VITAMIN D DEFICIENCY AS A NUTRITIONAL CHILD HEALTH DETERMINANT

by

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Abstract

**Vitamin D Deficiency as a Nutritional Child Health Determinant**

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**Objective:** This thesis aims to construct a framework for studying vitamin D deficiency in young Canadian children.

**Methods:** A practice based research network was created to collect vitamin D data from children 1-5 years of age in Toronto, Canada (TARGet Kids!). A cross-sectional pilot study was completed and a larger study proposed to determine the prevalence and predictors of low vitamin D.

**Results:** The prevalence of low vitamin D (<50nmol/L) in the pilot study was 32% (29/92, 95% CI: 22-42%). Using multivariable linear regression, lower vitamin D level was associated with lower milk volume, higher BMI and watching TV during snacks. A larger study involving 2400 children 1-5 years of age has been proposed.

**Interpretation:** Pilot data has suggested that 30-80% of toddlers in this setting have low vitamin D. A study to clarify these findings and form the basis of a large longitudinal vitamin D cohort has been proposed.
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Vitamin D Deficiency as a Nutritional Child Health Determinant

CHAPTER 1: BACKGROUND

The Determinants of Child Health

Considerable evidence has emerged in the past two decades to support the hypothesis that the potential to be healthy develops over the course of child development. Systematic differences in child health indicators have been reported for children by family income (1-4), parental education (5), family structure (6, 7), family conflict (8-10), maternal depression (11-16) and community violence (17-23) among others. Furthermore, there appears to be increased risk of poor health among children with multiple risk factors suggesting a dose response.(5, 24-27) Based on a survey of 102,353 parents of children 0-17 years of age, Halfon et al. have reported a 17 fold increase in risk of parental report of poor child health and 11 fold increased risk of overweight for children with ≥ 6 social risks relative to children with no risks.(5)

The Institute of Medicine has defined child health as, “the extent to which individual children or groups of children are able to or enabled to a) develop and realize their potential, b) satisfy their needs and c) develop the capacities that allow them to interact successfully with their biological, physical and social environments.”(28) As shown in Figure 1, this definition implies an inseparable connection between health and the innumerable biological, environmental, social and environmental factors that impact on a child’s health and development. Although much work has focussed on these influences on child health, surprisingly little attention has been paid to
nutritional factors and the interplay between nutrition and the other four major spheres of influence on child health.

*Child Nutrition as a Health Determinant*

There is growing evidence that systematic nutritional differences among young children may have lasting effects on health. The most well described example of this is iron deficiency in young children.

Iron deficiency has been a longstanding problem in developed and developing countries. It has been estimated that roughly 1/3 of toddlers and preschoolers in Canada have low iron stores.\(^{(29-31)}\) The most extensively studied cohort of children with iron deficiency comes from the work by Lozoff and colleagues. They studied the iron stores of a Costa Rican cohort of 185 children from infancy to adolescence to determine the effect of iron deficiency anemia (IDA) at 1-2 years of age on later health and development. They found that young children with IDA had significantly lower cognitive and motor test scores at baseline than children without iron deficiency anemia even after having anemia corrected.\(^{(32, 33)}\) When followed at 5, 11-14 and 15-19 years of age, the formerly iron deficient group consistently demonstrated a variety of verbal, cognitive and behavioural deficits relative to the non-iron deficient group without evidence of catch-up.\(^{(34-36)}\) Furthermore, the cognitive gap was widest among children from low-SES families implying an interaction between social and nutritional determinants of health.\(^{(35)}\)
In many ways, iron deficiency has provided a model for studying nutritional determinants of child health. First, iron deficiency is common in young childhood making it readily amenable to study using epidemiologic tools. Second, iron deficiency is preventable through dietary change so that population-based interventions hold promise to be effective. Third, iron deficiency has long-term health consequences making its prevention a potentially powerful means of improving population health.

Vitamin D Deficiency as a Potential Nutritional Determinant of Child Health

The same three characteristics (common, preventable and potential for later health consequences) may also be present in the context of vitamin D deficiency.

First, vitamin D deficiency as defined by the American Academy of Pediatrics (25-hydroxyvitamin D < 50 nmol/L) (37) is likely to be common in young childhood. Data from 133 children 6 to 23 months of age who were recruited from the Women, Infants and Children (WIC) programs in Alaska (58-61° N) suggested that 31% of children had 25-hydroxyvitamin D level < 62.5 nmol/L. (38) Ten percent of 84 breastfed 9 month old infants from Iowa City (41° N) were found to have 25-hydroxyvitamin D < 27.5 nmol/L. (39) In Boston (42° N), 12% of 365 mainly African American children age 8-24 months were found to have 25-hydroxyvitamin D < 50 nmol/L and 40% were < 75 nmol/L. (40) If these findings are generalizable to healthy young children in Toronto, Canada, vitamin D deficiency may be as common as iron deficiency.
Second, vitamin D deficiency in young childhood may be preventable. Several modifiable risk factors for vitamin D deficiency have been suggested in the literature for infants and adolescents. These include limited sun exposure, skin pigmentation, low milk intake and increased body mass index (BMI). However, little is known about risk factors for vitamin D deficiency in toddlers.

Third, vitamin D deficiency may have long-term health consequences. It has long been recognized that severe vitamin D deficiency (generally 25-hydroxyvitamin D level < 25nmol/L) results in rickets, an irreversible bowing of the long bones and deformity of the joints and teeth with well described long term implications on skeletal growth. While vitamin D fortification of cow’s milk and universal vitamin D supplementation of breast fed infants has dramatically reduced the prevalence of rickets in North America (estimated 2.9 cases per 100,000 in Canada), emerging population based cohort and case-control studies have suggested that less severe vitamin D deficiency (25-50nmol/L) in young childhood may be associated with other negative health outcomes.

Javaid et al. have reported that low circulating vitamin D levels (<27.5nmol/L) during the third trimester in 160 mothers in the UK was associated with significantly lower whole-body and lumbar spine bone-mineral content in their offspring at 9 years of age. Given the well documented correlation between maternal and infant vitamin D levels, lower vitamin D levels in utero, and likely in infancy, may impact on bone development raising concerns about
the potential impact of early low vitamin D on fracture risk during childhood as well as osteopenia and osteoporosis later in life.(61)

Hypponen et al., using data from a birth cohort of 12 058 children in Lapland, Finland, have suggested that children who were regularly supplemented with vitamin D in the first year had a reduced risk of developing type 1 diabetes relative to children who were not (relative risk 0.22 [95% CI 0.05-0.89]).(62) A recent meta-analysis of case-control studies on the same issue revealed a similar result [pooled odds ratio 0.71 (95% CI 0.6-0.84)].(63)

Vitamin D Measurement Issues

Several issues make measuring vitamin D in young children particularly challenging. First, vitamin D testing requires a blood sample. Blood is difficult to obtain from young children. Unlike in the United States, in Canada blood is not routinely taken at 1 year of age from healthy children for iron screening.(64) Several longitudinal studies have been launched by Health Canada including the Canadian Community Health Survey (65) and the Canadian Health Measures Survey (66) however neither have included serum sampling from children younger than 5 years of age. Thus, research that requires serum samples from young Canadian children must undertake to obtain such samples from individual children.

Second, measuring the vitamin D content of serum samples is also challenging. Vitamin D is a fat soluble 9,10-seco steroid which has a plethora of metabolites which have varying degrees of biological activity.(67) The two clinically relevant vitamin D metabolites are 25-hydroxyvitamin
D and 1,25-dihydroxyvitamin D which differ by one hydroxyl group (Figure 2). 1,25-dihydroxyvitamin D is the active form of vitamin D and its serum level is tightly regulated by plasma parathyroid hormone, serum calcium and phosphorous levels and is not reflective of vitamin D stores. However, circulating levels of 25-hydroxyvitamin D are thought to be reflective of vitamin D stores. To complicate matters further, 25-hydroxyvitamin D exists in two forms, 25-hydroxyvitamin D$_2$ and 25-hydroxyvitamin D$_3$ which can both be converted into biologically active 1,25-dihydroxyvitamin D.

A number of techniques have been developed to measure 25-hydroxyvitamin D. Early radioassays used a competitive binding protein which recognised both 25-hydroxyvitamin D$_2$ and 25-hydroxyvitamin D$_3$ to provide a total 25-hydroxyvitamin D level. However, due to the highly lipophilic nature of 25-hydroxyvitmain D, these assays have been shown to be sensitive to impurities contained in the serum sample which also bind to 25-hydroxyvitmain D. The radioimmunoassay is a refinement of this technique which uses an antibody co-specific for both 25-hydroxyvitmain D$_2$ and 25-hydroxyvitamin D$_3$ providing a total 25-hydroxyvitmain D level and is not as sensitive to competitive impurities. This method has been used for much of the work linking circulating 25-hydroxyvitamin D levels to various diseases.

More recently, advances in mass spectrometry have provided a direct method for determining levels of 25-hydroxyvitamin D in serum samples utilizing liquid chromatography followed by tandem mass spectrometry (LC-MS/MS). This method separately quantifies 25-hydroxyvitamin D$_2$ and 25-hydroxyvitamin D$_3$ which are added to quantify total 25-
hydroxyvitamin D. The LC-MS/MS method has been shown to be highly reproducible and, in general, is considered the gold standard method.(73) It is also becoming the analytic method of choice for clinical laboratories which are increasingly utilizing LC-MS/MS machines for clinical testing. Because of the aforementioned advantages, this method has been chosen for use in studies described in this thesis. However, it is slower, more costly and requires larger sample volumes than the radioimmunoassay.(73)

How these methods compare with each other is a highly contentious issue. Several studies have reported important discrepancies between results produced using different assays.(75-78) Theories to explain this variation have emerged which include variation in the assays’ abilities to equally detect 25-hydroxyvitamin D$_2$ and 25-hydroxyvitamin D$_3$, preparation of calibrants and the presence of 25-hydroxyvitamin D epimers which may interfere with some assays.(79, 80) The lack of an international vitamin D reference standard makes the comparison of the various methods very difficult.(76) Fortunately, vitamin D standard reference material is currently under development by the National Institute of Standards and Technology (NIST) using LC-MS/MS technology.(79) This reference standard will include various concentrations of 25-hydroxyvitmain D$_2$ and 25-hydroxyvitamin D$_3$ as well as samples containing 25-hydroxyvitamin D epimers and is expected to be available in the summer of 2009 (K. Phinney – personal communication).

The final difficulty with measuring vitamin D is that there is no consensus on what 25-hydroxyvitamin D serum level constitutes deficiency.(81, 82) Based on adult data supporting that parathyroid hormone levels and calcium reabsorption from bone are minimized at 25-
hydroxyvitamin D levels greater than 75 nmol/L, the Canadian Pediatrics Society has suggested that vitamin D levels in children below 75 nmol/L are insufficient. The American Academy of Pediatrics has suggested that serum 25-hydroxyvitamin D concentrations in infants and children should be above 50 nmol/L based largely on evidence that 400 IU per day of vitamin D is sufficient to maintain serum 25-hydroxyvitamin D above 50 nmol/L in exclusively breastfed infants.

In summary, nutritional indicators in young children fit well with the determinants of child health model. Vitamin D deficiency is likely to be a nutritional child health determinant because it may be common, preventable and may have lasting health consequences. To ameliorate such health consequences, we must first understand the prevalence of low vitamin D among young children as well as its determinants. However, this requires systematically measuring vitamin D levels as well as dietary and lifestyle exposures from young children. Several challenges to this exist including the difficulty in obtaining serum samples from young children, lack of consensus on what vitamin D level constitutes childhood deficiency and no consensus on the appropriate method for isolating 25-hydroxyvitamin D from serum.

*Thesis Objectives:*

The specific objectives of this thesis are:

1. Participate in the development of a community-based research platform to facilitate the collection of nutritional, behavioural and environmental data as well as serum samples
from healthy children 1-5 years of age (TARGet Kids!) in order to study vitamin D deficiency – Chapter 2.

2. Use TARGet Kids! to generate pilot data on the prevalence of vitamin D deficiency in urban Canadian toddlers and generate hypotheses regarding potentially modifiable risk factors for vitamin D deficiency in this population – Chapter 3.

3. Propose a larger study to determine prevalence, normative values and predictors of vitamin D deficiency using this pilot data and generate the foundation for a large long-term vitamin D cohort study – Chapter 4.
Figure 1: (28) The Determinants of Child Health

![Diagram showing the determinants of child health]

Institute of Medicine, 2004

Figure 2: (68) Vitamin D2 and D3 Structure

![Chemical structures of Vitamin D2 and D3]
CHAPTER 2:  
AN INTRODUCTION TO TARGET KIDS!

*The Need for a Primary Care Based Research Network*

Much has been written about the advantages of Primary Care Based Research Networks in the United States (PCBRN). The largest pediatric PCBRN is the American Academy of Pediatrics sponsored Pediatric Research in the Office Setting (PROS) which has been in existence since 1986 and includes over 1400 practitioners and 470 practice sites. Such networks have a well documented history of providing access to healthy, non-referred patient populations as well as the opportunity for large sample sizes and the possibility for follow-up of subjects in longitudinal studies.

In Ontario, the majority of children receive primary health care in community-based primary care physicians’ offices (pediatricians or family physicians). This is especially true for infants and toddlers, over 85% of whom see a physician for routine vaccination. Thus, physician’s offices would seem an ideal venue to collect data from young healthy children. As health is the reason parents bring their child to a doctor, primary care physicians are uniquely positioned to introduce parents and children to health research. Furthermore, as a result of their special relationship with their patients and families, primary care physicians may be highly successful at disseminating knowledge generated from such research.
Laboratory Measurement on Toddlers

Despite the demonstrated advantages of PCBRNs, no such network in Canada has been used to collect survey and laboratory data on healthy children. Other strategies have been tried. The Canadian Community Health Survey (2004) collected survey and anthropometric data on 2591 children under 4 years of age but no laboratory specimens were obtained(65). The Canadian Health Measures survey proposes to collect survey and blood samples from 1000 children older than 5 years of age using a travelling motorized laboratory.(66) There is no currently available infrastructure in Canada to facilitate the collection of laboratory data on large numbers of healthy toddlers.

TARGGet Kids Structure

In collaboration with the Childhood Obesity Research Team at the Hospital for Sick Children and The Canadian Laboratory Initiative on Pediatric Reference Intervals (CALIPER), the author along with Patricia Parkin and Catherine Birken have developed a PCBRN to determine the cross-sectional prevalence, predictors and health consequences of common early childhood nutritional disorders (including obesity, vitamin D deficiency and iron deficiency) in children 1 to 5 years of age. This network has been named TARGGet Kids! (Toronto Area Research Group). It is a partnership between the Pediatrics Outcomes Research Team and primary care providers
in the Section on Community Pediatrics of the Department of Pediatrics and the Department of Family and Community Medicine at the University of Toronto (see Figure 1).

The central unit of TARGet Kids! is the primary care group practice (see Figure 2). Participating group practices have been chosen for the following reasons:

1) They care for a population of children not previously represented within TARGet Kids! – either geographically or culturally.
2) They contain at least 3 physicians and see at least 5 children per day between 1 and 5 years of age.
3) They have interested and academically motivated clinicians.
4) They are located in Toronto.

For these reasons, the chosen TARGet Kids! participating group practices are:

Danforth Pediatrics
Clairhurst Pediatrics
Village Park Pediatrics
St. Joseph’s Hospital Family Medicine
Mount Sinai Hospital Family Medicine
St. Michael’s Hospital Family Medicine
Subject Recruitment and Data Management

Children are recruited, data collection instruments administered, anthropometric measurements taken and blood obtained all during the child’s doctor’s appointment by a trained research assistant and phlebotomist (see Figure 3). Laboratory samples are transported to and analyzed in a central laboratory (Hospital for Sick Children Core Laboratory) and survey data are collected centrally by the TARGet Kids! research co-ordinator (see figure 2). Standardized research assistant training, anthropometric measurement tool calibration and network oversight are also facilitated by the research co-ordinator.

Funding Opportunities

Funding to support the TARGet Kids! infrastructure has come from multiple sources each contributing to network costs and each benefiting from the existence of the network. Initial funding avenues have come from the CIHR through a Childhood Obesity Team Grant, the Hospital for Sick Children Foundation through the Pediatrics Outcomes Research Team (PORT), the CALIPER initiative and the Dairy Farmers of Ontario for vitamin D related research.

Timeline

Over the previous 2 years, the author has participated in obtaining start-up finding, recruiting practices and imbedding TARGet Kids! infrastructure. Preliminary data is now available. Publication of initial results and knowledge translation activities are foreseen in the third year (see figure 4).
Figure 1: TARGGet Kids! Partners

Warren McIsaac
Patricia Parkin  Catherine Birken  Jonathon Maguire

Figure 2: TARGGet Kids! Structure
Figure 3: TARGet Kids! Patient Flow

Subject Recruitment

Information package mailed to patient 2-3 weeks prior to routine well child doctor’s appointment

Research assistant approaches parent → Informed consent

Nutritional surveys administered to parent

Anthropometric measurement & blood collection

Well child MD visit

Figure 4: TARGet Kids! Timeline

Timeline

Funding

Network Creation

Data Collection

Publication

0 6 months 12 months 24 months

• TSUF
• DFO
• DPLM
• PORT
• CIHR

• Recruit practices
• Hire personnel
• Embed infrastructure

Refine Questions

Knowledge Translation
CHAPTER 3:
PREVALENCE AND PREDICTORS OF LOW VITAMIN D
IN URBAN CANADIAN TODDLERS - A Pilot Study

(Submitted to CMAJ – May 2009)

Introduction

The role of vitamin D for skeletal development and maintenance of normal serum
calcium levels is well established.(54) In its severest form, vitamin D deficiency in
childhood results in rickets.(53) Recent population-based cohort and case-control studies
suggest that suboptimal vitamin D status in childhood is also associated with several
chronic medical conditions later in life including low bone mass,(57, 61, 88) type 1
diabetes,(62, 63, 89-92) multiple sclerosis,(93-95) and a number of cancers.(96-102)

It is well established that 25-hydroxyvitamin D serum levels above 50 nmol/L in children
are sufficient to prevent rickets.(103) The American Academy of Pediatrics (AAP)
suggests that 25-hydroxyvitamin D levels in children should be above 50 nmol/L,
whereas, the Canadian Paediatric Society (CPS) has suggested that 25-hydroxyvitamin D
levels be above 75 nmol/L based on extrapolation from adult data.(43)

There is emerging evidence that young children in North America have vitamin D levels
significantly lower than either the AAP or CPS recommendations. Studies from three
regions in the United States suggest that 10 to 40 percent of infants are Vitamin D
insufficient.(38-40) Vitamin D levels of young urban Canadian children are not known.
With limited cutaneous production of vitamin D between November and March (52) and an annual incidence of rickets of 2.9 cases per 100,000 children (55), there is some concern that vitamin D levels in urban Canadian children may be lower than recommended.

In addition, while efforts have focused on the vitamin D status of breastfed infants in the first year of life, little is known about the vitamin D status of older infants as they transition from breast or formula feeding to a diet containing solid foods.

The primary objective of this study was to determine the prevalence of low vitamin D in a cohort of healthy two year old urban Canadian children during the winter and spring. A secondary objective was to explore biological, dietary and life-style factors related to the transition from breast or formula feeding to solid foods and their relationship to low vitamin D levels.

**Methods**

*Subjects and Design*

A cross-sectional study was performed with concurrent measurement of dietary and lifestyle exposures as well as 25-hydroxyvitamin D by blood test.
Healthy children between 24 and 30 months of age were recruited from a 3 physician (CT, SJ and MP) community-based pediatric group practice located in downtown Toronto, Canada (latitude 43.4° N) during a well child visit between November 2007 and May 2008. This practice is hospital and university affiliated and provides primary care for approximately 10,000 children.

Children included in the study had previously participated in a randomized controlled trial designed to evaluate the effectiveness of a bottle weaning educational intervention at 9 months of age.(104) As part of this trial, all parents received nutritional education at their child’s 9 month well child visit according to recommendations by the Canadian Paediatric Society and Health Canada.(105) Parents of children in the intervention group received education regarding the health benefits of bottle cessation and a step-wise protocol to discontinue bottle use. Standardized data collection at 9, 15 and 24 months of age as well as blood work at 24 months of age were components of this trial. Exclusion criteria were birth weight less than 2kg and acute or chronic illness (except for asthma).

Outcome variables

The primary outcome was prevalence of low vitamin D. Two estimates were calculated, based on the recommendations of the AAP (25-hydroxyvitamin D < 50 nmol/L)(37) and the CPS (25-hydroxyvitamin D < 75 nmol/L).(43) Blood was obtained at the time of the well child visit, and samples were transported daily on ice to the Biochemistry Laboratory at the Hospital for Sick Children. Serum samples were analyzed for 25-
21

hydroxyvitamin D as well as other measures of bone turnover including alkaline phosphatase and calcium.

25-Hydroxyvitamin D was measured using liquid chromatography tandem mass spectrometry which has been shown to compare favorably with radioimmunoassay methods for children older than 1 year of age. One instrument (Applied Biosystems 4000 Q TRAP® LC/MS/MS system) was used for all samples and the instrument was regularly calibrated according to the Vitamin D External Quality Assessment Scheme (DEQAS) which is an internationally recognized protocol. Intraassay imprecision was 3.2% and interassay imprecision was 6.8% at 135 nmol/L 25-hydroxy vitamin D. The assay was not calibrated against National Institutes of Health vitamin D reference standard NIST 972 because it was not available at the time.

Exposure variables

Data on factors that might influence vitamin D status were collected using a standardized data collection form completed by parents. These included: age, sex, birth weight, month of vitamin D collection, ethnicity, maternal age and education level, duration of breastfeeding, vitamin D supplementation when breast fed, cups of cow’s milk and juice daily (1 cup = 250mL), bottle use, daily multivitamin use, daily snacking (consumption of chips or sweets or > 2 glasses of pop daily), screen viewing time, meals or snacks with the TV on, weekly time outdoors, sun screen use and skin pigmentation using the Fitzpatrick scale. Nutritional survey questions were adapted from the Canadian
Community Health Survey. Body Mass Index (BMI) was calculated as weight in kilograms divided by height in meters squared. Weight was measured using a precision digital scale (±0.025% SECA, Hamburg Germany) and standing height was measured using a stadiometer (SECA, Hamburg Germany).

**Statistical analysis**

The prevalence of low vitamin D (based on the AAP and CPS definitions) was estimated along with 95% confidence intervals. Data were analyzed using SAS 9.0 for Windows (SAS Institute, Cary, NC). Fisher’s exact test and Student’s t-test were used to test for associations between low vitamin D status and categorical and continuous variables, respectively. Variables associated with low vitamin D at p values < 0.1 on univariate analysis were incorporated into a multi-variable logistic regression model. For the purpose of this analysis, the most conservative cut-off for vitamin D (25-hydroxyvitamin D < 50nmol/L) was used as the binary outcome. In addition, multi-variable linear regression modeling was used to assess for associations between potential predictor variables and vitamin D level (as a continuous measure).

This study was approved by the Research Ethics Board at the Hospital for Sick Children, Toronto, Canada and parents of all participating children consented to participate in the study.
Results

Study population

A total of 126 children met inclusion criteria between November 2007 and May 2008. Of these, 26 parents did not consent (21%) and 9 had insufficient blood quantity (7%). Therefore, 91 children (72%) had anthropometric data, survey data and blood work completed and were included in the final analysis. Child age ranged from 22.6 to 29.9 months (median 24.3 months) with 52% being male. Maternal age ranged from 21 to 44 years (median 34 years) and 86% of mothers reported having college or university education. Approximately 74% of mothers reported their ethnicity as Canadian, American or European, 13% as Latin American, Cuban or Portuguese, 9% as Asian or South East Asian and 4% as African or Caribbean.

Prevalence of low vitamin D

Median vitamin D level was 60 nmol/L with a range from 20 nmol/L to 126 nmol/L. Low vitamin D (<50 nmol/L) was found in 29 of 91 children (32%, 95% CI: 22%-42%). One child had a vitamin D level less than 25 nmol/L (1%, 95% CI: 0%-6%) and 75 of 91 children had vitamin D level less than 75nmol/L (82%, 95% CI: 73%-90%). Of note, there was no difference in median serum 25-hydroxy vitamin D between children in the intervention group compared with children in the control group of the previously conducted randomized controlled trial (59 nmol/L vs. 60 nmol/L, p=0.48).
Variables associated with low vitamin D

Comparison of demographic characteristics and blood indices between children with 25-hydroxyvitamin D concentrations <50 nmol/L versus ≥50 nmol/L are found in Tables 1 and 2 respectively. Children with low vitamin D were slightly younger (median 24.2 vs 24.4 months, p = 0.01), more likely to consume a smaller daily volume of cow’s milk (median 1.5 cups vs. 1.75 cups, p=0.05), have the TV on during snacks (10/29[37%] vs. 10/62[18%], p=0.05) and have higher BMI (median BMI 16.6 vs. 15.6 kg/m², p= 0.05).

Multivariable analysis

Results of the logistic regression analysis adjusted for slight differences in child age are shown in Table 3. Lower cow’s milk intake, higher BMI and snacking with the TV on were independently associated with low vitamin D.

These variables were also incorporated into a multiple linear regression model adjusted for age. Higher daily cow’s milk intake was associated with higher vitamin D level (6.9 nmol/L increase per cup of milk; 95% CI: 1.8-12.1), higher BMI was associated with lower vitamin D level (4.0 nmol/L decrease per kg/m²; 95% CI: 1.4-6.6) and snacking with the TV on was associated with lower vitamin D level (8.5 nmol/L decrease for children who snack with the TV on; 95% CI: 0.7 – 16.4).
**Interpretation**

This cross sectional study has shown that 32% of a cohort of healthy two year old urban children in Toronto, Canada had 25-hydroxyvitamin D levels below that recommended by the AAP (<50 nmol/L) and 82% had levels below that recommended by the CPS (<75 nmol/L) during the winter and spring. With regard to modifiable risk factors associated with low vitamin D, our findings suggest that lower cow’s milk intake, higher BMI and watching TV while snacking are associated with low vitamin D (25-hydroxyvitamin D < 50 nmol/L).

Other reports of the vitamin D status of young children in North American have come from various regions of the United States. Thirty one percent of 133 children 6 to 23 months of age recruited from the Women, Infants and Children (WIC) programs in Alaska (58-61° N) were found to have 25-hydroxyvitamin D < 62.5 nmol/L.(38) Ten percent of 84 breastfed 9 month old infants from Iowa City (41° N) were found to have 25-hydroxyvitamin D < 27.5 nmol/L.(39) In Boston (42° N ), 12% of 365 mainly African American children age 8-24 months were found to have 25-hydroxyvitamin D < 50 nmol/L and 40% were < 75 nmol/L.(40)

The prevalence of low vitamin D found in this cohort in Toronto is higher than reported for infants and toddlers in Boston (40) and Iowa (39) and similar to infants and toddlers in Alaska (38). This difference does not appear to be a function of latitude as Toronto, Canada is only 1.5 degrees north of Boston and 2.5 degrees north of Iowa but at least 14
degrees south of Alaska. This difference may be a function of season as we chose to measure vitamin D during the winter and spring and our data showed a trend towards lower vitamin D during the winter as compared with the spring (Figure 1). However, others have reported a paradoxical relationship between 25-hydroxyvitamin D and season in infants and toddlers with lower levels found April through September as compared with October through March. Differences in population may explain the difference; however, children in this study had lighter skin pigmentation and higher socioeconomic status as compared with children from Boston and Iowa, two factors thought to be associated with higher vitamin D levels. (39-41)

We suggest that age may partly be responsible for the higher prevalence of low vitamin D in this cohort. While Canadian guidelines support supplementation of breast fed infants with 400 IU of vitamin D per day during the first year of life (43, 105), the discontinuation of vitamin D supplementation when transitioning to low vitamin D containing solid foods may have a negative impact on vitamin D status as vitamin D dietary intake decreases resulting in a rebound of low vitamin D in the second and third years of life. Our finding that cow’s milk intake was a significant moderator of low vitamin D is consistent with this hypothesis as vitamin D fortified cow’s milk is likely the major dietary source of vitamin D for young children. (50) Others have reported a positive association between volume of cow’s milk intake and vitamin D in toddlers. Gordon et al. found a 7.75 nmol/L increase in 25-hydroxyvitamin D per cup of cow’s milk in toddlers 12 to 24 months of age which is similar to our finding of 6.9 nmol/L increase per cup. (40)
The inverse association between BMI and vitamin D that we report is consistent with that reported for adults, adolescents and toddlers.\textsuperscript{(40, 42, 47, 49, 110, 111)} It has been proposed that increased adiposity may decrease vitamin D bioavailability because of deposition of this fat soluble vitamin in fatty tissues.\textsuperscript{(111)} Interestingly, a recent report of a lack of association between BMI and 25-hydroxyvitamin D in young infants underscores the difference between infants and older children.\textsuperscript{(40)}

Our finding of an independent association between low vitamin D and snacking in a room with the television on is surprising given that eating meals with the TV on, daily screen time and snacking were not associated with low vitamin D. This variable may be a proxy measure for multiple factors which negatively influence vitamin D status such as reduced outdoor time, increased screen time and inactivity, and a vitamin D deficient diet.

Others have reported an association between low vitamin D, skin pigmentation and outdoor time in adolescents and breastfed infants.\textsuperscript{(38, 41, 47, 55, 59, 112)} Our data were not supportive of these associations which may indicate that low solar UVB radiation during winter and spring \textsuperscript{(52)} has blunted the effect of skin pigmentation and outdoor time on vitamin D.

This study is unique because it focused on a population of healthy (primarily Caucasian) children of educated mothers. The sampling of children at two years of age also allowed us to capture the nutritional effects of the transition from exclusive breast or formula
feeding to solid foods. It does, however, have a number of limitations. First, cross-
sectional designs can identify associations but cannot determine causality. Second, this
population is not generalizable to all urban Canadian children as it was primarily a
population of children with lightly pigmented skin, and born to highly educated mothers.
Third, children in this study had been enrolled in a randomized controlled trial at 9
months of age raising the possibility that the parental educational intervention to reduce
bottle use may in some way have influenced vitamin D status or variables associated with
low vitamin D. However, vitamin D status was the same in both intervention and control
groups and findings in the multivariable analysis did not differ when the intervention and
control groups were analyzed separately. Fourth, our convenience sample resulted in
relatively large confidence intervals around the prevalence point estimates. However, to
narrow the 95% confidence intervals to ± 5% would have required a sample size of
roughly 250 children. Lastly, with the exception of BMI, exposure variables were
collected by parental report which has well documented limitations.(113-119)

This study suggests that 2 year old urban Canadian children are at particular risk of
wintertime vitamin D levels below those recommended by the American Academy of
Pediatrics and the Canadian Paediatric Society. It also suggests that young children rely
on fortified cow’s milk to maintain their vitamin D status and that having higher BMI
makes this more difficult. These findings support recent American Academy of
Pediatrics recommendations for vitamin D supplementation beyond breastfeeding and
through childhood.(37)
Data are given as number (percentage) of each group unless otherwise indicated
†Data are given as median (min-max)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>25-OH Vit D &lt; 50 nmol/L</th>
<th>25-OH Vit D ≥ 50 nmol/L</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (months)†</td>
<td>24.2 (22.6-25.7)</td>
<td>24.4 (23.1-29.9)</td>
<td>0.01</td>
</tr>
<tr>
<td>Male sex</td>
<td>15 (52%)</td>
<td>32 (52%)</td>
<td>0.99</td>
</tr>
<tr>
<td>Season of visit</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>November – February</td>
<td>23 (79%)</td>
<td>38 (62%)</td>
<td>0.15</td>
</tr>
<tr>
<td>March - May</td>
<td>6 (21%)</td>
<td>23 (38%)</td>
<td></td>
</tr>
<tr>
<td>Birth weight (g) †</td>
<td>3364 (2140-4045)</td>
<td>3225 (2600-4578)</td>
<td>0.96</td>
</tr>
<tr>
<td>Maternal age (years) †</td>
<td>37 (23-44)</td>
<td>34 (21-42)</td>
<td>0.30</td>
</tr>
<tr>
<td>Maternal post secondary education</td>
<td>25 (86%)</td>
<td>53(85%)</td>
<td>0.99</td>
</tr>
<tr>
<td>Cow’s milk intake† [cups (250ml) daily]</td>
<td>1.5 (0.13-2.5)</td>
<td>1.75(0.44-3.13)</td>
<td>0.05</td>
</tr>
<tr>
<td>Juice intake† [cups (250ml) daily]</td>
<td>0.75 (0.13-2)</td>
<td>0.5 (0.13-2.5)</td>
<td>0.72</td>
</tr>
<tr>
<td>Bottle use</td>
<td>5 (19%)</td>
<td>17 (30%)</td>
<td>0.30</td>
</tr>
<tr>
<td>Daily snacking</td>
<td>14 (52%)</td>
<td>29 (51%)</td>
<td>0.99</td>
</tr>
<tr>
<td>Weight (kg) †</td>
<td>13 (10.4-14.5)</td>
<td>12.1 (10.3-17)</td>
<td>0.54</td>
</tr>
<tr>
<td>BMI (kg/m²) †</td>
<td>16.6 (13.3-18.8)</td>
<td>15.6 (12.8-19.3)</td>
<td>0.05</td>
</tr>
<tr>
<td>Daily screen time (minutes) †</td>
<td>60 (7-206)</td>
<td>42 (0-189)</td>
<td>0.32</td>
</tr>
<tr>
<td>TV on during meals</td>
<td>6 (22%)</td>
<td>12 (21%)</td>
<td>0.99</td>
</tr>
<tr>
<td>TV on during snacks</td>
<td>10 (37%)</td>
<td>10 (18%)</td>
<td>0.05</td>
</tr>
<tr>
<td>Breast feeding at 9 months Without D supplementation</td>
<td>13 (45%)</td>
<td>30 (48%)</td>
<td>0.65</td>
</tr>
<tr>
<td></td>
<td>4 (31%)</td>
<td>6 (20%)</td>
<td>0.70</td>
</tr>
<tr>
<td>Multivitamin use</td>
<td>6 (22%)</td>
<td>21 (37%)</td>
<td>0.22</td>
</tr>
<tr>
<td>Outdoor time (hours/week)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 - 4</td>
<td>16 (59%)</td>
<td>29 (51%)</td>
<td>0.49</td>
</tr>
<tr>
<td>≥ 5</td>
<td>11 (41%)</td>
<td>28 (49%)</td>
<td></td>
</tr>
<tr>
<td>Always uses sunscreen</td>
<td>17 (63%)</td>
<td>31 (54%)</td>
<td>0.49</td>
</tr>
<tr>
<td>Skin pigmentation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-3 Light skin</td>
<td>23 (85%)</td>
<td>47 (82%)</td>
<td>0.99</td>
</tr>
<tr>
<td>4-6 Dark skin</td>
<td>4 (15%)</td>
<td>10 (18%)</td>
<td></td>
</tr>
</tbody>
</table>

Table 1: Subject Characteristics
Table 2: Laboratory Values

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>25-OH Vit D &lt; 50 nmol/L n=29</th>
<th>25-OH Vit D ≥ 50 nmol/L n=62</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium (mmol/L)</td>
<td>2.55 (2.43-2.76)</td>
<td>2.50 (2.32-2.7)</td>
<td>0.13</td>
</tr>
<tr>
<td>Alkaline Phosphatase (IU/L)</td>
<td>198 (101-132)</td>
<td>205 (132-2418)</td>
<td>0.18</td>
</tr>
</tbody>
</table>

Data are given as median (min-max)

Table 3: Multivariable logistic regression model for low vitamin D (<50 nmol/L)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Odds ratio (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cow’s milk intake*</td>
<td>0.42 (0.2-0.98)</td>
<td>0.04</td>
</tr>
<tr>
<td>(per 250ml cup daily)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI*</td>
<td>1.5 (1.01-2.3)</td>
<td>0.05</td>
</tr>
<tr>
<td>(per kg/m²)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TV on during snacks</td>
<td>3.6 (1.1-11.7)</td>
<td>0.04</td>
</tr>
</tbody>
</table>

*Odds ratio for continuous variables represents the increase in odds per unit predictor

Figure 1: Vitamin D vs. Appointment Date Scatter Plot
CHAPTER 4:
PREVALENCE AND PREDICTORS OF LOW VITAMIN D IN URBAN CANADIAN TODDLERS – A CROSS-SECTIONAL STUDY PROPOSAL
(Funding successfully obtained from the Dairy Farmers of Ontario – September 2008)

Introduction

The role of vitamin D for skeletal development and maintenance of normal serum calcium levels is well established.\(^{(54)}\) In its severest form, vitamin D deficiency in childhood results in rickets.\(^{(53)}\) Recent population-based cohort and case-control studies suggest that suboptimal vitamin D status in childhood is also associated with several chronic medical conditions later in life including low bone mass,\(^{(57, 61, 88)}\) type 1 diabetes,\(^{(62, 63, 89-92)}\) multiple sclerosis,\(^{(93-95)}\) and a number of cancers.\(^{(96-102)}\)

It is well established that 25-hydroxyvitamin D serum levels above 50 nmol/L in children are sufficient to prevent rickets.\(^{(103)}\) The American Academy of Pediatrics (AAP) suggests that 25-hydroxyvitamin D levels in children should be above 50 nmol/L, whereas, the Canadian Paediatric Society (CPS) has suggested that 25-hydroxyvitamin D levels be above 75 nmol/L based on extrapolation from adult data.\(^{(43)}\)

There is emerging evidence that young children in North America have vitamin D levels significantly lower than either the AAP or CPS recommendations. Studies from three regions in the United States suggest that 10 to 40 percent of infants are Vitamin D insufficient.\(^{(38-40)}\)
Furthermore, pilot data from our group in Toronto, Canada has suggested that 32% of 91 urban Canadian 2 year olds had levels below that recommended by the American Academy of Pediatrics and up to 81% were below the CPS recommended value.(120) With limited cutaneous production of vitamin D between November and March (52) and an annual incidence of rickets of 2.9 cases per 100,000 children (55), there is reason for concern that vitamin D levels in urban Canadian children may be lower than recommended.

In addition, while efforts have focused on understanding the vitamin D status of breastfed infants in the first year of life, little is known about risk factors for vitamin D deficiency in older infants and children as they transition from breast or formula feeding to a diet containing solid foods. Preliminary data has suggested that increase body mass index (BMI) and lower cow’s milk intake may be associated with vitamin D deficiency while it is unclear as to whether season, skin pigmentation, screen time and other dietary factors such as consumption of snack foods affect vitamin D status in this population.(40, 120)

Objective

This study aims to determine the prevalence of sub-clinical vitamin D deficiency in a socioeconomically and culturally diverse cohort of healthy urban Canadian children 1-5 years of age as well as to generate normative values for vitamin D by age and season. A secondary aim is to clarify what effect BMI, cow’s milk intake, season, skin pigmentation, screen time and snacking have on the risk of vitamin D deficiency.
**Significance**

Understanding what vitamin D levels define the range of normal (5\textsuperscript{th} – 95\textsuperscript{th} percentile) for this population of young children will assist clinicians in determining at what vitamin D level interventions aimed at increasing vitamin D should be targeted. Furthermore, the identification of risk factors for vitamin D deficiency will assist clinicians, parents and policy makers with identifying which children are at risk of low vitamin D.

**Methods**

**Study Design**

A cross-sectional study with concurrent measurement of dietary, lifestyle, environmental and anthropometric exposures as well as 25-OH vitamin D level is proposed. Vitamin D deficiency will be defined as 25-hydroxy vitamin D level less than 25 nmol/L.(37)

**Study Population**

The target population will be healthy children 1-5 years of age living in Toronto who are attending a routine well child visit with a primary care physician. This study will recruit children from 5 large medical practices within TARGet Kids! research network. TARGet Kids! (Toronto Area Research Group for Kids) is a multi-disciplinary community based research network of collaborating primary care pediatricians and family physicians from the University of Toronto
Faculty of Medicine. Under the mandate of “health research for every child”, this network collects medical evidence on common health problems affecting urban Canadian children. Suitable children will be identified from the administrative database of their primary care physician one month prior to their 1, 2, 3, 4 and 5 year old well child doctor’s visit and an information package will be sent to each family. Families will be approached to participate during their corresponding doctor’s visit. Serum samples as well as parent completed standardized data collection instruments will be collected at the time of the well child visit.

*Inclusion criteria*

Healthy children between 1 and 5 years of age will be recruited. This age was chosen to capture the nutritional effects of the transition from breast or formula feeding to solid foods and their relationship to low vitamin D levels.

*Exclusion criteria*

Children with chronic illness with exception of asthma will be excluded. Children on medications known or suspected to affect vitamin D level or bone development will also be excluded. These include anti-seizure medications, systemic corticosteroids and anti-neoplastic medications. Children will be screened at the time of enrolment for exclusions.

*Outcome variables*
The primary exposure variable will be vitamin D deficiency which will be defined as 25-hydroxyvitamin D serum level less than 50 nmol/L as defined by the American Academy of Pediatrics. Under the direction of Dr. Khosrow Adeli who is an internationally recognized expert in pediatric clinical biochemical measurement and is Division Head of Clinical Biochemistry at the Hospital for Sick Children, total 25-hydroxy vitamin D will be measured from serum samples using isotope-dilution liquid chromatography-tandem mass spectrometry (LC/MS/MS) which has been validated for measurement of 25-hydroxyvitamin D in children older than 1 year of age.

The specific instrument that will analyze all samples is an Applied Biosystems 4000 Q TRAP LC/MS/MS machine. Extensive testing and validation of this machine has been performed against the radioimmunoassay (RAI) method. This testing has demonstrated an intraassay imprecision of 3.2% at a concentration of 135 nmol/L and an interassay imprecision of 11.9% at 24nmol/L, 6.8% at 135nmol/L and 4.9% at 390nmol/L which is well within acceptable limits for biochemical measurements. During this study, the instrument will be monitored using the UK DEQUAS external quality assessment scheme which is an internationally recognized vitamin D quality assessment protocol. This assay will also be calibrated against an NIH vitamin D reference standard (NIST 972).

Samples will be run in batches to minimize measurement variation by a single technologist (Man Khun Chan MSc, MLT) in the Division of Clinical Biochemistry, Hospital for Sick Children. Duplicate measurements using LC/MS/MS as well as the RAI method will be obtained on 10% of samples to determine actual measurement variation.
Additional blood tests as descriptors of bone metabolism will include: parathyroid hormone, alkaline phosphatase, calcium, phosphate and albumen as descriptors of bone metabolism. Total blood requirement per sample to run all tests (including 25-hydroxyvitamin D duplicates as well as parathyroid hormone, alkaline phosphatase, calcium, phosphate and albumin) is less than 3ml.

*Exposure variables*

Numerous descriptor variables will be collected to explore dietary, environmental, behavioral and anthropometric influences on vitamin D status using a standardized data collection instruments completed by each child’s parent.

The study instrument (Appendix 1) was adapted from the Canadian Community Health Survey ([http://www.statcan.gc.ca/concepts/health-sante/index-eng.htm](http://www.statcan.gc.ca/concepts/health-sante/index-eng.htm)). For both cases and controls the following descriptor variables will be collected: Age, sex, birth weight, month of vitamin D collection, health problems, medication use, country of birth, postal code, parental education level, dietary variety, breast feeding history, cow’s milk, yogurt and cheese intake, formula use, junk food and soda intake, vitamin D supplementation, multivitamin use, sun exposure and sun block use.

Skin pigmentation will be quantified by using the Fitzpatrick scale which is a validated skin pigmentation scale (107, 122) that has been used for quantification of skin pigmentation in young children (40, 120)).
Dietary variety and nutrition risk will be quantified using Nutristep\textsuperscript{TM}, a validated nutritional screening tool.(123)

Body mass index (BMI) will be calculated as weight in kilograms divided by height squared.(108, 109) Each child’s weight will be measured using a precision digital scale (±0.025% SECA, Hamburg Germany) calibrated weekly by kilogram weights. Standing height will measured using a weekly calibrated stadiometer (SECA, Hamburg Germany) for children older than 2 years of age or a length board for children younger than 2 years.

Child temperament and parenting style will be quantified using the Childhood Behaviour Questionnaire (CBQ). The CBQ is a validated questionnaire designed to assess temperament and parenting style in young children.(124)

Procedure

Children will be prospectively enrolled at the time of presentation for a 1, 2, 3, 4 or 5 year routine well child primary care physician visit between January 1, 2009 and December 31, 2010. Data collection instruments will be administered and checked for completeness by a trained research assistant at that time. 4ml of blood will be drawn by venipuncture following the physician visit. Specimens will be appropriately transported to the Department of Pediatric Laboratory Medicine at the Hospital for Sick Children twice daily for analysis.
The same standardized protocol will be used for administration of the data collection instruments across all TARGet Kids! sites. The scales and stadiometers used at all sites will be calibrated using the same calibration scheme which will include duplicate measurements on the 1st 4 children seen each day and weekly calibration of scales using kilogram weights. Every effort will be undertaken to ensure that data is collected in an identical fashion across all sites.

**Statistical analysis**

The primary outcome will be the prevalence of vitamin D deficiency (25-hydroxyvitamin D<25 nmol/L) along with 95% confidence intervals for each of 5 age groups (1, 2, 3, 4 and 5 years). Vitamin D level (as a continuous variable) will also be described by age group and season.

Secondary outcomes will include testing associations that were suggested in our pilot study between vitamin D deficiency and age, season, skin pigmentation, BMI, daily snacking, screen time and milk intake. Fisher’s exact test and Student’s t-test will be used to test for associations between vitamin D deficiency and categorical and continuous variables, respectively. Statistical significance will be adjusted for multiple hypotheses testing using the Bonferroni correction.

An exploratory analysis will be undertaken to assess associations between vitamin D deficiency and other measured exposure variables.

To assess for confounding and interaction between variables, variables associated with low vitamin D at p values < 0.2 on univariate analysis will be incorporated into a multi-variable
logistic regression model using the binary outcome (25-hydroxyvitamin D < 50nmol/L). In addition, step-wise multi-variable linear regression modeling will be used to assess for associations between these variables and vitamin D level (as a continuous measure).

**Sample size**

Pilot data from our group has informed us that the prevalence of vitamin D deficiency in children at 2 years of age in Toronto is 32% (95% CI 22%-42%). Using an assumed prevalence of vitamin D deficiency of 20% ($\alpha=0.05$, $\beta=0.2$), 480 children in each of 5 age groups (2400 total) would estimate the population prevalence of vitamin D deficiency in each age group to ±5%. Using Bonferroni’s correction for testing 7 hypothesis (p<0.007), this sample size (2400 children) would provide sufficient power (80%) to detect differences in exposures between those children with and without vitamin D deficiency with a minimum absolute difference of 8%.

**Feasibility**

On average 20 children aged 1 to 5 years are seen weekly in each practice x 48 weeks x 5 practices = 4800 children. Assuming that 50% of these children can be recruited (data from our pilot study for this study demonstrated a 72% recruitment rate (120)) and equal enrolment in each age category (1, 2, 3, 4, and 5 years of age), 2400 children would be recruited over 1 year.
Timeline

<table>
<thead>
<tr>
<th>Date</th>
<th>Events</th>
</tr>
</thead>
<tbody>
<tr>
<td>September 1, 2008</td>
<td>Commencement of project</td>
</tr>
<tr>
<td>November 1, 2008</td>
<td>Preparation of data collection forms</td>
</tr>
<tr>
<td></td>
<td>Preparation of patient recruitment material</td>
</tr>
<tr>
<td></td>
<td>Recruitment of phlebotomists and research assistants</td>
</tr>
<tr>
<td>December 1, 2009</td>
<td>Training of research assistants</td>
</tr>
<tr>
<td></td>
<td>Beta testing of study questionnaire</td>
</tr>
<tr>
<td></td>
<td>Orientation for participating medical practices</td>
</tr>
<tr>
<td>January 1, 2009</td>
<td>Commencement of patient recruitment</td>
</tr>
<tr>
<td>December 31, 2009</td>
<td>Completion of patient recruitment</td>
</tr>
<tr>
<td>March 1, 2010</td>
<td>Completion of data entry</td>
</tr>
<tr>
<td>June 1, 2010</td>
<td>Completion of data analysis</td>
</tr>
<tr>
<td>August 31, 2010</td>
<td>Preparation of abstracts for Pediatric Academic Societies Meeting (PAS)</td>
</tr>
<tr>
<td></td>
<td>and Canadian Pediatric Society (CPS) Annual Meeting</td>
</tr>
<tr>
<td>May/June 2011</td>
<td>Presentation at PAS and CPS annual meetings.</td>
</tr>
<tr>
<td></td>
<td>Manuscript completion and submission to prestigious medical journal.</td>
</tr>
</tbody>
</table>

Ethics

Informed consent will be obtained from each child’s parent prior to enrolment. Blood test results will be reported to each child’s primary care provider. This study has been approved by the Research Ethics Board at the Hospital for Sick Children, Toronto, Canada. (Appendix 2).
## Budget

### Budget Information For All Years of Support Requested

<table>
<thead>
<tr>
<th>Description</th>
<th>1st Year</th>
<th>2nd Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Personnel (Salaries, Fringe Benefits, etc.) – Research Assistants (1.85 FTE)</td>
<td>$33,500</td>
<td>$33,500</td>
</tr>
<tr>
<td>Phlebotomy</td>
<td>$9,000</td>
<td>$9,000</td>
</tr>
<tr>
<td>Supplies</td>
<td>$3,000</td>
<td>$3,000</td>
</tr>
<tr>
<td>Laboratory costs</td>
<td>$36,000</td>
<td>$36,000</td>
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<tr>
<td>Database development and management</td>
<td>$5000</td>
<td>$0</td>
</tr>
<tr>
<td>Data entry</td>
<td>$4,500</td>
<td>$4,500</td>
</tr>
<tr>
<td>Statistical analysis</td>
<td>$0</td>
<td>$3,000</td>
</tr>
<tr>
<td>Annual Total Costs</td>
<td>$91,000</td>
<td>$89,000</td>
</tr>
<tr>
<td>Amount Requested</td>
<td>$45,500</td>
<td>$44,500</td>
</tr>
</tbody>
</table>

### Budget Justification:

**Personnel:**
- Phlebotomist – to take venous blood samples - 1 sample per patient x 2400 @ $7.50/sample = $18,000
- Research assistants (1.85 Full time equivalents) – patient recruitment & data collection – 40 min per patient (avg) x 4800 pts approached @ $20.69/h = $67,000

**Supplies:**
- Sample tubes, needles, paper, stamps, printing costs - $2.50 per patient x 2400 = $6,000

**Laboratory costs:**
- $30 per sample x 2400 = $72,000

**Data management and statistical support:**
- Database management – Microsoft Access database creation = $5,000
- Data entry – 9 min per patient x 2400 @ $25/h = $9,000
- Data analysis – biostatistics support – 40h @ $75/h = $3,000

Total project cost = $180,000
Fifty percent of the funding for this project ($90,000) will come from the Canadian Institutes of Health Research through a grant for the CALIPER project (Canadian Laboratory Initiative on Pediatric Reference Interval Database) – Principal investigator Dr. M. Adeli, Department of Pediatric Laboratory Medicine, Hospital for Sick Children.

The main objective of the CALIPER project is to develop a database of patient demographics and laboratory results for pediatric biochemistry tests that are in urgent need of having accurate reference intervals established for the entire pediatric age range from birth to 18 years old. Critical gaps currently exist in accurate and up-to-date pediatric reference intervals (normal values) of laboratory tests performed in children and adolescents. These critical gaps in reference intervals have the clear potential of contributing to erroneous diagnosis or misdiagnosis of many diseases of childhood and adolescence. The database will be utilized to derive comprehensive reference intervals based on specific criteria including age and gender. The reference intervals will be shared among pediatric hospital laboratories across Canada. The major benefit of the CALIPER study is an accurate and reliable determination of what is "normal" considering a child's age and gender.
CHAPTER 5: DISCUSSION, FUTURE RESEARCH & CONCLUSIONS

Discussion:

This thesis has outlined an approach to studying vitamin D as a nutritional child health determinant using a model suggested by the study of iron deficiency in young children.(29-36) It has accomplished the three main objectives as outlined in Chapter 1: Participation in the development of a community-based research network to facilitate vitamin D data collection on healthy young children (Chapter 2); acquisition of pilot data on the prevalence of vitamin D deficiency among Canadian toddlers (Chapter 3); and the preparation of a larger cross-sectional study based on this pilot data to determine the prevalence and predictors of vitamin D deficiency among young Canadian children (Chapter 4).

This thesis documents the author’s participation in the development of TARGet Kids!, a novel practice based research network. Although other pediatric practice based research networks exist in North America, to our knowledge this is the only one in existence in Canada.(125, 126) This network has enabled the collection of vitamin D related data in young healthy Canadian children which is a necessary component to developing an understanding of the prevalence, causes and consequences of chronic low vitamin D levels in young children in Canada.

Leveraging the creation of TARGetKids!, funding was sought and obtained from the Hospital for Sick Children Research Institute Trainee Start-up Fund to generate pilot data on the prevalence and predictors of vitamin D deficiency in healthy 2 year old children. Data was collected over a 6 month period during the winter and spring of 2007-2008 and analyzed in late 2008 resulting in
a manuscript that was submitted for publication (Chapter 3). This work identified that 1/3 of
toddlers in this urban population had vitamin D levels lower than the American Academy of
Pediatrics recommendation and over 80% had levels lower than that recommended by the
Canadian Pediatrics Society. It also generated several hypotheses regarding modifiable risk
factors for low vitamin D including higher body mass index (BMI), lower cow’s milk intake and
snacking in front of the television.

These findings are significant for a number of reasons. First, although there are data from infants
with vitamin D deficient rickets, (55, 127, 128) there is a paucity of Canadian data on
subclinical vitamin D deficiency in young Canadian children. Second, while there are data from
2 US cities on vitamin D levels of children between 1 and 2 years of age (see Figure 1), to our
knowledge there have been no studies on the vitamin D levels of children in the third year of
life.(38, 40, 45)

We chose to study the vitamin D levels of children in the third year of life because we speculated
that the nutritional consequences of the transition from breast milk or formula to solid foods
during the first and second year would be most likely to manifest in the third year. Indeed, our
findings support this. Dietary and behavioural factors associated with lower vitamin D levels
such as BMI, volume of cow’s milk consumption and television viewing while snacking all are
consequences of varying degrees of success with this transition and could be targets for
intervention studies. Lastly, although BMI and milk intake have previously been identified as
being associated with vitamin D deficiency in children 1 to 2 years of age, television viewing
while snacking is a unique finding.(40) We speculate that this may be an indicator of an
unhealthy lifestyle representing a number of factors that are acting together to negatively influence vitamin D status such as a lack of sun exposure, increased sedentary behaviour and a low vitamin D containing diet.

As a first step, this pilot study demonstrated that such data can be collected on healthy Canadian young children through TARGet Kids! However, being a pilot study, it did not have the power necessary to provide sufficient confidence in the estimate of vitamin D deficiency prevalence nor was it intended to test the hypothesis it had suggested. Therefore, these pilot data were used to propose a larger cross-sectional study to determine the prevalence and predictors of vitamin D deficiency in healthy children between 1 and 5 years of age (Chapter 4) and test the hypotheses suggested by the pilot data.

This larger study aims to generate normative curves for 25-hydroxyvitamin D level by age and season and test the hypotheses that age, season, skin pigmentation, BMI, milk intake, screen time and snacking are associated with vitamin D deficiency. It will also form a cohort of 2400 children to be used for a future long-term cohort study to identify consequences of vitamin D deficiency (see below). Funding for this cross-sectional study has been obtained through an unrestricted grant from the Dairy Farmers of Ontario. Data collection commenced January 2009 and is expected to be complete in early to mid 2010.

Future Research:

We have suggested that vitamin D deficiency in young children is a measurable and common outcome. It also appears to have identifiable and modifiable predictors. These factors make it
ideal for future work. For example, predictors for vitamin D deficiency could be combined and prospectively evaluated to identify children at high risk of vitamin D deficiency without the need for a blood sample using clinical prediction tool methodology. Furthermore, interventions aimed at increasing vitamin D levels could be created and targeted to these high risk children. Finally, the cohort developed in the proposed large cross-sectional study could be used to identify health consequences associated with vitamin D deficiency.

Developing interventions aimed at decreasing vitamin D deficiency is an important long-term goal. However, identifying children who are likely to benefit from these interventions is a necessary prerequisite. Obtaining serum samples from every child to identify vitamin D deficiency is not a realistic screening plan. Not only is blood sampling painful, it is also technically challenging to take blood from young children and in the case of 25-hydroxyvitamin D measurement, it is costly. Based on costing associated with the preparation of this thesis, the estimated retail cost of phlebotomy and 25-hydroxyvitamin D testing is $75 per sample.

A strategy to predict vitamin D deficiency without the need for blood work would be useful. Clinical prediction rule methodology has been well established and has been used to identify such outcomes as ankle fractures and head injury using combinations of predictors.(129-137) Individual predictors of vitamin D deficiency identified through the proposed cross-sectional study could be combined using recursive partitioning or logistic regression to develop a survey based predictive tool that could be completed by parents of young children to identify children at risk of vitamin D deficiency. Such a tool could be derived using data from the cross-sectional study data and prospectively validated using children recruited through TARGet Kids!. 
Interventions aimed at decreasing vitamin D deficiency in children at risk of vitamin D deficiency could be tested using randomized controlled trial methodology which has been shown to be feasible using the TARGet Kids! network. Given that increased BMI is associated with lower vitamin D levels, interventions such as healthy weight and healthy active living strategies could be evaluated with vitamin D sufficiency as a potential outcome.

Furthermore, Vitamin D supplementation trials could be performed to identify what dose of orally administered vitamin D (number of International Units), of which type (D2 or D3), by which method (liquid or concentrated drops), and by which frequency (daily, weekly or monthly) is needed to maintain vitamin D levels at or above recommended levels.

Vitamin D fortification trials could also be performed using TARGet Kids!. Given that vitamin D levels are influenced by cow’s milk intake, children could be randomized to receiving regularly fortified cow’s milk (100IU / cup) or cow’s milk with increased vitamin D fortification (>100IU / cup). Vitamin D levels could be measured in each group to identify deficiency and estimate the risk of vitamin D excess. Indicators of toxicity such as high calcium levels in blood or urine could also be taken.

One of the major areas of interest in vitamin D research is the identification of long term consequences of subclinical vitamin D deficiency. The cohort generated in the proposed large cross-sectional study will be well positioned to contribute to this literature. In addition, the
effects of modification of vitamin D level through supplementation trials could be followed longitudinally.

This cohort could be used as a control group for case-control studies comparing disease incident vitamin D levels with vitamin D levels in healthy children. Given that profound vitamin D deficiency affects bone development,(68) one such study might test the hypothesis that vitamin D deficiency is associated with bone fracture risk in young children. Vitamin D levels could be obtained from a cohort of children who have fracture, such as those who present to the fracture clinic at the Hospital for Sick Children. Vitamin D deficiency could then be compared between children with fracture and children without fracture from our cross-sectional cohort to generate an odds ratio for vitamin D deficiency in children with fracture. Other similar studies could test hypothesis for associations between vitamin D deficiency and type 1 diabetes(62, 63) or leukemia(139-141) or demyelinating illness(94, 142-144) among others. Data for cases in these studies could come from disease incident cohorts at the Hospital for Sick Children.

Although case-control methodology is an efficient method for assessing relatively rare long term outcomes, there are several limitations. One is the assumption that vitamin D deficiency at the time of disease diagnosis is representative of chronic vitamin D status which may or may not be the case. Another is that the methodology assumes that vitamin D status is in no way influenced by the presenting disease itself which also may or may not be true.

A large, long term, longitudinal cohort study would circumvent these issues. The TARGet Kids cross sectional cohort provides a unique opportunity for the development of longitudinal cohort
study to identify health outcomes associated with vitamin D deficiency. The cohort could be followed through direct contact with participating children at stated intervals or through linkage via health insurance number (OHIP) to Ontario’s health administrative databases.

Ideally, yearly or biyearly direct follow up would generate rich data on outcomes related to vitamin D deficiency. Such outcomes could include persistent vitamin D deficiency defined as vitamin D deficiency on repeat testing or bone fractures or frequency of viral infections.(145) Although a costly undertaking, children could be followed in their primary physician’s office by TARGet Kids! personnel.

Follow up through health administrative databases, however, would be much more efficient. This could be accomplished by extracting the child’s OHIP number from the TARGet Kids! database and anonymously linking it to a unique individualized identifier (IKN) linked to Canadian Institutes for Health Information (CIHI) databases. TARGet Kids! data could be linked to hospital discharge data (the Discharge Abstract Database) which contains diagnostic and therapeutic data on hospitalizations or to Emergency Department utilization data found in the National Ambulatory Care Reporting System (NACRS) databases. Relatively short term outcomes which might be associated with vitamin D deficiency in young children could include Emergency Department visits for bone fracture or viral infections. Longer term outcomes could include hospitalizations for leukemia, multiple-sclerosis, type I diabetes or demyelinating illness. Long term adult outcomes could include myocardial infarction, stroke or breast or colon cancer.(68)
Conclusions:

This thesis has described an approach to studying Vitamin D deficiency as a nutritional child health determinant in young healthy Canadian children. It has outlined the author’s participation in the creation of TARGet Kids!, a novel Canadian practice based research network. It has produced pilot data on the prevalence and predictors of vitamin D deficiency which has suggested that vitamin D deficiency in young healthy children is common and has modifiable predictors. Based on this pilot data, a larger cross-sectional study has been proposed and funded. Finally, building on the ground work undertaken for this thesis, a number of future research projects have been outlined to predict vitamin D deficiency, intervene in its prevention and identify long term health consequences of early vitamin D deficiency.

<table>
<thead>
<tr>
<th>Study</th>
<th>Proportion low</th>
<th>Cutoff (nmol/L)</th>
<th>Age (months)</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maguire et al. 2009</td>
<td>32% (29/91)</td>
<td>&lt; 50</td>
<td>23-30</td>
<td>Toronto (43°N)</td>
</tr>
<tr>
<td>Gessner et al. 2003</td>
<td>31% (28/91)</td>
<td>&lt; 62.5</td>
<td>12-22</td>
<td>Alaska (58-61°N)</td>
</tr>
<tr>
<td>Gordon et al. 2008</td>
<td>14% (18/133)</td>
<td>&lt; 50</td>
<td>12-24</td>
<td>Boston (42°N)</td>
</tr>
</tbody>
</table>
Acknowledgments

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APPENDIX 1 -TARGGet Kids!
Nutrition and Health Questionnaire

(This sheet to be stored separately from study data)

Date: ______________________2008
       Month   Day   Year

1. Please provide contact information for you, your child, and your child’s doctor. You will only be contacted if your responses need to be clarified.

   a) Your name: ____________________________  
      (First)                        (Last)  
      Phone #: _______ - _______ - _______  
      Your relationship to the child:  
      □ Biological mother  
      □ Biological father  
      □ Adoptive mother  
      □ Adoptive father  
      □ Other: ______________________

   b) Your child’s name: ____________________________  
      (First)                        (Last)  
      Phone #: _______ - _______ - _______  
      □ SAME phone number as above

   c) Your child’s doctor’s name: Dr. ____________________________  
      (Initial)                        (Last)

2. Your postal code: _______ _______ _______ _______ _______

3. Your child’s date of birth: ____________________________  
      Month   Day   Year

4. Your child’s gender:  
      □ Female  
      □ Male

5. Would you like us to notify you of future CALIPER events?  
      □ Yes  
      □ No

6. We will share your child’s blood test results that could be of concern with your child’s doctor.  
   Would you like us to also share normal blood results with your child’s doctor (such as healthy levels of iron and vitamin D)?  
   □ Yes  
   □ No
Answer these questions for mother and father

7. Where were your child’s **biological parents** born?

<table>
<thead>
<tr>
<th>Mother</th>
<th>Father</th>
</tr>
</thead>
<tbody>
<tr>
<td>City</td>
<td>City</td>
</tr>
<tr>
<td>Country</td>
<td>Country</td>
</tr>
</tbody>
</table>

8. If not born in Canada, when did parents move here?

<table>
<thead>
<tr>
<th>Mother</th>
<th>Father</th>
</tr>
</thead>
<tbody>
<tr>
<td>City</td>
<td>City</td>
</tr>
</tbody>
</table>

9. What are the ages of the child’s parents?

<table>
<thead>
<tr>
<th>Mother</th>
<th>Father</th>
</tr>
</thead>
<tbody>
<tr>
<td>_______</td>
<td>_______</td>
</tr>
</tbody>
</table>

10. Are the child’s parents currently employed?

<table>
<thead>
<tr>
<th>Mother</th>
<th>Father</th>
</tr>
</thead>
<tbody>
<tr>
<td>☐ No</td>
<td>☐ No</td>
</tr>
<tr>
<td>☐ Yes</td>
<td>☐ Yes</td>
</tr>
<tr>
<td>☐ Part time employed</td>
<td>☐ Part time employed</td>
</tr>
<tr>
<td>☐ Full time employed</td>
<td>☐ Full time employed</td>
</tr>
<tr>
<td>☐ On parental leave</td>
<td>☐ On parental leave</td>
</tr>
</tbody>
</table>

11. What is the immigration status of the parents?

<table>
<thead>
<tr>
<th>Mother</th>
<th>Father</th>
</tr>
</thead>
<tbody>
<tr>
<td>☐ Canadian Citizen</td>
<td>☐ Canadian Citizen</td>
</tr>
<tr>
<td>☐ Landed immigrant</td>
<td>☐ Landed immigrant</td>
</tr>
<tr>
<td>☐ Refugee</td>
<td>☐ Refugee</td>
</tr>
</tbody>
</table>

12. What is the highest level of education completed by mother?  

| ☐ Public School | ☐ High school | ☐ College/University |

13. Do you consider your child’s biological mother to be healthy?

| ☐ Yes | ☐ No— **Please explain** | ☐ Unsure |

14. Do you consider your child’s biological father to be healthy?

| ☐ Yes | ☐ No— **Please explain** | ☐ Unsure |

15. Does mother usually wear a head scarf, or **hijab**?

| ☐ Yes | ☐ No |

16. Has your child’s **mother, father or sibling** (check those that apply) been diagnosed with:

<table>
<thead>
<tr>
<th>Condition</th>
<th>Mother</th>
<th>Father</th>
<th>Sibling</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multiple Sclerosis</td>
<td>☐ No</td>
<td>☐ Yes</td>
<td>☐ Who?</td>
</tr>
<tr>
<td>Diabetes</td>
<td>☐ No</td>
<td>☐ Yes</td>
<td>☐ Who?</td>
</tr>
<tr>
<td>Osteoporosis</td>
<td>☐ No</td>
<td>☐ Yes</td>
<td>☐ Who?</td>
</tr>
<tr>
<td>Heart disease</td>
<td>☐ No</td>
<td>☐ Yes</td>
<td>☐ Who?</td>
</tr>
<tr>
<td>Hypertension</td>
<td>☐ No</td>
<td>☐ Yes</td>
<td>☐ Who?</td>
</tr>
</tbody>
</table>
17. What is considered healthy and normal for one ethnic group may be different for other ethnic groups. For this reason, it is important for us to know your child’s ethnic origin. Please indicate the ethnic origin(s) of your child’s biological mother and biological father. You can provide more than one answer.

Ethnic origin(s) of your child’s biological mother
________________________________________________

Ethnic origin(s) of your child’s biological father
________________________________________________

18. What was the biological mother’s weight prior to her pregnancy? __________  □ pounds  □ kg

19. Was your child’s biological mother ill during her pregnancy?
☐ Yes— Please explain______________________________________________________________
☐ No
☐ Unsure

20. Did your child’s biological mother take any medications prescribed by a doctor during her pregnancy?
☐ Yes— Please explain______________________________________________________________
☐ No
☐ Unsure

21. Did your child’s biological mother take any vitamins or supplements during her pregnancy?
☐ No
☐ Prenatal multi-vitamin _______ times per _________ (day, week, month, year)
☐ Iron _______ times per _________ (day, week, month, year)
☐ Vitamin D _______ times per _________ (day, week, month, year)
☐ Unsure
☐ Other— Please explain______________________________________________________________

22. Did your child’s biological mother take any vitamins or supplements while breastfeeding?
☐ Mother did not breast feed
☐ No
☐ Prenatal multi-vitamin _______ times per _________ (day, week, month, year)
☐ Iron _______ times per _________ (day, week, month, year)
☐ Vitamin D _______ times per _________ (day, week, month, year)
☐ Unsure
☐ Other— Please explain______________________________________________________________

23. Please check all non-prescribed medications and substances that your child’s biological mother took during her pregnancy.
☐ Cold/flu medication
☐ Cigarettes
☐ Alcohol
☐ Other— Please explain______________________________________________________________
☐ None
☐ Unsure

24. Please specify the diet for your child’s biological mother during her pregnancy. Please check all that apply.
☐ Red meat (beef, veal, pork, lamb etc)
☐ Poultry (chicken, turkey, duck etc)
☐ Fish (salmon, halibut, haddock, cod, tuna etc)
☐ Shellfish (lobster, crab, shrimp etc)
☐ Eggs
☐ Milk
☐ Cheese
☐ Yogurt
☐ Margarine
☐ Honey
☐ Unsure
Questions about your child’s health

1. Which of the following best describes your child’s living arrangements?
   - Lives with 2 parents in the same household
   - Lives with 1 parent only
   - Lives alternating with 2 parents in different households
   - Other— Please explain

2. Aside from the child being assessed with this questionnaire, please list the date of birth for other children you have
   - No other children
   - Birth dates ___________ ___________ ___________ ___________ ___________ ___________

(The remaining questions are about your child being assessed with this questionnaire.)

3. Where was your child born? ________________ ___________________
   - City
   - Country

4. What was your child’s birth weight? _______Pounds ________Ounces (OR _________Grams)

5. Do you consider your child to be healthy?
   - Yes
   - No— Please explain

6. Does your child have a history of any illness, allergy or health condition?
   - Yes— Please explain
   - No

7. Has your child been ill within the past month?
   - Yes— Please explain
   - No

8. Has your child ever broken a bone?
   - Yes— Where was the bone?_______________________________________________________________
   - No

9. Does your child take any vitamins or supplements regularly?
   - No
   - Multivitamin _______ times per _________ (day, week, month, year)
   - Multivitamin with iron _______ times per _________ (day, week, month, year)
   - Iron _______ times per _________ (day, week, month, year)
   - Vitamin D _______ times per _________ (day, week, month, year)
   - Calcium _______ times per _________ (day, week, month, year)
   - Other— Please explain

10. Does your child regularly take any prescribed medications?
    - Yes – Which ones? ________________________________________________________________
    - No

11. Has your child taken any medication prescribed by a doctor in the past 2 weeks?
    - Yes – Which ones? ________________________________________________________________
    - No

12. Please check all non-prescribed medications or substances that your child has taken in the past 2 weeks.
    - Cold/flu medication
13. a) Has your child ever been breastfed?
   - Yes
   - No (SKIP TO QUESTION 15)
   - Unsure (SKIP TO QUESTION 15)

   b) **How long** has your child been breastfed? _____ months, _____ weeks, _____ days

   c) Did your child receive Vitamin D drops?
      - Yes
      - No (SKIP TO QUESTION 14)
      - Unsure (SKIP TO QUESTION 14)

   d) At what age did you stop giving the Vitamin D drops? _____ months

   e) How often did you give the Vitamin D drops? _____ times per _____ (day, week, month, year)

14. Is your child **currently** breastfeeding?
   - Yes
   - No— At what age did you stop breastfeeding? _____ months

15. Which scenario **best describes** your child?
   - My child received infant formula 80-100% of the time (was exclusively formula fed),
   - My child received breast milk 80-100% of the time (was exclusively breastfed).
   - My child received both breast milk and formula equally.
   - Unsure

16. How long has your child received infant formula? _____ months, _____ weeks, _____ days

17. Does your child **currently** use a bottle?
   - Yes
   - No

18. Does your child use a bottle during the day?
   - Yes— At what age **do you plan** to stop bottle use? _____ months.
   - No— At what age did you stop bottle use? _____ months.
   - My child has never used the bottle (breast to cup)

19. Does your child use a bottle in bed?
   - Never
   - Occasionally
   - Most of the time

20. In a typical week, besides parents, who usually feeds your child? **Check all that apply**
   - Other family members (ex. grandparents)
   - Licensed child care provider
   - Home child care provider (not licensed)
   - Nanny or babysitter
   - No one else
   - Other— Please explain

Is the person in the last question (question 21) a **vegan** (does not eat meat, poultry, fish/shellfish, dairy, eggs)?
   - Yes
   - No
21. Does your child have any food allergies, intolerances or food restrictions that have been confirmed by your child’s doctor?

☐ Yes - What are they? ____________________________________________________________

☐ No

22. Please specify your child’s diet in typical week. Please check all that apply.

☐ Breast milk
☐ Infant formula
☐ Red meat (beef, veal, pork, lamb, etc.)
☐ Poultry (chicken, turkey, duck, etc.)
☐ Fish (salmon, halibut, haddock, cod, tuna, etc.)
☐ Shellfish (lobster, crab, shrimp, etc.)
☐ Eggs
☐ Milk □ Skim □ 1% □ 2% □ Homo
☐ Cheese
☐ Yogurt
☐ Margarine
☐ Honey
☐ Vegetarian: does not eat red meat, poultry, fish or shellfish
☐ Vegan: does not eat red meat, poultry, fish, shellfish, eggs, dairy or honey

23. Please specify your child’s diet for the past 3 days. Please check all that apply.

☐ Breast milk
☐ Infant formula
☐ Red meat (beef, veal, pork, lamb, etc.)
☐ Poultry (chicken, turkey, duck, etc.)
☐ Fish (salmon, halibut, haddock, cod, tuna, etc.)
☐ Shellfish (lobster, crab, shrimp, etc.)
☐ Eggs
☐ Milk □ Skim □ 1% □ 2% □ Homo
☐ Cheese
☐ Yogurt
☐ Margarine
☐ Honey
☐ Unsure
☐ Vegetarian: does not eat red meat, poultry, fish or shellfish
☐ Vegan: does not eat red meat, poultry, fish, shellfish, eggs, dairy or honey

24. Circle how many cups of each drink your child has currently in a typical day. (1 cup=8 ounces=250 ml)

<table>
<thead>
<tr>
<th>Drink</th>
<th>0</th>
<th>½</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cow’s milk</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infant formula</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other milk (soy, rice, goat etc)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100% Juice (apple, orange etc)</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sweetened drinks (Kool aid, Sunny D)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tea</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soda or Pop</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

25. If your child drinks “other milk” (soy, rice, goat etc) what kind is it? ________________________________

26. At what age did you introduce OR do you plan to introduce:
a) Infant cereal  Age introduced______________  OR  Age plan to introduce______________
b) Cows milk  Age introduced______________  OR  Age plan to introduce______________
c) Juice  Age introduced______________  OR  Age plan to introduce______________

27. Circle how many servings of each food your child has in a typical day.
   Sweets or candy   0  ½  1  2  3  4  5+
   Chips or Fried snacks   0  ½  1  2  3  4  5+

28. Which of the following statements best describes the food eaten in your household in the past 12 months?
   □ You and other household members always had enough of the kinds of food you wanted to eat.
   □ You and other household members had enough to eat, but not always the kinds of food you wanted.
   □ Sometimes you and other household members did not have enough to eat.
   □ Often you and other household members didn’t have enough to eat.

29. In a typical week, how many times does your family eat the evening meal together?
   □ Zero (0 days)
   □ 1 day
   □ 2 days
   □ 3 days
   □ 4 days
   □ 5 days
   □ 6 days
   □ 7 days

Questions about screen time (time spent in a room with the TV, video/DVD on, or using a computer)

30. How many of the following are in your home:
   Televisions   ___________
   DVD/video players   ___________
   Computers   ___________
   Video game consuls (e.g. Playstation, Xbox)   ___________

31. Is there a television in your child’s bedroom?
   □ Yes— If yes, does your child share a bedroom with parents or siblings?  □ Yes  □ No
   □ No

32. Write in the number of minutes that your child spends awake in a room on a typical weekday AND weekend day with:

   A typical weekday  A typical weekend day
   The television on: ________ minutes ________ minutes
   Videos or a DVD on: ________ minutes ________ minutes
   Playing the computer: ________ minutes ________ minutes
   Playing video games: ________ minutes ________ minutes

33. Do you have household rules about watching television/videos/DVD?
   □ Yes
   □ No

34. Which meals does your child eat in a room with the television on for a typical weekday AND weekend day:
A typical weekday

<table>
<thead>
<tr>
<th>Activity</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breakfast</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lunch</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dinner</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A snack</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

A typical weekEND day

<table>
<thead>
<tr>
<th>Activity</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breakfast</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lunch</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dinner</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A snack</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

35. In the last **weekday**, how much time did you spend:
   a. Watching television? (not videos/DVDS) _________ minutes
   b. Watching videos or DVDs? __________ minutes
   c. Using the computer (not for work)? __________ minutes
   d. Playing video games? __________ minutes

Physical activity is any activity that increases your child’s heart rate and makes your child get out of breath some of the time. It can be done in sports, school activities, playing with friends, or walking to school.

36. Over a typical/usual week, on how many days is your child physically active for a total of at least 60 minutes per day?
   (Add up all the time your child spends in physical activity each day)
   □ None (zero days)
   □ 1 day
   □ 2 days
   □ 3 days
   □ 4 days
   □ 5 days
   □ 6 days
   □ 7 days

37. Has your child exercised vigorously in the past 24 hours (e.g. running, cycling, swimming, dancing, etc.)
   □ Yes
   □ No
   □ Unsure

38. On a typical weekday, how much time does your child spend outside or in a gymnasium for ‘recess’ or ‘unstructured free play during daycare or preschool’? ____________ minutes

39. Aside from time in daycare and preschool, on a typical weekday, how much time does your child spend outside in ‘unstructured free play’? ____________ minutes

40. When you or a caregiver is going for a walk with your child, how often does your child ride in a stroller or wagon?
   **Circle the best answer:**
   Never   25% of the time   50% of the time   75% of the time   Always

41. On a typical **weekday AND weekEND day**, how much time does your child spend in the following activities?
   **Put a check mark in the most appropriate box:**

<table>
<thead>
<tr>
<th>Time</th>
<th>&lt;1/2 hour</th>
<th>½ hour</th>
<th>1 hour</th>
<th>1 ½ hour</th>
<th>2 hour</th>
<th>&gt;2 hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>On a typical weekday</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Play in the yard or street</td>
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<td></td>
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<tr>
<td>Play in the park</td>
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</tbody>
</table>
### Going for a walk

<table>
<thead>
<tr>
<th>In organized physical activities (ex. swimming, soccer, gymnastics)</th>
</tr>
</thead>
</table>

### On a typical weekend day

<table>
<thead>
<tr>
<th>Play in the yard or street</th>
</tr>
</thead>
<tbody>
<tr>
<td>Play in the park</td>
</tr>
<tr>
<td>Going for a walk</td>
</tr>
</tbody>
</table>

| In organized physical activities (ex. swimming, soccer, gymnastics) |

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42. Is your child currently in a licensed daycare or preschool program?
- ☐ Yes – **Last week**, how many hours did your child attend daycare or preschool? ____________ hours
- ☐ No

43. Do you do physical activity in your leisure time (not as part of your job)?
- ☐ Yes ______ times per ☐ week for ☐ 0-15 min
- ☐ No ☐ month ☐ 16-30 min
  - ☐ 31-60 min
  - ☐ More than one hour

### Questions about sun exposure

44. How much time did your child spend outside last week?
- <1h
- 1h
- 2h
- 3h
- 4h
- 5h
- 6h
- >7h

45. In the summer, how often does your child play outside for at least 15 min with minimal clothing (without a shirt on or wearing only bathing suit)?
- Never
- 1d/week
- 2d/week
- 3d/week
- 4d/week
- 5d/week
- 6d/week
- Every day

46. When outside in the summer, how often does your child wear a hat?
- Never
- 25% of the time
- 50% of the time
- 75% of the time
- Always

47. When your child is outside in the summer, how often do you apply sun block to your child’s exposed skin?
- Never
- 25% of the time
- 50% of the time
- 75% of the time
- Always

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### Office use only

<table>
<thead>
<tr>
<th>ID number</th>
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<table>
<thead>
<tr>
<th><strong>Child</strong></th>
<th>Weight (kg)</th>
<th>Height (m)</th>
<th>Blood pressure (&gt;2 yrs)</th>
<th>Waist circumference (cm)</th>
<th>Skin type</th>
<th>Time last ate (Meal)</th>
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<table>
<thead>
<tr>
<th><strong>Parent</strong></th>
<th>Weight (kg)</th>
<th>Height (m)</th>
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</table>
APPENDIX 2: REB APPLICATION
PREVALENCE AND PREDICTORS OF LOW VITAMIN D IN URBAN CANADIAN TODDLERS
RESEARCH CONSENT FORM

Investigator(s):
Principle Investigator: Dr Patricia Parkin (416) 813-6933
Co-investigators: Dr Jonathon Maguire (416) 813-8157
Magda Mekky (416) 813-8144

Purpose of the Research:
Vitamin D is very important for bone health and normal calcium levels in the blood.

This purpose of this study is to help determine what healthy and “normal” ranges are for vitamin D in children. We may also be able to determine possible risk factors for low levels of vitamin D. This information will contribute to better assessment and treatment of children across Canada and possibly internationally.

Description of the Research:
When your child attends their scheduled doctor’s visit, a trained health professional will take a small blood sample. We will also ask you to complete a short questionnaire and we will record your child’s height, weight and waist circumference. That will be the end of your participation in the study.

Potential Harms, Discomforts or Inconvenience:
There may be a small amount of bleeding when blood is taken from a vein and there may be slight discomfort and bruising or redness that will usually disappear in a few days.

Potential Benefits:
Your child may benefit from having their vitamin D level measured as the results will be provided to your pediatrician. This could inform your pediatrician if your child might need a nutritional supplement (such as a vitamin).

Society may benefit from this study by having more accurate ranges of what healthy levels of vitamin D in young children should be which can more accurately identify children in need of treatment.

Confidentiality:
We will respect your privacy. No information about who your child is will be given to anyone or be published without your permission, unless required by law. For example, the law could make us give information about you if a child has been abused, if your child has an illness that could spread to others, if your child or someone else talks about suicide (killing themselves), or if the court orders us to give them the study papers.

The data produced from this study will be stored in a secure, locked location. Only members of the research team will have access to the data. This could include external research team members. Following completion of the research study, the data will be kept as long as required then destroyed as required by SickKids policy. Published study results will not reveal your identity.

We will give you a copy of this consent form.
We will reimburse you for all your reasonable out of pocket expenses for being in this study (e.g. babysitters, parking) if the visit is scheduled outside of your child’s regularly scheduled appointments.

**Participation:**
It is your choice to allow your child to take part in this study. You can stop at any time.
During this study we may create new tests, new medicines, or other things that may be worth some money. Although we may make money from these findings, we cannot give you or your child any of this money now or in the future because your child took part in this study.

During this study we may create new tests, new medicines, or other things that may be worth some money. Although we may make money from these findings, we cannot give you or your child any of this money now or in the future because your child took part in this study.

Participation in research is voluntary. If you choose not to participate, you and your family will continue to have access to quality care at SickKids and at your pediatrician’s office if needed. If you choose on behalf of your child to participate in this study you can take your child out of the study at any time. Again, you and your family will continue to have access to quality care at SickKids and at your pediatrician’s office.

**Sponsorship:**
Funding for this study is provided by the Dairy Farmers of Ontario and the Department of Pediatric Laboratory Medicine at the Hospital for Sick Children.

**Consent:**
By signing this form, I agree that:
1) You have explained this study to me. You have answered all my questions.
2) You have explained the possible harms and benefits (if any) of this study.
3) I know what I could do instead of having my child take part in this study. I understand that I have the right to refuse to let my child take part in the study. I also have the right to take my child out of the study at any time. My decision about my child taking part in the study will not affect my child’s health care at Sick Kids.
4) I am free now, and in the future, to ask questions about the study.
5) I have been told that my child’s medical records will be kept private except as described to me.
6) I understand that no information about my child will be given to anyone or be published without first asking my permission.
7) I agree, or consent, that my child___________________ may take part in this study.

____________________  ______________________________
Printed Name of Parent/Legal Guardian  Parent/Legal Guardian’s signature & date

____________________  __________________________________________
Printed Name of person who explained consent  Signature of Person who explained consent & date

____________________
Printed Witness’ name (if the parent/legal guardian does not read English)

If you have any questions about this study, please call Dr Patricia Parkin at (416) 813-6933.
If you have questions about your child’s rights as a subject in a study or injuries during a study, please call the Research Ethics Manager at 416-813-5718.