of whole milk (3.25% milk fat), and for older children low fat (2% or 1%) milk is recommended to reduce dietary fat, and lower a child’s risk of obesity (19, 20). In North America, children have steadily decreased their cow’s milk consumption over time. According to NHANES data, the percentage of pre-adolescent children who do not consume milk has risen from 12% in 1978 to 24% in 2008. In addition, the percentage of children consuming whole milk (3.25% fat) has decreased from 60% to 32% in the same timeframe (21).

In North America, the incidence of childhood overweight and obesity has doubled over the past 30 years (22). Several factors play into the accumulation of excess adiposity in childhood, including sugar-sweetened beverage consumption, which is often substituted for cow’s milk (23, 24). Various studies have suggested a relationship between reduced serum 25-hydroxyvitamin D and childhood overweight/obesity (25-32). A possible inverse link between consuming high fat milk and adiposity in children has also been explored, but the volume children drink of milk may modify this relationship (33-37).

The purpose of this thesis was to evaluate the following research objectives: 1) to determine the relationship between the fat content of cow’s milk consumed and serum 25-hydroxyvitamin D in early childhood (Study 1), 2) to determine the relationship between percent fat content of milk and 25-hydroxyvitamin D and BMI z-score (zBMI) simultaneously (Study 2), and 3) to explore the roles of volume of milk consumed and age in these relationships (Studies 1 & 2).

The following literature review (Chapter 2) is divided into three sections: vitamin D, cow’s milk, and childhood adiposity. Chapters 3 and 4 expand on two studies: Study 1: *Milk fat*
content and vitamin D in early childhood, and Study 2: The relationship between milk fat content, vitamin D and adiposity in early childhood. A general review and discussion of results can be found in Chapter 5, and Chapter 6 concludes the thesis.
CHAPTER 2: LITERATURE REVIEW

2.1 Vitamin D

2.1.1 Structure and function

Vitamin D is a fat-soluble steroid that takes two forms, vitamin D$_2$, or ergocalciferol; and vitamin D$_3$, or cholecalciferol (38). Vitamin D$_2$ is made from ultraviolet irradiation of ergosterol found in yeast and fungi, and is commonly produced synthetically for food fortification. Vitamin D$_3$ is synthesized in the skin as a result of ultraviolet irradiation of 7-dehydrocholesterol, or synthetically from sources such as wool. Vitamin D$_3$ is also commonly added to foods for fortification (39). Vitamins D$_2$ and D$_3$ differ in structure and possibly function; vitamin D$_3$ is known to have a longer half-life and bind more tightly to vitamin D-binding protein in the blood, thus raising blood concentrations more effectively (Figure 1 (40)) (39). Once consumed, both D$_2$ and D$_3$ are hydroxylated in the liver to create 25-hydroxyvitamin D followed by a second hydroxylation in the kidney to create 1,25-hydroxyvitamin D (calcitriol), the biologically active form of vitamin D (38).

**Figure 1:** Structures of vitamin D$_2$ (ergocalciferol) and vitamin D$_3$ (cholecalciferol).
The main function of vitamin D is to regulate calcium and phosphate homeostasis in the body; thus it is predominantly active in bone health and mineral balance. Vitamin D promotes calcium and phosphorous absorption in the digestive tract when necessary to ensure healthy bone ossification, as well as aiding in osteoclast and osteoblast activity to mineralize and recycle bone tissue when appropriate (41). Other functions of vitamin D include neuromuscular, immune and cell growth maintenance; thus, it is a critical nutrient to skeletal and overall health (42, 43).

2.1.2 Fat solubility

Vitamin D is a fat soluble vitamin and it is absorbed in the intestinal tract similarly to fat. Fat ingestion stimulates bile acid secretion, which breaks down fats and allows for lipolytic enzymes to work on a greater surface area of fats and fat-soluble vitamins. Micelles then carry fats and fat-soluble vitamins via passive diffusion into the bloodstream (44). Thus, the presence of fat in the intestine is believed to assist its absorption through the intestinal lumen and promote its secretion into the bloodstream (45). Vitamin D is then stored in adipose or liver tissue (38).

The ideal amount or type of fat for optimal vitamin D absorption, as well as maintenance of serum 25-hydroxyvitamin D levels, is unknown. One study determined that people who consumed a vitamin D supplement with a fat-containing meal had serum 25-hydroxyvitamin D 32% higher than those who consumed a supplement with a fat-free meal (45); similarly, another study established that people who consumed a vitamin D supplement with their largest meal of the day had higher serum 25-hydroxyvitamin D (46). Tangpricha et al. found no difference between the effect of vitamin D-fortified skim (0.1% fat) milk, whole (3.25% fat) milk and corn oil on toast on serum 25-hydroxyvitamin D (47). Finally, an analysis of the effects of consuming fat with a vitamin D supplement determined that there was no difference between fat free, low fat and high fat meals on serum 25-hydroxyvitamin D long-term; however, the low-fat group had the highest initial absorption of vitamin D (48).
2.1.3 **Sources**

2.1.3.1 **Endogenous synthesis**

Humans can synthesize vitamin D in the skin when exposed to ultraviolet B (UVB) rays between 290 and 320 nm (49). A molecule known as 7-dehydrocholesterol, or pre-vitamin D₃, naturally occurs subcutaneously both in the dermis and epidermis. Pre-vitamin D₃ is thermally activated by the sun and undergoes a series of double bond rearrangements within the molecule to form vitamin D₃. Next, it is delivered to the dermal capillary bed to be linked to vitamin D binding protein (DBP), where it is then released to the bloodstream for circulation (50). To prevent vitamin D toxicity, other compounds are created as a result of UVB irradiation such as lumisterol and tachysterol, which are inactive metabolites that are released into circulation (51).

2.1.3.2 **Exogenous sources**

2.1.3.2.1 **Food**

Food sources of naturally occurring vitamin D include fatty fish, and small amounts are found in mushrooms and egg yolks (52). A complete list of food sources of vitamin D can be found in Appendix B: Food Sources of Vitamin D.

2.1.3.2.2 **Fortification**

In Canada, both cow’s milk and margarine are fortified with vitamin D. In effort to combat low vitamin D levels, the Food and Drug Regulation states that cow’s milk must be fortified with 35-45 IU of vitamin D per 100 mL, and margarine is required to contain 530 IU/100g (equivalent to 75 IU/15 mL tablespoon) (53). Infant formula is also fortified with between 40 and 80 IU/100 kcal (54). Despite this legislation, several researchers have conducted tests to examine the vitamin D content in cow’s milk products available to North American consumers and have identified less vitamin D than the label value (18, 55, 56).
2.1.3.2.3 Supplementation during childhood

In Canada, children’s supplements are available containing doses of 200 to 600 IU of vitamin D$_2$ or D$_3$; higher doses are accessible with a prescription (57). Health Canada recommends that “all breastfed, healthy term babies receive a daily vitamin D supplement of 400 IU per day” (14). Children who are exclusively formula-fed receive 400 IU vitamin D/litre of fortified infant formula (7), so children consuming at least 1 litre of formula per day do not require supplementation. The American Academy of Pediatrics (AAP) “encourage[s] parents of infants who are either breastfed or consuming less than 1 litre of infant formula per day to give their infants an oral vitamin D supplement” (58). During childhood, the AAP recommends that “children who are consuming less than [1000 mL] per day of vitamin D-fortified formula or milk, should receive a vitamin D supplement of 400 IU a day,” and “those taking certain medications and with chronic diseases such as cystic fibrosis, may need higher doses of vitamin D” (59). Neither Health Canada nor the CPS have published guidelines for vitamin D supplementation of Canadian children older than 1 year of age.

2.1.4 Health implications of low vitamin D levels in early childhood

2.1.4.1 Functions in growth and development

Vitamin D is best known for its role in bone health and prevention of rickets during childhood. As bones progress through the stages of development and maturation, vitamin D plays an important role in bone mineralization supporting the availability of calcium and phosphorous in the blood (41). Biologically active vitamin D (calcitriol or 1,25-hydroxyvitamin D) stimulates duodenal and jejunal absorption of calcium and phosphorous (43). These two minerals are the main constituents of hydroxyapatite, which is responsible for bone strength and integrity (60). Calcitriol also plays a role in balancing both osteoblastic activity, to enhance bone mineralization, and osteoclastic activity, which aids in bone turnover to promote overall bone
Calcitriol and parathyroid hormone (PTH) are involved in a feedback loop to maintain calcium homeostasis. Vitamin D and calcium in the blood suppress PTH secretion, but when PTH levels are high, calcium is resorbed into the bloodstream in the distal tubule of the kidney and liberated from bone (Figure 2 (62)) (63, 64). Other functions of vitamin D essential to child growth and development include cell growth and metabolism, and immune system maintenance (7).

![Figure 2: Parathyroid hormone (PTH), vitamin D, and calcium in extracellular fluid (ECF) feedback loop.](image_url)

2.1.4.2 Recommended concentrations of serum 25-hydroxyvitamin D

The most commonly used, and believed to be the most accurate, measure of vitamin D in the blood is 25-hydroxyvitamin D (65). The National Institutes of Health and AAP state that the adequate serum 25-hydroxyvitamin D level in children is >50 nmol/L, to support bone and
overall health in most individuals (41, 66). However, CPS argues that the optimal level is >75 nmol/L, a level at which “parathyroid hormone production and calcium reabsorption from bone are minimized, and intestinal calcium absorption is stabilized” (7). These recommendations were primarily based on optimizing bone health in children.

2.1.4.3 Levels of 25-hydroxyvitamin D in children

Survey data on Canadian children’s serum 25-hydroxyvitamin D levels were collected in 2011 by the Canadian Health Measures Survey, and show that 11% of three to five year olds were insufficient, which was defined as <50 nmol/L (17). In the United States, the National Health and Nutrition Examination Survey determined in 2006 that 63% of children aged 1-5 had serum 25-hydroxyvitamin D levels <75 nmol/L, and 14% had levels <50 nmol/L (16). Both studies are comprehensive measures of representative samples of the North American population.

2.1.4.4 Consequences of vitamin D deficiency (<30 nmol/L)

The Institute of Medicine (IOM) has determined that persons with serum 25-hydroxyvitamin D 30-50 nmol/L are considered vitamin D insufficient, and a concentration <30 nmol/L is “associated with vitamin D deficiency” (41). The CPS, however, suggests the cutoff for vitamin D deficiency is <25 nmol/L (7). Rickets is the main manifestation of vitamin D deficiency in children; it is a condition in which the formation of collagen and bone fails, and usually develops between 3 and 18 months of age (66). When vitamin D is deficient in the body, calcium and phosphorus levels also drop, thus diminishing their availability to mineralize collagen matrices for bone production. The most prominent symptom of rickets is skeletal deformities. Low serum calcium can also result in seizures, growth failure, fatigue, and/or compromised immune function (4, 67, 68). Data from the Canadian Paediatric Surveillance
Program identified that between 2002 and 2004, there were 104 cases of rickets in Canada in children younger than 7 years of age (69).

2.1.4.5 Consequences of vitamin D insufficiency (<50 or <75 nmol/L)

Serum 25-hydroxyvitamin D levels of either 30-50 or 25-75 nmol/L, depending on reference cutoffs suggested by the IOM or CPS, respectively, mark vitamin D insufficiency. Several studies have identified cross-sectional associations between vitamin D insufficiency and various health outcomes in children, such as upper respiratory tract infections (8), asthma (9) and overweight/obesity (10, 11). Populations identified as being at risk for insufficiency and/or deficiency include breastfed infants not receiving a supplement (breast milk contains a concentration of vitamin D reflective of the mother’s serum levels, but does not provide adequate amounts daily to the child (66, 70)), people with limited sun exposure, people with dark skin, and people who have compromised intestinal absorptive abilities (41). Longitudinal investigations are needed to link child vitamin D health to such outcomes; so far, a developing field of research shows promising evidence for inverse relationships between vitamin D levels and the manifestation of type 1 diabetes (71), type 2 diabetes (72), multiple sclerosis (73), and glucose intolerance (74). Though the biological mechanisms in these relationships are poorly understood, vitamin D is believed to have an ability to act as an immunosuppressor, which can act beneficially in autoimmune disorders such as diabetes and multiple sclerosis (75). Additionally, Chiu and colleagues (74) observed an association between low serum vitamin D levels and impaired beta cell function, as well as insulin resistance.

2.1.5 Nutritional recommendations

Standards have been developed in both Canada and the United States by the Food and Nutrition Board of the Institute of Medicine to determine the recommended amount of vitamin D to be consumed daily (14, 38). Appropriate intake levels were established as the Estimated
Average Requirement (EAR), which is the amount thought to satisfy the needs of 50% of the healthy population; Recommended Dietary Allowance (RDA), the amount needed to meet the needs of 97% of the healthy population; and Tolerable Upper Level (UL), over which level adverse health effects may occur if consumed (76).

2.1.5.1 Dietary reference intake values

Dietary reference intake values, or the DRIs, for vitamin D can be found in Table 1 (14, 38, 43). These values were empirically determined based on observations in healthy adults. A daily intake of 400 IU, which is the EAR, of vitamin D per day is successful to maintain serum 25-hydroxyvitamin D levels at 40 nmol/L, 600 IU/day, or the RDA for children 1-8 years old, can maintain serum 25-hydroxyvitamin D at 50 nmol/L, and 800-1000 IU/day are required to increase serum 25-hydroxyvitamin D to 75 nmol/L (77). These calculations resulted from a mixed-model approach designed by the Institute of Medicine, based on a person who derives essentially all vitamin D from the diet, and has minimal sun exposure (78).

Table 1: DRIs for vitamin D.

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Adequate Intake (AI), per day</th>
<th>Estimated Average Requirement (EAR), per day</th>
<th>Recommended Dietary Allowance (RDA), per day</th>
<th>Tolerable Upper Level (UL), per day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infants 0-6 months</td>
<td>400 IU</td>
<td>N/A</td>
<td>N/A</td>
<td>1000 IU</td>
</tr>
<tr>
<td>Infants 7-12 months</td>
<td>400 IU</td>
<td>N/A</td>
<td>N/A</td>
<td>1500 IU</td>
</tr>
<tr>
<td>Children 1-3 years</td>
<td>N/A</td>
<td>400 IU</td>
<td>600 IU</td>
<td>2500 IU</td>
</tr>
<tr>
<td>Age Group</td>
<td>Vitamin D Level</td>
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<td>-----------------</td>
<td></td>
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<tr>
<td>Children 4-8 years</td>
<td>N/A</td>
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</tr>
<tr>
<td></td>
<td>400 IU</td>
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<tr>
<td></td>
<td>600 IU</td>
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<td></td>
<td>3000 IU</td>
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<tr>
<td>Children and adults 9-70 years</td>
<td>N/A</td>
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<tr>
<td></td>
<td>400 IU</td>
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<td></td>
<td>600 IU</td>
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<td></td>
<td>4000 IU</td>
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<tr>
<td>Adults &gt;70 years</td>
<td>N/A</td>
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<tr>
<td></td>
<td>400 IU</td>
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<td>800 IU</td>
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<td></td>
<td>4000 IU</td>
<td></td>
<td></td>
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<tr>
<td>Pregnancy and lactation</td>
<td>N/A</td>
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<td></td>
<td>400 IU</td>
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<td>4000 IU</td>
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</tbody>
</table>

2.1.6 Measurement of vitamin D stores

Although calcitriol (1,25-hydroxyvitamin D) is the active form of vitamin D in the blood, it is not a reliable indicator of body stores of vitamin D because of its short half life and may be elevated even when serum 25-hydroxyvitamin D is low (79). 25-hydroxyvitamin D has been suggested as the most accurate measure of vitamin D levels, as it is reflective of both dietary intake and sun exposure (42).

Serum 25-hydroxyvitamin D is a combination of both 25-hydroxyvitamin D$_2$ and 25-hydroxyvitamin D$_3$, so both of these must be quantified to attain an accurate measure of 25-hydroxyvitamin D. The majority of 25-hydroxyvitamin D in the blood is bound to vitamin D-binding protein (DBP), so measurement systems need to be capable of removing vitamin D from the binding protein (80).

There are various ways to measure vitamin D in the blood; liquid chromatography-tandem mass spectrometry (LC-MS/MS) is considered the gold standard method due to its high reproducibility. However, this procedure is very costly, complex, and lacks efficiency (81, 82). A quicker, cheaper, and more convenient method of measuring 25-hydroxyvitamin D in the blood is using a 2-step competitive chemiluminescence assay (LIAISON 25 OH Vitamin D TOTAL;
DiaSorin) (83). In this procedure, serum undergoes an initial incubation with anti-vitamin D particles that release 25-hydroxyvitamin D from DBP, and the freed vitamin D then binds to a solid phase antibody. Next, a second incubation involves isoluminol derivative-conjugated 25-hydroxyvitamin D, a tracer used to release light during the chemical reaction that follows, and all excess material is removed with a wash cycle (84). Reagents for the chemiluminescent reaction are added, and light is subsequently measured using a photomultiplier, in relative light units. This measure is inversely proportional to the amount of 25-hydroxyvitamin D in the sample (85). Specifically, the DiaSorin LIAISON 25 OH Vitamin D TOTAL has an intra-assay imprecision of 7.2% at a concentration of 213 nmol/L, and an inter-assay imprecision of 4.9% at 32 nmol/L, 8.9% at 77 nmol/L and 17.4% at 213 nmol/L, all of which are within adequate limits for biochemical measurements (86, 87).

2.1.7 Determinants of serum 25-hydroxyvitamin D

2.1.7.1 Skin pigmentation

Melanin, the molecule found in skin responsible for pigmentation, has the ability to influence UVB ray absorption and thus endogenous vitamin D synthesis (88). Pre-vitamin D, or 7-dehydrocholesterol, is primarily found in the epidermis, despite being present in both the dermis and epidermis layers of the skin. Thus, the bulk of its conversion to vitamin D through UV irradiation occurs in the epidermis; this is also the layer of the skin that contains melanocytes, which produce melanin (89). Melanin acts as a light filter and therefore determines the amount of UVB rays that are able to infiltrate and react with pre-vitamin D molecules in the skin (90). This mechanism lies in melanin’s ability to absorb UVB rays in the 290-320 nm range, which is comparable to that of pre-vitamin D, so it acts as a competitor (49, 51).

A review of the reported cases of rickets in the United States between 1986 and 2003 identified that 83% of cases were in African American or black children (91). It has been
suggested that populations with dark skin pigmentation may benefit from taking a vitamin D supplement at all times, especially those who are pregnant or lactating (92). A study of NHANES data showed that mothers with darker skin pigmentation were more likely to enter pregnancy with low vitamin D levels. This problem is exacerbated in their infants because of the low vitamin D content in breast milk, low UVB absorption due to high levels of melanin in the infant’s skin and sub-optimal vitamin D supplementation (93).

2.1.7.2 Adiposity

Several studies have identified an association between higher adiposity and lower 25-hydroxyvitamin D (26, 31, 32, 93, 94). Other notable factors explored in conjunction with low vitamin D in children are increased screen time and decreased physical activity (11), elevated triglyceride levels (30), and heightened c-reactive protein (95), which are themselves associated with adiposity in children. A genetic study using a cohort of over 42,000 adults investigated the causal link between low vitamin D levels and adiposity, which suggested that excess adiposity might be the initial trigger for vitamin D levels to fall, although numerous other factors such as diet and time spent outdoors may perpetuate both issues (96).

It has been hypothesized that in individuals with excess adipose tissue, vitamin D is made unavailable due to its fat soluble properties and it is sequestered into adipose tissues (97). High amounts of fat in the body render vitamin D’s accessibility in serum for cells, bones and other tissues (38, 97). Another possible mechanism involves PTH, in which low levels of vitamin D in the blood stimulate a rise in PTH, which causes an influx of calcium into adipocytes, where it causes lipogenesis to occur (98-100).

2.1.7.3 Supplementation

Vitamin D supplementation for all children is recommended by some regulatory health agencies, such as the AAP (59), but not others such as CPS and Health Canada (7, 14). It has
been established that children who supplement with vitamin D are less likely to develop rickets (6, 101). Various investigations have found that in older children, vitamin D supplementation is associated with higher levels of serum 25-hydroxyvitamin D and higher bone mineral density (13, 102).

### 2.1.7.3.1 Supplementation during breastfeeding

Breast milk is said to be the gold standard in terms of nutrition for infants, being complete in all ways except for one nutrient: vitamin D (66, 103). Without maternal supplementation, breast milk contains approximately 25-80 IU/L of vitamin D, which is insufficient for an infant’s daily vitamin D requirements (104). Reports of children who are breastfed and not supplemented with vitamin D show that they are at risk for vitamin D deficiency and developing rickets (69, 91, 105, 106). A study exploring all Canadian reports of rickets between 2002 and 2004 found that 94% of children with the condition had been breastfed and not supplemented with vitamin D (69).

High dose supplements (>2000 IU/day) for lactating women have been proposed to support the vitamin D needs of both the mother and child (104, 107-109). However, further research is needed to determine the safety of this approach and considering other factors such as season and skin pigmentation (66). There are no professional recommendations for mothers to supplement during breastfeeding with vitamin D; instead, breastfed children are to be supplemented with 400 IU/day of the nutrient, regardless of any infant formula intake (7, 59).

### 2.1.7.4 Sun exposure and latitude

Children receiving low amounts of sun exposure are at an elevated risk for vitamin D deficiency. In a light-skinned adult, spending 10-15 minutes in direct sunlight and wearing minimal clothing can generate between 10 000 and 20 000 IU of vitamin D. Darker-skinned individuals require 5-10 times that amount of exposure for approximately the same dose (110).
There are a variety of factors that further influence sun exposure and its ability to adequately influence vitamin D synthesis: season, sunscreen use, latitude, and type and amount of clothing worn (111).

During October-April in locations exceeding a latitude of 41°N, vitamin D is inadequate when sourced from UVB rays alone (12). In addition, the AAP states that children younger than 6 months should have no sun exposure, thus limiting dermal vitamin D synthesis. Older children are advised to wear sunscreen and avoid sun exposure through clothing, hats and shade, for the primary purpose of sunburn and heat stress protection; secondarily, for melanoma risk minimization later in life (112, 113). Hence, it is difficult even in summer months for young children to receive any vitamin D from the sun. For children living north of the 55th parallel (Edmonton and further north), the CPS recommends higher-dose supplements of 800 IU (compared to 400 IU) from October to April (7).

Higher daily screen time and lower daily outdoor play (114, 115), as is becoming increasingly common, is believed to manifest in a matrix of consequences such as increased adiposity (116), decreased sun exposure (117), and likely an increased consumption of processed foods low in nutritional value (118, 119); all of which are factors that may influence a child’s vitamin D status. A study using NHANES data by Turer et al. in 2012 identified associations between lower serum 25-hydroxyvitamin D, higher zBMI, higher screen time, and lower physical activity (11).

2.1.7.5 Cow’s milk consumption

Cow’s milk consumption has been identified as the main dietary source of vitamin D among North American children (13-15). Despite efforts to encourage children to drink milk, consumption patterns in children may not foster vitamin D sufficiency (120). Maguire et al. identified cow’s milk intake as a predictor of vitamin D stores; each cup of the beverage
increased children’s serum 25-hydroxyvitamin D levels by approximately 4 nmol/L (95% CI 2.5-5 nmol/L) (13). Various other analyses have explored the predictors of low serum 25-hydroxyvitamin D in children; low cow’s milk intake is a common predictor (121-123). The odds of vitamin D deficiency among toddlers who consumed milk were significantly lower than non-milk drinkers in a study by Gordon et al. in 2008 (121, 122). Finally, two studies found that milk-avoiding children are more likely to fracture a bone and have overall compromised bone health (124, 125).

2.2 Cow’s milk

2.2.1 Vitamin D fortification

Cow’s milk in North America has been fortified with vitamin D since the 1930’s in effort to reduce the incidence of rickets (126), and in 1965, Canada established regulations to mandate the addition of vitamin D to all cow’s milk (127). However, these protocols did not tightly regulate the amount of vitamin D added to fluid milk, or the method by which it was fortified. In 1993, the Canadian Food and Drug Regulations placed acceptable limits on vitamin addition to cow’s milk, as well as testing procedures to standardize the fortification taking place in each dairy facility across Canada (53, 126).

2.2.1.1 Milk Processing

Fresh milk is processed in a specific fashion to preserve its freshness, nutrient quality, and chemical stability once harvested. Prior to pasteurization, milk is stored at less than 7°C to prevent spoilage, until it is standardized to achieve its desired fat content. Milk is processed through centrifugal separators to remove the desired amount of fat, which creates two portions: the skim portion, which is 0.01% fat, and cream, which is approximately 40% fat. Then, a quantity of cream may be added back to the skim portion to achieve the final percent fat content of milk (128).
Next, various methods may be applied to accomplish the fortification process. First, the “continuous flow” method requires a concentrated vitamin solution to be prepared, which is pumped into large quantities of milk and regulated by a meter to ensure proper rate of flow. The vitamin concentrate solution is added after fat has been skimmed off milk, but before pasteurization. This is to ensure the longevity of fat-soluble vitamins; however, vitamin concentrates are not sterile, so pasteurization is necessary. Homogenization also takes place after vitamin addition to ensure adequate distribution throughout the entire quantity of milk. Similarly, the “batch” method requires a large quantity of milk to be mixed with a vitamin concentrate solution, but instead of a line feeding the vitamin into milk and thus regulation of its distribution via rate of flow, a measured quantity of solution is added to a known quantity of milk. This is followed by mechanical agitation to ensure proper circulation of the solution in the milk.

Other methods use a vitamin premix, which is available in liquid concentrates, powders, or beads and is used to fortify evaporated milk and milk powders. Regardless of the method used to fortify milk, the protocol mandates that procedures are strictly followed and machinery is consistently inspected to ensure an adequate supply of vitamins, proper function, and to record routine examinations. Quality control checks are regularly implemented by the Canadian Food Inspection Agency to ensure compliance (129).

Next, milk undergoes high temperature short time (HTST) pasteurization, in which the milk is required to reach at least 71.6°C for 15 seconds, to destroy pathogens. Finally, homogenization not only ensures mixing of vitamin solution when the continuous flow method is employed, but it also regulates fat globule size to prevent fat from floating to the surface of milk, and ensure that globules remain evenly dispersed throughout the fluid. This is accomplished by forcing the fluid through a filter to break up large fat globules (128).
2.2.1.2 Legal requirements

According to the Canadian Food and Drug Act, cow’s milk “shall contain added vitamin D in such an amount that a reasonable daily intake of the milk contains not less than 300 IU and not more than 400 IU of vitamin D” (129). The Canadian Food Inspection Agency has clarified this to standardize fortification at 31.7 to 51.6 IU/100 mL fluid milk (18). In the United States, vitamin D fortification is not mandatory in cow’s milk; however, the majority of milk is fortified with 400 IU per quart, or 100 IU per 8-ounces (approximately 237 mL) (56, 130). This amount is comparable to the Canadian standards.

Despite efforts to tightly regulate the amount of vitamin D added to milk in Canada and the United States, various studies have identified shortcomings in fortified milk available to consumers. A study at the University of Guelph analyzed samples of skim (0.1% fat), 2% and whole (3.25% fat) milk to determine actual quantities of vitamin D added to milk found in grocery stores. Within the collected sample only 20% of skim, 40% of 2% milk, and 20% of whole milk met the guidelines for vitamin D fortification; the majority of samples were over-fortified and thus did not fall within acceptable limits (18). Another study took place over a 4-year period in New York State and found that of 648 samples collected, 47.7% of milk was under-fortified. No statistically significant disparities were identified between varying milk fat content of samples (131). Similarly, a study examining milk samples from across the United States found no statistically significant differences in vitamin D content between different fat contents of milk; however, milk tended to be over-fortified within their sample (55). Although not statistically significant, in all three studies the vitamin D content of cow’s milk varied by milk fat content (18, 55, 131).
2.2.2 Cow’s milk consumption in early childhood

Based on Canadian Community Health Survey (CCHS) data in 2004, 88% of Canadian children aged 1-3 had consumed milk on the previous day; older children (ages 4-8) were less likely to drink milk, with 80% of boys and 71% of girls reporting consumption (15). NHANES data show that between 2005 and 2010 in the U.S.A., females aged 4-8 were consuming an average of 279 mL of milk per day, while on average, males were drinking 312 mL of milk per day (132). Overall cow’s milk consumption in American children under 5 years old has decreased by 7% from 1976-2006 (133); during the same period the proportion of pre-adolescent children who do not consume milk doubled from 12% to 24% (21).

2.2.2.1 Dietary recommendations

Health Canada is responsible for publishing Eating Well with Canada’s Food Guide (EWCFG), which stipulates that children aged 2-8 consume 2 servings of milk and/or dairy alternatives per day. Dairy alternatives are defined as yogurt, kefir, cheese, or a fortified soy beverage (134). Cheese and yogurt may be made with vitamin D fortified milk, but a serving designated by EWCFG does not provide an adequate quantity of vitamin D (one serving of cheese typically provides 0 IU of vitamin D; one serving of yogurt provides between 0 and 70 IU (52)). Thus, to ensure sufficient vitamin D intake, the CPS and AAP suggest children to consume 2-250 mL cups of milk per day to a maximum of 500-720 mL; children consuming less should consume a vitamin D supplement (59, 135).

Various health agencies stress the importance of high-fat milk in early childhood. Generally, children should be offered cow’s milk initially between 9 and 12 months of age, depending on developmental milestones (135). The AAP and National Institutes of Health state that children younger than 2 years should only receive whole (3.25% fat) milk, to ensure adequate calorie intake for proper growth and development (20). Older children, however, may
switch to 2%, 1% or skim to help achieve a low fat diet, as “diets high in fat may contribute to heart disease, obesity, and other health problems later in life” (19). However, a recent meta-analysis by de Souza et al. analyzed the effects of dietary saturated fat, which is present in fat-containing dairy products, and discovered no association with all-cause mortality, cardiovascular disease, type 2 diabetes, or stroke (136). Parents are advised by the CPS to wait until age 5 to introduce skim milk. Whole, 2% or 1% milk are recommended between age 2 and 5 years of age (137).

2.2.3 Nutrient composition

Human breast milk is regarded as the “gold standard” of nutrition for infants because of its nutrition and bioactive molecule composition. Specifically its distribution of calories from carbohydrates (45-50%), fat (50%), and protein (6%) makes it a complete and balanced source of nutrition. Cow’s milk has a similar macronutrient distribution: approximately 50% of calories come from carbohydrate, 30% from fat, and 20% are sourced from protein (138). Comparatively, cow’s milk is an ideal source of nutrition during early childhood.

Nutrition facts tables for skim, 1%, 2%, and whole cow’s milk are shown in Figure 2 (139). The main carbohydrate in milk is in the form of lactose. Dairy fats are composed in the following distribution: 65% saturated, 29% monounsaturated, and 6% polyunsaturated. Milk proteins are made up of approximately 82% casein and 18% whey. Naturally occurring vitamins in milk, that are present in high quantities, include thiamin, riboflavin, pantothenic acid, vitamin B6, and vitamin B12. Minerals such as calcium, potassium, selenium and phosphorous are also found in cow’s milk (140).
2.2.3.1 Role of cow’s milk in body function, growth and development

Carbohydrates are the body’s most prominent and readily available source of energy for daily activities, brain function, and growth. Glucose is the primary carbohydrate substrate used in the body, and is supplied by milk in the form of lactose. When lactose is broken down by lactase in the intestine, it releases the two monomers glucose and galactose into the body’s circulation (140).

Fat is an essential part of a child’s diet for a number of reasons; predominantly, children need to store energy for constant growth and activity. Another major function of fat in infancy and early childhood is brain development. Fat also aids in the absorption of fat-soluble vitamins; without it, micronutrient deficiencies could inflict a number of health complications (141).

Milk is also an excellent source of protein. During childhood, protein fuels growth of the cells that comprise organs, muscles and the skin. Since milk proteins contain whey, they play an important role in immune function; whey proteins constitute immunoglobulins (140). Casein, which makes up the majority of protein in milk, is a key factor in micelle formation. Micelles are responsible for transporting nutrients and other molecules throughout the body (142).
Finally, substantial micronutrients in milk include calcium and phosphorous, which aid in bone development (as discussed in section 2.1.4.1). Vitamin B12 is also very prominent in cow’s milk (8 ounces provides 47% of the DRI), and is integral to protein metabolism and blood function (140).

2.3 Childhood adiposity

2.3.1 Adiposity measurement

The human body is composed of a variety of tissues: for example, nervous, muscular, connective, and adipose tissues. Adipose tissue, or subcutaneous and visceral fat, contributes to a child’s overall weight (143). There are various techniques to measure the proportion of adipose tissue in the body. DXA (dual x-ray absorptiometry) is a method that involves x-rays to quantify body fat (144); another method called the “bod pod” uses air displacement technology to measure adipose tissue (145). Though highly accurate and sophisticated, both of these methods are costly, time consuming, and relatively invasive. More time-efficient methods, though less precise, include skinfold measurements, bioelectrical impedance, and the Body Mass Index (BMI) (146).

Body mass index is a screening tool for weight status and provides information to suggest whether an individual has a healthy body weight, overweight, or obese. BMI is calculated using the formula: weight (kg) divided by height (m$^2$) (147). In children aged 2 to 19 years, BMI-for-age, or a BMI z-score (zBMI), is used to adjust a child’s weight-for-height ratio to their age. For children younger than 2 years, weight-for-length is used and interpreted in the same way as zBMI (148). According to Flegal & Ogden, “a BMI z-score or percentile represents a measure of weight, adjusted for height, sex, and age, relative to a smoothed reference distribution, and not simply a measure of weight and height for a child” (147). It is the recommended indicator to use
for weight status screening in children (149), and correlates with body fat; it is known as a predictor of future obesity and health issues (150).

The World Health Organization (WHO) Growth Standards provides a set of zBMI-for-age curves, which were derived from a multi-ethnic sample of children gathered by the WHO in 2006. These children were breastfed and raised in a healthy environment based on standards such as non-smoking households and receiving routine health care (151). Curves are segmented into percentiles, or z-scores that represent standard deviations from the mean (50th percentile). When interpreting a growth curve, z-scores, which correspond to percentiles, are used to determine a child’s growth in reference to the sample cohort of children collected by the WHO. For example, curves are divided into z-scores -2, -1, 0, 1, and 2 (147, 149). Growth curves for children 0-5 years can be found in Appendix E. Table 2 provides standardized references used to interpret a child’s zBMI or weight-for-length score derived from growth curves (149, 152).

**Table 2: zBMI or weight-for-length cut-off points.**

<table>
<thead>
<tr>
<th>Z-SCORE</th>
<th>INTERPRETATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;-3</td>
<td>Severe thinness</td>
</tr>
<tr>
<td>-2 to -3</td>
<td>Thinness</td>
</tr>
<tr>
<td>-2 to 1</td>
<td>Healthy</td>
</tr>
<tr>
<td>1 to 2</td>
<td>Overweight</td>
</tr>
<tr>
<td>&gt;2</td>
<td>Obese</td>
</tr>
</tbody>
</table>

### 2.3.2 Obesity statistics and trends

Over the past 30 years, the proportion of North American children classified as overweight or obese has risen; consequently, the proportion of children of healthy weights has decreased. According to NHANES data, 5% of American children aged 2-5 in 1974 were obese. By 2008, that percentage rose to 10.4 (153). In Canada, 1 in 7 children and adolescents is obese.
at the current time (154). Data from the Canadian Health Measures Survey (CHMS) show that boys rather than girls, and adolescents rather than younger children are more likely to be obese (155). The most recent cycle of CHMS in 2011 determined that 19.7% of children aged 5-11 were overweight, and 13.1% were obese (a total of 32.8% of children were overweight or above) (156). Children of Aboriginal descent are at a heightened risk of overweight/obesity, at 41% (157). However, since 2007, obesity rates in children have stabilized (158).

2.3.3 Adiposity and cow’s milk consumption

Cow’s milk consumption during childhood has been examined in relation to adiposity in a number of analyses, which have produced mixed results. Some longitudinal studies suggest that a higher volume of milk consumed increases energy in the diet, thus resulting in weight gain (159, 160). However, several observational studies have identified that children who consumed above average quantities of milk and dairy alternatives per day (usually ≥3 servings/day) had lower body mass scores than those who did not meet average dairy consumption (161-163). Two longitudinal analyses showed that children who drank the least amount of milk during preschool gained the most weight later in childhood and adolescence (164, 165). Some researchers hypothesize that the mechanism behind this relationship lies in calcium found in milk (166-168); calcium is known to regulate intracellular lipid metabolism and triglyceride storage (98). Increased dietary calcium is believed to inhibit lipogenesis and foster lipolysis (99).

2.3.3.1 Adiposity and dairy fat consumption

A variety of studies have explored the relationship between cow’s milk fat intake and adiposity in children. Theoretically, higher milk fat consumed would be related to higher energy intake, and thus heightened body fat in children. However, contrary to many hypotheses, studies have often revealed that children typically exhibit an inverse relationship between dairy fat consumption and adiposity. One study identified that preschool children who drank high-fat milk
had higher diet quality in comparison to children who drank water, fruit juice, or a mix; the same study found an inverse association in children aged 6-11 years between high-fat milk consumption and zBMI (34). Another analysis showed that choosing lower fat milk (1% or 2%) instead of whole (3.25%) at age 2 did not reduce the incidence of overweight at age 3 (169). A longitudinal study examined children at ages 2 and 4, and found that drinking lower fat milk (skim or 1%) placed children at a higher risk for overweight in comparison to children drinking higher fat (2% or 3.25%) milk (170). In older children, cross-sectional observations at age 11 and 13 years identified that greater intake of full-fat milk (3.25%) was associated with lower body fat percentage (36). One hypothesis has suggested that a reverse-causality situation may exist where parents of children with more adiposity choose lower fat milk to reduce weight gain (34).

Conversely, other studies have identified either no relationship, or a positive association between milk fat intake and adiposity in children (171-173). Mixed results are common when an analysis utilizes reported dietary intake, because measurement techniques are often inconsistent between studies, and dietary measurement errors are frequent. Further, small sample sizes, lack of adjustment for confounding factors, and cross-sectional study designs may contribute to inconsistencies in results. Thus, the relationship between milk fat intake and body composition remains unclear.

2.4 Summary of literature review

Vitamin D has an important role for healthy childhood growth and development. Further, childhood obesity has become more prevalent in recent years, and appears to have a relationship with serum 25-hydroxyvitamin D levels in both adults and children. The majority of North Americans consume cow’s milk during early childhood. It is the main dietary source of vitamin D and an important source of calories. Low serum 25-hydroxyvitamin D has been documented to
be associated with higher BMI in children and adults, thus the role that cow’s milk consumption plays in affecting children’s vitamin D stores and adiposity is important to understand.

Current guidelines state that children over the age of 2 years should consume lower fat milk to maintain a healthy body weight. However, there is little evidence to support that this recommendation leads to lower adiposity. Further, there is growing evidence that it may paradoxically compromise children’s vitamin D status, as well as their risk of obesity. It is important to understand the optimal fat content of milk for children to consume, that results in optimal vitamin D stores and healthy weights, to provide an evidence based foundation for future guidelines about milk fat consumption for children.
CHAPTER 3: MILK FAT CONTENT AND VITAMIN D IN EARLY CHILDHOOD

3.1 Abstract

Objectives

Current guidelines for cow’s milk consumption in children over age 2 suggest reduced fat milk (1% or 2%), to lower the risk of obesity. Given that cow’s milk is the main dietary source of vitamin D for North American children and vitamin D is fat-soluble, we hypothesized children’s 25-hydroxyvitamin D concentration to be positively associated with percent fat content of cow’s milk. The primary objective was to determine the relationship between percent fat content of cow’s milk consumed and serum 25-hydroxyvitamin D concentration; secondarily, to determine if the relationship between volume of milk and 25-hydroxyvitamin D was modified by milk fat content.

Methods

A cross-sectional study of children 12-72 months of age who participated in the TARGetKids! practice based research network. Multivariable linear regression was used to test the association between percent milk fat content and child 25-hydroxyvitamin D, adjusted for clinically relevant covariates. The interaction between volume of milk consumed and percent milk fat content was examined.

Results

2857 children were included in the analysis. Percent fat content of milk was positively associated with 25-hydroxyvitamin D (p=0.03). The interaction between volume of milk consumed and milk fat content was statistically significant (p=0.005), suggesting that the relationship between milk volume and 25-hydroxyvitamin D was modified by milk fat content. Each 1% higher fat content was associated with a 2.79 nmol/L (95% CI 1.22-4.39) higher 25-hydroxyvitamin D. Children who drank skim milk needed to consume 2.5 cups (95% CI 2.38-2.54) of milk to have
similar 25-hydroxyvitamin D as children who drank 1 cup of whole milk. Children who consumed skim milk had 2.05 (95% CI 1.73-2.42) times higher odds of 25-hydroxyvitamin D concentration <50 nmol/L than children who consumed whole milk.

**Conclusion**

Recommendations for children to drink lower fat milk (1% or 2%) may compromise serum 25-hydroxyvitamin D levels, and may require further study to ensure optimal health in young North Americans.

### 3.2 Background

Vitamin D is a fat soluble steroid that is important for children’s growth and development (41). Low vitamin D intake in early childhood is known to result in health complications, including rickets (4, 5). Cow’s milk has been identified as the main dietary source of vitamin D for children in North America (13-15). In the United States and Canada, cow’s milk is fortified with roughly 100 IU of vitamin D per 250 mL of milk (18). Current professional guidelines from the National Institutes of Health and the American Academy of Pediatrics recommend that children between 1 and 2 years of age consume 2 cups of whole milk (3.25% milk fat) per day, and for older children low fat (2% or 1%) milk is recommended to reduce dietary fat and lower obesity risk (19, 20). However, vitamin D is a fat soluble hormone and dietary fat is believed to facilitate vitamin D absorption in the GI tract (45, 174, 175).

It is currently unknown whether consumption of lower fat milk may have the unintended consequence of lower 25-hydroxyvitamin D serum levels in children. We hypothesized that children who consume milk with higher percent fat content will have higher serum 25-hydroxyvitamin D concentration relative to children who consume milk with a lower percent fat content. Our primary objective was to evaluate the relationship between the percent fat content of cow’s milk consumed and serum 25-hydroxyvitamin D concentration in early childhood. Our
secondary objective was to determine if the known relationship between volume of milk consumed and 25-hydroxyvitamin D is modified by the fat content of milk consumed.

3.3 Methods

3.3.1 Participants and Design

This cross-sectional study was conducted through the TARGetKids! (The Applied Research Group for Kids) practice-based research network. TARGetKids! is a collaboration between researchers in the Faculty of Medicine at the University of Toronto and primary care practitioners in the university’s Department of Paediatrics and Department of Family and Community Medicine (176).

Healthy children aged 12-72 months were recruited during routine primary healthcare visits at nine primary healthcare practices located in Toronto, Ontario, Canada (latitude 43.4°N) between September 2008 and August 2014. Exclusion criteria were conditions affecting growth (e.g. failure to thrive), severe developmental delay or other chronic health conditions (except for asthma).

3.3.2 Measurements

Clinical data was collected using a standardized, parent completed questionnaire based on the Canadian Community Health Survey (177) by trained research assistants at each of the participating practices. Research assistants took physical measurements of each child, and trained phlebotomists collected venous blood samples. Blood samples were analyzed at the Mount Sinai Services Laboratory (mountsinaiservices.com) in Toronto, Canada.

The primary exposure was percent fat content of milk consumed. This was measured by the following question: “Please specify your child’s diet for the past 3 days: skim, 1%, 2%, or whole milk.”
The primary outcome was serum 25-hydroxyvitamin D concentration as a continuous variable which was measured using a 2-step competitive chemiluminescence assay (LIAISON 25 OH Vitamin D TOTAL; DiaSorin) (83). This technique has been demonstrated to have an intra-assay imprecision of 7.2% at a concentration of 213 nmol/L, and an inter-assay imprecision of 4.9% at 32 nmol/L, 8.9% at 77 nmol/L and 17.4% at 213 nmol/L, all of which are within acceptable limits for biochemical measurements (86, 87). Our secondary outcomes were serum 25-hydroxyvitamin D concentration cutoffs of <50 nmol/L and <75 nmol/L, based on recommendations from the Institute of Medicine and the Canadian Paediatric Society, respectively (7, 178).

Clinically relevant covariates, which might be confounders of the relationship between percent fat content of milk consumed and serum 25-hydroxyvitamin D, were identified through a literature review and pre-specified. These included: children’s age, sex, skin pigmentation, BMI z-score (zBMI), daily vitamin D supplementation, daily volume of milk consumed, date of blood collection, non-cow’s milk consumption, and median neighbourhood family income (179). The Fitzpatrick scale was used to assess skin pigmentation, an acceptable method for skin pigmentation quantification used in dermatological research (180, 181). Weight was measured with a precision digital scale (±0.025%; SECA); child length using a calibrated length board for children under 2 years, and older children’s heights were measured with a stadiometer (SECA, Germany). Growth curves from the World Health Organization were utilized for zBMI calculation (182-184). Child vitamin D supplementation was measured as currently taking a multivitamin and/or vitamin D supplement daily. All vitamin D containing supplements in Canada marketed for children contain 400 IU or 10 micrograms per daily dose (185). Volume of milk consumed per day was measured by the question “How many cups of milk does your child drink in a typical day.” Postal codes were used to generate after-tax median family income using
the Statistics Canada Postal Code Conversion File, which was based on the 2011 Canadian census (186).

### 3.3.3 Statistical analysis

Descriptive statistics were performed for our primary exposure, outcome and covariates. Univariate linear regression was used to test the unadjusted association between percent fat content of milk consumed (primary exposure) and child 25-hydroxyvitamin D concentration (primary outcome). After examining residual plots, 25-hydroxyvitamin D was positively skewed and necessitated a log transformation. For our primary analysis, we used multivariable linear regression adjusted for pre-specified biologically plausible sociodemographic, dietary, and environmental factors (outlined above). All covariates were included in the final model regardless of statistical significance to prevent biased regression coefficients and falsely inflated $R^2$ values (187). To adjust for season, a sinusoidal function was applied to the date of blood sample collection (188).

For our secondary analysis, we explored whether the relationship between volume of milk consumed and 25-hydroxyvitamin D concentration was modified by percent fat content of milk. We accomplished this by adding an interaction term between percent fat content of milk and volume of milk consumed to our primary model. This interaction was tested at a significance level of $\alpha=0.05$. We also repeated the primary and secondary analysis using multivariable logistic regression to explore the relationship between our primary exposure and 25-hydroxyvitamin D cutoffs of 50 and 75 nmol/L (7, 78).

To assess multicollinearity, the variance inflation factor (VIF) was used (189). All covariates had VIF values <3. Missing data was assumed to satisfy the missing at random criteria, so multiple imputation was used on 50 datasets to minimize bias from missing data.
No variable contained more than 11% missing information. Data analysis was performed using R version 3.1.1.

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human patients were approved by the Research Ethics Boards at the Hospital for Sick Children and St. Michael’s Hospital. Written informed consent was obtained from all parents of participating children.

3.4 Results

Of the 6320 children who consented to participate in the TARGetKids! cohort, blood samples were obtained and analyzed for 2857 participants, who were included in the analysis. Characteristics of children with and without blood sampling appeared clinically similar (see Table 3). The mean age of participants was 2.8 years, and 52.7% were male. Whole milk (3.25% fat) was consumed by 48.5% of children, followed by 2% milk consumed by 34.8%, 1% milk consumed by 12.0%, and skim milk consumed by 4.6% of children respectively. On average, children drank 2.1 cups of milk per day. The mean serum 25-hydroxyvitamin D concentration was 87 nmol/L, and 54.1% of children were consuming a daily vitamin D supplement. Thirty seven percent of children had serum 25-hydroxyvitamin D concentrations <75 nmol/L and 5.9% <50 nmol/L.

For our primary analysis, multivariable linear regression identified that percent fat content of milk was positively associated with serum 25-hydroxyvitamin D (p=0.03). Each 1% increase in milk fat content was associated with a 1.52% (95%CI: 0.83-2.21%) higher 25-hydroxyvitamin D (see Table 4). For example, children who drank whole milk had a 4.1 nmol/L (95%CI: 3.57-4.74) higher median 25-hydroxyvitamin D than children who drank the same volume of 1% milk (see Figure 3). Covariates positively associated with serum 25-hydroxyvitamin D included vitamin D supplementation (p<0.001), light skin pigmentation (p<0.001), higher volume of
cow’s milk consumption (p<0.001), lower zBMI (p=0.01) and median neighbourhood family income between $80,000 and $150,000 (p=0.03).

For our secondary analysis (see Table 4), the interaction between daily volume of milk consumption and percent fat content of the milk consumed was also statistically significant (p=0.005). This suggested that milk fat content was an effect modifier of the relationship between volume of milk consumed and serum 25-hydroxyvitamin D. For children who consumed 1 cup of milk per day, those drinking milk with 1% higher fat content had a 2.79 nmol/L (95% CI 1.22-4.39) higher median serum 25-hydroxyvitamin D (see Figure 4). Children who drank 1% milk needed to consume 2.5 cups (95% CI 2.38-2.54) of milk to have the same 25-hydroxyvitamin D as children who drank 1 cup of whole milk (3.25% fat).

Examination of 25-hydroxyvitamin D at <50 nmol/L revealed that the odds ratio for serum 25-hydroxyvitamin D <50 nmol/L was 1.25 (95% CI 1.14-1.35) per 1% lower fat content of milk consumed. For example, children drinking 1% milk had 2.05 (95% CI 1.73-2.42) times higher odds of serum 25-hydroxyvitamin D concentration <50 nmol/L than children drinking whole milk. When we assessed 25-hydroxyvitamin D at <75 nmol/L, we did not identify an association between percent fat content of milk consumed and serum 25-hydroxyvitamin D concentration (OR=1.07, 95% CI 0.98-1.16.)

3.5 Discussion

In this study we have identified a relationship between higher fat content of milk and higher serum 25-hydroxyvitamin D in early childhood. Children who consumed whole milk had a 4.1 nmol/L higher median 25-hydroxyvitamin D concentration than children consuming the same volume of 1% milk. Further, milk fat content appeared to modify the relationship between volume of milk consumed and serum 25-hydroxyvitamin D. For children drinking 1 cup of milk, each 1% increase in percent fat content of milk was associated with a 2.79 nmol/L higher serum
25-hydroxyvitamin D, which is similar to the effect of an additional cup of milk (13). Children who drank 1% milk needed to consume more than double the volume of milk to have the same 25-hydroxyvitamin D as children who drank whole milk. We also found that children who drank 1% milk had a 2-fold increased odds of serum 25-hydroxyvitamin D less than 50 nmol/L relative to children drinking whole milk.

Dietary fat is known to stimulate bile acid secretion, breaking down lipid globules and allowing lipolytic enzymes to work on a greater surface area, thus enhancing fat-soluble vitamin absorption into the bloodstream (191). Given that vitamin D is a fat soluble vitamin, we hypothesized that higher percent fat content of milk may be associated with higher serum 25-hydroxyvitamin D concentration in children. Results from our primary and secondary analyses were consistent with this hypothesis.

A few studies have examined the effect of dietary fat on supplemental doses of vitamin D, which yielded inconsistent results on the relationship between dietary fat and serum vitamin D concentration in adults (45, 47, 48). For example, Tangpricha et al. observed no difference between the effects of vitamin D-fortified skim (0.1% fat) milk, whole (3.25% fat) milk and corn oil on toast on serum 25-hydroxyvitamin D levels (47); however, Dawson-Hughes et al. found a 32% increase in serum 25-hydroxyvitamin D between participants who consumed a vitamin D supplement with a fat containing meal compared to a fat free meal (45). To our knowledge, the relationship between the percent fat content of cows milk and 25-hydroxyvitamin D concentration in children has not previously been studied.

Children over age 2 are recommended to consume reduced-fat milk. While this recommendation was intended to have a positive effect on childhood obesity (20, 41, 169, 170), our results suggest that it may have the unintentional effect of reducing children’s vitamin D stores. Children consuming milk with a lower percent fat content may benefit from vitamin D
supplementation, particularly those with other risk factors for vitamin D deficiency (59). We hope that findings from this study will generate dialogue around current guidelines on milk fat consumption for children.

Strengths of our study include data from a large, ethnically diverse, healthy cohort of urban North American children. The relatively large sample size, combined with clinically rich data has allowed us to have sufficient power to account for numerous biologically plausible potential confounders of the identified relationships.

Limitations of our study include the cross-sectional design; thus, causation cannot be concluded from our results. Parent-reported questionnaire data may have been affected by recall bias. The majority of children consumed moderate amounts (about two cups per day) of higher fat milk (2% or 3.25% fat), with fewer children drinking low fat (skim or 1%) milk, or very high or low milk volumes, which may have limited our statistical power at the extremes. Our population was from one urban North American urban setting and may not be representative of other urban populations of children. Overall 54% of participants consumed a daily vitamin D containing supplement, which may have resulted in a relatively high mean serum 25-hydroxyvitamin D concentration.

### 3.6 Conclusion

We have identified an association between higher fat content of milk and higher 25-hydroxyvitamin D in early childhood. We also found that the association between volume of milk consumed and 25-hydroxyvitamin D was modified by percent fat content of milk such that higher percent fat content of milk appeared to potentiate the effect of milk consumption on 25-hydroxyvitamin D serum concentration. Current recommendations for lower milk fat consumption may be negatively affecting vitamin D status in early childhood.
Table 3: Characteristics of children who participated in study 1 and nonparticipants

<table>
<thead>
<tr>
<th>Child Characteristics</th>
<th>Children with blood sample n= 2857</th>
<th>Children without blood sample n= 3463</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, months, mean ± SD</td>
<td>33.9 ± 16.3</td>
<td>33.6 ± 16.8</td>
</tr>
<tr>
<td>Sex, males, no. (%)</td>
<td>1506 (53)</td>
<td>1810 (52)</td>
</tr>
<tr>
<td>Child 25(OH)D, nmol/L, mean</td>
<td>86.7 ± 30.0</td>
<td>N/A</td>
</tr>
<tr>
<td>Percent fat content of milk, no. (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skim</td>
<td>131 (5)</td>
<td>120 (3)</td>
</tr>
<tr>
<td>1%</td>
<td>344 (12)</td>
<td>311 (9)</td>
</tr>
<tr>
<td>2%</td>
<td>996 (35)</td>
<td>1256 (36)</td>
</tr>
<tr>
<td>Homo</td>
<td>1386 (49)</td>
<td>1776 (51)</td>
</tr>
<tr>
<td>Child zBMI, mean ± SD</td>
<td>0.2 ± 1.0</td>
<td>0.2 ± 1.0</td>
</tr>
<tr>
<td>Skin pigmentation, Fitzpatrick scale ≤3, no. (%)</td>
<td>2247 (79)</td>
<td>2606 (86)</td>
</tr>
<tr>
<td>Cow’s milk, cups/day, mean ± SD</td>
<td>2.1 ± 1.1</td>
<td>2.0 ± 1.0</td>
</tr>
<tr>
<td>Child vitamin D daily supplementation, yes, no. (%)</td>
<td>1547 (54)</td>
<td>1660 (48)</td>
</tr>
<tr>
<td>Median Neighbourhood Family Income, n=3188, no. (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Less than $30,000</td>
<td>177 (6)</td>
<td>188 (5)</td>
</tr>
<tr>
<td>$30,000-79,999</td>
<td>2111 (74)</td>
<td>2493 (72)</td>
</tr>
<tr>
<td>$80,000-$149,999</td>
<td>333 (12)</td>
<td>464 (13)</td>
</tr>
<tr>
<td>$150,000 or more</td>
<td>23 (1)</td>
<td>43 (1)</td>
</tr>
<tr>
<td>Ethnicity, no. (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mixed Western,</td>
<td>1889 (66)</td>
<td>2415 (75)</td>
</tr>
<tr>
<td>East/South East Asian</td>
<td>277 (10)</td>
<td>327 (10)</td>
</tr>
<tr>
<td>Southwest Asian</td>
<td>207 (7)</td>
<td>206 (6)</td>
</tr>
<tr>
<td>African/Caribbean</td>
<td>116 (4)</td>
<td>114 (4)</td>
</tr>
<tr>
<td>Mixed Western/Non-Western</td>
<td>132 (5)</td>
<td>137 (4)</td>
</tr>
</tbody>
</table>
Table 4: Multivariable linear regression results for study 1.

<table>
<thead>
<tr>
<th>Child characteristics</th>
<th>Without Interaction†</th>
<th>With Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% Change in 25(OH)D</td>
<td>95% CI</td>
</tr>
<tr>
<td></td>
<td>95% CI</td>
<td>Change in median 25(OH)D, nmol/L</td>
</tr>
<tr>
<td>Percent Fat Content of Milk</td>
<td>1.52</td>
<td>0.83, 2.21</td>
</tr>
<tr>
<td>Age (month)</td>
<td>-0.01</td>
<td>-0.10, 0.10</td>
</tr>
<tr>
<td>Sex (males)</td>
<td>-1.77</td>
<td>-4.11, 0.60</td>
</tr>
<tr>
<td>BMI z-score</td>
<td>-1.57</td>
<td>-2.76, -0.40</td>
</tr>
<tr>
<td>Season (sine month)</td>
<td>0.57</td>
<td>-1.90, 2.33</td>
</tr>
<tr>
<td>Skin pigmentation (Fitzpatrick scale ≤3)</td>
<td>9.48</td>
<td>5.87, 13.20</td>
</tr>
<tr>
<td>Volume of milk consumed (cups/day)</td>
<td>3.61</td>
<td>2.33, 4.81</td>
</tr>
<tr>
<td>Child vitamin D daily supplementation (yes)</td>
<td>11.26</td>
<td>8.55, 14.0</td>
</tr>
<tr>
<td>Non-cow’s milk consumption (yes)</td>
<td>-3.07</td>
<td>-7.23, 1.21</td>
</tr>
<tr>
<td>Median Neighbourhood Family Income</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Less than $30,000</td>
<td>-4.57</td>
<td>-9.34, 0.50</td>
</tr>
<tr>
<td>$30,000-79,999</td>
<td>4.41</td>
<td>0.5, 8.55</td>
</tr>
<tr>
<td>$80,000-149,999</td>
<td>-2.71</td>
<td>-15.21, 11.63</td>
</tr>
<tr>
<td>$150,000 or more</td>
<td>N/A</td>
<td></td>
</tr>
</tbody>
</table>

†The interaction analyzed was between percent fat content of milk and volume of milk consumed.
Figure 4: Adjusted* association between percent fat content of milk consumed and child serum 25-hydroxyvitamin D.

*Adjusted for age, sex, BMI z-score, season of serum collection, skin pigmentation, daily vitamin D supplementation, non-cow’s milk consumption, and median neighbourhood family income. Shaded areas indicate 95% CI.
**Figure 5:** Adjusted* association between volume of milk consumed (cups/day) and serum 25-hydroxyvitamin D (nmol/L) with interaction.†

*Adjusted for age, sex, BMI z-score, season of serum collection, skin pigmentation, daily vitamin D supplementation, non-cow’s milk consumption, and median neighbourhood family income.

†The interaction analyzed was between percent fat content of milk and volume of milk consumed.
CHAPTER 4: THE RELATIONSHIP BETWEEN MILK FAT CONTENT, VITAMIN D, AND ADIPOSY IN EARLY CHILDHOOD

4.1 Abstract

Background and Objectives
Fortified cow’s milk is an important source of vitamin D and dietary fat for children. Guidelines recommend reduced milk fat consumption to reduce childhood obesity, yet the relationship between lower milk fat, vitamin D stores and adiposity is unclear. The objective of this study was to determine the association between percent fat content of milk and both zBMI and 25-hydroxyvitamin D; secondly, to explore if volume of milk consumed modified this relationship.

Methods
A cross-sectional study of healthy urban children 12-72 months of age was conducted. Adjusted bivariate linear regression was used to test the association between percent milk fat content and child 25-hydroxyvitamin D and zBMI concurrently. The interaction between volume of milk consumed and percent milk fat content was examined to explore how milk volume might modify these relationships.

Results
2745 children were included in the analysis. Percent fat content of milk was positively associated with 25-hydroxyvitamin D (p=0.006), and negatively associated with zBMI (p<0.0001). Children who drank whole milk (3.25% fat) had 5.4 nmol/L (95%CI 4.32-6.54) higher median 25(OH)D concentration and 0.72 lower (95%CI 0.68-0.76) zBMI score than children who drank 1% milk. Volume of milk consumed potentiated the effect of percent fat content of milk on 25-hydroxyvitamin D (p=0.003) but not on zBMI (p=0.77).

Conclusion
Whole milk consumption was associated with higher serum 25-hydroxyvitamin D and lower adiposity in early childhood. Current guidelines for reduced milk fat consumption in childhood may require further study to achieve desired outcomes.

4.2 Introduction

Vitamin D is an essential nutrient for healthy bone development and the prevention of rickets (4, 5). In North America, the main dietary source of vitamin D is vitamin D fortified cow’s milk (13-15). In children older than 2 years of age, 2 servings of reduced fat (1% or 2%) milk are recommended each day by the National Institutes of Health and American Academy of Pediatrics to limit fat and cholesterol intake and reduce the incidence of childhood obesity (14, 20, 134, 192).

Childhood obesity in North America has tripled in the past 30 years (153) while children’s consumption of whole cow’s milk (3.25% fat) has halved over the same period (21). Several studies have suggested an association between higher milk fat consumption and lower risk of childhood obesity (33-37). Further, adiposity and serum 25-hydroxyvitamin D in children are known to manifest in an inverse relationship (25-32). The role that milk fat consumption has on serum 25-hydroxyvitamin D is less clear, and the milk fat concentration that maximizes 25-hydroxyvitamin D and minimizes adiposity in children is currently unknown.

The primary objective of this study was to examine the association between percent fat content of milk and both 25-hydroxyvitamin D and Body Mass Index (BMI) in healthy preschool children. The secondary objective was to explore how the volume of milk consumed might modify the relationship between percent fat content of milk, 25-hydroxyvitamin D and adiposity.
4.3 Methods

This cross-sectional study was performed through the TARGetKids! (The Applied Research Group for Kids) practice based research network. TARGetKids! is a collaboration between researchers in the Faculty of Medicine at the University of Toronto and primary care practitioners in the university’s Department of Paediatrics and Department of Family and Community Medicine (176).

Healthy children aged 12 to 72 months were recruited during routine primary healthcare visits at nine primary health care practices located in Toronto, Ontario, Canada (latitude 43.4°N) between September 2008 and August 2014. Exclusion criteria were conditions affecting growth (e.g. failure to thrive), severe developmental delay or other chronic health conditions (except for asthma).

Trained research assistants collected clinical data using a standardized, parent-completed questionnaire based on the Canadian Community Health Survey (179), as well as anthropometric measurements. Trained phlebotomists collected venous blood from each child, which was analyzed at the Mount Sinai Services Laboratory (mountsinaiservices.com) in Toronto, Canada.

The primary exposure was percent fat content of milk consumed by each child as a continuous variable. This was measured by the question, “Please specify your child’s diet for the past 3 days: skim, 1%, 2%, or whole milk.” The mean value was used for subjects consuming more than one percent fat content of milk.

There were two primary outcomes: serum 25-hydroxyvitamin D, and BMI z-score. Serum 25-hydroxyvitamin D was measured using a 2-step competitive chemiluminescence assay from venous blood (LIAISON 25 OH Vitamin D TOTAL; DiaSorin) (83); this method had an intra-assay imprecision of 7.2% at a concentration of 213 nmol/L, and an inter-assay imprecision of 4.9% at 32 nmol/L, 8.9% at 77 nmol/L and 17.4% at 213 nmol/L, all of which are within
adequate limits for biochemical measurements (86, 87). BMI z-score (zBMI), which is an age and sex standardized measure of adiposity in children, was calculated using weight (kg) divided by height (m$^2$) based on the World Health Organization (WHO) growth standards (182-184). Height measurements were obtained using a calibrated length board for children younger than 2 years, and a stadiometer for older children (SECA, Germany). Weight was measured using a precision digital scale (±0.025%; SECA).

Biologically plausible factors that might affect the relationship between percent fat content of milk consumed and both serum 25-hydroxyvitamin D and zBMI were determined a priori through a literature review and included as potential confounding variables. These included age, sex, daily vitamin D supplementation, minutes of daily outdoor free play, minutes of daily screen time, volume of milk consumed daily, volume of sugar-sweetened beverages consumed daily, maternal BMI, skin pigmentation, after-tax median neighborhood family income, maternal ethnicity, and date of serum collection. Daily vitamin D supplementation was measured as currently taking a multivitamin and/or vitamin D supplement daily. In Canada, all vitamin D containing supplements for children contain 400 IU or 10 micrograms per daily dose (185). Daily outdoor free play was measured by the question “On a typical weekday, how much time does your child spend outside in ‘unstructured free play’.” Daily screen time was quantified with the question “On a typical day how many minutes did your child spend in a room with a TV, video games, computer games, or handheld device games on.” Volume of milk, as well as sugar-sweetened beverages, consumed daily was measured by the question “How many cups of each drink does your child have currently in a typical day.” Trained research assistants measured maternal BMI at the time of child primary care visits, using the standard formula (182, 183). The Fitzpatrick scale was used to evaluate skin pigmentation, which is commonly used in dermatological research (180, 181). Postal codes were used to obtain after-tax median family
income using the Statistics Canada Postal Code Conversion File, which used the 2011 Canadian census (186). Maternal ethnicity was classified into one of six geographic based categories (193).

4.3.1 Statistical Analysis

To determine the association between percent fat content of milk (primary exposure) and the two continuous primary outcomes, serum 25-hydroxyvitamin D and zBMI, bivariate multivariable regression was used (194). This model was adjusted for clinically relevant sociodemographic, dietary and environmental covariates determined a priori (listed above); all of which remained in the model regardless of statistical significance to prevent incorrect standard errors and biased $R^2$ values (187). Using this model, we compared the bivariate outcomes among children who drank whole (3.25% fat) milk to 1% milk (it was expected that few children of this age would consume skim (0.1% fat) milk). Residual plot examination of 25-hydroxyvitamin D revealed a positive skew, which normalized with log transformation. A sinusoidal function was applied to date values to adjust for seasonal influences on 25-hydroxyvitamin D.

For the secondary analysis, an interaction term between volume and percent fat content of milk consumed was added to the primary model to explore possible effect modification by the volume of milk consumed. This interaction was tested at a significance level of $\alpha=0.05$.

Multicollinearity was evaluated using variance inflation factor (VIF); all covariates’ VIF remained under 2 (189). As data was assumed to be missing at random, multiple imputation was used on 50 datasets to minimize bias from missing data (187, 195). All analyses were performed using R version 3.1.1 (190).

The Research Ethics Board of both The Hospital for Sick Children and St. Michael’s Hospital approved this study and consent was attained from all parents/guardians.
4.4 Results

Of 5301 children who consented to participate in the TARGetKids! cohort, 2745 had venous blood samples taken and 25-hydroxyvitamin D testing. Children included and not included in the analysis appeared clinically similar (Table 5). The mean age of children was 2.8 years, and 53% were male. The average serum 25-hydroxyvitamin D concentration was 87 nmol/L, and the mean zBMI was 0.2 (SD 1.0). Average daily milk consumption was 2.1 cups per child, and 56% of participants took a daily vitamin D supplement. Forty-nine percent of children drank whole milk (3.25% fat); 35% consumed 2% milk, 12% drank 1% milk and 4% consumed skim milk (0.1% fat). Overweight children (zBMI > 1) constituted 21% of the sample and 5% were obese (zBMI > 2) (149). Thirty eight percent of children had serum 25-hydroxyvitamin D concentration less than 75 nmol/L, and 5.9% less than 50 nmol/L (7, 59).

Results of the primary analysis are shown in Table 6. In the adjusted bivariate linear regression model, percent fat content of milk was positively associated with 25-hydroxyvitamin D (p=0.006), and negatively associated with zBMI (p<0.0001). Each 1% higher fat content of milk was associated with a 1.67 nmol/L (95% CI 1.01 to 3.05) higher median 25-hydroxyvitamin D (Figure 6). The average child who drank whole (3.25% fat) milk had median 25-hydroxyvitamin D concentration 5.43 nmol/L (95% CI 4.32 to 6.54) higher than a child who drank 1% milk. Further, children who drank whole milk had 2.25 fold lower odds (95% CI 1.28 to 3.99) of 25-hydroxyvitamin D <50 nmol/L compared to children who drank 1% milk. Covariates positively associated with 25-hydroxyvitamin D included higher volume of milk consumption and daily vitamin D supplementation; southwest Asian maternal ethnicity was negatively associated with 25-hydroxyvitamin D (p=0.01).

Each 1% increase in fat content of milk was associated with a 0.22 (95% CI 0.18 to 0.26) lower zBMI (Figure 6). For example, the average child who drank whole milk (3.25% fat) had a
0.72 (95% CI 0.68 to 0.76) unit lower zBMI score than a child who consumed 1% milk. Further, children who drank whole milk had 2.43 (95% CI 1.69 to 3.49) fold lower odds of overweight, and 3.21 (95% CI 1.76 to 5.88) fold lower odds of obesity than children who drank 1% milk. Factors positively associated with zBMI in the adjusted model included male gender (p=0.005), higher volume of milk consumption (p=0.0007), and higher maternal BMI (p<0.0001); covariates negatively associated with zBMI included daily vitamin D supplementation (p=0.005) and higher daily screen time (p=0.005).

For the secondary analysis an interaction between volume of milk and percent fat content of milk was added to the primary model to evaluate whether volume of milk consumed modified the relationship between percent fat content of milk and both 25-hydroxyvitamin D and zBMI (Table 6). The interaction between percent fat content of milk and volume of milk consumed was statistically significant for 25-hydroxyvitamin D (p=0.003) but not zBMI (p=0.77) (Figure 7). Each cup of milk consumed was associated with a 4.97 nmol/L (95% CI 3.09 to 6.92) higher 25-hydroxyvitamin D and a statistically non-significant 0.08 (95% CI 0.01 to 0.14) unit higher zBMI for a given fat content of milk. Children who drank 1 cup of whole milk each day had a similar 25-hydroxyvitamin D as children who drank 2.9 cups (95% CI 2.85 to 3.04) of 1% milk, but had zBMI score 0.79 (95% CI 0.64 to 0.94) units lower.

4.5 Discussion

In this study we have identified an association between percent fat content of milk and both 25-hydroxyvitamin D and zBMI in early childhood. Children who consumed whole milk (3.25% fat) had a median serum 25-hydroxyvitamin D concentration 5.4 nmol/L higher than children who consumed 1% milk, which is comparable to consuming 1 additional cup of milk per day (196). Children who drank a given volume of whole milk also had a 0.72 unit lower zBMI score than children drinking 1% milk, which is comparable to the difference between healthy weight
and overweight (197). Interventions for childhood obesity have considered a 0.5 change in zBMI to be meaningful suggesting that the magnitude of this association may be clinically important (198, 199). It also appeared that percent fat content of milk potentiated the association between volume of milk consumed and 25-hydroxyvitamin D but not zBMI. When analyzing the outcomes associated with either 1% milk or whole (3.25% fat) milk, children who consumed 1 cup of whole milk each day had a similar 25-hydroxyvitamin D concentration as children who consumed 2.9 cups of 1% milk but a 0.79 unit lower zBMI.

The National Institutes of Health and American Academy of Pediatrics recommend that children older than 2 years of age consume 2 servings of reduced fat milk (1% or 2%) each day to maintain a lower-fat diet. Our findings raise the possibility that this recommendation may paradoxically decrease children’s vitamin D status and increase their adiposity, which may not be desirable outcomes. Several mechanisms have been described, which may explain findings from this study. The positive association observed between milk fat and serum 25-hydroxyvitamin D may be a result of increased intestinal vitamin D absorption since vitamin D is fat soluble vitamin (45), or a reflection of children’s adiposity, as adiposity and serum 25-hydroxyvitamin D have an inverse relationship in children (25-32). The negative association between milk fat consumption and adiposity may be the result of heightened satiety following higher milk fat consumption thus reducing total caloric intake as reported by others (34, 37). Alternatively, a reverse-causality scenario may exist where overweight children transition to lower fat milk to reduce further gains in adiposity (34).

Findings from the current study raise the possibility that reduced fat milk may compromise both serum 25-hydroxyvitamin D and zBMI. Should these associations prove to be causal, young children may benefit from consuming higher fat content milk (i.e. whole milk) rather than low fat milk (1% or skim) to maximize 25-hydroxyvitamin D and minimize risk of overweight (33-
37). As both vitamin D status and adiposity have a significant impact on children’s growth and development, these findings may have important implications for health maintenance at a population level.

Strengths of our study include a large, healthy, culturally diverse cohort of young children with data on milk consumption, vitamin D status and adiposity. In addition, the sample size and rich clinical data allowed sufficient power to control for a broad range of potential confounders. Further, the analytic approach allowed modeling both 25-hydroxyvitamin D and zBMI as simultaneous outcomes, which co-exist in children.

Limitations include the cross sectional nature of our study; thus, causality cannot be inferred between the exposure and outcomes. Data collection for milk consumption was by parent report, which may be subject to recall bias. Our population of urban North American children may not be representative of all urban children. Due to the small number of children consuming low volumes of skim milk and large volumes of whole milk, power to detect effects may have been lower at the extremes of milk consumption.

Current guidelines for reduced milk fat consumption for children over the age of 2 years are intended to reduce the risk of childhood obesity, but may have the paradoxical effect of limiting 25-hydroxyvitamin D concentration and increasing adiposity. Should these findings prove causal, consumption of milk with higher fat content may be helpful in optimizing both serum 25-hydroxyvitamin D and adiposity. Longitudinal and interventional studies are needed.
### Table 5: Characteristics of children who participated in study 2 and nonparticipants

<table>
<thead>
<tr>
<th>Covariates</th>
<th>Children with 25(OH)D measured N=2745</th>
<th>Children without 25(OH)D measured N=2556</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, months, mean ± SD</td>
<td>33.9 ± 16.5</td>
<td>35.9 ± 16.6</td>
</tr>
<tr>
<td>Sex, males, n (%)</td>
<td>1448 (53)</td>
<td>1327 (52)</td>
</tr>
<tr>
<td>Child 25(OH)D, nmol/L, mean ± SD</td>
<td>87.0 ± 30.0</td>
<td>N/A</td>
</tr>
<tr>
<td>Percent fat content of milk consumed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skim (0.1%), n (%)</td>
<td>123 (4)</td>
<td>111 (4)</td>
</tr>
<tr>
<td>1%, n (%)</td>
<td>335 (12)</td>
<td>316 (12)</td>
</tr>
<tr>
<td>2%, n (%)</td>
<td>954 (35)</td>
<td>1002 (39)</td>
</tr>
<tr>
<td>Whole (3.25%), n (%)</td>
<td>1333 (49)</td>
<td>1127 (44)</td>
</tr>
<tr>
<td>Child zBMI, mean ± SD</td>
<td>0.2 ± 1.0</td>
<td>0.22 ± 1.0</td>
</tr>
<tr>
<td>zBMI &lt; -1, n (%)</td>
<td>315 (11)</td>
<td>279 (11)</td>
</tr>
<tr>
<td>-1 &lt; zBMI &lt; 1, n (%)</td>
<td>1860 (68)</td>
<td>1755 (69)</td>
</tr>
<tr>
<td>1 &lt; zBMI &lt; 2, n (%)</td>
<td>435 (16)</td>
<td>406 (16)</td>
</tr>
<tr>
<td>2 &lt; zBMI, n (%)</td>
<td>128 (5)</td>
<td>111 (4)</td>
</tr>
<tr>
<td>Skin pigmentation (Fitzpatrick scale ≤3), n (%)</td>
<td>2213 (83)</td>
<td>2005 (87)</td>
</tr>
<tr>
<td>Cow’s milk consumption volume (cups/day), mean ± SD</td>
<td>2.1 ± 1.1</td>
<td>2.0 ± 1.0</td>
</tr>
<tr>
<td>Child vitamin D daily supplementation (yes), n (%)</td>
<td>1491 (56)</td>
<td>1361 (55)</td>
</tr>
<tr>
<td>Ethnicity, n=2537</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mixed Western, n (%)</td>
<td>1830 (72)</td>
<td>1873 (77)</td>
</tr>
<tr>
<td>Mixed Western/Non-Western, n (%)</td>
<td>128 (5)</td>
<td>98 (4)</td>
</tr>
<tr>
<td>East/Southeast Asian, n (%)</td>
<td>266 (10)</td>
<td>264 (10)</td>
</tr>
<tr>
<td>Southwest Asian, n (%)</td>
<td>193 (8)</td>
<td>142 (6)</td>
</tr>
<tr>
<td>African, n (%)</td>
<td>109 (4)</td>
<td>72 (3)</td>
</tr>
<tr>
<td>Other, n (%)</td>
<td>11 (0)</td>
<td>6 (0)</td>
</tr>
<tr>
<td>Median Neighbourhood Family Income, n=2644</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;$30,000, n (%)</td>
<td>167 (7)</td>
<td>130 (5)</td>
</tr>
<tr>
<td>$30-79,999, n (%)</td>
<td>2039 (80)</td>
<td>1851 (78)</td>
</tr>
<tr>
<td>$80-150,000, n (%)</td>
<td>319 (12)</td>
<td>369 (15)</td>
</tr>
<tr>
<td>&gt;$150,000, n (%)</td>
<td>23 (1)</td>
<td>32 (1)</td>
</tr>
<tr>
<td>Daily screen time (mins), mean ± SD</td>
<td>71 ± 75</td>
<td>69 ± 67</td>
</tr>
<tr>
<td>Daily free play (mins), mean ± SD</td>
<td>61 ± 60</td>
<td>56 ± 51</td>
</tr>
</tbody>
</table>
Table 6: Bivariate Multivariable Linear Regression: Percent fat content of milk in association with 25-hydroxyvitamin D and zBMI

<table>
<thead>
<tr>
<th>Child characteristics</th>
<th>Change in median 25(OH)D, nmol/L (95% CI)</th>
<th>% Change in 25(OH)D, (95% CI)</th>
<th>P Value</th>
<th>Parameter estimate of zBMI (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent fat content of milk consumed, per 1% increase</td>
<td>1.67 (1.01, 3.05)</td>
<td>2.04 (0.82, 2.50)</td>
<td>0.006</td>
<td>-0.221 (-0.26, -0.18)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Age (months)</td>
<td>0 (-0.07, 0.08)</td>
<td>0 (-0.06, 0.07)</td>
<td>0.95</td>
<td>-0.0003 (0, 0)</td>
<td>0.80</td>
</tr>
<tr>
<td>Sex (males)</td>
<td>-1.70 (-3.92, 0.40)</td>
<td>-2.08 (-3.22, 0.33)</td>
<td>0.10</td>
<td>0.110 (0.03, 0.19)</td>
<td>0.005</td>
</tr>
<tr>
<td>Date of serum collection</td>
<td>0.74 (-0.80, 2.63)</td>
<td>0.90 (-0.65, 2.16)</td>
<td>0.33</td>
<td>-0.0083 (-0.062, 0.045)</td>
<td>0.76</td>
</tr>
<tr>
<td>Skin pigmentation (Fitzpatrick scale &lt;3)</td>
<td>3.52 (2.02, 10.52)</td>
<td>4.29 (1.66, 8.62)</td>
<td>0.05</td>
<td>0.037 (-0.09, 0.16)</td>
<td>0.57</td>
</tr>
<tr>
<td>Volume of milk consumption (cups/day)</td>
<td>2.84 (2.02, 5.13)</td>
<td>3.46 (1.66, 4.20)</td>
<td>&lt;0.0001</td>
<td>0.065 (0.03, 0.10)</td>
<td>0.0007</td>
</tr>
<tr>
<td>Child vitamin D daily supplementation (yes)</td>
<td>9.26 (8.33, 13.88)</td>
<td>11.29 (6.83, 11.38)</td>
<td>&lt;0.0001</td>
<td>-0.113 (-0.19, -0.03)</td>
<td>0.005</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mixed Western</td>
<td>Reference value</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mixed Western/Non-Western</td>
<td>-1.38 (-7.69, 4.08)</td>
<td>-1.69 (-6.30, 3.35)</td>
<td>0.58</td>
<td>-0.105 (-0.29, 0.08)</td>
<td>0.26</td>
</tr>
<tr>
<td>East/Southeast Asian</td>
<td>-1.46 (-5.82, 4.08)</td>
<td>-1.78 (-4.78, 1.66)</td>
<td>0.35</td>
<td>-0.019 (-0.13, 0.10)</td>
<td>0.74</td>
</tr>
<tr>
<td>Southwest Asian</td>
<td>-5.70 (-12.19, -1.00)</td>
<td>-6.95 (-10.00, -0.82)</td>
<td>0.01</td>
<td>-0.156 (-0.33, 0.02)</td>
<td>0.08</td>
</tr>
<tr>
<td>African/Caribbean</td>
<td>-2.18 (-9.52, 4.08)</td>
<td>-2.66 (-7.80, 3.35)</td>
<td>0.45</td>
<td>0.184 (-0.03, 0.40)</td>
<td>0.09</td>
</tr>
<tr>
<td>Median Neighbourhood Family Income</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;$30,000</td>
<td>-2.42 (-7.69, 3.05)</td>
<td>-2.96 (-6.30, 2.50)</td>
<td>0.34</td>
<td>0.073 (-0.09, 0.23)</td>
<td>0.37</td>
</tr>
<tr>
<td>$30-79,999</td>
<td>Reference value</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$80-150,000</td>
<td>3.26 (0.07, 8.33)</td>
<td>3.98 (0.06, 6.83)</td>
<td>0.05</td>
<td>0.036 (-0.08, 0.15)</td>
<td>0.56</td>
</tr>
<tr>
<td>&gt;$150,000</td>
<td>-1.86 (-14.79, 11.63)</td>
<td>-2.27 (-12.12, 9.53)</td>
<td>0.74</td>
<td>-0.271 (-0.69, 0.15)</td>
<td>0.21</td>
</tr>
<tr>
<td>Daily screen time (mins)</td>
<td>-0.01 (-0.03, 0.01)</td>
<td>-0.01 (-0.02, 0.01)</td>
<td>0.48</td>
<td>-0.001 (-0.002, -0.0002)</td>
<td>0.005</td>
</tr>
<tr>
<td>Daily free play (mins)</td>
<td>0.02 (-0.01, 0.03)</td>
<td>0.02 (-0.005, 0.02)</td>
<td>0.16</td>
<td>0.0005 (0, 0)</td>
<td>0.17</td>
</tr>
<tr>
<td>Sugar sweetened beverages (cups/day)</td>
<td>-1.46 (-5.82, 2.02)</td>
<td>-1.78 (-4.78, 1.66)</td>
<td>0.39</td>
<td>0.068 (-0.06, 0.19)</td>
<td>0.28</td>
</tr>
<tr>
<td>Maternal BMI</td>
<td>-0.16 (-0.50, 0.20)</td>
<td>-0.20 (-0.41, 0.16)</td>
<td>0.36</td>
<td>0.033 (0.02, 0.04)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Percent fat content of milk * Volume of milk consumed</td>
<td>-1.59 (-2.62, -0.55)</td>
<td>-1.94 (-3.20, -0.67)</td>
<td>0.003</td>
<td>0.006 (-0.01, 0.03)</td>
<td>0.77</td>
</tr>
</tbody>
</table>
**Figure 6:** Adjusted* association between percent fat content of milk, 25-hydroxyvitamin D, and zBMI.

*Adjusted for age, sex, date of serum collection, skin pigmentation, daily vitamin D supplementation, volume of milk consumption (cups/day), maternal ethnicity, daily screen time (minutes), daily outdoor play (minutes), maternal BMI, sugar sweetened beverage consumption (cups/day), and median neighbourhood family income.
Figure 7: Adjusted* association between percent fat content of milk, 25-hydroxyvitamin D (a), and zBMI (b), with effect modification by volume of milk consumed. The interaction between volume of milk and percent fat content of milk was statistically significant for 25-hydroxyvitamin D, but not statistically significant for zBMI.

*Adjusted for age, sex, date of serum collection, skin pigmentation, daily vitamin D supplementation, volume of milk consumption (cups/day), maternal ethnicity, daily screen time
(minutes), daily outdoor play (minutes), maternal BMI, sugar sweetened beverage consumption (cups/day), and median neighbourhood family income.
CHAPTER 5: OVERALL DISCUSSION

In this thesis I have attempted to determine the relationship between the fat content of cow’s milk consumed and serum 25-hydroxyvitamin D in early childhood; second, to determine the relationship between percent fat content of milk and 25-hydroxyvitamin D and zBMI simultaneously, and finally explore the role of volume of milk consumed in these relationships. I have tested hypotheses determined a priori through sequential, pre-specified data analysis plans (see Appendices C and D). This process was beneficial to ensure statistical integrity, maintain transparency and reproducibility of my findings. I identified that higher fat milk was positively associated with 25-hydroxyvitamin D concentration and negatively associated with zBMI in early childhood. The fat content of milk that children consumed modified the effect of volume of milk on serum 25-hydroxyvitamin D, but not zBMI.

Given that vitamin D is a fat soluble vitamin, I hypothesized that higher milk fat consumption would be associated with higher vitamin D stores in children. Fat is known to stimulate bile acid secretion, which helps break down and disperse fat and fat soluble vitamins, to micelles for uptake into the bloodstream (45). In Study 1: Milk fat content and vitamin D in early childhood, I identified that higher milk fat was associated with higher 25-hydroxyvitamin D levels in children. Children who consumed lower fat milk needed to consume higher quantities of milk to have a similar 25-hydroxyvitamin D concentration as those who drank a lower volume of higher fat milk. Further, children who consumed lower fat milk had double the odds of 25-hydroxyvitamin D <50 nmol/L, which is considered ‘insufficient’ by the Institute of Medicine (41, 43).

Several studies have examined the relationship between dietary fat and vitamin D status in adults with heterogeneous results. A randomized controlled trial (RCT) by Tangpricha et al. demonstrated that dietary fat is not necessary for vitamin D₂ absorption in adults (47); another
RCT demonstrated that increased dietary fat had a positive effect on vitamin D₃ absorption in the gut, but little impact on 25-hydroxyvitamin D levels (48). Two other RCTs revealed that serum 25-hydroxyvitamin D was highest in people who consumed a fat-containing meal, as opposed to those who consumed a fat-free meal (45, 200). A prospective cohort study found that adults who consumed a vitamin D supplement with their largest meal of the day increased the impact of supplementation on serum 25-hydroxyvitamin D levels (46). All of these studies relied on small sample sizes (n ≤ 62) of adults, and administered high-dose supplements (50 000 IU), which are not typical of the vitamin D intake of children or adults. To my knowledge, no study has yet evaluated the effect of milk fat content on 25-hydroxyvitamin D in children. My study was novel in demonstrating a positive association between percent fat content of milk and serum 25-hydroxyvitamin D in children and that milk fat content modified the effect of volume of milk on serum 25-hydroxyvitamin D. My study was also unique in including an ethnically diverse sample who consumed a variety of milk fat and milk volume, with a relatively large sample size and detailed clinically relevant information which allowed adjustment for numerous potential confounders.

Current guidelines recommend that children over the age of 2 years consume lower fat (1% or 2%) milk to reduce their risk of health complications and obesity (19). These guidelines aimed to limit childhood obesity through reduced milk fat consumption (201-203). However, existing research has largely identified an inverse relationship between milk fat consumption and adiposity in children (33-37). Therefore, in Study 2: The relationship between milk fat content, vitamin D, and adiposity in early childhood, I hypothesized that increased percent fat content of milk would be associated with both higher 25-hydroxyvitamin D concentration (based on my results from Study 1) as well as lower zBMI scores in children.
In Study 2, I identified that higher percent fat content of milk was associated with higher serum 25-hydroxyvitamin D and lower zBMI in children aged 12-72 months. Children drinking 1% milk were twice as likely to be vitamin D insufficient (<50 nmol/L), had 2.4 fold higher odds of overweight, and 3.2 fold higher odds of obesity when compared to children drinking whole (3.25%) milk. My finding that children who drank whole milk had a 0.72 unit lower zBMI score than children drinking 1% milk may be clinically meaningful, noting that the difference between healthy weight and overweight is a zBMI of 1 (152). Further, in pediatric obesity interventions, a 0.5 decrease in zBMI is considered meaningful because it is associated with significant changes in a child’s metabolic risk factors (198, 199).

I chose bivariate linear regression to examine the associations between milk fat content and two outcomes concurrently (25-hydroxyvitamin D and zBMI). This is an appropriate method when two outcomes are correlated within the same individual as 25-hydroxyvitamin D and adiposity have been shown to be (25, 26, 28, 29, 31, 32). Another advantage of bivariate regression is reducing the risk of type 1 error relative to completing two separate regression analyses (204).

Studies 1 and 2 utilized similar samples of participants, but yielded slightly different results when determining the association between percent fat content of milk and 25-hydroxyvitamin D. There are a few reasons for this discrepancy. A small number of children included in study 1 were excluded from study 2 due to missing zBMI data. Second, missing data from children across both samples was supplied using multiple imputation, which estimates and assigns new data to participants with missing information, but varies marginally with each estimate. Finally, due to the inclusion of predictors of zBMI in the statistical model in study 2 which were absent in the model used in study 1, results were adjusted in a somewhat different manner in each study.
Although causality in the relationship between milk intake and adiposity remains unclear, several hypotheses regarding the biological mechanism have been proposed. One hypothesis is that higher fat milk may increase satiety and displace calories from other more calorically dense foods resulting in an overall lower energy intake (34, 37). Other hypotheses include reverse causality such that parents of children with higher zBMI may choose lower fat milk to minimize further weight gain (34). However, should the first of these mechanisms be true, switching to lower fat milk may exacerbate further increases in adiposity.

The research that I have completed in this thesis has led to a number of questions. The mechanisms by which milk fat is positively associated with serum 25-hydroxyvitamin D and negatively associated with zBMI are unclear. As TARGetKids! has collected longitudinal data on children throughout childhood, a longitudinal study could identify temporality, which is one of the conditions by which causality is established (205). For example, temporality could be established by identifying whether increases or decreases in milk fat intake precede or follow changes in childhood adiposity. Also, understanding the reasons parents choose a particular milk fat concentration for their children may provide insight about reversed causality. Further, by collecting more detailed dietary information, we could evaluate whether calories are displaced in children who consume higher fat content milk. Another question is whether cow’s milk fat has a positive effect on other outcomes of childhood such as height or cognitive development. Understanding these associations could be helpful in providing a holistic approach to inform future guidelines about milk fat consumption in childhood. Ultimately, interventional research including randomized controlled trials is needed to demonstrate cause-and-effect in the relationships between cow’s milk fat, adiposity, and vitamin D in children. Such studies would be most informative in moving this field forward.
Strengths of the studies completed in this thesis include a large, healthy and ethnically diverse population of children. TARGetKids! is currently the largest childhood cohort in Canada. There are a few comparable European cohorts (Avon Longitudinal Study of Parents and Children, Born in Bradford); however, this analysis would not be possible in Europe due to lacking legislation for vitamin D fortification in cow’s milk. The large sample size provided sufficient statistical power to execute my analytical plan. Detailed clinical data collected through TARGetKids! allowed me to control for many potential confounders which added strength to my findings by reducing the likelihood of confounding from measured covariates. Sophisticated statistical analysis plans were developed a-priori based on biologically plausible conceptual models to test specific hypotheses. As pre-specified hypotheses were investigated using double-sided tests in each of my studies, the probability of type 1 error was preserved at p<0.05.

Limitations of this work include the cross-sectional nature of this study; causation cannot be concluded due to study design. As cross-sectional studies assess relationships at one point in time, they cannot explain temporality and cannot assign cause and effect to the exposure and outcome variables. This research may also be subject to a number of biases, including recall and selection bias. Bias has the effect of influencing results to be different than the truth. Recall bias, which occurs when collected information is recalled inaccurately from the past, is a possibility in my dietary intake data since parent completed questionnaires were used for data collection. For example, parents of obese children may recall milk intake differently than parents of healthy weight children. However, questionnaires were based on the CCHS, which has been used extensively on similar populations (177). Selection bias, which occurs when sample characteristics are different from population parameters, is also a possibility. Parents who consented for their children to participate in TARGetKids!, particularly those who contributed a blood sample, may be different from those who did not. However, when I compared sample
attributes between participants who did and did not contribute a blood sample, the groups appeared clinically similar. Although the TARGetKids! cohort is from Toronto, Canada’s most ethnically diverse city, my results may not be generalizable to children from other urban centres. Characteristics of my study population such as skin pigmentation (82% of analyzed children were fair-skinned), ethnicity (66% were of Western descent), and daily vitamin D supplementation (54% of children were supplemented) may be different from other populations. Further, children included in my studies receive routine primary healthcare and may be different from children who do not receive primary healthcare. Finally, children who consumed very high or very low volumes of milk were infrequent in this population (9% of children consumed <0.5 cups of milk, and 2% drank 5+ cups of milk per day); thus, power to detect effects in these children may have been lower.

To my knowledge, no other research has attempted to determine the milk fat concentration that is associated with optimizing both serum 25-hydroxyvitamin D and adiposity. I hope this research will foster dialogue among health professionals, professional associations and parents about current milk fat recommendations and stimulate further research.
CHAPTER 6: CONCLUSION

Through this thesis, I have identified that percent fat content of milk was positively associated with serum 25-hydroxyvitamin D and negatively associated with zBMI in healthy children aged 12-72 months. Further, percent fat content of milk appeared to modify the relationship between volume of milk consumed and 25-hydroxyvitamin D, but not zBMI. Children who consumed 1% milk had higher odds of 25-hydroxyvitamin D concentration <50 nmol/L, overweight, and obesity in comparison to children who consumed whole (3.25% fat) milk. This information may be helpful to parents, health care practitioners, and policymakers for optimizing both vitamin D and adiposity in early childhood. Further research is needed to determine the mechanism by which milk fat is related to higher serum 25-hydroxyvitamin D and lower adiposity in children.
CHAPTER 7: APPENDICES

7.1 APPENDIX A: TARGETKIDS PARTICIPANT CONSENT FORM

PARENT/GUARDIAN RESEARCH CONSENT FORM

TARGet Kids! Measuring nutrition in young preschool-aged children in the primary care practice setting

Investigator(s):
TARGet Kids!:
Principle Investigator:
Dr Patricia Parkin (416) 813-7654 ext. 301544

Co-investigators:
Dr Catherine Birken (416) 813-7654 ext. 301544
Dr Jonathon Maguire (416) 813-7654 ext. 302129

Purpose of the Research:
Your child’s physician is a member of TARGet Kids! (The Applied Research Group) which is a network of SickKids child health researchers and community doctors dedicated to improving the health of young children.

With the aim of “health research for every child,” this network will collect medical evidence on common health problems affecting urban Canadian children. We have a special focus on measuring the nutritional health of children from early childhood to adolescence. This is the first group in Canada to study children in community settings with a goal to promote wellness and prevent disease. We are inviting you and your child(ren) to participate in this exciting new study. This study aims to collect information on nutrition in healthy children. Depending on your child’s age at the time of participation, you will be asked to complete a set of questionnaires.

TARGet Kids questionnaires include:

Nutrition and Health Questionnaire (NHQ) – an age specific TARGet Kids questionnaire designed to capture important information about health, for example, physical activity and vitamin use.

NutriSTEP™ (NS) – designed to assess nutrition in children.

Infant Behavior (IBQ) / Early Childhood Behavior (ECBQ) / Children’s Behavior Questionnaire (CBQ) – designed to assess temperament, activity and parenting.

Nipissing District Developmental Screen (NDDS) – a developmental screening tool used in Ontario at the 18 month routine primary health care visit. The NDDS includes questions about vision, hearing, and communication.

Infant Toddler Checklist (ITC) – designed to assess communicative development including emotion, use of gestures, use of sounds, and understanding of words.

Parenting Stress Index (PSI) – designed to assess stress in the parent-child system.

We will also ask your child to have blood tests to measure his/her nutritional health. This will be based on things such as cholesterol, iron, and vitamin D levels. These blood tests will be obtained and processed by experts from the Mount Sinai Hospital Services team.

Lastly, we would like to understand more about your child’s readiness for school. A questionnaire called the Early Development Instrument (EDI) was developed at McMaster University with the help of principals and kindergarten teachers. It asks questions about five areas of child development: 1) physical health and well-being; 2) social knowledge and
competence; 3) emotional health and maturity; 4) language and cognitive development; and 5) general knowledge and communication skills. The EDI is collected every few years in most schools in Toronto, Ontario, and across Canada. This research is being done to better understand and measure children’s developmental health as they enter school.

**Description of the Research:**
If you agree to participate the following will occur at each of your child’s regularly scheduled annual visits between birth and adolescence. No additional visits to your doctor are required. We will ask you to complete study questionnaires (described above)
We will record your child’s height/length, weight, waist circumference and blood pressure. We will also record your height, weight and waist circumference.
A trained health professional experienced with pediatric blood collection will take a small blood sample from your child.
We will obtain information on your child’s medical history from your doctor’s records. For example, your child’s height, weight, head circumference measurements, gestational age at birth, birth weight and information on previous sick visits.
We may offer a developmental assessment conducted by trained psychometrist
We may ask your child to wear an accelerometer to measure their physical activity. in this case, you will be provided with an accelerometer and we will ask you to record the time your child wakes up, goes to bed and takes the accelerometer off.
We may also collect additional health information about you and your child using an OHIP number.
Depending on your child’s age, we will contact your child’s school to collect EDI information. If this has not already been routinely collected, all the necessary information and documents will be provided for the teacher to complete an EDI questionnaire on your child.

**Potential Harms, Discomforts or Inconvenience:**
We will collect a small blood sample (up to 9-12 ml of blood) from your child’s arm using a needle. Topical anesthetic cream (EMLA or Ametop) will be offered to minimize discomfort from venipuncture. There may be slight discomfort, bruising or redness that will usually disappear in a few days. Blood collection is usually a quick process (about 5 minutes) and at other times it can require a little more time. While the amount of blood that will be collected is small, the impact that it will have in terms of helping sick children is immeasurable.

**Potential Benefits:**
Your child may benefit from participating in the TARGet Kids study by having the NutriSTEP™ results available to you and to your child’s doctor, who will discuss the results with you in more detail. The NutriSTEP™ questionnaire will provide you with guidance about your child's nutrition and how it may be improved. We will also give you some helpful handouts to take home with healthy living tips for your child. Society may benefit from the nutrition study if the questionnaire is found to be useful for doctors in their offices. This quick questionnaire may help guide doctors to make useful recommendations to improve their patients’ health.
Your child may benefit from participating in the TARGet Kids! study by having his or her blood measured as the results will be provided to your child’s doctor. This could inform your doctor of whether your child might need a nutritional supplement or changes in diet or lifestyle. In addition to knowing one has helped sick children across Canada and the world, participants themselves (and/or family member or friend) could potentially be a patient at SickKids or other paediatric health centre, and benefit directly from the results obtained from this study.

**Confidentiality:**
We will respect your privacy. No information about who your child is will be given to anyone outside of the research team or be published without your permission, unless required by law.
For example, the law could make us give information about you if a child has been abused, if your child has an illness that could spread to others, if your child or someone else talks about harming themselves or others, or if the court orders us to give them the study papers. Information about your child may be shared with researchers at other agencies without any identifying information (such as your child's name) and only if approved by the Research Ethics Board. SickKids Clinical Research Monitors, employees of the funders or the regulator of the study may see your questionnaire responses or your child’s blood test results to check on the study. By signing this consent form, you agree to let these people look at this information.

The data produced from this study will be stored in a secure, locked location. Only members of the research team (and maybe those described above) will have access to the data. Following completion of the research study, the data will be kept as long as required, then destroyed as required by SickKids policy. Published study results will not reveal you or your child’s identity. For those participants who completed the Parenting Stress Index (PSI) questionnaire, the following information will be shared with Psychological Assessments Resources group (distributor of PSI questionnaire): child’s month and year of birth, gender, parents’ ages, ethnicity, relationship to a child and marital status, highest level of education completed by mother and data collected through PSI questionnaire. Identifying information will not be shared with Psychological Assessments Resources.

We will give you a copy of this consent form.

Reimbursement:
We will reimburse you for all your reasonable out of pocket expenses for being in this study, (eg. babysitters, parking) if the visit is scheduled outside of your child’s regularly scheduled appointments.

Participation:
It is your choice to allow your child to take part in this study. You can stop at any time. During this study, we may create new tests, new medicines, or other things that may be worth some money. Although we may make money from these findings, we cannot give you or your child any of this money now or in the future because your child took part in this study. Participation in research is voluntary. If you choose not to participate, you and your family will continue to have access to quality care at SickKids and at your child’s doctor’s office if needed. If you choose, on behalf of your child, to participate in this study, you can take your child out of the study at any time. Again, you and your family will continue to have access to quality care at SickKids and at your child’s doctor’s office if needed.

New information that we get while we are doing this study may affect your decision to take part in this study. If this happens, we will tell you about this new information. And we will ask you again if you still want to be in the study. If your child becomes ill or is harmed because of study participation, we will treat your child for free.

Your signing this consent form does not interfere with your legal rights in any way. The staff of the studies, the hospital, and the doctor’s office are still responsible, legally and professionally, for what they do.

Conflict of Interest
None of the people involved in this study have a conflict of interest. This means that they will not benefit personally or financially from this study.

Sponsorship:
Funding for the TARGet Kids study is provided by The Hospital for Sick Children Foundation’s grant to our research program, the Pediatric Outcomes Research Team (PORT) and the Canadian Institute for Health Research (CIHR).

**Future Research:**
In the future, our research team may approach you to participate in other studies with the aim of improving children’s health. The research will be explained to you and your consent we will ask for your consent at that time.

**Consent:**
By signing this form, I agree that:

1) You have explained these studies to me. You have answered all my questions.
2) You have explained the possible harms and benefits (if any) of these studies.
3) I understand that I have the right to refuse to let my child take part in the study/studies. I also have the right to take my child out of the study/studies at any time. My decision about my child taking part in the study/studies will not affect my child’s health care.
4) I am free now, and in the future, to ask questions about the study.
5) I have been told that my child’s questionnaires, blood test results and medical records will be kept private unless it is described to me.
6) I understand that no information about my child will be given to anyone unless required by law.
7) I understand the publication of the results from this study will not identify me or my child in any way.

I agree, or consent, that my child___________________ may take part in this study.

<table>
<thead>
<tr>
<th>Printed Name of Parent/Legal Guardian</th>
<th>Parent/Legal Guardian’s signature</th>
<th>date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Printed Name of person who explained consent</td>
<td>Signature of Person who explained consent</td>
<td></td>
</tr>
<tr>
<td>date</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Printed Witness’ name (if parent/legal guardian is unable to read consent form)</th>
<th>Witness’ signature</th>
<th>date</th>
</tr>
</thead>
</table>

If you have any questions about the TARGet Kids study, please call Dr Patricia Parkin at (416) 813-7654 ext 301544. If you have questions about your child’s rights as a subject in a study or about injuries during a study, please call the Research Ethics Manager at 416-813-5718.
### 7.2 APPENDIX B: FOOD SOURCES OF VITAMIN D

<table>
<thead>
<tr>
<th>Food</th>
<th>Serving Size</th>
<th>Vitamin D (IU)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vegetables and Fruit</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Orange juice, fortified with vitamin D</td>
<td>125 mL (½ cup)</td>
<td>50</td>
</tr>
<tr>
<td><strong>Grain Products</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>This food group contains very little of this nutrient.</td>
<td></td>
</tr>
<tr>
<td><strong>Milk and Alternatives</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soy beverage, fortified with vitamin D</td>
<td>250 mL (1 cup)</td>
<td>123</td>
</tr>
<tr>
<td>Milk (3.3 % homo, 2%, 1%, skim, chocolate milk)</td>
<td>250 mL (1 cup)</td>
<td>103-105</td>
</tr>
<tr>
<td>Skim milk powdered</td>
<td>24 g (will make 250 mL of milk)</td>
<td>103</td>
</tr>
<tr>
<td>Soy beverage, fortified with vitamin D</td>
<td>250 mL (1 cup)</td>
<td>88</td>
</tr>
<tr>
<td>Yogurt (plain, fruit bottom), fortified with vitamin D</td>
<td>175 g (3/4 cup)</td>
<td>58-71</td>
</tr>
<tr>
<td><strong>Meat and Alternatives</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Egg, yolk, cooked</td>
<td>2 large</td>
<td>57-88</td>
</tr>
<tr>
<td>Pork, various cuts, cooked</td>
<td>75 g (2 ½ oz)</td>
<td>6-60</td>
</tr>
<tr>
<td>Deli meat (pork, beef, salami, bologna)</td>
<td>75 g (2 ½ oz)/ 3 slices</td>
<td>30-54</td>
</tr>
<tr>
<td>Beef live, cooked</td>
<td>75 g (2 ½ oz)</td>
<td>36</td>
</tr>
<tr>
<td><strong>Fish and Seafood</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salmon, sockeye/red, canned, cooked or raw</td>
<td>75 g (2 ½ oz)</td>
<td>530-699</td>
</tr>
<tr>
<td>Salmon, humpback/pink, canned, cooked or raw</td>
<td>75 g (2 ½ oz)</td>
<td>351-497</td>
</tr>
<tr>
<td>Salmon, coho, raw or cooked</td>
<td>75 g (2 ½ oz)</td>
<td>326-421</td>
</tr>
<tr>
<td>Snapper, cooked</td>
<td>75 g (2 ½ oz)</td>
<td>392</td>
</tr>
<tr>
<td>Salmon, chinook, raw or cooked</td>
<td>75 g (2 ½ oz)</td>
<td>319-387</td>
</tr>
<tr>
<td>Whitefish, lake, cooked</td>
<td>75 g (2 ½ oz)</td>
<td>369</td>
</tr>
<tr>
<td>Mackerel, Pacific, cooked</td>
<td>75 g (2 ½ oz)</td>
<td>342</td>
</tr>
<tr>
<td>Salmon, Atlantic, raw or cooked</td>
<td>75 g (2 ½ oz)</td>
<td>181-246</td>
</tr>
<tr>
<td>Salmon, chum/keta, raw or cooked</td>
<td>75 g (2 ½ oz)</td>
<td>203-221</td>
</tr>
<tr>
<td>Mackerel, canned</td>
<td>75 g (2 ½ oz)</td>
<td>219</td>
</tr>
<tr>
<td>Herring, Atlantic, pickled</td>
<td>75 g (2 ½ oz)</td>
<td>210</td>
</tr>
<tr>
<td>Trout, cooked</td>
<td>75 g (2 ½ oz)</td>
<td>150-210</td>
</tr>
<tr>
<td>Herring, Atlantic, cooked</td>
<td>75 g (2 ½ oz)</td>
<td>161</td>
</tr>
<tr>
<td>Roe, raw</td>
<td>30 g (1 oz)</td>
<td>145</td>
</tr>
<tr>
<td>Sardines, Pacific, canned</td>
<td>75 g (2 ½ oz)</td>
<td>144</td>
</tr>
<tr>
<td>Halibut, cooked</td>
<td>75 g (2 ½ oz)</td>
<td>144</td>
</tr>
<tr>
<td>Tuna, albacore, raw or cooked</td>
<td>75 g (2 ½ oz)</td>
<td>82-105</td>
</tr>
<tr>
<td>Mackerel, Atlantic, cooked</td>
<td>75 g (2 ½ oz)</td>
<td>78</td>
</tr>
<tr>
<td>Tuna, white, canned with water</td>
<td>75 g (2 ½ oz)</td>
<td>60</td>
</tr>
</tbody>
</table>
### Fats and Oils

<table>
<thead>
<tr>
<th>Description</th>
<th>Serving Size</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cod liver oil</td>
<td>5 mL (1 tsp)</td>
<td>427</td>
</tr>
<tr>
<td>Margarine</td>
<td>5 mL (1 tsp)</td>
<td>25</td>
</tr>
<tr>
<td><strong>Other</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Goat’s milk, fortified with Vitamin D</td>
<td>250 mL (1 cup)</td>
<td>100</td>
</tr>
<tr>
<td>Rice, oat, almond beverage, fortified with Vitamin D</td>
<td>250 mL (1 cup)</td>
<td>88-90</td>
</tr>
</tbody>
</table>

**Source:** "Canadian Nutrient File 2010"


Accessed October 6, 2015
### 7.3 APPENDIX C: DATA CREATION PLAN FOR STUDY 1

#### Cross-sectional Study Dataset Creation Plan

<table>
<thead>
<tr>
<th>Name of Study</th>
<th>Milk Fat Content in Association With Child 25(OH)D</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PI and P&amp;B Contacts</strong></td>
<td></td>
</tr>
<tr>
<td>• Jonathon Maguire</td>
<td></td>
</tr>
<tr>
<td>• Shelley Vanderhout</td>
<td></td>
</tr>
</tbody>
</table>

#### Short Description of Research Question

**Primary:**
Among children aged 12 to 72 months, is consumption of higher fat milk associated with higher serum 25(OH)D?

**Secondary:**
Does the volume of milk consumed by children modify the relationship between milk fat content and serum 25(OH)D?

#### List of Datasets Used

- TARGet Kids! Baseline pre-migration (before September 2011)
- TARGet Kids! Baseline post-migration
- TARGet Kids! Lab data
- TARGet Kids! Follow-up (for subjects with missing baseline lab data)

#### Defining the Cohort

**TARGet Kids! Cohort**
Patients aged 12 to 72 months who drink milk and attend well-child visits at a primary care pediatrician’s or family physician’s office

**Exclusions in Cohort (in order):**
- Children with associated health conditions affecting growth (e.g. failure to thrive, cystic fibrosis)
- Children with an chronic condition(s) except for asthma
- Children with severe developmental delay
- Families who are not fluent in English
- Children born premature (children born < 34 weeks gestation)

#### Size of Primary Cohort

- Number of children recruited from December 2008 to September 2014 who filled out the NHQ, have completed blood work for 25(OH)D and drink cow’s milk (NHQ #27: selected “Milk” in response to “Please specify your child’s diet for the past 3 days. Please check all that apply.”)

#### Time Frame Definitions

**Accrual Start/End Dates**
- Beginning of Accrual Period for cohort: December 2008
- End of Accrual Period for cohort: September 2014

#### Variable Definitions

**Main Exposure or Risk Factor**

<table>
<thead>
<tr>
<th>Primary Exposure: Cow’s milk consumption, as average percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>NHQ (27) Please specify your child’s diet for the past 3 days. Please check all that apply.</td>
</tr>
<tr>
<td>• Milk</td>
</tr>
<tr>
<td>• Skim</td>
</tr>
<tr>
<td>• 1%</td>
</tr>
<tr>
<td>• 2%</td>
</tr>
<tr>
<td>• Homo</td>
</tr>
<tr>
<td>Continuous variable</td>
</tr>
</tbody>
</table>

**Secondary Exposure:**

- Volume of milk consumed

<table>
<thead>
<tr>
<th>NHQ (28) Circle how many cups of each drink your child has currently in a typical day, if none then circle 0.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Categorical variable (0, 1/2, 1, 2, 3, 4, 5+)</td>
</tr>
</tbody>
</table>
**Baseline Characteristics**

- Age (years)
- Sex (M/F)
- zBMI
- 25(OH)D (nmol/L)
- Others (list):
  - Season (Month)
  - Skin pigmentation (Fitzpatrick scale ≤3)
  - Cow’s milk consumption (cups/day)
  - Child daily vitamin D supplementation (yes)
  - Family Income

**Outcome Definition(s)**

- **Primary Outcome**: Child serum 25(OH)D level laboratory value
  - Continuous variable (mean nmol/L)
- **Secondary Outcome**:
  - Categorical variable (<50 AAP, <75 CPS nmol/L)

**Covariates for primary analysis**

1. Age (years)
   - Continuous variable
2. Sex
   - Categorical variable
3. Skin pigmentation [reference value: 3]
   - Based on the Fitzpatrick's scale
   - Categorical variable (≤3, >3)
4. Season of serum collection
   - Sine (month)
5. Non-cow’s milk consumption
   - (NHQ #28) *Circle how many cups of each drink your child has currently in a typical day, if none then circle 0.*
     - Soy milk
     - Other milk (rice, goat, etc.)
   - Categorical variable (1/2, 1, 2, 3, 4, 5+ of either of the above variables)
6. Child vitamin D supplementation [reference value: yes]
   - (NHQ #18) *Does your child take any vitamins or supplements regularly?*
   - Vitamin D (dose) _______ days per week
   - Multivitamin _______ times per week
   - Categorical variable
   - If answer yes to any, value=yes
   - If answer no to all, value=no
7. Child zBMI (direct measurement in primary care office)
   - Continuous variable
8. Family income
   - Categorical variable (Less than $30,000, $30,000-$79,999, $80,000-$150,000, More than $150,000)
9. Non-cow’s milk consumption
   - Categorical variable (Yes, No)
Analysis Plan

1. Descriptive Statistics
   a. Table 1 of N (%) for baseline characteristics of population
   b. Table 2 of N (%) for study covariates

2. Examine data to determine outliers

3. Use variance inflation factor (VIF) to assess multicollinearity in covariates
   a. Remove variables with VIF >5 before performing multivariable analysis.

4. Primary analysis
   a. Run linear regression model to determine association between type of milk consumption and child 25(OH)D (nmol/L) (table 2)
   b. Run multiple linear regression model to determine association between type of milk consumption and child 25(OH)D, and factors known or suspected to affect their relationship (table 3)
      i. Child 25(OH)D status (nmol/L) = Type of Milk Consumption + Age + Sex + Skin Pigmentation + Season + Child zBMI + Child vitamin D supplementation + Volume of Child cow’s milk consumption + Family income + Non-cow’s milk consumption
   c. Run multiple logistic regression model to determine association between type of milk consumption and child 25(OH)D, and factors known or suspected to affect their relationship (table 3a&b)
      i. Child 25(OH)D status (<50 nmol/L) = Type of Milk Consumption + Age + Sex + Skin Pigmentation + Season + Child zBMI + Child vitamin D supplementation + Volume of Child cow’s milk consumption + Family income + Non-cow’s milk consumption
      ii. Child 25(OH)D status (<75 nmol/L) = Type of Milk Consumption + Age + Sex + Skin Pigmentation + Season + Child zBMI + Child vitamin D supplementation + Volume of Child cow’s milk consumption + Family income + Non-cow’s milk consumption
   d. Validate model using bootstrap.

5. Secondary analysis
   a. Run multiple linear regression model to determine association between type of milk consumption and child 25(OH)D, with interaction term for volume of milk and type of milk, and factors known or suspected to affect their relationship (table 4)
      i. Child 25(OH)D status (nmol/L) = Type of Milk Consumption + Type of milk*Volume of milk consumed + Age + Sex + Skin Pigmentation + Season + Child zBMI + Child vitamin D supplementation + Volume of Child cow’s milk consumption + Family income + Non-cow’s milk consumption
## Cross-sectional Study Dataset Creation Plan

<table>
<thead>
<tr>
<th>Name of Study</th>
<th>Percent Fat Content of Milk in Association with Child zBMI and 25(OH)D</th>
</tr>
</thead>
<tbody>
<tr>
<td>PL and P&amp;B Contacts</td>
<td></td>
</tr>
<tr>
<td>Primary:</td>
<td>To evaluate the relationship between percent fat content of milk consumed and both zBMI and serum 25-hydroxyvitamin D in early childhood.</td>
</tr>
<tr>
<td>Secondary:</td>
<td>1. Does the volume of milk consumed modify the relationships between percent fat content of milk and zBMI, as well as 25-hydroxyvitamin D, in early childhood?</td>
</tr>
<tr>
<td></td>
<td>2. Does child age, before or after age 2, modify the relationships between percent fat content of milk and zBMI, as well as 25-hydroxyvitamin D, in early childhood?</td>
</tr>
<tr>
<td></td>
<td>3. What is the fat content required for a child to be categorized as:</td>
</tr>
<tr>
<td></td>
<td>a. 25-hydroxyvitamin D &lt;50 and BMI &gt;97th percentile?</td>
</tr>
<tr>
<td></td>
<td>b. 25-hydroxyvitamin D &lt;50 and BMI &gt;85th percentile?</td>
</tr>
<tr>
<td></td>
<td>c. 25-hydroxyvitamin D &lt;75 and BMI &gt;97th percentile?</td>
</tr>
<tr>
<td></td>
<td>d. 25-hydroxyvitamin D &lt;75 and BMI &gt;85th percentile?</td>
</tr>
</tbody>
</table>

### Short Description of Research Question
- TARGet Kids! Baseline pre-migration (before September 2011)
- TARGet Kids! Baseline post-migration
- TARGet Kids! Lab data
- TARGet Kids! Follow-up (for subjects with missing baseline lab data)

### Defining the Cohort
- TARGet Kids! Cohort: Children aged 12-72 months attending well-child visits, who meet inclusion criteria and have blood analysis for 25-hydroxyvitamin D.
- Exclusions in Cohort (in order):
  - Children with associated health conditions affecting growth (e.g., failure to thrive, cystic fibrosis)
  - Children with an chronic condition(s) except for asthma
  - Children with severe developmental delay
  - Families who are not fluent in English
  - Children born premature (children born < 34 weeks gestation)

### Time Frame Definitions
- Accrual Start/End Dates:
  - Beginning of Accrual Period for cohort: December 2008
  - End of Accrual Period for cohort: September 2014

### Variable Definitions
- NHQ (27) Please specify your child’s diet for the past 3 days. Please check all that apply.
  - Milk
  - Skim
  - 1%
  - 2%
  - Homo
- Continuous variable
- NHQ (28) Circle how many cups of each drink your child has currently in a typical day, if none then circle 0.
### Covariates for primary analysis

1. **Age (years)**  
   - Continuous variable
2. **Sex**  
   - Categorical variable
3. **Skin pigmentation [reference value: 3]**  
   - Based on the Fitzpatrick’s scale  
   - Categorical variable (≤3, >3)
4. **Season of serum collection**  
   - Categorical variable
5. **Child vitamin D supplementation [reference value: yes]**  
   - (NHQ #18) *Does your child take any vitamins or supplements regularly?*  
   - Vitamin D (dose) ______ days per week  
   - Multivitamin ______ times per week  
   - Categorical variable  
   - If answer yes to any, value=yes  
   - If answer no to all, value=no
6. **Volume of milk consumption**  
   - Categorical variable (1/2, 1, 2, 3, 4, 5+)
7. **Ethnicity**  
   - Categorical variable
8. **Median neighbourhood family income**  
   - Categorical variable
9. **Screen time**  
   - Continuous variable (minutes/day)
10. **Sugar sweetened beverage consumption**  
    - Categorical variable (1/2, 1, 2, 3, 4, 5+)
11. **Unstructured free play**  
    - Continuous variable (minutes/day)

### Baseline Characteristics

- Age (years)
- Sex (M/F)
- zBMI
- 25(OH)D (nmol/L)
- Others (list):
  - Season (Month)
  - Skin pigmentation (Fitzpatrick scale ≤3)
  - Cow’s milk consumption (cups/day)
  - Child daily vitamin D supplementation (yes)
  - Ethnicity
  - Median neighbourhood family income
  - Unstructured free play (mins/day)
  - Sugar sweetened beverage consumption (cups/day)
  - Screen time (mins/day)

### Outcome Definition(s)

- **Primary Outcomes:**  
  - Child serum 25(OH)D level laboratory value  
    - Continuous variable (mean nmol/L)
  - Child zBMI  
    - Continuous variable
12. Maternal BMI
Continuous variable

Analysis Plan

1. Descriptive Statistics
   a. Table 1 of N (%) for baseline characteristics of population
   b. Table 2 of N (%) for study covariates
2. Examine data to determine outliers
3. Primary analysis
   a. Run bivariate linear regression model to determine association between percent fat content of milk and zBMI and 25-hydroxyvitamin D.
      i. Percent fat content of milk = zBMI, 25-hydroxyvitamin D (nmol/L)
   b. Run bivariate multiple linear regression model to determine association between percent fat content of milk and zBMI and 25-hydroxyvitamin D, factors known or suspected to affect their relationship
      i. Percent fat content of milk + child vitamin D supp + season + age + sex + ethnicity + income + screen time + sugar sweetened bev + unstructured free play + skin pigmentation + daily volume of milk consumed + maternal BMI = zBMI, 25-hydroxyvitamin D (nmol/L)
   c. Validate model using bootstrap.
4. Use variance inflation factor (VIF) to assess multicollinearity in covariates
   a. Remove variables with VIF >5 before performing multivariable analysis.
5. Secondary analysis
   a. Run bivariate multiple linear regression model to determine association between percent fat content of milk and zBMI and 25-hydroxyvitamin D, with interaction term for volume of milk consumed, and factors known or suspected to affect their relationship
      i. Percent fat content of milk + child vitamin D supp + season + age + sex + ethnicity + income + screen time + sugar sweetened bev + unstructured free play + skin pigmentation + daily volume of milk consumed + percent fat content of milk*daily volume of milk consumed + maternal BMI = zBMI, 25-hydroxyvitamin D (nmol/L)
Appendix E: WHO BMI-for-Age Curves

Source: World Health Organization [http://www.who.int/childgrowth/standards/cht_bfa_boys_z_0_5.pdf?ua=1]
Retrieved October 8, 2015
BMI-for-age GIRLS
Birth to 5 years (z-scores)

Source: World Health Organization [http://www.who.int/childgrowth/standards/cht_bfa_girls_z_0_5.pdf?ua=1]
Retrieved on October 8, 2015
CHAPTER 8: REFERENCES


147. Flegal KM, Ogden CL. Childhood obesity: are we all speaking the same language? Advances in nutrition. 2011;2(2):159S-66S.


