CONSUMPTION OF NON-COW’S MILK AND 25-HYDROXYVITAMIN D LEVELS IN EARLY CHILDHOOD

by

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A thesis submitted in conformity with the requirements for the degree of Master of Science
Graduate Department of Nutritional Sciences
University of Toronto

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2015

Abstract

Non-cow’s milk includes goat’s milk and plant-based milk beverages. Vitamin D fortification of non-cow’s milk is voluntary in North America. The effect of non-cow’s milk on serum 25-hydroxyvitamin D levels is unclear. Through two studies, I aimed to determine the association between non-cow’s milk consumption (both animal and plant based) and 25-hydroxyvitamin D serum levels in early childhood. Children ages 1-6 years were recruited through the TARGet Kids! practice based research network between 2008 and 2013. Non-cow’s milk consumption, 25-hydroxyvitamin D serum levels and clinically relevant covariates were collected. In study 1, I identified a dose dependent association between higher non-cow’s milk consumption and lower 25-hydroxyvitamin D levels. In study 2, I demonstrated that consumption of plant-based milk beverages was associated with lower 25-hydroxyvitamin D; the magnitude of this association was both smaller and in the opposite direction to the relationship of both cow’s milk and goat’s milk and 25-hydroxyvitamin D.
Acknowledgments

Completing my master’s has been an invaluable learning experience, and it would not have been possible without the incredible people that have supported me along the way. I would first like to thank my supervisor, Dr. Jonathon Maguire, for believing in my potential, for inspiring me to have big dreams, and for always humbly reminding me that success is possible through hard work. His enthusiasm and optimistic outlook on life was extremely motivating and contagious; I have learned to walk into every opportunity with optimism.

I am so grateful for the amazing TARGet Kids! research team that I had the opportunity to work with. This includes the entire team who helped make this project possible, including the practitioners, research manager, coordinators, assistants, and the children and families who have been generous with their time to participate in the study. Thank you for being the most encouraging, supportive, and inspiring group of people, and for always providing excellent feedback. I would also like to acknowledge my wonderful committee members, Dr. Gerald Lebovic and Dr. Mary L’abbe for their endless support and guidance.

I would like to express the greatest thanks to the most encouraging group of friends and lab mates that I have been blessed with. To Jessica Omand, my amazing academic and career mentor from day 1, thank you for your ongoing support, positivity, and for creating big shoes for me to fill. Shelley Vanderhout, my last year of master’s would not have been the same without you. You were a great source of joy in my life this past year, and I’m excited knowing that we will likely cross career paths again as dietitians. To the Vantage family and the OPC crew, your support has been incredible. And a heartfelt gratitude goes to my mum, dad, and my sister Monica for being my greatest cheerleaders.

Finally, I would like to acknowledge the Ontario Ministry of Training, Colleges, and Universities for the Ontario Graduate Scholarship and the Canadian Institutes of Health Research, for providing funding for this project.
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<td>AAP</td>
<td>American Academy of Paediatrics</td>
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<tr>
<td>AI</td>
<td>Adequate Intake</td>
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<td>BMI</td>
<td>Body Mass Index</td>
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<tr>
<td>CCHS</td>
<td>Canadian Community Health Survey</td>
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<tr>
<td>CHMS</td>
<td>Canadian Health Measures Survey</td>
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<tr>
<td>CI</td>
<td>Confidence Interval</td>
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<tr>
<td>CPS</td>
<td>Canadian Paediatric Society</td>
</tr>
<tr>
<td>DRI</td>
<td>Dietary Reference Intakes</td>
</tr>
<tr>
<td>EAR</td>
<td>Estimated Average Requirement</td>
</tr>
<tr>
<td>FDR</td>
<td>Food and Drug Regulations</td>
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<tr>
<td>FNB</td>
<td>Food and Nutrition Board</td>
</tr>
<tr>
<td>IOM</td>
<td>Institute of Medicine</td>
</tr>
<tr>
<td>IU</td>
<td>International Units</td>
</tr>
<tr>
<td>nmol/L</td>
<td>Nanomoles Per Litre</td>
</tr>
<tr>
<td>NHANES</td>
<td>National Health and Nutrition Examination Survey</td>
</tr>
<tr>
<td>OR</td>
<td>Odds Ratio</td>
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<tr>
<td>OZ</td>
<td>Ounce</td>
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<tr>
<td>PTH</td>
<td>Parathyroid Hormone</td>
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<tr>
<td>Acronym</td>
<td>Definition</td>
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<tr>
<td>RDA</td>
<td>Recommended Dietary Allowance</td>
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<td>SD</td>
<td>Standard Deviation</td>
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<tr>
<td>TSP</td>
<td>Teaspoon</td>
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<tr>
<td>UL</td>
<td>Tolerable Upper Intake Level</td>
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<tr>
<td>UVB</td>
<td>Ultraviolet B</td>
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<tr>
<td>VIF</td>
<td>Variance Inflation Factor</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
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<td>25(OH)D</td>
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Student Contributions

- Conceptualized and designed the research studies
- Recruited study participants into TARGet Kids! at St. Michael’s Hospital family health centre site (410 Sherbourne St.)
- Successfully obtained Research Ethics Board approval from St. Michael’s Hospital and The Hospital for Sick Children to contact parents and caregivers for retrospective data collection on type and brand of non-cow’s milk
- Performed statistical analysis using SAS 9.3 and R 3.0.3
- Presented research findings at two national research conferences: Canadian Nutrition Society (St. John’s, Newfoundland) and Canadian Paediatric Society (Montreal, Quebec)
- Chosen as one of six to orally present at the Hospital for Sick Children Research Day 2014 out of over 100 applicants
- Presented at St. Michael’s Hospital Research Day and placed top 3 in the poster presentation competition
- Wrote thesis
- Submitted research study (Study 2) to JAMA Pediatrics
Chapter 1
Introduction

1 Introduction

Nutrition plays a crucial role in a child’s growth and development (1-3). Nutritional habits and behaviors that occur in early childhood are thought to influence the development and outcome of health throughout life (1, 2, 4-6). This underlies the importance of determining the predictors of nutritional child health, in order to lead to the prevention of negative health outcomes resulting from poor nutrition.

There is a growing body of literature stating the importance of vitamin D on child health. Vitamin D is known to play a role in cellular functions and skeletal health through the regulation of calcium and phosphorous metabolism (7). The Canadian Pediatric Society (CPS) recommends a 25-hydroxyvitamin D level above 75 nmol/L (8), and the Institute of Medicine (IOM) and American Academy of Pediatrics (AAP) recommend a 25-hydroxyvitamin D level above 50 nmol/L to optimize bone health and prevent rickets resulting from vitamin D deficiency (7, 9, 10). Low levels of this fat-soluble vitamin have been suggested to be involved in a number of chronic disease processes outside of skeletal health (i.e. rickets), including cancer, cardiovascular, infectious, and autoimmune diseases, which underlies the important role of vitamin D in optimizing overall health (7, 11). Despite efforts to reduce vitamin D deficiency through the mandatory fortification of cow’s milk and updated recommendations for vitamin D intake and status, vitamin D deficiency is still considered a pandemic (12, 13), and is estimated to have an economic burden of $14.4 billion per year in Canada (14).

Emerging studies have identified modifiable determinants of vitamin D deficiency, which suggest that vitamin D deficiency in early childhood may be preventable. These include skin pigmentation, low sunlight exposure, increased body mass index (BMI), and low cow’s milk intake (10, 15-24).

Fortified cow’s milk is the main dietary source of vitamin D in children (21, 25-27). Unlike cow’s milk, the fortification of non-cow’s milk beverages is voluntary (24, 28-30). These beverages are increasingly apparent on supermarket shelves, and parents may choose non-cow’s
milk beverages for their children because of perceived health benefits. However, the association between the consumption of non-cow’s milk and child health outcomes, specifically vitamin D, has not been studied. It is unclear whether the consumption of non-cow’s milk offers health advantages or alternatively increases the risk of nutritional inadequacy in early childhood.

The aims of this thesis are: 1) to determine whether there is an association between total daily consumption of non-cow’s milk and serum 25-hydroxyvitamin D concentration in a population of healthy urban children attending routinely scheduled well-child doctor’s visits; 2) to explore how consumption of cow’s milk might modify this association; 3) to explore the association between daily intake of non-cow’s milk and cow’s milk; 4) to determine whether the relationship between non-cow’s milk consumption and children’s 25-hydroxyvitamin D is different for goat’s milk and plant-based milk beverages; and 5) to explore how these relationships might be different than the relationship between cow’s milk consumption and 25-hydroxyvitamin D.

The thesis is organized into 7 sections. Chapter 1 provides an overview of the literature and the purpose of this thesis. Chapter 2 provides the literature review. Chapter 3 and 4 describe the studies: Consumption of non-cow’s milk and 25-hydroxyvitamin D levels in early childhood and Type of non-cow’s milk consumption and 25-hydroxyvitamin D levels in early childhood. Chapter 5 is an overall discussion and chapter 6 describes the conclusions and future directions. Chapter 7 provides the references and chapter 8 the appendices.
Chapter 2  
Literature Review

2  Literature Review

2.1  Vitamin D

2.1.1  Structure and function

Vitamin D (calciferol) is a fat-soluble seco steroid that is stored in the body’s adipocytes or enters the liver for metabolism (31). There are two forms of vitamin D from diet and supplements: vitamin D₂ (ergocalciferol) and vitamin D₃ (cholecalciferol) (7). Vitamin D₂ and D₃ have different side chain structures (vitamin D₂ contains an additional methyl group and a double bond in the side chain), but serve the same biological function (7) (See Figure 1). They are also metabolized through the same process (7). Vitamin D₂ is found in fungi, yeast, and plant-based sources, whereas vitamin D₃ is synthesized naturally in human skin and ingested through dietary intake of animal based foods (7, 32). The activation of both vitamins D₂ and D₃ is initiated by two enzyme mediated hydroxylation reactions (7). The reactions occur first in the liver, followed by in the kidney (7). The enzyme, 25-hydroxylase, mediates the formation of 25-hydroxyvitamin D (25(OH)D) in the liver (7). Next, the enzyme, 1α-hydroxylase, mediates the formation of 1,25-dihydroxyvitamin D (1,25(OH)₂D), the active form of vitamin D (calcitriol) (7).

Calcitriol undergoes many biological activities in the body that are crucial for health (31, 33, 34). These include the regulation of calcium and phosphorus metabolism for bone growth and bone maintenance, in addition to the regulation of cell differentiation and cell proliferation (7, 31). Vitamin D has also been suggested to play roles outside of endocrine functions (optimizing bone health through the maintenance of calcium homeostasis), such as cancer, diabetes, respiratory conditions, cardiovascular disease, metabolic syndrome, infections, and autoimmune diseases (7, 33-35). However, the associations are not clear and further research is warranted.
2.1.2 **Sources of Vitamin D**

There are two ways to obtain vitamin D. This includes endogenous synthesis of vitamin D through the absorption of sunlight by skin, and exogenous synthesis of vitamin D through the consumption of dietary sources (7).

2.1.2.1 **Endogenous synthesis**

The cutaneous synthesis of vitamin D in the human body is initiated when the pre-curser molecule, 7-dehydrocholesterol, is converted to pre-vitamin D₃ upon sunlight exposure (7, 37). The photochemical process occurs upon exposure of epidermis and dermis layers of skin to ultraviolet B (UVB) radiation with wavelength 290 to 315 nm (7, 37). Pre-vitamin D₃ converts to vitamin D₃ through thermal isomerization. Vitamin D₃ enters into the capillary bed and attaches to vitamin D binding protein (7). The process of thermal isomerization also activates non-vitamin D forms, such as lumisterol and tachysterol, thereby limiting vitamin D₃ formation and preventing vitamin D toxicity (7, 38, 39). There are several factors, including skin pigmentation, seasonality, and latitude, which may influence the cutaneous synthesis of vitamin D.
D (discussed below in section 2.1.6).

It has been suggested that sunlight exposure of face, arms, legs or back without sunscreen for 5 to 30 minutes between 10AM and 3PM at a minimum of 2 times per week would allow sufficient endogenous synthesis of vitamin D (32). The Canadian Paediatric Society recommends sunlight exposure for infants and children in limited intervals (less than 15 minutes per day), in order to prevent potential skin damage (8).

2.1.2.2 Exogenous sources

Natural Sources

Vitamin D is naturally present in very few foods. Food sources include fatty fish (i.e. salmon, mackerel, tuna, etc.) and fish liver oil, which contain higher levels of vitamin D. Mushrooms and egg yolk contain lower levels of vitamin D (7). See Appendix 1.

Fortification

The government of Canada implemented the mandatory fortification of vitamin D to cow’s milk and margarine as a public health intervention to prevent vitamin D deficiency rickets, osteomalacia, and osteoporosis (29, 30, 40). Cow’s milk is required to contain 35-45 international units (IU) of vitamin D per 100 mL and margarine is required to contain 530 IU per 100 g (29, 30, 40). The Food and Drugs Regulations of Canada (FDR) also mandates the fortification of infant formula, which must contain 40-80 IU of vitamin D per 100 kcal (29). Despite the mandated legislations, the fortified vitamin D content may not reflect the actual amount that is stated on the nutrition label. Various studies have shown variability between fortified vitamin D levels and their respective nutrition labels (30, 41-47).

Supplementation

Vitamin D supplementation may be necessary when adequate levels of vitamin D are not obtained through sunlight exposure or dietary intake to prevent vitamin D deficiency (48-50). In Canada, Vitamin D supplements are found as vitamin D$_2$ or D$_3$ and contain a range between 8 IU to 1000 IU per daily dose serving (51). Any single dosages greater than 1000 IU of vitamin D per day must be prescribed by a physician (52). The Canadian Paediatric Society recommends vitamin D supplementation of 400 IU/day for all exclusively breastfed, healthy, term infants, until the infant is able to obtain 400 IU/day independent of the supplement (8). The Dietitians of
Canada recommends both exclusively and partially breastfed infants to receive a supplement of 400 IU until they reach 2 years of age (53). Exclusively formula fed infants are not recommended a vitamin D supplement, as infant formula is fortified with 40-80 IU of vitamin D per 100 kcal (29, 53). An updated report from the Infant Feeding Joint Working Group (a collaboration between Health Canada, Canadian Paediatric Society, Dietitians of Canada, Breastfeeding Committee for Canada, and Public Health Agency of Canada) published a statement to recommend a supplement of 400 IU for infants not exclusively formula-fed, regardless of their formula intake (54). This was based on the assumption that the upper level of 1000 IU/day would likely not be exceeded (54). In Canada, there are currently no recommendations for vitamin D supplementation in children over the age of 1 (8). The AAP recommends a vitamin D supplement of 400 IU/day for exclusively and partially breastfed infants drinking less than 1000 mL/day of vitamin D fortified formula or milk (55).

2.1.3 Laboratory measurement of vitamin D status

Calcitriol (biologically active form of vitamin D) has a short half-life (approximately 15 hours) and is very tightly regulated (56). Serum levels of calcitriol may appear normal or elevated even when 25-hydroxyvitamin D levels are considered low (31, 56). Therefore, the preferred biomarker for determining vitamin D status is suggested to be serum 25-hydroxyvitamin D (7). It has the longest half-life in comparison to other vitamin D derivatives (approximately 2-3 weeks) and reflects total vitamin D (vitamin \( \text{D}_2 \) and vitamin \( \text{D}_3 \)) from both endogenous synthesis and exogenous sources (7, 31, 56).

2.1.3.1 Vitamin D measurement issues

There are various techniques to measure 25-hydroxyvitamin D (57). Radioassays were first used to determine 25-hydroxyvitamin D using competitive binding protein; however, this method has been replaced by other refined techniques due to its sensitivity to impurities and labor intensity (58-60). Other techniques include immunoassay methods and chromatography-based assays that use mass spectrometry and UV absorbance (61). Examples of commonly used methods include the Liquid Chromotography-Tandem Mass spectrometry (LC-MS/MS), which has been suggested to have high reproducibility, and considered as the gold standard method for measuring 25-hydroxyvitamin D in clinical laboratories (57, 62). Despite its advantages, the LC-MS/MS is costly, and its requirement for larger sample values hinders feasibility of use (57,
The DiaSorin LIAISON 25-hydroxyvitamin D TOTAL Assay is another commonly used method in clinical laboratories to measure and link vitamin D sufficiency to various health outcomes. This method has been shown to have the same specificity as the DiaSorin 25-hydroxyvitamin D radioimmunoassay, which has been used to define 25-hydroxyvitamin D reference levels (63). The LIAISON 25-hydroxyvitamin D TOTAL Assay is also known to provide quick measurement results and is the only fully automated assay that measures total 25-hydroxyvitamin D levels (63).

Studies have shown discrepancies between the 25-hydroxyvitamin D measurements obtained by different assay techniques (64-71). The variation may be due to the differences in the assays’ capabilities to detect vitamin D epimers and vitamin D metabolites (25-hydroxyvitamin D$_2$ and 25-hydroxyvitamin D$_3$) (71-73). To assess the dissimilarities, proficiency testing programs have been established, such as the Vitamin D External Quality Assessment Scheme (DEQAS) (74). Using the DEQAS, the National Institute of Standards and Technology and National Institutes of Health Office of Dietary supplements established the Vitamin D Metabolites Quality Assurance Program (VitDQAP) and a vitamin D Standard Reference Material to improve accuracy of 25-hydroxyvitamin D measurements across various assays (61).

2.1.4 Vitamin D and child health outcomes

2.1.4.1 Recommended 25-hydroxyvitamin D serum concentrations

There are several recommendations and definitions for serum 25-hydroxyvitamin D concentration levels in children. The CPS defined 25-hydroxyvitamin D status based on levels that would minimize parathyroid hormone production (PTH) and calcium reabsorption from the bone, while maintaining optimal calcium absorption from the gut (8). The CPS defined 25-hydroxyvitamin D below 25 nmol/L as deficient, between 25-75 nmol/L as insufficient, and between 75-225 nmol/L as optimal (8). The IOM and AAP suggest that a 25-hydroxyvitamin D concentration level above 50 nmol/L will meet the needs of 97.5% of the population to optimize bone health and prevent rickets (7, 9, 10). Specifically, the IOM classified 25-hydroxyvitamin D below 30 nmol/L as deficient and below 50 nmol/L as insufficient (7). Depending on the cut-off used, the prevalence of vitamin D deficiency in a population varies; therefore, there has been suggestion for a consensus in defining reference values for 25-hydroxyvitamin D concentration in children (7, 75, 76). This may allow proper identification of vitamin D deficient children and
thus, allow for appropriate treatment (e.g. supplementation).

### 2.1.4.2 Serum 25-hydroxyvitamin D concentrations in children

There is consistent evidence suggesting that North American children have lower 25-hydroxyvitamin D levels than values recommended by the AAP and CPS. The Canadian Health Measures Survey (CHMS) is a national survey that was established in 2007 to collect information on the health of Canadians through household interviews and direct physical measurements (77, 78). This includes the measurement of serum 25-hydroxyvitamin D concentrations. According to the CHMS Cycle 2 (2009-2011), children 3-5 years of age had a mean serum 25-hydroxyvitamin D level of 74 nmol/L, and children 6-11 had a mean of 67 nmol/L (79). Furthermore, 11% and 24% of children 3-5 years of age and 6-11 years of age had insufficient (<50 nmol/L) 25-hydroxyvitamin D levels, respectively (79). In the most recent CHMS (2012-2013), approximately 20% of children had insufficient serum 25-hydroxyvitamin D concentrations (<50 nmol/L) (80). Currently, there is no Canadian data on 25-hydroxyvitamin D concentrations of children under the age of 3 years (77).

Similarly, the National Health and Nutrition Examination Survey (NHANES) is a national survey that aims to collect nutrition and health data of children and adults in the United States through interviews and physical measurements (81). Serum 25-hydroxyvitamin D levels of children 6-11 years were collected between 2001-2006 and between 2003-2006 for children 1-5 years (75, 82). According to the 2001-2006 NHANES data, the mean serum 25-hydroxyvitamin D levels for children ages 1-5 years was 70 nmol/L and the mean serum 25-hydroxyvitamin D level for children ages 6-11 years was 66 nmol/L (75, 82). Specifically, for children ages 1-5, 14% to 15% of children had serum 25-hydroxyvitamin D levels <50 nmol/L, and approximately 62% had serum 25-hydroxyvitamin D levels <75 nmol/L (75, 82). For children ages 6-11, 20% to 21% had serum 25-hydroxyvitamin D levels <50 nmol/L, and approximately 72% of children had serum 25-hydroxyvitamin D levels <75 nmol/L (75, 82).

### 2.1.4.3 Vitamin D deficiency

The main cause of vitamin D deficiency is lack of sunlight exposure and lack of vitamin D intake through dietary sources (including supplementation) (32, 83, 84). This suggests that this disease is preventable with appropriate sunlight exposure and adequate vitamin D intake (85, 86). Vitamin D deficiency leads to suboptimal building of the bone in children, and calcium, and
phosphorus metabolism (7, 32, 84). When adequate levels of vitamin D are not achieved through sunlight or dietary intake, levels of 25-hydroxyvitamin D in the body decrease (9). This causes PTH levels to increase as a result of decreased calcium and phosphorus absorption from dietary intake (7, 32, 84). The increased PTH works to maintain calcium levels in the body, eventually causing demineralizing of the bone (7, 32, 84). In children, severe vitamin D deficiency resulting in skeletal deformities, osteomalacia, and damaged growth plates is often presented as rickets (7, 13, 86). Symptoms of rickets include growth failure, irritability, hypocalcemic seizures, fractures, and increased susceptibility to respiratory infections (84, 87, 88). Despite global efforts to eradicate vitamin D deficiency rickets with vitamin D fortification of cow’s milk, vitamin D deficiency rickets is still remains present in Canada (13, 22, 89). Specifically, the annual incidence rate is 2.9 cases per 100,000 children (13). Vitamin D deficiency is estimated to have an economic burden of $14.4 billion per year in Canada (14).

The prevalence of suboptimal or deficient vitamin D status in children is a concern that is not just limited to bone health and endocrine systems (7, 32). Vitamin D receptors have been shown to be present outside the bone, intestine, and kidney, in tissues and cells all throughout the body, suggesting that vitamin D may play a role in autoimmune diseases, chronic diseases, and certain cancers (12, 32, 90-92). Studies have suggested suboptimal levels of vitamin D to be involved in non-skeletal pathologies, such as respiratory infections, cardiovascular disease, cancer, obesity, and asthma, all of which may contribute heavily to the economic burden of the healthcare system (93-100).

In a systematic review and meta-analysis of observational studies and randomized controlled trials, 25-hydroxyvitamin D concentration had inverse associations with risks of all cause mortality (relative risk: 1.44, 95% CI: 1.34 to 1.55) and cause specific mortality, including cardiovascular (relative risk: 1.43, 95% CI: 1.25 to 1.64), cancer (relative risk: 1.25, 95% CI: 1.10 to 1.43), and non-cardiovascular or non-cancer death (relative risk: 1.34, 95% CI: 1.13 to 1.60) in adults (101). Furthermore, vitamin D₃ supplementation appeared to reduce the risk of mortality (101).

There is also evidence that suggest inverse associations between vitamin D status and non-bone related health outcomes in children. This includes upper respiratory tract infections and asthma, two of the most frequently seen health problems in children. In a cohort of children from New
Zealand, infants at 3 months of age with 25-hydroxyvitamin D levels in cord blood below 25 nmol/L were found to have a 2-fold higher risk of vital respiratory tract infections in comparison to infants with 25-hydroxuvitamin D levels in cord blood above 75 nmol/L (102). In addition, 25-hydroxyvitamin D levels in cord blood were inversely associated with risk of wheezing throughout early childhood (102). In a cross-sectional study of American children, children with 25-hydroxyvitamin D levels below 75 nmol/L had increased use of corticosteroids and increased airway limitations than children with vitamin D levels above 75 nmol/L (103). In a systematic review and meta-analysis of high-dose vitamin D supplementation in pediatric asthma, there was a statistically significant reduction in asthma exacerbation (104). These various studies present the importance of further research to clarify the relationship between vitamin D and various health conditions in children. Additional research may ultimately provide the necessary information to develop a consensus on vitamin D guidelines, such as serum cut-offs values, as well as effective nutrition intervention strategies (e.g. vitamin D supplementation) that attempt to improve 25-hydroxyvitamin D status in children.

2.1.5 Nutritional Recommendations

Since 1995, the Food and Nutrition Board (FNB) of Health Canada and the United States IOM have established guidelines for recommended intakes of vitamins and minerals for a healthy population (105). Dietary reference intakes (DRI) are the result of this collaboration. DRIs have been established for specific sex and age groups.

2.1.5.1 Dietary Reference Intakes for Vitamin D

The DRIs for vitamin D were first established in 1997 to provide vitamin D intake levels that would support bone growth and maintenance and to prevent the risk of adverse health outcomes from consuming excess vitamin D (48, 106). These nutrient reference values assume a healthy population within a normal BMI range, receiving minimal sunlight exposure (48). The latter is due to the potential risks associated with UVB exposure and skin cancer, in addition to the association between sunlight exposure and vitamin D status being potentially dependent on skin pigmentation and sunscreen use (48). The updated DRI for vitamin D was released by the IOM in November 2010, along with the DRI for calcium. The recommended values for vitamin D are based on maintaining serum 25-hydroxyvitamin D levels above a specific targeted range, which is suggested to be sufficient for skeletal health (48). The Estimated Average Requirement (EAR)
was established to meet the needs of approximately 50% of the population, using a serum 25-hydroxyvitamin D level of 40 nmol/L (48). Similarly, the Recommended Dietary Allowance (RDA) was established using a serum 25-hydroxyvitamin D level of 50 nmol/L, suggested to meet the needs of 97.5% of the population (48). For children under the age of 1, an EAR was not established due to insufficient data; thus, an Adequate Intake (AI) level was developed instead (105). The AI for children under the age of 1 was based on maintaining serum 25-hydroxyvitamin D levels between 40-50 nmol/L (48). The tolerance upper intake level (UL) levels were set to avoid exceeding serum 25-hydroxyvitamin D levels of 125-150 nmol/L.

### 2.1.5.2 Dietary Reference Intakes for Children

The AI for children 0-12 months of age is 400 IU. The UL is 1000 IU for children 0-6 months of age, and 1500 IU for children 7-12 months of age (48). The EAR and RDA for children 1-8 years of age is 400 IU and 600 IU, respectively. The UL for children 1-3 years is 2500 IU, and for children 8 years, the UL is 3000 IU (48) (Table 1).

It is important to note the findings of children’s vitamin D intake from the Canadian Community Health Survey 2.2 (CCHS). On average, children 1-8 years of age consumed less than the AI from food intake alone (49). They consumed around 248 IU/day, with vitamin D fortified milk as the main dietary source of vitamin D (49). Among these children, 85% of children 1-3 years of age and 94% of children 4-8 years of age had inadequate vitamin D intake levels (107). When supplement intake was considered, levels of inadequacy decreased to 10% among 1-3 year olds and 15% among 4-8 year olds (107).
### Table 1: Vitamin D Dietary Reference Intakes for Children

<table>
<thead>
<tr>
<th>Children (males and females)</th>
<th>Vitamin D</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AI (IU/day)</td>
</tr>
<tr>
<td>0-6 month old</td>
<td>400</td>
</tr>
<tr>
<td>7-12 month old</td>
<td>400</td>
</tr>
<tr>
<td>1-3 year old</td>
<td>-</td>
</tr>
<tr>
<td>4-8 year old</td>
<td>-</td>
</tr>
</tbody>
</table>

### 2.1.6 Determinants of 25-hydroxyvitamin D in children

#### 2.1.6.1 Skin pigmentation

Skin pigmentation is determined by the amount of melanin in the skin’s epidermal layer (7, 32). The higher the level of melanin, the darker the skin pigmentation (7). Melanin absorbs ultraviolet B (UVB) radiation, which thereby hinders the cutaneous synthesis of vitamin D from sunlight exposure (7, 32, 108). Several studies have shown an association between lower 25-hydroxyvitamin D concentrations and darker skin pigmentation (13, 20, 32, 109, 110).

A study looking at the prevalence of vitamin D deficiency among children enrolled in NHANES found that 87% of African-American children and 52% of Latino children had vitamin D deficiency (defined as 25-hydroxyvitamin D levels <50 nmol/L), in comparison to the 27% that were deficient among white children (111). Similarity, a study looking at vitamin D deficiency rickets in Canadian children found that there was a higher incidence of vitamin D deficiency rickets among children who had darker skin pigmentation (13).

#### 2.1.6.2 Seasonality and latitude

Endogenous synthesis in the skin through ultraviolet B (UVB) exposure is a major source of vitamin D, and can be stored in the liver and adipose tissues for later use when there is limited
sunlight for cutaneous synthesis to occur (i.e. during winter season) (7, 32, 112-114). Ultraviolet B radiation is unable to penetrate through clouds and is only available at certain latitudes, seasons, and time of day (32, 33, 115, 116). It is suggested that cutaneous synthesis of vitamin D is most efficient during non-winter months, as the amount of UVB photons that reach the earth’s surface is plentiful (32, 83, 116).

Latitude is also a determining factor of 25-hydroxyvitamin D levels. Specifically, the zenith angle of the sun determines the number of solar UVB photons that reach the earth (22, 32, 117). When this angle shifts to become more oblique, the ozone absorbs the UVB photons before the photons reach the earth’s surface, due to the longer distance the photons need to travel (11). At higher latitudes, the zenith angle is more oblique (11). In other words, as latitude increases, endogenous synthesis of vitamin D through UVB decreases. Earlier in vitro studies have suggested that during winter months in North America, obtaining vitamin D through endogenous synthesis is very limited at latitudes above 40 degrees North and 40 degrees South (7, 116, 118). Data from studies suggest that low 25-hydroxyvitamin D levels are typically seen in children residing at latitudes above 40 degrees (17, 20). Furthermore, endogenous synthesis through UVB exposure may be more limited in northern climates where skin is more likely to be covered due to colder temperatures (17). This may also be specific to the type of clothing that is worn. For instance, thicker clothing (e.g. winter jacket) worn during wintertime may block UVB radiation from reaching the skin surface (118-120). Endogenous synthesis may also be limited during summertime, when sunscreen is used to protect the skin from deoxyribonucleic acid (DNA) damage through ultraviolet A (UVA) and UVB waveband exposure (7, 10).

2.1.6.3 Body Mass Index

Higher Body Mass Index (BMI) may be a potential risk factor for vitamin D deficiency, and there is emerging evidence on the association between higher BMI and lower 25-hydroxyvitamin D levels (21, 23, 32, 111, 112, 121-124). The inverse association may be related to the sequestration of fat-soluble vitamin D in adipose tissues, which thereby decreases the bioavailability of the vitamin (32, 121). Studies have shown increasing levels of 25-hydroxyvitamin D with weight loss among obese children and women (125, 126). Data from obese adolescents with vitamin D deficiency have suggested that this association may be confounded by factors such as low vitamin D intake from dietary sources, as well as limited sun
exposure due to low physical activity (127, 128).

According to the NHANES 2003-2006 data, the prevalence of vitamin D deficiency (25-hydroxyvitamin D level <50 nmol/L) among 6-18 year olds was 21%, 29%, 34%, and 49% for children in the healthy-weight, overweight, obese, and severely obese BMI (defined using Centers for Disease Control and Prevention age and gender specific BMI percentile cut points) categories (111). Furthermore, there were higher odds of having vitamin D deficiency for overweight (OR: 1.7, 95% CI: 1.2 to 2.4), obese (OR: 1.8, 95% CI: 1.3 to 2.5), and severely obese (OR: 2.3, 95% CI: 1.1 to 4.5) children than healthy weight children (111). This inverse association between BMI and vitamin D has also been identified among 25-30 month old Canadian children (21).

### 2.1.6.4 Breast milk with vitamin D supplementation

Exclusive breastfeeding is recommended for infants for the first 6 months; however, it contains approximately 10 IU vitamin D/250 mL and is considered to be a poor source of vitamin D (129-132). Therefore, a vitamin D supplement is suggested for all breastfed children (54). Specifically, both the CPS and AAP recommended a vitamin D supplement of 400 IU/day for all breastfed children until the child is consuming 1 L of vitamin D fortified formula or whole cow’s milk (8, 9). Health Canada also recommends a vitamin D supplement of 400 IU/day for exclusive and partially breastfed children, irrespective of the amount of formula consumption (54). Maguire et al. found vitamin D supplementation to be a modifiable determinant of higher 25-hydroxyvitamin D status in Canadian children (18). Further, there has been suggestion for supplementing lactating women with vitamin D, as this may allow nursing infants to receive breast milk with higher vitamin D concentration. Hollis and Wagner have suggested that a high daily vitamin D intake (4000 IU) taken by lactating women may meet the needs of both the mother and the nursing infant, while a daily vitamin D intake of 2000 IU or lower may only meet the needs of the mother, and not the nursing infant (133). The efficacy and safety of this approach is not yet clear; further research is needed (9, 54, 133).

Exclusive breastfeeding without vitamin D supplementation is suggested to be a risk factor for low 25-hydroxvitamin D concentration in infants (13, 15-17, 134-138). In a Canadian study, breastfeeding was considered to be the most frequent risk factor for vitamin D deficiency rickets (13). Of the children identified as having vitamin D deficiency rickets, 94% were breastfed (13).
Additionally, a study looking at children in the United States found breastfed children without supplementation at a 10-fold increased risk of vitamin D deficiency (defined as 25-hydroxyvitamin D < 50 nmol/L) in comparison to exclusively bottle fed children (17).

### 2.1.6.5 Cow’s milk intake

Cow’s milk has been suggested to be the main dietary source of vitamin D in early childhood (21, 25, 49) and found to be a modifiable determinant of higher 25-hydroxyvitamin D levels in children (18). Low milk consumption has been found to be associated with lower serum 25-hydroxyvitamin D levels in children (17, 20, 21, 82, 111).

According to the Canadian Health Measures Survey (CHMS Cycle 2), children consuming milk once or more per day had higher serum 25-hydroxyvitamin D levels than children consuming milk less than once per day (79). Specifically, 25% of children consuming milk once or more per day had serum 25-hydroxyvitamin D levels below 50 nmol/L, in comparison to 40% of children below 50 nmol/L who were drinking milk less than once per day (79). Similarly, children not consuming milk daily and weekly had higher odds (OR: 2.9, 95% CI: 2.1 to 3.9) of having 25-hydroxyvitamin D deficiency (<37.5 nmol/L), according to the 2001-2004 NHANES data (82).

Other studies show consistent findings. A Canadian study found lower cow’s milk consumption to be associated with lower 25-hydroxyvitamin D concentrations in 2-year-old children (18). In Boston, toddlers 12-24 months old had decreased odds (OR: 0.5, 95% CI: 0.3 to 0.8) of having vitamin D deficiency (<37.5 nmol/L), and a higher 25-hydroxyvitamin D concentration with increasing cups of cow’s milk consumed (17). This was also seen in adolescents 11-18 years of age. Adolescents had decreased odds (OR: 0.8, 95% CI: 0.6 to 0.9) of having vitamin D deficiency, and higher 25-hydroxyvitamin D concentration for every cup of cow’s milk consumed (20).

### 2.1.6.6 Other factors

A number of studies have found other factors that may be associated with 25-hydroxyvitamin D status. Increasing age may be associated with lower 25-hydroxyvitamin D due to the skin’s decreasing capacity to produce vitamin D through cutaneous synthesis with increasing age (139-143). Ethnicity and immigration status may also play a role in vitamin D status, in which
children identified as an ethnic minority or non-western immigrant may have lower 25-hydroxyvitamin D status (144-147). Reasons for lower 25-hydroxyvitamin D status may be related to darker skin pigmentation and coverage of skin for religious or cultural purposes, both of which may inhibit endogenous synthesis of vitamin D through sunlight (109, 110, 118-120). However, the associations are not conclusive in children and there is need for more research to clarify the determinants of 25-hydroxyvitamin D status in this specific population.

2.2 Non-cow’s milk

Non-cow’s milk consists of goat’s milk (contains vitamin D$_3$, if fortified) and plant-based milk beverages (contains vitamin D$_2$, if fortified), derived from nuts, legumes, seeds, grains, or potatoes (28). This includes soy, almond, rice, coconut, hemp, and oat milk, among others.

2.2.1 Consumption

Consumption of non-cow’s milk appears to be a growing trend. The parents’ decision to choose these products for their children may vary, and may potentially be related to health (e.g. galactosemia, lactose intolerance, cow’s milk allergy), cultural, religious, personal, or lifestyle (e.g. vegetarian) reasons, which are indications for use of soy formulas in infants (148). The literature on non-cow’s milk consumption is scarce and it is unclear whether consuming these beverages offers health benefits or leads to adverse health effects.

2.2.2 Recommended Intake

Breast milk is recommended for the first 6 months of life and infant formula with iron is recommended if breast milk is not an option (54, 149). Introduction of pasteurized whole cow’s milk is not recommended until 9 to 12 months of age in order to reduce the risk of iron deficiency, and suggested to continue until 2 years of age (54, 148-152). The same recommendations for cow’s milk apply for goat’s milk, which has a similar protein profile to cow’s milk (129, 150, 153). Children are also recommended to not consume more than 750 mL of cow’s milk per day, as excessive intake of may lead to displacement of other foods containing important nutrients for growth, such as iron (150, 154, 155). Maguire et al. found that 500 mL of cow’s milk per day appears to maintain 25-hydroxyvitamin D levels without compromising serum ferritin levels in children (154). Consuming above that amount appears to lead to a trade off between vitamin D and iron stores in children (154).
For children 0 to 2 years of age, the consumption of non-cow’s milk is discouraged (54, 149, 156). It is suggested that non-cow’s milk- both fortified and not fortified- do not serve as nutritionally adequate alternatives to breast milk, whole cow’s milk, or commercial infant formula to support proper growth and development of children (54, 105, 157). Non-cow’s milk beverages generally contain less calories, fat, protein, and may also be a poor source of iron, calcium and vitamin D, even after fortification (54, 129, 148, 149) (Please see Table 2). Therefore, infants drinking plant-based beverages in replacement of breast milk as their main milk source may be at risk of failure to thrive and nutritional deficiencies (54, 156, 158-161). However, infants older than 6 months may consume full fat, fortified, unflavoured soy milk beverage as complimentary food (54, 149). For infants who are unable to consume dairy (indications include galactosemia and health, religious, or cultural reasons), soy formulas are recommended until 12 months of age (162, 163). If consumption of soy formula continues past 12 months of age, second- stage soy formulas (for infants >6 months) should be given between 12-24 months to ensure adequate nutrition is being provided (54, 148, 162). Despite concerns regarding phytoestrogen content present in soy-based formulas, there is insufficient evidence to draw conclusions of their potential adverse health effects (162, 163).

For children over the ages of 2, Eating Well with Canada’s Food Guide and United States Department of Agriculture’s MyPlate recommends a fortified soy beverage if cow’s milk is not consumed (164, 165). Other plant-based milk beverages are not listed as potential milk alternatives, as they contain lower levels of protein (156). If other plant-based milk beverages are being consumed, parents are recommended to choose products labeled as fortified and containing a minimum of 6 g of protein per 250 mL cup (148). Parents are also recommended to provide alternate sources of protein (i.e. beans, lentils, fish, poultry, eggs, tofu, nuts, lean meats) if lower protein containing beverages are chosen as the child’s milk source (148).

2.2.3 Legislation

Unlike cow’s milk, the fortification of non-cow’s milk is not mandatory in Canada (28-30, 166). Thus, the nutrient profile of non-cow’s milk beverages may vary within the different types of milk, and also between various brands (129, 148, 167) (Table 2). In the United States, the vitamin D fortification of all milk types, including cow’s milk, goat’s milk, and plant-based milk beverages is voluntary, unless stated as fortified (30, 166).
2.2.3.1 Goat’s milk

The nutrient content of goat’s milk is similar to that of cow’s milk (129, 168) (Table 2). Both milks are animal based and contain vitamin D₃. However, the fortification of goat’s milk is also voluntary (29). If stated as fortified, goat’s milk is required to contain 35-45 international units (IU) of vitamin D per 100 mL (29, 30, 169).

2.2.3.2 Plant-based milk beverages

Cow’s milk naturally contains vitamin B₁₂, riboflavin, and zinc; however, these vitamins and minerals are not naturally present in plant-based milk beverages. If stated as fortified, plant-based milk beverages must contain vitamin A, vitamin B₁₂, riboflavin, zinc, calcium, and vitamin D (28). Specifically, plant-based milk beverages are required to contain 34 IU of vitamin D per 100 mL (28-30, 169). These products are often labeled as vegetarian-and vegan-friendly (170-174). If products are labeled as vegetarian, contents must only come from plant-based ingredients (175). Therefore, if plant-based milk beverages are stated as fortified, they appear to contain vitamin D₂, which is derived from plant origins (176). Additionally, plant-based milk beverages containing less than 2.5g of protein 100 mL should state “not a source of protein” on the product label (28).
### Table 2: Nutritional profile of milk types per 250 mL cup serving*

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Breast milk (whole)</th>
<th>Cow’s milk (whole)</th>
<th>Goat’s milk (whole)</th>
<th>Soy</th>
<th>Almond</th>
<th>Rice</th>
<th>Coconut</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kcal)</td>
<td>182</td>
<td>155</td>
<td>178</td>
<td>70-150</td>
<td>30-130</td>
<td>120-140</td>
<td>45-110</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>11.0</td>
<td>8.0</td>
<td>11.0</td>
<td>0-4.0</td>
<td>2-5</td>
<td>1-3</td>
<td>4-10</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>18.0</td>
<td>12.0</td>
<td>11.0</td>
<td>3-23</td>
<td>1-23</td>
<td>25-29</td>
<td>1-10</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>3.0</td>
<td>8.0</td>
<td>9.0</td>
<td>6-8</td>
<td>1-2</td>
<td>0-2</td>
<td>0-2</td>
</tr>
<tr>
<td>Iron (mg)</td>
<td>0.08</td>
<td>0.13</td>
<td>0.8-2.1</td>
<td>0.3-2.1</td>
<td>0-0.6</td>
<td>0-0.3</td>
<td></td>
</tr>
<tr>
<td>Riboflavin (mg)</td>
<td>0.09</td>
<td>0.46</td>
<td>0.36</td>
<td>0.38-0.40</td>
<td>0.40</td>
<td>0.40</td>
<td></td>
</tr>
<tr>
<td>Vitamin B12 (ug)</td>
<td>0.13</td>
<td>1.14</td>
<td>0.18</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>0.60</td>
</tr>
<tr>
<td>Calcium (mg)</td>
<td>83</td>
<td>291</td>
<td>345</td>
<td>88-330</td>
<td>330</td>
<td>22-330</td>
<td>0-22</td>
</tr>
<tr>
<td>Vitamin D (IU)</td>
<td>10</td>
<td>108</td>
<td>10-100</td>
<td>0-90</td>
<td>0-90</td>
<td>0-90</td>
<td>0</td>
</tr>
</tbody>
</table>

*This table is not representative of all products

Source:


Chapter 3
Consumption of non-cow’s milk beverages and 25-hydroxyvitamin D levels in early childhood*

3 Consumption of non-cow’s milk

3.1 Abstract

Background: Vitamin D fortification of non–cow’s milk beverages is voluntary in North America. The effect of consuming non–cow’s milk beverages on serum 25-hydroxyvitamin D levels in children is unclear. I studied the association between non–cow’s milk consumption and 25-hydroxyvitamin D levels in healthy children. I also explored whether cow’s milk consumption modified this association and analyzed the association between daily non–cow’s milk and cow’s milk consumption.

Methods: In this cross-sectional study, children 1–6 years of age attending routinely scheduled well-child doctor’s visits were recruited. Survey responses, and anthropometric and laboratory measurements were collected. The association between non–cow’s milk consumption and 25-hydroxyvitamin D levels was tested using multiple linear regression and logistic regression. Cow’s milk consumption was explored as an effect modifier using an interaction term. The association between daily intake of non–cow’s milk and cow’s milk was explored using multiple linear regression.

Results: A total of 2831 children were included. The interaction between non–cow’s milk and cow’s milk consumption was statistically significant (p = 0.03). Drinking non–cow’s milk beverages was associated with a 4.2-nmol/L lower 25-hydroxyvitamin D level per 250-mL cup consumed among children who also drank cow’s milk (p = 0.008). Children who drank only non–cow’s milk were at higher odds of having a 25-hydroxyvitamin D level below 50 nmol/L than children who drank only cow’s milk (odds ratio 2.7, 95% confidence interval 1.6 to 4.7).

Conclusions: Consumption of non–cow’s milk a beverage was associated with lower serum 25-hydroxyvitamin D levels in early childhood. This association was modified by cow’s milk consumption, which suggests a trade-off between consumption of cow’s milk fortified with

* Published in Canadian Medical Association Journal:
higher levels of vitamin D and non–cow’s milk with lower vitamin D content.

3.2 Background

Goat’s milk and plant-based milk beverages made from soy, rice, almond, coconut, hemp, flax, or oats (herein called “non-cow’s milk”) are increasingly available on supermarket shelves. Many consumers may be switching from cow’s milk to these non-cow’s milk beverages (177-179). Parents may choose non-cow’s milk beverages for their children because of perceived health benefits. However, it is unclear whether they offer health advantages or, alternatively, whether they increase the risk of nutritional inadequacy.

In the United States and Canada, cow’s milk is required to contain about 40 International Units (IU) of vitamin D per 100 mL, making it the major dietary source of vitamin D for children (10, 18, 21, 29, 30). The only other food source with mandatory vitamin D fortification in Canada is margarine, which is required to contain 53 International Units of vitamin D per 2 teaspoons (10g)(30). Fortification of non-cow’s milk beverages with vitamin D is also possible, but it is voluntary in both countries. Furthermore, there is little regulation on vitamin D content, even if such beverages are fortified (29, 30, 180). Therefore, vitamin D intake from non-cow’s milk beverages may be variable. I hypothesized that children’s vitamin D stores, measured by 25-hydroxyvitamin D, may be lower among children who consume non-cow’s milk beverages.

3.2.1 Objectives

The primary objective of this study was to test the association between total daily consumption of non-cow’s milk and serum 25-hydroxyvitamin D concentration in a population of healthy urban children attending routinely scheduled well-child doctor’s visits. The secondary objectives were to explore how consumption of cow’s milk might modify this association and to study the association between daily intake of non-cow’s milk and cow’s milk.

3.3 Methods

Study design and participants

A cross-sectional observational study was conducted through the TARGet Kids! (The Applied Research Group for Kids) practice-based research network in Toronto (latitude 43.4°N). TARGet Kids! is a collaboration between child health researchers in the Faculty of Medicine at
the University of Toronto and primary care physicians in the university’s Department of Paediatrics and Department of Family and Community Medicine (181). Children 1-6 years of age were recruited from seven pediatric or family medicine primary care practices during routinely scheduled well-child doctor’s visits between December 2008 and September 2013. Children who had a condition affecting growth (e.g. failure to thrive, cystic fibrosis), chronic illnesses (excluding asthma) or severe developmental delay were excluded.

3.3.1 Measurements

Data were collected from parents by a trained research assistant at each participating practice using a standardized data collection form adapted from the Canadian Community Health Survey (182). The research assistants also obtained physical measurements of the children. Venous blood sampling was performed by a trained phlebotomist and daily samples were sent to the Clinical Biochemistry Laboratory at Mount Sinai Hospital in Toronto (183).

The total serum 25-hydroxyvitamin D level, the primary outcome, was determined with the use of a competitive two-step chemiluminescence assay (LIAISON 25-hydroxyvitamin D TOTAL Assay; Diasorin). This machine has been extensively tested and validated to demonstrate 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, values which are well within acceptable limits for biochemical measurements (73, 184). My primary exposure variable was total consumption of non-cow’s milk per day. This amount was determined from the response to the following standardized question on the data collection form: “How many 250-mL cups of non-cow’s milk (soy, rice, goat, etc.) does your child have currently in a typical day?”.

The research assistants collected data on the following pre-specified covariates hypothesized to influence the association between total consumption of non-cow’s milk and 25-hydroxyvitamin D levels: age, sex, Body Mass Index (BMI) z-score, daily vitamin D supplementation, consumption of cow’s milk, consumption of margarine, skin pigmentation, outdoor play time, and date of laboratory testing. Weight was measured with a scale for children under the age of 2 years and a precision digital scale (Seca model 703, measurement accuracy ± 0.025 %; Seca) for older children. The length of children under the age of 2 was measured with a calibrated length board; the height of older children was measured using a calibrated stadiometer (Seca). BMI
was calculated using the standard formula (weight/height², where weight is measured in kilograms and height in measured in meters) (185, 186). The BMI z-score was calculated with the use of the World Health Organization growth curves (186). These curves are recommended for use among this age group in Canada as they reflect optimal growth in children (187, 188). Daily vitamin D supplementation was determined from the data collection form as the daily use of a vitamin D supplement or multivitamin; all children’s over-the-counter multivitamins in Canada contain a dose of 400 International Units (52). The amount of cow’s milk consumed daily was determined from the response to the following question on the form: “How many 250-mL cups of cow’s milk does your child have currently in a typical day?”. Margarine consumption (Yes/No) was determined according to whether the child ate margarine in the 3 days before the well-child doctor’s visit. The research assistants used the Fitzpatrick scale, a skin pigmentation classification system used commonly in dermatological research, to measure skin pigmentation (189). Outdoor playtime was the number of hours per week spent outside, as reported on the form.

3.3.2 Statistical Analyses

Descriptive statistics were performed for the primary exposure and outcome variables and the covariates. A univariate linear regression model was used to determine the unadjusted association between the primary exposure (total consumption of non-cow’s milk), and my primary outcome (25-hydroxyvitamin D level).

For my primary analysis, I developed a multiple linear regression model to test the association between total consumption of non-cow’s milk (measured in 250-mL cups per day) and 25-hydroxyvitamin D levels, adjusted for pre-specified and clinically relevant covariates (listed in the preceding section). To account for the seasonal effect on 25-hydroxyvitamin D, a sinusoidal function was applied to the date of laboratory testing. All covariates were included in the final model, regardless of statistical significance to avoid biased regression coefficients and standard errors and artificially inflated R² values (190). To explore whether consumption of cow’s milk modified the association between the primary exposure and outcome, I tested the interaction between total consumption of non-cow’s milk and cow’s milk consumption at a significance level of α = .05. I also explored the risk of having a low 25-hydroxyvitamin D level (<50nmol/L) using an adjusted logistic regression model (7, 9).
For my secondary analysis, I developed a multiple linear regression model to explore the association between non-cow’s milk consumption and cow’s milk consumption (measured in 250-mL cups per day), adjusted for age and sex. To ensure the variables in the models produced independent effects, multicollinearity for all covariates was assessed using the variance inflation factor. The factor is a measure of the degree of inflation in the standard errors of regression coefficients when multicollinearity exists (i.e. variance inflation factor >5) (191, 192). All variance inflation factors were below 1.9.

Residual plots of serum 25-hydroxyvitamin D against total consumption of non-cow’s milk were used to assess linearity of the association between the primary exposure and the primary outcome. The outcome variable, 25-hydroxyvitamin D level, was positively skewed and was log transformed, which resulted in a normal distribution. Model checking using residual analysis on the transformed outcome indicated a good fit. Bootstrap validation was performed on the linear regression model to identify the likelihood of overfitting. No variable had more than 12% missing data. However, to overcome biases that can result from missing data, multiple imputation was performed (190). Models were run on 50 imputed data sets and the results of the individual analysis were combined to obtain valid statistical inferences in the final analysis (190).

All statistical analyses were conducted with the use of SAS 9.3 for Windows and R 3.0.3. This study was approved by the Hospital for Sick Children and St. Michael’s Hospital Research Ethics Boards. Consent was obtained from the parents of all children participating in the study.

3.4 Results

Of the 4523 children who met the inclusion criteria and for whom consent was obtained, 2831 were included in the study because they had undergone laboratory testing (Figure 2). Baseline characteristics of the participants and nonparticipants are shown in Table 3. The participants appeared to have lighter skin pigmentation and higher vitamin D supplementation than the children not included in the analysis. Otherwise, the two groups appeared clinically similar. The mean age of included children was 2.9 years (standard deviation 1.5), and 52.6 % were male. Vitamin D supplementation was noted for 52.4% of children, and median 25-hydroxyvitamin D level was 80 (interquartile range 66-99) nmol/L. Among the participants whose milk consumption was known, 85.4% drank cow’s milk daily and 12.3% drank non-cow’s milk daily.
The 25-hydroxyvitamin D level was below 50 nmol/L in 11.0% of the children who only drank non-cow’s milk and in 4.7% of those who drank only cow’s milk.

In the univariate analysis, each 250 mL cup of non-cow’s milk consumed was associated with a 3.1% (p=0.005) lower 25-hydroxyvitamin D level. For example, an increase in non-cow’s milk consumption from 0 to 1 cup was associated with a 2.5 nmol/L (95% CI 0.9 to 5.2 nmol/L) lower median 25-hydroxyvitamin D level.

Results for my primary analysis using multiple linear regression are shown in Table 4. The interaction between total consumption of non-cow’s milk and consumption of any cow’s milk was statistically significant (p=0.03), which suggested that drinking cow’s milk was an effect modifier of the association between total non-cow’s milk consumption and 25-hydroxyvitamin D levels (Figure 4). After adjusting for clinically relevant covariates, I found that each additional 250-mL cup of non-cow’s milk beverage consumed by children who also drank cow’s milk was associated with a 5.1% (p=0.008) lower 25-hydroxyvitamin D and a drop in the median 25-hydroxyvitamin D level of 4.2 nmol/L (95% CI 1.1 to 7.1) (Table 4). Statistically significant covariates (p< 0.001) included vitamin D supplementation, which was associated with higher 25-hydroxyvitamin D levels, and having dark skin pigmentation, which was associated with lower 25-hydroxyvitamin D.

In the logistic regression model, children who drank only non-cow’s milk beverages were at higher odds of having a 25-hydroxyvitamin D level below 50 nmol/L than children who drank only cow’s milk (odds ratio 2.7, 95% CI 1.6 to 4.7).

In my secondary analysis, the age- and sex- adjusted multiple linear regression showed an inverse association between total daily intake of non-cow’s milk and cow’s milk (Figure 5). Each additional 250-mL cup of non-cow’s milk consumed was associated with a 0.5 cup lower consumption of cow’s milk in both the unadjusted and adjusted regression analyses (95% CI 0.4 to 0.6 cup in the adjusted analysis).
Figure 2: Selection of participants for the study

Children with parental consent who met the inclusion criteria  
$n=4523$

Excluded $n=1692$  
(no laboratory testing)

Included in the analysis  
$n=2831$

Data on milk consumption available  
$n=2468$

No data on milk consumption  
(imputed for the analysis)  
$n=363$
Table 3: Characteristics of children who participated in the study and nonparticipants

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Participants n = 2831*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Only cow’s milk n= 1950</td>
</tr>
<tr>
<td>Age, yr, mean ± SD</td>
<td>3.0 ± 1.4</td>
</tr>
<tr>
<td>Sex, male, no. (%)</td>
<td>1025 (52.6)</td>
</tr>
<tr>
<td>BMI z score, median (IQR)</td>
<td>0.23 (-0.44 to 0.88)</td>
</tr>
<tr>
<td>No. of cups† per day, mean ± SD</td>
<td></td>
</tr>
<tr>
<td>Non-cow’s milk</td>
<td>0</td>
</tr>
<tr>
<td>Cow’s milk</td>
<td>2.0 ± 1.1</td>
</tr>
<tr>
<td>Margarine consumption, no. (%)</td>
<td>587 (30.1)</td>
</tr>
<tr>
<td>Vitamin D supplementation, no. (%)</td>
<td>1020 (52.3)</td>
</tr>
<tr>
<td>Skin pigmentation, ‡ no. (%)</td>
<td></td>
</tr>
<tr>
<td>Light (type I or II)</td>
<td>1003 (51.4)</td>
</tr>
<tr>
<td>Medium type (III or IV)</td>
<td>766 (39.3)</td>
</tr>
<tr>
<td>Dark (type V or VI)</td>
<td>107 (5.5)</td>
</tr>
<tr>
<td>Outdoor play, h/wk, mean ± SD</td>
<td>5.0 ± 2.3</td>
</tr>
<tr>
<td>Serum 25(OH)D level, nmol/L, median (IQR)</td>
<td>81 (67 to 100)</td>
</tr>
<tr>
<td>Serum 25(OH)D level &lt;50 nmol/L, no. (%)</td>
<td>92 (4.7)</td>
</tr>
</tbody>
</table>
Note: 25(OH)D= 25-hydroxyvitamin D, BMI= body max index, IQR= Interquartile range, SD= standard deviation, NA= not available.
* 363 children not included because of missing data on milk intake (imputed for analysis)
† 1 cup= 250 mL
‡ Determined using Fitzpatrick scale
Table 4: Association between consumption of non-cow’s milk and 25-hydroxyvitamin D levels

<table>
<thead>
<tr>
<th>Variable</th>
<th>Unadjusted analysis</th>
<th>Adjusted analysis*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% change in 25(OH)D level (95% CI)</td>
<td>Change in Median 25(OH)D nmol/L (95% CI)</td>
</tr>
<tr>
<td>Daily consumption of non-cow’s milk (per cup§)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drinks cow’s milk</td>
<td>-4.7 (-8.4 to -0.8)</td>
<td>-4.7 (-6.7 to -0.6)</td>
</tr>
<tr>
<td>Does not drink cow’s milk</td>
<td>-0.3 (-3.3 to 2.8)</td>
<td>-0.2 (2.8 to 2.2)</td>
</tr>
<tr>
<td>Age (per additional year)</td>
<td>-0.2 (-1.1 to 0.7)</td>
<td>-0.2 (-0.9 to 0.5)</td>
</tr>
<tr>
<td>Sex (male v. female)</td>
<td>-0.04 (-2.6 to 2.6)</td>
<td>-0.03 (-2.1 to 2.1)</td>
</tr>
<tr>
<td>BMI z score</td>
<td>-0.2 (-1.5 to 1.0)</td>
<td>-0.2 (-1.2 to 0.8)</td>
</tr>
<tr>
<td>Cow’s milk consumption (no v.yes)</td>
<td>-6.4 (-9.9 to -2.7)</td>
<td>-5.1 (-7.9 to -2.2)</td>
</tr>
<tr>
<td>Margarine consumption (no v.yes)</td>
<td>-0.9 (-3.7 to 2.0)</td>
<td>-0.7 (-3.0 to 1.6)</td>
</tr>
<tr>
<td>Vitamin D supplementation (yes v.no)</td>
<td>8.5 (5.7 to 11.3)</td>
<td>6.8 (4.6 to 9.1)</td>
</tr>
<tr>
<td>Skin pigmentation (v. type III or IV)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Light (I or II)</td>
<td>1.8 (-0.9 to 4.6)</td>
<td>1.5 (-0.7 to 3.7)</td>
</tr>
<tr>
<td>Dark (V or VI)</td>
<td>-14.0 (-18.6 to -9.0)</td>
<td>-14.0 (-14.9 to 7.2)</td>
</tr>
<tr>
<td>Seasonal effect¶</td>
<td>Sine function</td>
<td>Cosine function</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>1.3 (-0.5 to 3.1)</td>
<td>1.0 (-1.2 to 0.3)</td>
<td>0.2</td>
</tr>
<tr>
<td>-4.3 (-6.0 to -2.5)</td>
<td>-3.4 (-4.8 to -2.0)</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Note: 25(OH)D= 25-hydroxyvitamin D, BMI= body max index z-score, IQR= Interquartile range, SD= standard deviation.
*Adjusted for all other variables in the table
†Median level= 80.0 nmol/L
‡ p values (t test) represent significance of % change in 25(OH)D level.
§1 cup= 250 mL
¶The seasonal amplitude was 10% and peaked in June 26th. Seasonal effect was tested using a likelihood ratio test and was statistically significant (p<0.001).
Figure 3: Adjusted association between total consumption of non-cow’s milk beverages and serum 25-hydroxyvitamin D levels among children drinking only non-cow’s milk and among those drinking both non-cow’s milk and cow’s milk.

Screened areas = 95% confidence intervals.
Figure 4: Adjusted association between daily consumption of non-cow’s milk beverages and daily consumption of cow’s milk
3.5 Discussion

More than 10% of children in my study drank non-cow’s milk beverages on a regular basis. I identified an independent association between non-cow’s milk consumption and lower 25-hydroxyvitamin D in early childhood, and the association appeared to be modified by cow’s milk consumption. Children who drank only non-cow’s milk were more than twice as likely as children who drank only cow’s milk to have a 25-hydroxyvitamin D level below 50 nmol/L. Among children who drink both types of milk, each additional cup of non-cow’s milk beverage consumed was associated with a 5% lower 25-hydroxyvitamin D level. This association is consistent with my finding of an inverse association between non-cow’s milk and cow’s milk consumption, and suggests a trade-off between consumption of cow’s milk fortified with higher levels of vitamin D and non-cow’s milk with lower vitamin D content.

Fortified cow’s milk has been identified as the main dietary source of vitamin D in early childhood (18, 21, 25-27, 193), with about a 5 nmol/L higher 25-hydroxyvitamin D per 250-mL cup of cow’s milk consumed (17, 154, 194). Substitution of cow’s milk with non-cow’s milk beverages that have lower vitamin D content could put children at unnecessary risk of complications from low dietary vitamin D intake. Several case studies have identified severe rickets from vitamin D deficiency in children who did not drink cow’s milk and had not been taking vitamin D supplements (158, 195-197).

One strategy to increase vitamin D intake is vitamin D supplementation. Alternatively, standardized legislated vitamin D content for all milk products, including non-cow’s milk beverages, could level the vitamin D gradient between children who drink cow’s milk and those who drink non-cow’s milk products (198). According to the Food and Drug Regulations of Canada, the term milk can be used only on products obtained from a cow fortified with vitamin D (29). Thus, non-cow’s milk products are commonly labeled as “beverages” without the inclusion of the term milk, and therefore fall outside the scope of legislation for vitamin D content and monitoring.

Strengths of my study include a relatively large sample from an ethnically diverse population of healthy young urban children with rich clinical and laboratory data, which allowed me to take into account a range of clinically important potential confounders.
Limitations of my study include the cross-sectional design, from which causality cannot be determined. Parent reported measurement of survey data may have been susceptible to measurement error. Children included in the analysis had lighter skin pigmentation and higher vitamin D supplementation than the nonparticipants had; thus, my findings may not be generalizable to children from other urban areas or nonurban children who may be at higher risk of vitamin D deficiency. However, median 25-hydroxyvitamin D concentration was similar to the median levels in other population-based studies of this age group (25, 199).

3.6 Conclusion

I identified a dose-dependent association between consumption of non-cow’s milk in early childhood and lower serum levels of 25-hydroxyvitamin D. This association was modified by cow’s milk consumption, which suggests a trade-off between the consumption of cow’s milk fortified with higher levels of vitamin D and non-cow’s milk beverages with lower vitamin D content. My findings may be helpful for healthcare providers caring for children who drink non-cow’s milk beverages because of an allergy to cow’s milk, lactose intolerance or dietary preference. Improved education regarding nutrition labels is important to ensure that non-cow’s milk products fortified with vitamin D are being chosen by caregivers, and improved package labeling may help parents make informed decision about healthy milk beverages for their children. Standardization of vitamin D content of both cow’s and non-cow’s milk products would make decisions simpler. Future research on the type and brand of non-cow’s milk is needed to understand better the association between consumption of specific types of non-cow’s milk and serum 25-hydroxyvitamin D levels in childhood.
Chapter 4
Type of non-cow’s milk consumption and 25-hydroxyvitamin D levels in early childhood

4 Type of non-cow’s milk consumption and 25-hydroxyvitamin D levels in early childhood

4.1 Abstract

Background: I have previously identified a dose dependent relationship between higher consumption of non-cow’s milk and lower vitamin D levels in early childhood. It is unclear whether this is true for both animal based (goat’s milk) and plant-based milk beverages. My objectives were to: 1) determine whether the relationship between non-cow’s milk consumption and children’s 25-hydroxyvitamin D is different for goat’s milk and plant-based milk beverages; and 2) compare these associations to the relationship between cow’s milk consumption and 25-hydroxyvitamin D.

Methods: In this cross sectional study, children 1-6 attending routine primary healthcare visits were recruited. Survey responses, anthropometric and laboratory measurements were collected. The association between cow’s milk, goat’s milk and plant-based milk beverages with 25-hydroxyvitamin D was determined using an adjusted multiple linear regression model.

Results: There were 2711 children included. Goat’s milk consumption appeared to have a trend towards higher 25-hydroxyvitamin D level per cup (p=0.2) and plant-based milk beverage consumption was associated with a lower 25-hydroxyvitamin D level (3 nmol/L lower 25-hydroxyvitamin D per cup, p=0.01). The magnitude of the effect of plant-based milk consumption on children’s 25-hydroxyvitamin D was lower than both cow’s milk (p<0.0001) and goat’s milk (p=0.01).

Conclusions: Plant-based milk beverage consumption was associated with lower 25-hydroxyvitamin D levels in early childhood. This association was significantly lower in magnitude than the association between cow’s milk and goat’s milk with 25-hydroxyvitamin D, respectively.
### 4.2 Background

Adequate levels of vitamin D are important for children’s health and development (9, 48). Vitamin D plays a role in calcium absorption, optimizing bone development and preventing rickets (9, 17, 106, 200). Vitamin D has also been suggested to be involved in a number of disease processes including respiratory (94, 95, 201-205), cardiovascular (9, 17, 106, 200), and autoimmune conditions (22, 201, 206-208). In children, the main dietary source of vitamin D is fortified cow’s milk (18, 25, 26, 48, 49, 209). In Canada, cow’s milk is required to contain 40 IU of vitamin D$_3$ per 100 mL (29, 30). The consumption of 2 cups of cow’s milk per day in childhood has been recommended by a number of organizations internationally (164, 210, 211).

In recent years, the commercial availability and interest of milk alternatives has been growing. Types of non-cow’s milk beverages include plant-based milk (i.e. soy, almond, rice, hemp, etc.) and animal based milk (i.e. goat’s milk). Despite the increasing consumption of non-cow’s milk by children, the health benefits are unclear.

Unlike cow’s milk, vitamin D fortification of non-cow’s milk is voluntary in Canada (29, 30). If stated as fortified, goat’s milk is required to be fortified with 40 IU of vitamin D per 100 mL (28, 30, 169) and plant-based milk beverages with 34 IU vitamin D per 100 mL (29). In the United States, the vitamin D fortification of all milk types, including cow’s milk, goat’s milk, and plant-based milk beverages is voluntary (30, 166).

I have previously identified a dose dependent relationship between higher consumption of non-cow’s milk beverages and lower serum 25-hydroxyvitamin D levels in early childhood (212). However, it is unclear whether this is true for both animal based and plant-based non-cow’s milk beverages. I hypothesized that the effect of animal based non-cow’s milk (i.e. goat’s milk) on children’s 25-hydroxyvitamin D levels may be similar to cow’s milk because both are fortified to the same levels (40 IU/100 mL) and both are fortified with vitamin D$_3$. I also hypothesized that the effect of plant-based milk beverages on serum 25-hydroxyvitamin D may be lower than cow’s milk because when stated as fortified, plant-based milk beverages contain less vitamin D than cow’s milk (34 IU/100 mL), and contain vitamin D$_2$, which may influence serum 25-hydroxyvitamin D less than vitamin D$_3$ (213-217).
4.2.1 Objectives

The primary objective of this study was to determine whether the relationship between non-cow’s milk consumption and children’s 25-hydroxyvitamin D is different for goat’s milk and plant-based milk beverages. I also aimed to explore how these relationships might be different than the relationship between cow’s milk consumption and 25-hydroxyvitamin D.

4.3 Methods

4.3.1 Study design and participants

This was a cross-sectional observational study of healthy children 1-6 years of age seen for routine primary healthcare. Children were recruited through the TARGGet Kids! practice based research network between December 2008 and September 2013. Children were recruited from one of seven pediatric or family medicine primary care practices in Toronto, Canada (latitude 43.4°N) during a scheduled well child doctor’s visit. TARGGet Kids! is a collaboration between primary care physicians at the Department of Paediatrics and the Department of Family and Community Medicine at the University of Toronto, and child health researchers in the Faculty of Medicine at the University of Toronto (181). Children who had health conditions affecting growth (e.g. failure to thrive), chronic illnesses (excluding asthma), or severe developmental delay were excluded. Additionally, children drinking a combination of goat’s milk and plant-based milk beverages were excluded to capture the individual effect of each non-cow’s milk type.

4.3.2 Measurements

Survey data were administered to parents and collected by trained research assistants at each primary healthcare practice. Anthropometric data were obtained by trained research assistants. Trained phlebotomists performed child venous blood sampling and samples were sent daily to the Mount Sinai Services Laboratory in Toronto (183).

My primary outcome was total serum 25-hydroxyvitamin concentration. This was measured using a competitive two-step chemiluminescence assay (Diasorin LIAISON 25-hydroxyvitamin D TOTAL). Through extensive testing and validation, this assay has demonstrated values well within acceptable limits for biochemical measurements: 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 (73, 184).

My primary exposure variable was daily volume of milk consumed (cups/day) for each type of milk: 1. Cow’s milk, 2. Goat’s milk, 3. Plant-based milk beverages. Total consumption of cow’s milk was measured by response to the following standardized question: “How many 250-mL cups of cow’s milk does your child have currently in a typical day?”. Consumption of goat’s milk and plant-based milk beverages were measured by response to the following question: “How many 250-mL cups of non-cow’s milk (soy, rice, goat, etc.) does your child have currently in a typical day?”. Parents who indicated greater than 0 cups of non-cow’s milk were contacted by telephone or email to determine the specific type of non-cow’s milk consumed (goat’s milk vs. plant-based milk beverages).

Data for clinically relevant covariates, hypothesized a-priori to influence the association between type of milk consumption and serum 25-hydroxyvitamin D included: age, sex, Body Mass Index z-score (z-BMI), daily vitamin D supplementation, margarine consumption (which is fortified with 53 IU vitamin D per 10 mL (10g) in Canada (29, 30)), skin pigmentation, outdoor play time, and date of laboratory testing. Children’s weight was measured using a precision digital scale (± 0.025 %; SECA, Hamburg, Germany) and height was measured using a calibrated stadiometer (SECA). BMI was calculated as weight in kilograms divided by height in meters squared (185, 218). Body Mass Index z-score was calculated using World Health Organization growth standards (186-188). Daily vitamin D supplementation was measured as the daily use of a vitamin D supplement and/or multivitamin. All children’s over-the-counter multivitamins in Canada contain a dose of 400 International Units (52). Margarine consumption was measured as consumption of margarine in the previous 3 days. Trained research assistants measured skin pigmentation using the Fitzpatrick scale, a skin pigmentation classification system used commonly in dermatological research (189). Outdoor playtime was defined as the number of hours spent outside playing per week.

4.3.3 Statistical Analyses

Descriptive statistics were performed for the primary exposure variable, outcome variable and covariates. A univariate linear regression model was developed to determine the unadjusted association between the primary exposures (milk type consumed) and the primary outcome (25-
hydroxyvitamin D). Consumption of each milk type (cow’s milk, goat’s milk, plant-based milk beverage) was included in the model as an independent variable. For my primary analysis, a multiple linear regression model was developed to determine the independent effect of cow’s milk consumption (cups/day), goat’s milk consumption (cups/day), and plant-based beverage consumption (cups/day) on children’s 25-hydroxyvitamin D level, adjusted for pre-specified covariates listed above. To avoid biased regression coefficients and standard errors and artificially inflated $R^2$ all covariates were included in the final model, irrespective of their level of statistical significance (190). A sinusoidal function was applied to the date of laboratory testing to account for seasonal variation of 25-hydroxyvitamin D.

Multicollinearity was assessed for all covariates using the variance inflation factor (VIF). All variables had VIF< 1.1 and were considered to produce independent effects. The linearity between the primary exposure (milk type consumed) and primary outcome (25-hydroxyvitamin D) was assessed using residual plots of the primary exposure against the primary outcome. As 25-hydroxyvitamin D was positively skewed, it was log-transformed, resulting in a normal distribution. Residual analysis was conducted on the log-transformed outcome variable, which indicated a good fit. Bootstrap validation was performed on the linear regression model to identify the likelihood of overfitting. No variable had more than 12% missing data, and multiple imputation was conducted to handle potential biases that can result from missing data (190). The statistical computing programs, SAS 9.3 for Windows and R 3.0.3 were used to conduct the statistical analyses. This study was approved by the Hospital for Sick Children and St. Michael’s Hospital Research Ethics Boards and parents of all children included in the study consented to participation in the study.

4.4 Results

Of the 4523 children who had parental consent to participate, 2831 children had laboratory testing. Of these, 114 children were excluded because milk type data was unavailable, and 6 were excluded who drank a combination of goat’s milk and plant-based milk beverages, in order to delineate the independent association between each type of milk and 25-hydroxyvitamin D levels. Thus, 2711 children were included in the study (Figure 6). Characteristics of the participants and nonparticipants were clinically similar (Table 5). Approximately half of the participants were male (53%) and the mean age was 2.9 years (standard deviation 1.5). Vitamin
D supplementation was reported in 53% of children, and median 25-hydroxyvitamin D level was 80 nmol/L (interquartile range 66-99). Among the participants, 85% drank cow’s milk, 1% drank goat’s milk and 6% drank plant-based milk beverages, and 8% drank no milk.

In the unadjusted analysis, cow’s milk consumption was associated with a higher 25-hydroxyvitamin D level (p<0.0001) and goat’s milk consumption was associated with a non-significant 3.7% higher 25-hydroxyvitamin D level (p=0.20). In contrast, plant-based milk beverage consumption was associated with a lower 25-hydroxyvitamin D level (p=0.04).

Results of the primary analysis were similar to the univariate analysis. Please see Table 6 and Figure 7. In the adjusted multiple linear regression model, each 250-mL cup of cow’s milk consumed was associated with a 3.7% higher 25-hydroxyvitamin D level (p=0.0001). For example, an increase in cow’s milk from 0 to 1 cup was associated with a higher median 25-hydroxyvitamin D level of 3.0 nmol/ (95% CI: 2.1 to 3.9). Each cup of goat’s milk consumed was associated with a non-significant 4.0% higher 25-hydroxyvitamin D level (p=0.2) and each cup of plant-based milk beverage consumed was associated with a 4.0% lower 25-hydroxyvitamin D level (p=0.01). For example, an increase in plant-based milk consumption from 0 to 1 cup was associated with a higher median 25-hydroxyvitamin D level of 3.2 nmol/L (95% CI: 0.7 to 5.6). Statistically significant covariates included vitamin D supplementation, which was associated with higher serum 25-hydroxyvitamin D level and dark skin pigmentation, which was associated with a lower serum 25-hydroxyvitamin D level (p<0.001).

Comparing the relationship of each milk type on 25-hydroxyvitamin D level (see Figure 7) revealed that the slope of cow’s milk and 25-hydroxyvitamin D level was similar to the slope of goat’s milk and 25-hydroxyvitamin D (p=0.9). In contrast, the slope of plant-based milk beverages on 25-hydroxyvitamin D level was statistically lower than the slope of both cow’s milk (p<0.0001) and goat’s milk (p=0.01).
Figure 5: Subject participation

Children with parental consent who met the inclusion criteria

\[ n = 4523 \]

\[ \text{Excluded} \ n = 120 \]

\[ n = 114 \text{ Unknown non-cow’s milk type} \]

\[ n = 6 \text{ Combined goat’s milk and plant-based milk beverage} \]

\[ \text{Children with no laboratory testing} \]

\[ \text{Excluded} \ n = 1692 \]

Children with laboratory testing

\[ n = 2831 \]

\[ \text{Excluded} \ n = 120 \]

\[ n = 114 \text{ Unknown non-cow’s milk type} \]

\[ n = 6 \text{ Combined goat’s milk and plant-based milk beverage} \]

Children included in analysis

\[ n = 2711 \]
Table 5: Characteristic of children who participated in the study and nonparticipants by milk type

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Participants n= 2711&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cow’s milk n= 2302</td>
</tr>
<tr>
<td>Age, yr, mean ± SD</td>
<td>3.1 ± 1.5</td>
</tr>
<tr>
<td>Sex, male, no. (%)</td>
<td>1214 (53)</td>
</tr>
<tr>
<td>BMI z score, median (IQR)</td>
<td>0.2 (-0.4 to 0.9)</td>
</tr>
<tr>
<td>Cow’s milk, no. (%)</td>
<td>2302 (100)</td>
</tr>
<tr>
<td>Non-cow’s milk, no. (%)</td>
<td>92 (5)</td>
</tr>
<tr>
<td>No. of cups† per day, mean ± SD</td>
<td>2.1 ± 1.1</td>
</tr>
<tr>
<td>Cow’s milk</td>
<td>0.04 ± 1.1</td>
</tr>
<tr>
<td>Non-cow’s milk</td>
<td></td>
</tr>
<tr>
<td>Margarine consumption, no. (%)</td>
<td>694 (30)</td>
</tr>
<tr>
<td>Vitamin D supplementation, no. (%)</td>
<td>1207 (53)</td>
</tr>
<tr>
<td>Skin pigmentation, ‡ no. (%)</td>
<td>1167 (53)</td>
</tr>
<tr>
<td></td>
<td>Medium type (III or IV)</td>
</tr>
<tr>
<td>--------------------------</td>
<td>-------------------------</td>
</tr>
<tr>
<td></td>
<td>Dark (type V or VI)</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Outdoor play, h/wk,</td>
<td></td>
</tr>
<tr>
<td>mean ± SD</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum 25(OH)D level,</td>
<td></td>
</tr>
<tr>
<td>nmol/L, median (IQR)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: 25(OH)D = 25-hydroxyvitamin D, z-BMI = body mass index z-score, IQR = Interquartile range, SD = standard deviation, NA = not available.
† 1 cup = 250 mL
‡ Determined using Fitzpatrick scale
a Total n does not add up due to children drinking combined milk types and missing values
b Only based on n=110 nonparticipants who had laboratory testing
Table 6: Adjusted association between milk type and 25-hydroxyvitamin D levels

<table>
<thead>
<tr>
<th>Adjusted analysis*</th>
<th>% change in 25(OH)D level (95% CI)</th>
<th>Change in median, † 25(OH)D level nmol/L (95% CI)</th>
<th>p value‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daily consumption of milk (per cup §)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cow’s milk</td>
<td>3.7 (2.6 to 4.9)</td>
<td>3.0 (2.1 to 3.9)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Goat’s milk</td>
<td>4.0 (-1.5 to 9.8)</td>
<td>3.2 (-1.2 to 7.9)</td>
<td>0.2</td>
</tr>
<tr>
<td>Plant’s milk</td>
<td>-4.0 (-7.0 to -0.9)</td>
<td>-3.2 (-5.6 to -0.7)</td>
<td>0.01</td>
</tr>
<tr>
<td>Age (per year)</td>
<td>-0.6 (-1.5 to 0.3)</td>
<td>-0.5 (-1.2 to 0.2)</td>
<td>0.2</td>
</tr>
<tr>
<td>Sex (male)</td>
<td>-0.05 (-2.6 to 2.5)</td>
<td>-0.04 (-2.1 to 2.0)</td>
<td>0.97</td>
</tr>
<tr>
<td>BMI z-score</td>
<td>-0.07 (-1.4 to 1.2)</td>
<td>-0.06 (-1.1 to 1.0)</td>
<td>0.9</td>
</tr>
<tr>
<td>Margarine consumption (yes)</td>
<td>1.4 (-4.2 to 1.5)</td>
<td>1.1 (-3.4 to 1.2)</td>
<td>0.3</td>
</tr>
<tr>
<td>Vitamin D supplementation (yes)</td>
<td>9.6 (6.8 to 12.5)</td>
<td>7.7 (5.4 to 10.0)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Skin pigmentation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Light (type I or II)</td>
<td>1.4 (-1.4 to 4.2)</td>
<td>1.1 (-1.2 to 3.4)</td>
<td>0.3</td>
</tr>
<tr>
<td>Dark (type V or VI)</td>
<td>-15.0 (-19.7 to -10.1)</td>
<td>-12.0 (-15.8 to -8.1)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Seasonal effect ¶</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sin function</td>
<td>0.7 (-1.1 to 2.6)</td>
<td>-0.6 (-0.9 to 2.1)</td>
<td>0.4</td>
</tr>
<tr>
<td>Cos function</td>
<td>-4.4 (-6.3 to -2.5)</td>
<td>-3.51 (-5.0 to -2.0)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Outdoor play (per additional hour)</td>
<td>0.2 (-0.4 to 0.8)</td>
<td>0.1 (-0.4 to 0.6)</td>
<td>0.6007</td>
</tr>
</tbody>
</table>

Note: 25(OH)D= 25-hydroxyvitamin D, CI= confidence Interval, SD= standard deviation
*Adjusted for all other variables in the table
† Median= 80.0 nmol/L
‡ p values (t test) represent significance of % change in 25(OH)D level
§ 1 cup= 250 mL
¶The seasonal amplitude was 10% and peaked in June 23rd. Seasonal effect was tested using a likelihood ratio test and was found to be statistically significant (p<0.0001).
Figure 6: Adjusted association between milk consumption and children’s 25-hydroxyvitamin D levels, by milk type
4.5 Discussion

I identified an independent association between plant-based milk beverage consumption and lower 25-hydroxyvitamin D level in early childhood (3 nmol/L lower 25-hydroxyvitamin D per cup). The magnitude of this association was both smaller and in the opposite direction to the relationship of both cow’s milk and goat’s milk on 25-hydroxyvitamin D.

The nutrient content of goat’s milk is similar to that of cow’s milk, with the exception that the vitamin D fortification of goat’s milk is voluntary (29, 129, 168). Both milks are animal based and contain vitamin D₃. Vitamin D fortification of plant-based milk beverages is also voluntary (28). If stated as fortified, they appear to contain vitamin D₂, which is derived from plant origins (176). Plant-based milk beverages are often labeled as vegetarian or vegan, with legislation mandating they exclusively contain plant-based ingredients like vitamin D₂ (170-175).

One explanation for the difference in 25-hydroxyvitamin D levels between animal and plant based milk consumption may be a difference in the biological potency of vitamin D₂ relative to vitamin D₃. There has been considerable debate about whether vitamin D₂ is as effective as vitamin D₃ in raising serum 25-hydroxyvitamin D concentration in humans (213-215, 217, 219, 220). Some studies have found vitamin D₂ to have a biological potency less than one third of vitamin D₃ (216) while other studies have argued that they are biologically equivalent (221-223). One possible explanation for decreased biological potency of vitamin D₂ is a lower affinity of vitamin D₂ for the vitamin D binding protein (DBP), which may result in more rapid clearance of vitamin D₂ from circulation (216, 217, 220).

Another explanation for my findings may be the differences in regulatory requirements for vitamin D fortification of plant-based and animal-based milk. Vitamin D fortification of goat’s milk falls under Canada’s Food and Drug Regulations, whereas plant-based milk beverages do not (29). Instead, an Interim Marketing Authorization was established by Health Canada in 1997 for plant-based milk beverages to initiate an amendment to Canada’s Food and Drug Regulations, but this has not yet been put into action (28). Vitamin D fortification of cow’s milk and goat’s milk, which both fall under the Food and Drug Regulations, may be more strictly regulated and monitored than plant-based milk beverages.
Among children drinking plant-based milk beverages, 24% were under the age of 2 years, despite professional recommendations by the Infant Feeding Joint Working Group in Canada (Canadian Paediatric Society, Health Canada, Dietitians of Canada, Breastfeeding Committee for Canada, and Public Health Agency of Canada) discouraging plant-based milk beverage consumption before 2 years of age (54, 149, 156). These recommendations suggest that both fortified and unfortified plant-based milk beverages do not serve as nutritionally adequate alternatives to breast milk, whole cow’s milk, or commercial infant formula to support proper growth and development of children (54, 105, 157). For children older than 2, Canada’s Food Guide and United States Department of Agriculture’s MyPlate recommends fortified soy beverage consumption if cow’s milk is not consumed (164, 165, 224). Other plant-based milk beverages are not listed as potential milk alternatives (156, 165).

Declaring the vitamin D content on the Nutrition Facts table of food products is currently voluntary under Health Canada’s regulations (225). If vitamin D content is stated, only the % daily value is required to be shown, without the absolute amount in micrograms or international units. Although parents are recommended to choose milk products that are fortified with vitamin D (148), caregivers may find it challenging to discern vitamin D fortified from non-fortified milk beverages based on current labeling requirements.

Vitamin D supplementation is one way to ensure children consuming plant-based milk beverages meet their vitamin D requirements; however, studies suggest that those at higher risk for nutritional inadequacy are less likely to take nutritional supplements (226, 227). Supplement use has been associated with higher socio-economic statuses and healthier lifestyles in adults (226, 227). It is possible that health conscious caregivers may be choosing plant-based milk beverages for perceived health benefits and may also be more likely to supplement their children. This would explain the high vitamin D supplement use among children who drank plant-based milk beverages (66%), in comparison to those who drank cow’s milk (53%) in my study. However, despite the higher vitamin D supplement use, plant-based milk drinkers had lower 25-hydroxyvitamin D levels. Alternative strategies that may be more effective include mandating universal vitamin D fortification for all non-cow’s milk beverages similar to cow’s milk (30, 228) and increasing awareness of foods that are fortified with vitamin D. Healthcare providers play an important role in educating consumers and caregivers about dietary sources of vitamin D and food product nutrition labeling.
Strengths of my study include the relatively large sample size with rich clinical and laboratory data. This allowed me to take into account a range of clinically important potential confounders. To my knowledge, this is the first study to examine the association between consumption of specific non-cow’s milk types and children’s vitamin D stores.

Limitations of my study include the cross-sectional observational design, from which causality cannot be determined. A small number of children were drinking goat’s milk (n=30). With a higher sample size of children drinking goat’s milk, there may have been more power to detect a significant association between goat’s milk consumption and children’s serum 25-hydroxyvitamin D levels. Parent reported measurement of survey data and retrospective data collection of non-cow’s milk type may have been susceptible to measurement error. Further, only children with laboratory testing were included which may limit generalizability. Parents who were more inclined to agree to have their child’s blood tested may be more health conscious. Lastly, I was unable to account for consumption of vitamin D fortified vs. unfortified non-cow’s milk types, which may have influenced the observed relationships.

### 4.6 Conclusion

Consumption of plant-based milk beverages was associated with lower 25-hydroxyvitamin D levels in early childhood, and the magnitude of this association was lower than the effect of both cow’s milk on 25-hydroxyvitamin D and goat’s milk on 25-hydroxyvitamin D. Future studies are needed to clarify this association, and to elucidate the reason for the blunted effect of plant based milk beverage consumption on children’s 25-hydroxyvitamin D levels. Determining whether this difference is due to a greater potency of vitamin D₃ than vitamin D₂ or due to regulatory differences between the types of commercially available milk may be helpful to policy makers as they update nutrition labeling and fortification processes (229, 230). Standardizing the fortification of vitamin D across all milk types would assist caregivers in choosing healthy milk products for their children.
Chapter 5
Overall discussion

This thesis documents my work in testing the \textit{a priori} generated hypotheses that non-cow’s milk may influence children’s serum 25-hydroxyvitamin D levels less than cow’s milk through a series of two projects. In my first study, I have identified an independent association between non-cow’s milk consumption and lower 25-hydroxyvitamin D levels in early childhood. Next, I have identified an independent association between plant-based milk beverages and lower 25-hydroxyvitamin D in early childhood. The magnitude of this association was both smaller and in the opposite direction to the relationship of both cow’s milk and goat’s milk and 25-hydroxyvitamin D.

My thesis has accomplished the main objectives as outlined in Chapter 1: 1) to determine whether there is an association between total daily consumption of non-cow’s milk and serum 25-hydroxyvitamin D concentration in a population of healthy urban children attending routinely scheduled well-child visits (Chapter 3); 2) to explore how consumption of cow’s milk might modify this association (Chapter 3); 3) to explore the association between daily intake of non-cow’s milk and cow’s milk (Chapter 3); 4) to determine whether the relationship between non-cow’s milk consumption and children’s 25-hydroxyvitamin D is different for goat’s milk and plant-based milk beverages; and 5) to explore how these relationships might be different than the relationship between cow’s milk consumption and 25-hydroxyvitamin D (Chapter 4).

To my knowledge, these are the first studies to examine the effect of non-cow’s milk consumption on children’s 25-hydroxyvitamin D level. I have identified a dose dependent association between higher non-cow’s milk consumption and lower vitamin D levels among children ages 1-6. This association was modified by consumption of cow’s milk, and was consistent with my finding of an inverse association between non-cow’s milk and cow’s milk consumption. This suggests there is a trade-off between consumption of cow’s milk fortified with higher levels of vitamin D and non-cow’s milk with lower vitamin D content.

After conducting Study 1, I realized the importance of determining the effect of specific non-cow’s milk types on children’s 25-hydroxyvitamin D levels. The TARGet Kids! Nutrition and Health Questionnaires did not extensively collect information on the various types and brands of
non-cow’s milk that children were consuming. Through the submission of an amendment to the TARGet Kids! protocol, I was able to obtain approval from the Hospital for Sick Children and St. Michael’s Hospital Research Ethic Boards to contact families with children who consume non-cow’s milk to delineate how the type of non-cow’s milk may be associated with children’s 25-hydroxyvitamin D level.

With the addition of this data, I have identified an independent association between plant-based milk beverages and lower 25-hydroxyvitamin D in early childhood. The magnitude of this association was both smaller and in the opposite direction to the relationship of both cow’s milk and goat’s milk and 25-hydroxyvitamin D.

More than 10% of children in my study drank non-cow’s milk beverages on a regular basis. Fortified cow’s milk has been identified as the main dietary source of vitamin D in early childhood (21, 25-27). Substitution of cow’s milk with non-cow’s milk beverages that have lower vitamin D content could put children at unnecessary risk of complications from low dietary vitamin D intake. Assessing the dietary intake of Canadian children suggest they may not be meeting their recommended vitamin D intakes. In the CCHS 2.2, the mean dietary intake of children 1-8 was 248 IU/day (49), and in a Montreal study, 95% of children were consuming less than the EAR (199). Whether children from my study are meeting their recommended vitamin D intakes is unknown.

Among children drinking non-cow’s milk, over 20% children were under the age of 2, despite professional recommendations by the Infant Feeding Joint Working Group in Canada. These recommendations suggest that both fortified and unfortified plant-based milk beverages do not serve as nutritionally adequate alternatives to breast milk, whole cow’s milk, or commercial infant formula to support proper growth and development of children (54, 105, 157).

For children older than 2 years, Canada’s Food Guide and United States Department of Agriculture’s MyPlate recommends fortified soy beverage consumption if cow’s milk is not consumed (164, 165, 224). Other plant-based milk beverages are not listed as potential milk alternatives, as they contain lower levels of protein (156, 165). If other plant-based milks are being consumed, parents are recommended to choose products labeled as fortified and containing a minimum of 6 g of protein per 250 mL cup (148). Parents are also recommended to
provide alternate sources of protein (i.e. beans, lentils, fish, poultry, eggs, tofu, nuts, lean meats) if lower protein containing beverages are chosen as the child’s milk source (148).

In the CCHS 2.2, 35% and 41% of children ages 1-3 and 4-8, respectively, were being supplemented with vitamin D (49, 227). In my studies, over 60% of children ages 1-6 drinking only non-cow’s milk were being supplemented with vitamin D, and over 50% of children drinking only cow’s milk were being supplemented. Whether these children were drinking fortified non-cow’s milk is unknown. The higher proportion of children taking vitamin D supplements in my study may be related to the nature of the TARGet Kids! research platform, in which children’s serum 25-hydroxyvitamin D levels are measured; thus, participating physicians are aware of the children’s 25-hydroxyvitamin D levels and may be providing recommendations for vitamin D supplementation.

One explanation for the difference in 25-hydroxyvitamin D levels between animal and plant based milk consumption may be a difference in the biological potency of vitamin D₂ relative to Vitamin D₃. There has been considerable debate about whether vitamin D₂ is as effective as vitamin D₃ in raising serum 25-hydroxyvitamin D concentration in humans (213-215, 217, 219, 220).

Another explanation for my findings may be the differences in regulatory requirements for vitamin D fortification non-cow’s milk (goat’s milk and plant-based milk beverages) and cow’s milk. Vitamin D fortification of goat’s milk falls under Canada’s Food and Drug Regulations, whereas plant-based milk beverages do not (29). Thus, vitamin D fortification of cow’s milk and goat’s milk, which both fall under the Food and Drug Regulations, may be more strictly regulated and monitored than plant-based milk beverages.

Despite the strict regulations and monitoring of milk under Canada’s Food and Drug Regulations, various studies have found non-compliance with the mandated fortification levels over the years (30, 41-47). There was variability in the vitamin D content between various cow’s milk, as well as variability in the measured vitamin D content and the amount stated on the Nutrition Facts table (30, 41-47). It is unclear whether this variability and incompliance is also seen with plant-based milk beverages, especially given the fact that it does not fall under the Food and Drug Regulations strict regulations and monitoring.
My findings raise the question about the effectiveness of current food labeling practices. Declaring the vitamin D content on the Nutrition Facts table of food products is currently voluntary under Health Canada’s regulations (225). The % DV are based on a 2000-calorie diet for healthy adults (231). Further, the current % DV for vitamin D is based on the 1997 AI for vitamin D of 200 IU, rather than the updated 2010 RDA for vitamin D of 600 IU for children and adults over the age of 1 (231). For instance, the vitamin D content in 1 cup of cow’s milk is currently represented as containing 45% DV, rather than 15% DV, which would accurately represent the vitamin D content based on the current and updated dietary reference intake for vitamin D (231). Although parents are recommended to choose milk products that are fortified with vitamin D (148), caregivers may find it challenging to discern vitamin D fortified from non-vitamin D fortified milk beverages based on current labeling requirements.

Health Canada has proposed changes to the current Nutrition Facts table, including the mandatory declaration of vitamin D as a % DV and in absolute amounts (micrograms) (230). Additionally, a footnote explaining the interpretation of % DV has been proposed (230). This outlines the important role of healthcare professionals in educating consumers and caregivers about dietary sources of vitamin D and nutrition label reading to ensure children’s dietary recommendations are being met. Thus, it is also important for healthcare providers to be aware of current and potentially changing fortification practices (229).

Vitamin D supplementation is one way to ensure children consuming non-cow’s milk beverages meet their vitamin D requirements; however, studies suggest that those at higher risk for nutritional inadequacy are less likely to take nutritional supplements (226, 227). Supplement use has been associated with higher socio-economic statuses and healthier lifestyles in adults (226, 227). It is possible that health conscious caregivers may be choosing non-cow’s milk beverages for perceived health benefits and may also be more likely to supplement their children. This would explain the high vitamin D supplement use among children who drank non-cow’s milk in comparison to those who drank cow’s milk in my study. Despite the higher vitamin D supplement use, children who drank non-cow’s milk had lower 25-hydroxyvitamin D levels. Other strategies to improve vitamin D intake include mandating universal vitamin D fortification for all non-cow’s milk beverages similar to cow’s milk (30, 228) and increasing awareness of foods that are fortified with vitamin D. This is especially important, given the decline in children’s cow’s milk consumption over the years (232). Healthcare providers play a
crucial role in educating consumers and caregivers about dietary sources of vitamin D and food product nutrition labeling.

Overall strengths of my two studies include a relatively large sample size with rich clinical and laboratory data, which allowed me to take into account a range of clinically important potential confounders. To my knowledge, these studies are the first to examine the association between non-cow’s milk consumption and children’s vitamin D stores.

Limitations include the cross-sectional observational design, from which causality cannot be determined. Parent reported measurement of survey data may have been susceptible to recall bias. Only children with laboratory testing were included which may limit generalizability. Parents who were more inclined to agree to have their child’s blood tested may be more health conscious. Children included in the analysis had lighter skin pigmentation and higher vitamin D supplementation than the nonparticipants had; thus, my findings may not be generalizable to children from other urban areas or nonurban children who may be at higher risk of vitamin D deficiency. However, median 25-hydroxyvitamin D concentration was similar to the median levels in other population-based studies of this age group (25, 199). Lastly, I was unable to account for consumption of vitamin D fortified vs. unfortified milk types, which may have influenced the observed relationships.
Chapter 6
Conclusions and future directions

This thesis described the association between non-cow’s milk consumption and children’s 25-hydroxyvitamin D levels through the completion of two projects. I identified an independent association between non-cow’s milk consumption and lower 25-hydroxyvitamin D among children ages 1-6 enrolled in TARGet Kids!. This association was modified by consumption of cow’s milk, and was consistent with my finding of an inverse association between non-cow’s milk and cow’s milk consumption. Through further investigation, I found an independent association between plant-based milk beverages and lower 25-hydroxyvitamin D in early childhood. The magnitude of this association was both smaller and in the opposite direction to the relationship of both cow’s milk and goat’s milk and 25-hydroxyvitamin D.

My studies have contributed to the limited body of literature on the health effects of non-cow’s milk consumption. There is future research needed to elucidate the association between animal based and plant-based non-cow’s milk consumption and serum 25-hydroxyvitamin D levels in children. Therefore, my future plan is to investigate whether the observed association is related to differences in the biological activity of vitamin D$_3$ (present in cow’s milk and goat’s milk) and vitamin D$_2$ (present in plant based milk beverages) in raising serum 25-hydroxyvitamin D or by differences in vitamin D fortification between the milk types by measuring the actual vitamin D content of non-cow’s milk and cow’s milk in a laboratory setting. The vitamin D$_3$ and vitamin D$_2$ content of various types and brands of non-cow’s milk and cow’s milk will be measured using high performance liquid chromatography. I will determine whether the amount of vitamin D content stated on the nutrition label accurately represents the measured amount in cow’s milk, goat’s milk, and plant-based milk beverages. Such measurements will allow us to see whether this differs by the specific type or brand of non-cow’s milk. These findings will provide insight on the effectiveness of the current statement for voluntary vitamin D fortification of non-cow’s milk and may inform public health policy makers as they update nutrition labeling and fortification processes (229, 230). Additionally, the findings will aid policy makers in initiating an amendment to the FDR’s current regulations for non-cow’s milk, which will hopefully lead to universal vitamin D fortification for all non-cow’s milk beverages similar to cow’s milk.
References


27. Cole CR, Grant FK, Tangpricha V, Swaby-Ellis ED, Smith JL, Jacques A, et al. 25-


53. Eat Right Ontario. What you need to know about Vitamin D. In: Nutrients, editor.


73. Singh RJ, Taylor RL, Reddy GS, Grebe SK. C-3 epimers can account for a significant proportion of total circulating 25-hydroxyvitamin D in infants, complicating accurate measurement and interpretation of vitamin D status. The Journal of clinical endocrinology and metabolism. 2006;91(8):3055-61.


133. Hollis BW, Wagner CL. Vitamin D requirements during lactation: high-dose maternal supplementation as therapy to prevent hypovitaminosis D for both the mother and the nursing infant. The American journal of clinical nutrition. 2004;80(6 Suppl):1752S-8S.


Appendices
### Appendix 1: Examples of Food sources containing 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><strong>Vegetables</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shiitake mushrooms, cooked</td>
<td>125 mL (½ cup)</td>
<td>21</td>
</tr>
<tr>
<td><strong>Juice</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Orange juice, fortified with</td>
<td>125 mL (½ cup)</td>
<td>50</td>
</tr>
<tr>
<td>vitamin D</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Grain Products</strong></td>
<td></td>
<td>This food group contain very little of this nutrient.</td>
</tr>
<tr>
<td><strong>Milk and Alternatives</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Milk</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.3% homo, 2%, 1%, skim,</td>
<td>250 mL (1 cup)</td>
<td>103-105</td>
</tr>
<tr>
<td>chocolate milk</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skim milk powder</td>
<td>24 g (will make 250 mL of milk)</td>
<td>103</td>
</tr>
<tr>
<td><strong>Alternatives</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Goat's milk, fortified with</td>
<td>250 mL (1 cup)</td>
<td>100</td>
</tr>
<tr>
<td>vitamin D</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rice, oat or almond beverage,</td>
<td>250 mL (1 cup)</td>
<td>90</td>
</tr>
<tr>
<td>fortified with vitamin D</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soy beverage, fortified with</td>
<td>250 mL (1 cup)</td>
<td>90</td>
</tr>
<tr>
<td>vitamin D</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Meat and Alternatives</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Salmon</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atlantic, raw or cooked</td>
<td>75 g (2 ½ oz)</td>
<td>181-246</td>
</tr>
<tr>
<td>Chinook, raw or cooked</td>
<td>75 g (2 ½ oz)</td>
<td>319-387</td>
</tr>
<tr>
<td>Chum/keta, raw or cooked</td>
<td>75 g (2 ½ oz)</td>
<td>203-221</td>
</tr>
<tr>
<td>Coho, raw or cooked</td>
<td>75 g (2 ½ oz)</td>
<td>326-421</td>
</tr>
<tr>
<td>Humpback/pink, raw, canned,</td>
<td>75 g (2 ½ oz)</td>
<td>351-497</td>
</tr>
<tr>
<td>or cooked</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sockeye/red, raw, canned or</td>
<td>75 g (2 ½ oz)</td>
<td>530-699</td>
</tr>
<tr>
<td>cooked</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Tuna</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Albacore, raw or cooked</td>
<td>75 g (2 ½ oz)</td>
<td>82-105</td>
</tr>
<tr>
<td>White, canned with water</td>
<td>75 g (2 ½ oz)</td>
<td>60</td>
</tr>
</tbody>
</table>
### Other Fish and Seafood

<table>
<thead>
<tr>
<th>Item</th>
<th>Serving Size</th>
<th>Calories</th>
</tr>
</thead>
<tbody>
<tr>
<td>Halibut, cooked</td>
<td>75 g (2 ½ oz)</td>
<td>144</td>
</tr>
<tr>
<td>Herring, Atlantic, cooked</td>
<td>75 g (2 ½ oz)</td>
<td>161</td>
</tr>
<tr>
<td>Herring, Atlantic, pickled</td>
<td>75 g (2 ½ oz)</td>
<td>210</td>
</tr>
<tr>
<td>Mackerel, Atlantic, cooked</td>
<td>75 g (2 ½ oz)</td>
<td>78</td>
</tr>
<tr>
<td>Mackerel, canned</td>
<td>75 g (2 ½ oz)</td>
<td>219</td>
</tr>
<tr>
<td>Mackerel, Pacific</td>
<td>75 g (2 ½ oz)</td>
<td>342</td>
</tr>
<tr>
<td>Roe, raw</td>
<td>75 g (2 ½ oz)</td>
<td>145</td>
</tr>
<tr>
<td>Sardines, Atlantic, canned</td>
<td>75 g (2 ½ oz)</td>
<td>70</td>
</tr>
<tr>
<td>Sardines, Pacific, canned</td>
<td>75 g (2 ½ oz)</td>
<td>144</td>
</tr>
<tr>
<td>Snapper, cooked</td>
<td>75 g (2 ½ oz)</td>
<td>392</td>
</tr>
<tr>
<td>Swordfish, cooked</td>
<td>75 g (2 ½ oz)</td>
<td>594</td>
</tr>
<tr>
<td>Trout, cooked</td>
<td>75 g (2 ½ oz)</td>
<td>150-210</td>
</tr>
<tr>
<td>Whitefish, lake, cooked</td>
<td>75 g (2 ½ oz)</td>
<td>369</td>
</tr>
</tbody>
</table>

### Meats and alternatives

<table>
<thead>
<tr>
<th>Item</th>
<th>Serving Size</th>
<th>Calories</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef, brisket, cooked</td>
<td>75 g (2 ½ oz)</td>
<td>33</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>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>
</tbody>
</table>

### Fats and Oils

<table>
<thead>
<tr>
<th>Item</th>
<th>Serving Size</th>
<th>Calories</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>
</tbody>
</table>
Appendix 2: TARGet Kids! Consent Form

PARENT/GUARDIAN LETTER OF INFORMATION and CONSENT FORM
(for participants 1-5 years)

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

Principal Investigator at St. Michael’s Hospital:
Dr. Jonathon Maguire, Department of Pediatrics, St. Michael’s Hospital
Telephone# (416) 813-2129 (available Mon - Friday 9am to 5pm)

Overall Study Principal Investigator:
Dr. Patricia Parkin, Division of Paediatric Medicine, The Hospital for Sick Children
Telephone# (416) 813-6933 (available Mon - Friday 9am to 5pm)

Co-investigators:
Dr. Catherine Birken, Division of Paediatric Medicine, The Hospital for Sick Children
Telephone# (416) 813-6933 (available Mon - Friday 9am to 5pm)
Dr. Brian McCrindle, Heart Centre, the Hospital for Sick Children
Telephone# (416) 813-3527

Study Coordinator
Marina Khovratovich, Division of Paediatric Medicine, The Hospital for Sick Children
Telephone# (416) 813-2129 (available Mon-Friday 9am to 5pm)

Funding:
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).

Before you decide whether or not your child will take part, it is important that you read and understand this research consent form. This form provides all the information we think you will need to know in order to decide whether or not you wish to have your child take part in this study. You must be sure to understand the possible benefits and risks in order to make an informed decision. If you wish, please discuss it with family members, friends, the study physician, your child’s treating physician or your family physician. If, after reading this document, there is anything you do not understand about this study, please ask the study physician or the study personnel. If all your questions are answered to your satisfaction and you decide to have your child take part, you will be asked to sign this consent document. You should not sign this form until you are sure you understand everything on this form. You will be given a signed and dated copy of this consent form to keep for your records.

If the investigator will also be your child’s treating doctor, this will be discussed with you.
Purpose of the Research:
Your child’s physician is a member of TARGet Kids! (Toronto Area 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 birth to 10 years of age. 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 initiative.

This study aims to collect information on nutrition in healthy children 0-10 years of age. Nutrition will be measured using the Nutrition Screening Tool for Every Preschooler (NutriSTEP™) which is a 17 item questionnaire that was developed by dieticians and parents. Parents can fill out the questionnaire for their children. We would like to see how the questionnaire relates to growth measures in children and how easy it is to use the NutriSTEP™ in the Canadian doctor’s office. We will ask you to complete a short questionnaire on your child’s personality/behaviour so that we can learn more about the relationship between personality and nutrition in children. We will also ask your child to have blood tests to measure his/her nutritional health, such as cholesterol, iron, and vitamin D. These blood tests will be obtained and processed by experts from the Mount Sinai Services team.

The goal of TARGet Kids! project is to collect information of 2400 children from different sites across Toronto. We anticipate that 500 of those kids will be recruited from St Michael’s Hospital.

Description of the Research:
If you agree for your child to participate in this study, the following tests/assessments will be performed:

- Questionnaires: (i) NutriSTEP the Nutrition Screening Tool for Every Preschooler and for Toddler, a 17 item parent report of nutrition for young children; (ii) Nutrition and Health Questionnaire - a demographics, dietary, and physical activity questionnaire that focuses on nutrition, physical activity, sedentary behaviours in your child; (iii) the Infant Behavior Questionnaire, Early Childhood Behavior Questionnaire, Children’s Behavioural Questionnaire (CBQ); (iv) Nipissing District Developmental Tool; (v) Infant Toddler Checklist; (vi) Parenting Stress Index will be administered by the research assistant. Questionnaires will take approximately 25 minutes to complete at each visit. These assessments are not a part of standard care and are being administered only for research purposes.

- Physical Examination: Your child’s height/ length, weight, waist circumference and blood pressure will be recorded annually up to 10 years of age. Aside from waist circumference, these measures are part of the normal annual visit for children. We will also record your height, weight and waist circumference. All of these measurements will occur at each of your child’s regularly scheduled annual visits between 1 and 10 years of age.

- Blood Collection: A trained health professional experienced with pediatric blood collection will take a small blood sample from your child to measure laboratory tests related to nutrition such as vitamin levels, iron levels, and cholesterol. These measurements will occur annually during your child’s regularly scheduled visits between 1 and 10 years of age.

- Collection of Health Information: We will obtain your child’s previous measurements (height, weight, head circumference, blood pressure) using previous records from your physician. We will collect Nipissing District Developmental Screen (NDDS) from your child medical record in case it was completed at his/her 18 months appointment. We may also collect additional health information on your child using an OHIP number. No additional visits to your doctor are required.
Collection of Health information from Ontario’s Health Administration Databases
As your child grows and develops, we would like to obtain information on their health using information routinely collected by the Ontario health care system. This information is housed in multiple health administration databases. In order to do this, we will use your child’s OHIP number to link data securely to such databases.

Privacy and Confidentiality using Health Administration Data
Health information held in Ontario’s health administration databases is used solely for research and statistical purposes. All data are kept confidential to protect the privacy of individuals through the use of multiple secure methods. By signing this form, you are authorizing access to your medical records by the study personnel and the St. Michael’s Hospital Research Ethics Board. Such access will be used only for the purpose of verifying the authenticity and accuracy of the information collected for the study, without violating your confidentiality to the extent permitted by applicable laws and regulations.

The data collected for the study will be kept for 7 years after completion of the project and will be securely destroyed after that.

Future Research:
Although your child will only be followed for this study until 10 years of age at your family physician’s office, your child will be followed further into the future. We would like to see how characteristics, lifestyles, and nutritional habits of young children under 10 affect future health outcomes in adulthood such as stroke, heart disease and diabetes.

In the future, our research team may approach you to participate in other studies with the aim of improving children’s health, which may include prevention of diabetes, heart disease, Vitamin D deficiency and other health conditions. The research will be explained to you and your consent will be asked for at that time.

Potential Harms, Discomforts or Inconvenience:
We will collect a small blood sample (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 the blood draw. 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. Participating in this project will lengthen your child’s doctor’s visit by up to 25 minutes.

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 with your permission. This could inform your doctor if your child might need a nutritional supplement or changes in diet or lifestyle. In addition to knowing one has helped understand dietary and physical activity habits and related health measures such as growth and laboratory tests, your child may be helping other children in Canada and the world. Participants themselves (and/or family member or friend) could potentially be a patient at a paediatric health centre, and benefit directly from the results obtained from this study.
Alternatives to Participation:
Participation in this study is completely voluntary, and declining to participate will in no way affect your care at this or any other health care facility.

Privacy and Confidentiality:
All persons involved in the study, including the study investigators and coordinators (hereby referred to as “study staff”), the study sponsor (Canadian Institute of Health Research), are committed to respecting your privacy. They will make every effort to keep your personal health information private and confidential in accordance with all applicable privacy legislations, including the Personal Health Information Protection Act (PHIPA) of Ontario.

Personal health information is any information that could be used to identify you and includes your:
• name,
• address,
• date of birth,
• new or existing medical records, that includes types, dates and results of medical tests or procedures.

Any personal identifying information (such as your child’s name) will be “de-identified” by replacing personal identifying information with a “study number”. The study coordinator and principal investigator here at St. Michael’s Hospital are in control of the study code key, which is needed to connect the study data to your child. The link between the study number and your child’s personal identity will be safeguarded by the St. Michael’s Hospital principal investigator.

We will respect your privacy. No information about who your child is will be given to anyone outside of the study or be published without your permission, unless required by law. All information collected during this study, including your child’s personal health information, will be kept confidential and will not be shared with anyone outside the study 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.

SickKids Clinical Research Monitors, employees of the funders (PORT), or the regulator may see your questionnaire responses or your child’s blood test results to monitor 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 at the Hospital for Sick Children. Only members of the research team (and maybe those described above) will have access to the data. This could include external research team members.

You or your child will not be named in any reports, publications, or presentations that may come from this study.

If you decide to withdraw from the study, the information that was collected before your child left the study will still be used. No new information will be collected without your permission, unless required for your child's safety.

Publication of Results
The results of this research study will be presented at various conferences, and will be published in scientific journals. Your name will not appear in any presentation or publication.
Costs to Participation and Reimbursement:
Participants in this study will not be reimbursed. Participation in this study may result in added costs (e.g. parking expenses) for which you will not be reimbursed for.

Compensation for Injury:
If your child suffers an injury from participating in this study, medical care will be provided to your child in the same manner as you would ordinarily obtain any other medical treatment. In no way does signing this form waive your legal rights nor release the study doctor(s), or involved institutions from their legal and professional responsibilities.

Participation and Withdrawal:
Participation in this study is totally voluntary. If you prefer for your child to not take part, you do not have to give a reason. You and your child will continue to have access to customary care at St. Michael’s Hospital or any other hospital you choose to visit. If you choose to take part in the study, but later change your mind you can withdraw participation at any time, without giving a reason and without any effect on the care that your child or your family may receive at St. Michael’s Hospital. If you decide to withdraw from the study, the information that was collected before your child left the study will still be used. No new information will be collected without your permission, unless required for your child’s safety.

New Findings or Information:
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.

Research Ethics Board Contact
If you have any questions regarding your rights as a research participant, you may contact the Chair of the Research Ethics Board at your hospital.
• St. Michael’s, 416-864-6060 ext 2557 during business hours.
• The Hospital for Sick Children, 416-813-5718

Study Contact
You may ask the study doctor and his/her staff any questions you may have about this study at any time.

If you have any questions or would like additional information please contact:
• Marina Khovratovich at 416-813-2129
• Dr. Jonathon Maquire, Principal Investigator at St. Michael’s Hospital at (416) 813-2129 (Mon-Fri: 9AM-5PM) or 416 864-5431 (Hospital Locating)
• Or Dr. Patricia Parkin, Overall Principal Investigator at the Hospital for Sick Children, at (416) 813-6933
Statement of Consent:

Study Title: TARGGet Kids! Measuring nutrition in young preschool-aged children in the primary care practice setting

By signing this form, I acknowledge that:
1) The study has been explained to me and all my questions have been answered to my satisfaction.
2) I have been informed of the alternatives to my child participating in this study, including the right not to participate and the right to withdraw my child from the study without compromising the quality of medical care at St. Michael's for me and for my other members of my family.
3) The potential risks, harms and discomforts have been explained to me and I also understand the benefits (if any) of participating in the research study.
4) I understand that I have not waived my legal rights (and the legal right's of my child) nor released the investigators, sponsors, or involved institutions from their legal and professional duties.
5) I know that I may withdraw, or in the future, any questions ask questions about the study.
6) I acknowledge that I may be asked to participate in future studies.
7) I have been assured that the records relating to me and my child's care will be kept confidential and that no information will be released or printed that would disclose personal identity without my permission, unless require by law.
8) I have been given sufficient time to read and understand the above information.

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

Name of Child Participant

Future Use of Study Data and Identifying Information
I consent that study data may be used for future studies without contacting me if all identifying information is removed so that the data cannot be identified as my child's (i.e. de-identified).

Yes No Initial ______

Communication With Your Child's Family Physician
Your family physician will be contacted if there are any abnormal results. Would you like us to communicate normal results of your testing with your family physician?

Yes No Initial ______

Printed Name of Parent/Legal Guardian Parent/Legal Guardian’s signature Date

I consent to providing my height, weight and waist circumference as required by the study.

Printed Name of Parent/Legal Guardian Parent’s/ Legal Guardian’s Signature Date

I have explained the study to the above participant explained the nature and purpose, the potential benefits, and possible risks associated with participation in this research study. I have answered all questions.

Name & Position of Person Obtaining Consent (Print) Signature of Person Obtaining Consent Date

Optional Consent Form A

Study Title: TARGGet Kids! Measuring nutrition in young preschool-aged children in the primary care practice setting
Accelerometry project for children between 1 and 5 years of age

Purpose of the Research
Within the TARGGet Kids network we would like to measure physical activity of children between 1 and 5 years of age. There is a growing evidence base for the association between physical activity and obesity in school aged children and only very few studies investigating physical activity levels and health measures in preschool aged children. TARGGet Kids! is currently collecting parent reported data on physical activity factors in preschool children and has a great opportunity to measure children’s physical activity in this age group through our already established platform. Accelerometry is the gold standard measure for physical activity in children, but has not been evaluated in the preschool age group.

Description of the Research
If you agree for your child to participate in the accelerometry part of the TARGGet Kids project your child will be provided with accelerometer - device that measures child’s physical activity. You will be asked to put it on your child during day and night for 1 full week (7 days). You will also be asked to record child waking and bed time, any time when the device was not on the child and reason for keeping it off. You will be provided with the information letter for yourself and child daycare or school (if needed). Information letter provides description on how accelerometer should be worn, instructions on how to return device back to the study team and study manager’s contact information in case questions arise during your child’s participation in our project.

Potential Harms, Discomfort, Inconvenience
The child might experience slight discomfort while wearing accelerometer, however device is small and has been approved to be used in both children and adults.

Potential Benefits
Upon completion of your child’s participation in accelerometry part of the TARGGet Kids study we will be providing you with daily steps count of your child, which is one of the indicators of physical activity in small children.

Alternatives to Participation
Participation in the accelerometry part of the project is optional. Declining to participate will in no way affect your care at this or any other health care facility or your participation in the TARGGet Kids study overall.

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

Name of Child Participant

Printed Name of Parent/Legal Guardian Parent/Legal Guardian’s signature Date

I have explained the study to the above participant explained the nature and purpose, the potential benefits, and possible risks associated with participation in this research study. I have answered all questions.

Name & Position of Person Obtaining Consent (Print) Signature of Person Obtaining Consent Date

Consent Form Version – TARGGet Kids! - main June 19, 2013 Page 7 of 8
Optional Consent Form B

Study Title: TARGet Kids! Measuring nutrition in young preschool-aged children in the primary care practice setting
Developmental assessments for children between 1 and 4.5 years of age

Purpose of the Research
Within the TARGet Kids network we have an opportunity to provide developmental assessment to our participants and to determine potential predictors for developmental delay.

Description of the Research
If you agree for your child to participate in this part of the project, you will be asked to return for an appointment with the TARGet Kids psychometrist. The appointment will be scheduled at the time convenient for you to attend. Developmental assessment takes approximately 1 hour and requires one of child’s parents or legal guardians to be present in the room.

Potential Harms, Discomfort, Inconvenience
You will be asked to return to your the clinic for one additional visit, which will take 1 hour of your time

Potential Benefits
Results of your child’s developmental assessment will be provided to you and your child’s physician with appropriate recommendations for follow up (if needed)

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

Name of Child Participant

Printed Name of Parent/Legal Guardian Parent/Legal Guardian’s signature Date

I have explained the study to the above participant explained the nature and purpose, the potential benefits, and possible risks associated with participation in this research study. I have answered all questions.

Name & Position of Person Obtaining Consent (Print) Signature of Person Obtaining Consent Date