Appendix 2: Full Research Protocol

DO IT! Trial: Vitamin D Outcomes and Interventions in Toddlers

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SUMMARY

Background: There is consistent evidence that many North American children older than 1 year have vitamin D serum levels lower than the American Academy of Pediatrics and the Canadian Paediatric Society recommend. Data from the 2001-2004 National Health and Nutrition Examination Survey (NHANES) indicated that 65-70% of children had vitamin D levels <75 nmol/L. However, it is not known what health benefits would be realized if children reached these levels. Observational data has demonstrated an association between low wintertime vitamin D levels and increased risk of viral upper respiratory tract infection (URTI), asthma-related hospitalizations and use of anti-inflammatory medication. However, it is not currently known whether vitamin D supplementation mediates UTRI risk and risk of asthma exacerbations in preschoolers. A randomized controlled trial is urgently needed to determine whether wintertime vitamin D supplementation could result in reductions in viral URTI and asthma, two of the most common and costly illness of early childhood.

Objective: This study will compare the effect of 'standard dose' (400 IU/day) vs. 'high dose' (2000 IU/day) orally supplemented vitamin D in achieving reductions in laboratory confirmed URTI and asthma exacerbations during the winter in preschool aged Canadian children. We also aim to assess the effect of 'high dose' wintertime vitamin D supplementation on vitamin D serum levels as well as RSV, adenovirus and influenza viruses as causes of URTI. Data from this RCT will be used to perform an economic analysis of the cost vs. the benefit of routine 'high dose' wintertime vitamin D supplementation for preschoolers.

Methods: This study is a pragmatic, active controlled, randomized trial. Over 4 successive winters we will recruit 750 healthy children 1-5 years of age from their primary care physician's office. Participating primary care practices are part of a CIHR funded primary healthcare research network called TARGet Kids! Recruited children will be randomly assigned to 'standard dose' (400 IU/day) vs. 'high dose' (2000 IU/day) supplemental vitamin D per day by mouth for 4 months (200 children per group). These doses of vitamin D are within the Health Canada safe dosage limit for children older than 1 year. Parents of enrolled children will be asked to obtain a nasal swab from their child and complete a symptom checklist with each URTI. Respiratory viruses will be identified using RT-PCR technology Families will be contacted monthly by telephone to ascertain the number of asthma exacerbations and to remind them to collect suspected URTI samples and complete symptom checklists. Unscheduled physician visits for URTIs and asthma exacerbations will be recorded by research assistants in each primary physician practice. In April or May, a blood sample will be taken from each child to determine 25-hydroxyvitamin D serum levels using a competitive two-step chemiluminescence assay. The primary analysis will be a comparison of laboratory confirmed URTI rate (per child) between study groups using a Poisson regression model. Secondary analyses will include a comparison of vitamin D serum levels, asthma exascerbations and the frequency of RSV, adenovirus and influenza viruses between arms. Furthermore, a cost effectiveness analysis on the effect of wintertime vitamin D supplementation of preschoolers will be undertaken using the net benefit regression approach.

Significance: Because of Canada's latitude and little exposure of young children to vitamin D generating UVB radiation for much of the year, understanding vitamin D deficiency and associated preventable health problems is of great importance to Canada's children and parents. Identifying whether vitamin D supplementation of preschoolers can lead to reductions in wintertime viral URTIs and asthma attacks and what dose is optimal could offer a significant benefit in reducing population wide morbidity and associated health care and societal costs. This information will assist parents, clinicians and policy makers in determining practice recommendations and health policy related to supplementation of vitamin D in healthy Canadian pre-schoolers.

SECTION 1: INTRODUCTION AND IMPORTANCE

A. Introduction

Evidence from observational studies suggests that low vitamin D levels early in life may be related to common child health outcomes frequently seen by primary care physicians including viral upper respiratory tract infections (URTI) and asthma.[1] Data from our group [2] and others have repeatedly demonstrated that most urban preschoolers living in temperate climates have vitamin D serum levels lower than values recommended by the American Academy of Pediatrics (AAP) and the Canadian Paediatric Society (CPS).[3, 4] Yet there are no Canadian recommendations for supplementation of children older than 1 year. Furthermore, **it is not known whether vitamin D supplementation leads to measureable improvement in child health outcomes nor what dose is needed to achieve optimal outcomes in preschoolers.**

We have developed a CIHR funded primary-care practice based research network called TARGet Kids! to conduct observational and interventional studies in preschoolers to improve child health outcomes through primary prevention. TARGet Kids! is the only child health primary care research network in Canada. In this two year *multi-centre pragmatic randomized controlled trial* we will leverage established TARGet Kids! infrastructure **to determine whether wintertime high dose vitamin D supplementation of preschoolers improves two common and costly child health outcomes, viral URTI and asthma exacerbations.**

B. Importance

Because of Canada's northern latitude, understanding vitamin D deficiency and associated health problems is of *major importance to Canadian children and their parents*. Observational studies have suggested that low vitamin D levels may be implicated in two of the most common health and burdensome issues during early childhood: URTI and asthma exacerbations. These two conditions place an enormous burden on Canada's health care system and economy. Viral URTI and exacerbations of asthma combined make up over 30% of all emergency department visits for children in Canada.[5] If vitamin D supplementation of preschoolers makes even a small contribution to improving these common childhood health problems, measures to increase vitamin D levels may significantly reduce population wide morbidity and associated health service costs.

TARGet Kids! infrastructure and collaborations make this team unique in Canada and position it ideally to carry out this randomized controlled trial on vitamin D supplementation of Canadian preschoolers.

SECTION 2: OBJECTIVES AND HYPOTHESIS

The **primary objective** is to compare the effect of high dose orally supplemented vitamin D (2000 IU/day) with standard dose vitamin D supplementation (400 UI/day) in achieving a reduction in laboratory confirmed viral URTI during the winter in healthy preschoolers 1 to 5 years of age.
We hypothesize that preschoolers supplemented with 2000 IU/day will have a reduction in wintertime viral URTI.

Secondary objectives include comparing high dose (2000 IU/day) vs. standard dose (400 IU/day) vitamin D supplementation for the following secondary outcomes: a) Specific viral infections including influenza, adenovirus and respiratory syncytial virus (RSV); b) Asthma exacerbations in preschoolers with asthma or recurrent wheezing; c) Direct and indirect economic costs associated with URTIs; d) Vitamin D deficient serum levels as defined by the AAP and CPS. **We hypothesize that the likelihood of these secondary outcomes will be lower in children supplemented with 2000 IU/day of vitamin D.**

SECTION 3: BACKGROUND

A. Vitamin D biochemistry

Vitamin D is a fat soluble steroid with two clinically relevant metabolites: 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D.[6, 7] 1,25-dihydroxyvitamin D is the active form of vitamin D and its serum level is tightly regulated by parathyroid hormone (PTH), serum calcium and phosphorous levels and is not reflective of vitamin D stores. **Circulating levels of 25-hydroxyvitamin D are reflective of vitamin D stores and is commonly measured to determine vitamin D serum level.[8]**

B. Sources of vitamin D

Vitamin D can be synthesized in the skin by exposure to sunlight or can be ingested from dietary sources.[6] When exposed to solar ultraviolet B radiation the skin converts 7-dehydrocholesterol to vitamin D3.[9] However, north of 42°N, there is little production of vitamin D from the skin between November and March.[10] **Therefore, in temperate climates where exposure to sunlight is limited for a significant part of the year, vitamin D levels fall dramatically during the winter and dietary sources of vitamin D become extremely important for avoiding deficiency**.[11] Few foods aside from fatty fish, which are not regularly consumed by preschoolers, naturally contain vitamin D.[1] Data from the Canadian Community Health Survey and other sources suggest that the majority of vitamin D that preschoolers ingest is from vitamin D fortified cow's milk which contains 100 IU of vitamin D per cup.[12-14] **Unfortunately, the amount of cow's milk that preschoolers typically drink is insufficient to receive enough vitamin D to avoid wintertime deficiency**.[2, 14-17] Further, efforts to increase cow's milk consumption in this population may increase the prevalence of iron deficiency.[18-22]

C. Recommended vitamin D serum levels and supplementation for preschoolers

It is well established that 25-hydroxyvitamin D serum levels above 50 nmol/L in children are sufficient to prevent rickets.[23] Therefore, the American Academy of Pediatrics (AAP) suggests that 25-hydroxyvitamin D levels in children be above 50 nmol/L.[3] Data from adults suggest that serum levels above 75 nmol/L are required to minimize calcium resorbtion from bone and maximize intestinal calcium absorption.[24] Therefore, the Canadian Paediatric Society (CPS) suggests that optimal 25-hydroxyvitamin D level for children is above 75 nmol/L.[4]

The recommended vitamin D dietary allowance (RDA) for children older than 1 year has been set at 600 IU/day by both Health Canada and the Institute of Medicine. Because children who consume less than 1000 ml of vitamin D fortified cow's milk per day (which includes most preschoolers)[2, 25] are unlikely to receive this amount of dietary vitamin D, the AAP recommends routine vitamin D supplementation of 400 IU/day for all children ingesting < 1000 ml/day of vitamin D-fortified formula or cow's milk.[3] The CPS has no recommendation for routine vitamin D supplementation for Canadian children older than 1 year.[4] **To reflect current AAP recommendation and standard practice in our population, our 'standard dose' arm has been chosen as 400 IU/day.**

D. Low vitamin D levels in North American children:

There is consistent evidence, from both single center studies [14, 28, 29] and national surveys[15, 16, 25], that North American children older than 1 year have vitamin D serum levels lower than AAP or CPS recommendations (See Table 1). Data from the 2001-2004 National Health and Nutrition Examination Survey (NHANES) indicated that 70% of children 1 to 11 years had vitamin D levels < 75 nmol/L.[15, 16] Data from the 2007-2009 Canadian Health Measures Survey suggested that 51% of children age 6-11 years had vitamin D levels < 75 nmol/L. Studies of pre-school aged children from Boston (42°N), Toronto (43°N), St. John's (47°N), Calgary (51°N), Edmonton (53°N) and Alaska (58-61°N) have all found significant rates of vitamin D deficiency in infants and toddlers using various definitions.[12, 14, 28-30] (See Table 1)

E. Potential child health consequences of sub-optimal vitamin D status

Severe vitamin D deficiency (generally 25-hydroxyvitamin D level < 25nmol/L) results in rickets[1], an irreversible bowing of the long bones and deformity of the joints and teeth with well described long term implications for skeletal growth.[11] Important health policy recommendations including 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 to be 2.9 cases per 100,000 children in Canada).[31] The more recent observation that the vitamin D receptor (VDR) is expressed in many tissues in the body in addition to the skeletal and endocrine systems has suggested that vitamin D may be acting in other ways.[6] Population based retrospective cohort and case-control studies have suggested that **less severe vitamin D deficiency (25-75nmol/L) in children may be associated with several other adverse health outcomes.**[32-39]

Viral Upper Respiratory Tract Infection

Viral upper respiratory tract infections (URTI) are the most common infectious disease in North America.[40] URTI is the most common reason for emergency department visits and unscheduled outpatient visits in Canada comprising 10% of emergency department visits for children under 10 years of age.[5, 41, 42] Preschoolers have the highest incidence of URTI of any age group, **occurring one to two times per month** per child during the winter and higher among children who attend daycare.[40, 43-51] Influenza, RSV and adenovirus, which collectively comprise 25% of respiratory infections in children, are the most common viruses that lead to febrile illness, acute otitis media, outpatient visits and hospitalization.[43, 52, 53] Roughly 50% of preschoolers with URTI are brought to medical attention resulting in an additional outpatient physician visit every 2 to 3 months during the winter with 1% requiring hospitalization.[43, 52, 54-56] Several groups have estimated the direct and indirect costs of URTI in preschoolers to be between $261 and $276 per URTI with influenza being the most costly virus, contributing $809 per URTI.[45, 54] The collective cost of URTI in children under 5 years of age has been estimated to be $1.8 billion annually in the US.[57] Evidence supporting a causal connection between low vitamin D serum level and URTI comes from multiple sources.

Temporality: Both vitamin D levels and viral URTI show a remarkably similar seasonal oscillation. R. Edgar Hope-Simpson hypothesized that a "seasonal stimulus" must affect the pathogenesis of influenza and Cannel et al. hypothesized that this seasonal stimulus may be related to seasonal oscillation of vitamin D levels.[58, 59]

Biological plausibility: Basic science has uncovered the role of vitamin D on the innate immune system.[60] The primary site for human contact with respiratory viruses is the upper respiratory tract mucosa.[61] It has been shown that vitamin D is constitutively converted to its active form 1,25-hydroxyvitamin D in respiratory epithelium.[62] The mucosa of the upper airway is protected from infection by a complex set of peptides which have direct antimicrobial properties and contribute to innate immunity.[63, 64] These peptides include defensins and calethicidin which have direct antiviral properties.[65-67] Furthermore, respiratory tract macrophages are stimulated to produce these peptides *in vitro* by the presence of 25-hydroxyvitamin D and *in vivo* through vitamin D supplementation [68-71].

Epidemiologic Association: Observational studies have supported an association between viral infections and vitamin D level in both adults and children. A post hoc analysis of a 3-year randomized controlled trial of vitamin D supplementation for bone loss in 208 post menopausal African American women found that 26 patients in the placebo group vs. 8 in the intervention group reported having a URTI (P=0.002).[72, 73] Ginde et al., using data from NHANESIII found that the odds of having a recent URTI in Americans 12 years of age or older was 25% higher for people with 25-hydroxyvitamin D < 75 nmol/L relative to those > 75 nmol/L.[74] Laaksi et al. found that young male Finnish soldiers with 25-hydroxyvitamin D levels below 40 nmol/L had nearly double the number of absent days from duty due to respiratory infections than soldiers with levels above 40 nmol/L.[75]

Sebetta et al., in recent prospective cohort study of 198 adults in Connecticut demonstrated that 25-hydroxyvitamin D concentrations > 95 nmol/L were associated with a two-fold reduction in URTI over a single winter.[76] Camargo et al., using data from a New Zealand birth cohort, found that infants with 25-hydroxyvitamin D levels in cord blood below 25 nmol/L were at 2-fold higher risk of viral respiratory tract infection at 3 months of age than infants with cord blood levels above 75 nmol/L.[77]

Randomized controlled trial (RCT) evidence: To our knowledge, there are no RCTs that have examined the effect of vitamin D supplementation on health outcomes in preschoolers. Three RCTs have examined the effect of vitamin D supplementation on URTI, two in adults and one in older children. Li-Ng and colleagues randomized 162 adults to 2000 IU per day of vitamin D or placebo for 3 months during the winter in Long Island, NY and recorded the frequency of URTI symptoms (without laboratory viral confirmation) using a bi-weekly online questionnaire. They found no difference in the incidence of reported URTI between vitamin D and placebo groups (48 URTIs vs 50 URTIs, p=0.57).[78] As the authors point out, the lack of effect may have resulted from a relatively small difference in follow-up vitamin D levels in the vitamin D (88 nmol/L) vs. placebo group (63 nmol/L). Recently, Laaski et al. randomized 164 male Finnish army recruits to 400 IU of vitamin D per day vs. placebo between October and March.[79] Their primary outcome, mean number of days absent from duty due to URTI in the vitamin D vs. placebo group, demonstrated a trend towards reduced absenteeism (2.2 days vs. 3.0 days, p=0.096). However, the dose of vitamin D chosen (400 IU per day), may not have been sufficient to raise 25-hydroxyvitamin D levels enough to impact significantly on viral URTI (72 nmol/L in the intervention group vs. 51 nmol/L for placebo). In the third trial, Urashima and colleagues randomized 167 six to 15 year old schoolchildren to 1200 IU per day of vitamin D or placebo for four winter months in Tokyo, Japan.[80] Their primary outcome, laboratory confirmed Influenza A infection, showed a statistically significant reduction in the vitamin D group vs. control group (11% vs. 19%, p=0.04). However, the authors did not measure baseline or follow-up vitamin D serum levels so it is unclear whether the positive effect was due to an increase in vitamin D levels. **To maximize our likelihood of finding a treatment effect we have chosen our 'high dose' arm to include any reasonable supplementation dose while keeping total vitamin D intakes below the Tolerable Upper Intake Level as recommended by Health Canada (2500 IU/day for preschoolers).[81, 82] As such, 2000 IU/day has been chosen as the 'high dose' arm.**

Asthma

Asthma is the most common chronic illness of childhood. Preschoolers bear the highest burden of asthma which affects 13% of children under 5 years of age compared with 8% of children under 18 years in Ontario.[83] Asthma exacerbations are the most common non-surgical cause for hospitalization of children in Canada and it costs the Canadian health care system over $300 million annually.[83-86] An association between vitamin D deficiency and asthma exacerbation was initially proposed to explain the observation that low vitamin D levels and asthma exacerbations are both more common in temperate climates and more common during the winter months.[87, 88] Recent basic science and epidemiological research has supported a connection between vitamin D serum level and asthma.

Biological plausibility: In vitro studies have demonstrated that the VDR is present in bronchial smooth muscle cells and that many asthma-associated genes are expressed following stimulation of lung tissue with vitamin D.[89, 90] Case-control studies have found associations between asthmatic individuals and polymorphisms in the VDR and other vitamin D related genes.[91-95]

Epidemiologic Association: Cross-sectional data from NHANESIII found a strong correlation between airway resistance and vitamin D levels in adults.[96] An examination of incident vitamin D levels in a cohort of 1024 seven to ten year old American children with mild-to-moderate asthma identified that

children with 25-hydroxyvitamin D levels below 75 nmol/L had increased odds of emergency department visits and hospitalizations relative to children with vitamin D levels above 75 nmol/L over a period of 4 years (OR 1.5, p=0.01).[97] Furthermore, two cross-sectional studies of American and Costa Rican children suggested that children with vitamin D levels below 75 nmol had increased airway resistance and increased use of inhaled and oral steroids relative to children with vitamin D levels above 75 nmol/L.[98, 99] **Whether these effects are due to vitamin D deficiency or are mediated through a vitamin D related reduction in viral URTIs is not clear.**

Randomized controlled trial (RCT) evidence: To our knowledge, no RCT has examined the effect of vitamin D on preschool asthma exacerbations. In the trial by Urashima and colleagues of vitamin D supplementation of Japanese schoolchildren, asthma exacerbations were measured as a secondary outcome.[80] They found a statistically significant decrease in asthma exacerbations in children receiving 1200 IU of vitamin D per day vs. placebo (2 vs. 12 of 167 children, RR 0.17, p=0.006). Interestingly, the reduction in URTI was stronger among children with asthma than those without (RR 0.17, p=0.006) suggesting that the reduction in exacerbations of asthma may be mediated through reduced viral URTIs among vitamin D supplemented children.

F. Summary of Background

There is substantial evidence that many North American preschoolers have vitamin D serum levels significantly lower than both Canadian and American guidelines suggest. However, these guidelines are not based on child health outcome data but on expert opinion and extrapolation from adult outcomes. **It is not known whether vitamin D supplementation of preschoolers leads to measureable improvement in important child health outcomes nor what vitamin D serum level is sufficient to maximize health outcomes.** There are wide gaps in knowledge of the potential health consequences associated with low vitamin D level in young children despite multiple calls for such research.[3, 4, 23, 100, 101] Existing basic science and epidemiologic observational studies have made a strong case for a connection between low vitamin D levels and annual wintertime viral URTI epidemics and asthma exacerbations. **A randomized controlled trial to test the effect of high dose orally supplemented vitamin D during the wintertime on these outcomes in Canadian preschoolers is urgently needed.**

SECTION 4: METHODS

A. Study design

A multi-centre, pragmatic, active controlled, blinded, parallel group, superiority randomized trial will be conducted over two winters. The primary purpose of this trial is to inform health policy and secondarily to contribute to an explanation of the causal relationship between vitamin D and child health outcomes. Therefore, according to the framework described by Thorpe (co-investigator) and other leading trial methodologists, this trial has been designed along the pragmatic end of the pragmatic-explanatory continuum.[102] Specifically, eligibility criteria, participant compliance, follow-up intensity, and primary analysis will follow pragmatic approaches ("Does this intervention work under usual conditions?"); follow-up of outcomes will follow approaches mid-way along the pragmatic-explanatory continuum ("Can this intervention work under ideal conditions?"). This protocol has been designed following the 2010 SPIRIT guidelines (Defining Standard Protocol Items for Randomized Trials).[103, 104] and trial results will be reported according to the 2008 CONSORT guidelines for pragmatic trials.[105]

B. Participants

Study Setting

Healthy children aged 1 to 5 years will be recruited during a routine well child doctor's visit at a TARGet Kids! participating academic pediatric or family medicine group practice in Toronto, Canada

September through November over four years. TARGet Kids! is a collaboration between academic health outcomes researchers in the Faculty of Medicine at the University of Toronto and primary care physicians from the Department of Pediatrics and the Department of Family and Community Medicine at the University of Toronto.

Eligibility Criteria

Inclusion Criteria are 1) Healthy children by parental report; 2) Have reached their 1st birthday but not past their 6th birthday; 3) Present to a TARGet Kids! practice for routine primary healthcare prior to viral season (September through November); 4) Parents provide informed consent to participate; 5) Enrolled in TARGet Kids!

Exclusion criteria are 1) Children with gestational age < 32 weeks as they are a high risk population for respiratory tract infection and asthma; 2) Children with chronic illness (except for asthma) on parental report which is known to interfere with vitamin D metabolism and increase the risk of respiratory infection; 4) Children with a sibling participating in the study to reduce clustering effects

Baseline Descriptor Variables

Baseline descriptor variables will be obtained by standardized research assistant completed data collection form adapted from the Canadian Community Health Survey.[106] The following will be collected: Age, sex, birth weight, enrolment date, ethnicity, maternal age, education and health, duration of breastfeeding, current and past vitamin D supplementation, dietary vitamin D intake using 3 day dietary recall, bottle use, daily multivitamin use, influenza immunization status, screen viewing time, physical activity, outdoor time, and sun exposure. In addition, height and weight will be measured using standardized techniques and skin pigmentation will be measured using a narrow-band reflectometer (Dermaspectrometer, Cortex Technology).[107-110] This data was be collected as part of "TARGet Kids!", and will be linked to this study. This is to avoid the inconvenience and redundancy of asking parents to complete the questionnaires twice and complete the height and weight measurements twice. A venous blood sample will be obtained to document baseline 25-hydroxyvitamin D levels.

C. Intervention

Children will be randomly assigned to one of two groups: 'standard dose' of 400 IU/day vitamin D or 'high dose' of 2000 IU/day vitamin D (see Figure 1: DOIT! Trial Schematic). **The 'standard dose' of 400 IU/day has been chosen because this is the AAP recommended supplementation dose and reflects current practice in our population.[3, 27] The 'high dose' of 2000 IU/day has been chosen to include any reasonable supplementation dose while keeping total vitamin D intake below the Tolerable Upper Intake Level as recommended by Health Canada (2500 IU/day for preschoolers).[81, 82]**

Parents of children in each group will be instructed to administer 1 drop of the provided solution to their child by mouth once daily, at any time of day, each day from the time of enrolment (September-November) over the winter until seen in follow-up (April-May) 4-8 months later. The duration of the intervention (4-8 months) has been chosen to mimic the routine practice of vitamin D supplementation of preschoolers over the Canadian winter. A drop based formulation has been chosen to facilitate ease of administration to young children. Vitamin D3 containing solutions will be provided in kind by Ddrops™. Actual vitamin D concentrations provided to study participants will be verified using established analytical techniques on a subset of provided solutions.[111, 112]

Concomitant interventions prohibited include over the counter multivitamins which contain vitamin D, over the counter vitamin D preparations and prescription vitamin D. **As this is a pragmatic trial, no specific strategies will be introduced to improve adherence.** To monitor adherence at the end of the trial, parents will be asked to return bottles and the amount of vitamin D administered will be calculated based on the volume of solution remaining. No specific criteria will be developed for

discontinuation or modification of the interventions, as the doses of vitamin D are within the safe and recommended dosages for children.[81]

D. Randomization

Stratification, Blocking and Sequence Generation

Children will be randomized to 'standard dose' or 'high dose' groups. Block randomization will occur by study site with blocks of varying sizes.[103] Block randomization ensures that group sizes are similar at the end of each block. This is particularly important in a study of vitamin D and URTI, as date of enrolment (time) may be an important covariate. Sequence generation stratified by clinic site will be performed by the Pharmacy Department at The Hospital for Sick Children using a computer random number generator.

Allocation Concealment Mechanism

Allocation concealment is the process that prevents any trial participant or investigator from knowing the treatment to which subjects will be assigned, and prevents selection bias. Allocation concealment will be achieved by having the Pharmacy Department prepare the vitamin D preparations in sealed, serially numbered bottles identical in appearance and weight.

Implementation

The Research Pharmacist will generate the allocation sequence; Research Assistants at each clinic site will enrol and assign participants to the interventions.

E. Duration of treatment

As 25-hydroxyvitamin D levels are reported to stabilize within 8 weeks,[113, 114] and respiratory viruses tend to circulate in Canada November through April,[115] children will be recruited September through November and parents will be asked to provide the daily study supplement from the time of enrolment through the winter until seen in follow-up 4 to 8 months later in April or May.

F. Outcomes

Primary Outcome

The primary outcome will be the number of laboratory-confirmed viral URTIs per child over the winter months. Because of the wide variety of respiratory viruses and URTI illness presentations, signs and symptoms of viral URTI are non-specific and may not be reliably measured by parental report.[116, 117]

Secondary Outcomes

Secondary outcomes include parent reported URTI, viral agent (specifically influenza, adenovirus and RSV which are the most likely viruses to cause illness), wheezing episodes among children with asthma, physician-diagnosed otitis media and pneumonia. Direct health services related costs as well as indirect costs to families from URTI will be calculated using an economic analysis described below. Follow-up 25-hydroxyvitamin D level will be measured in order to determine the vitamin D dose vs. serum response relationship. Finally, change in 25-hydroxyvitamin D level from baseline will be determined to document that an improvement in health outcomes is mediated through an increase in 25-hydroxyvitamin D serum level.

G. Outcome measurement

Viral isolation

Parents of enrolled children will be asked to obtain a nasal swab from their child and complete a symptom checklist with each URTI. Parental obtained nasal swabs have been shown to be as effective in detecting respiratory viruses as health professional obtained nasopharyngeal sampling but less invasive and better tolerated.[118] Parents will be instructed to place the swab in provided viral transport media and refrigerate until couriered to the study laboratory within 24 hours.[43, 119] Reverse transcriptase polymerase chain reaction (RT-PCR) will be performed on each sample which has a sensitivity of 95% and specificity of 100% for the identification of respiratory RNA viruses

which is more sensitive than viral culture and comparable to immunofluorescence.[120, 121] Samples will be tested for 18 common respiratory viruses including influenza A and B, adenoviruses, respiratory syncytial virus (RSV), picornaviruses (enteroviruses and rhinoviruses), coronovirus, metapneumovirus and parainfluenza virus using the ID-Tag™ RVP assay using the Luminex xMAP™ system (Cat# R019A0105, TM Bioscience Corp., Toronto, ON).[122-124] This is the same assay and machine that is routinely used in the Ontario public health laboratories.

Parent reported URTI

Parental reported URTI symptoms will be defined as two or more of fever (>38°C), cough, runny nose, sore throat, headache, vomiting, feels unwell, muscle aches, ear ache or infection, poor appetite, not sleeping well, cranky/fussy, low energy or crying more than usual from a validated parent completed symptom checklist (CARIFS) collected with the viral sample. CARIFS has been developed, validated and extensively used by our team and others.[125-127] A new URTI cannot commence until ≥ 3 symptom free days since the end of a previous URTI.[43]

Asthma exacerbation

Asthma will be defined as parental report of asthma plus confirmation from the child's medical record of 2 or more episodes of wheeze requiring the prescription of inhaled asthma controlling medications.[128, 129] Asthma exacerbation will be defined as a wheezing episode in children with asthma as obtained from parent completed symptom checklist based on the International Study of Asthma and Allergies in Childhood (ISAAC).[129]

Serum vitamin D level

Blood for serum levels will be drawn by trained pediatric phlebotomists from the antecubital vein for determination of 25-hydroxyvitamin D. Specimens will be sent to the Clinical Biochemistry Laboratory at the Mount Sinai Hospital with the study requisition, where they will be processed according to standard procedures. Under the direction of Dr. Reinhold Vieth who is an internationally recognized expert in vitamin D biochemistry and director of the Bone and Mineral Laboratory at Mount Sinai Hospital, total 25-hydroxyvitamin D will be measured from serum samples using a competitive two-step chemiluminescence assay which has been validated for measurement of 25-hydroxyvitamin D in children older than 1 year of age.[130, 131] The specific instrument that will analyze all samples is a Diasorin LIAISON® 25-hydroxyvitamin D TOTAL.[132] This technique and instrument have been chosen to be consistent with national vitamin D surveys from both Canada and the United states.[16, 25] Extensive testing and validation of this machine has been performed and has demonstrated an intraassay imprecision of 7.2% at a concentration of 213 nmol/L and an interassay imprecision of 4.9% at 32 nmol/L, 8.9% at 77 nmol/L and 17.4% at 213 nmol/L, values which are well within acceptable limits for biochemical measurements. 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. [133]

Other outcome measures

Physician-diagnosed otitis media, pneumonia, emergency department visits and hospitalizations will be collected by monthly telephone call and confirmed by review of the child's clinic medical record.

H. Duration and frequency of follow-up

Parents will receive a monthly telephone call by a research assistant reminding them to collect nasal samples and complete symptom checklists. This methodology has been used successfully by our group and others. [43, 45, 119, 126] In addition, parents will be asked to return to their child's physician's office in April or May for measurement of 25-hydroxyvitamin D level by blood test and the completion of a post-intervention survey. **I. Co-interventions**

The following potential co-interventions will also be measured at the follow-up visit: Influenza vaccination, dietary vitamin D intake measured by research assistants using a 3 day dietary

recall,[106] over the counter vitamin and mineral supplementation (ie. calcium containing multivitamins), herbal remedies (ie. echinacea) and hours per week in daycare.

J. Blinding

Parents, attending physicians, laboratory personnel, and study personnel conducting the outcome assessments, data analysts and investigators will be blind to the group allocation. Study medication will be supplied in bottles that look identical, and the appearance, consistency and taste of the drops will be similar. Group allocation will be concealed until the final data analysis is performed.

K. Target sample size

The sample size was based on asymptotic methods for a likelihood ratio test assuming a Poisson distributed outcome and was confirmed by simulation studies. All sample size and power calculations assume a 5% Type I error probability (two-sided). If we assume an average of one URTI per month during the winter months[40, 43-51] among children receiving the standard dose of vitamin D, we would therefore expect an average of four URTI per child over the winter. A sample size of 300 per group would give 90% power to detect a reduction in the average number of URTI per winter of one URTI. This sample size will retain sufficient power if the seasonal incidence of URTI is lower. Similarly, larger differences will be detected with power exceeding 90%. We believe that, even one fewer URTI over the winter would be a clinically important outcome, especially to families with young children. Although the study is not powered to detect reductions of specific kinds of infections, a conservative estimate is that at least 25% of children will have one of RSV, Adenovirus or Flu which collectively result in the greatest burden of illness in this population,[43, 52, 53] this sample size gives 80% power to detect a 50% absolute reduction in this composite outcome.

Preliminary TARGet Kids! data has suggested an 80% retention rate [134, 135] therefore, to accommodate a 20% loss to follow-up, 375 children will be recruited to each group (750 total).

L. Feasibility

Since September 2008, over 3500 children age 1-5 years have been recruited through TARGet Kids! practices with collection of non-invasive measures including completion of questionnaires (demographics, SES, family/lifestyle factors) and anthropometric measures (height, weight and waist circumference). Since February 2009 venous blood samples have been collected from over 1500 children.[134] TARGet Kids! is now operating out of six sites with over 100 children per month being recruited with phlebotomy. Therefore, recruitment of 200 children over three months (September – November) in 4 separate years for this study will be feasible.

M. Statistical analysis

Baseline characteristics will be summarized by appropriate descriptive statistics. Although randomization is expected to balance the covariates, variables that demonstrate, by chance, a potentially clinically meaningful imbalance, will be considered as possible adjusting covariates.

All outcomes will be analyzed following the *intention-to-treat* principle**.**[136] **The primary analysis of the primary outcome** will assess the effect of vitamin D supplementation on laboratory-confirmed URTI. Mean (per child) URTI rates will be computed for each group. A Poisson regression model will be used to make the statistical comparison between the groups. *Variable length of follow-up time* will be accounted for by using a suitable offset (logarithm of observation duration) in the Poisson model. If there is evidence of overdispersion, a negative-binomial model may be considered. **The secondary analysis of the primary outcome** will use survival methods to examine time to first URTI and the Andersen-Gill extension of the Cox model will be used to analyze recurrent URTI events.[137] The rationale for conducting the trial over 4 seasons is to increase the chance of a sufficiently active viral season to assess a protective effect of the intervention. This will also allow us to examine the consistency of the effect over the 4 seasons as strains of viruses vary from year to year.

Analysis of secondary outcomes will use standard methods for continuous data (ie. vitamin D level) using means, ANOVA and linear regression with and without adjustment for baseline group differences. The incidence of binomial secondary outcomes such as specific viral infections will be summarized descriptively. Since the incidence of some infections may be low, logistic regression analyses will only be performed to statistically assess the treatment effect when there are sufficient events (between 30 and 60). Subgroup analyses will be conducted for children with asthma. Frequency of asthma exacerbations will be analyzed similarly to the primary outcome.

N. Economic Analysis

Cost-consequence and cost-effectiveness analyses will be conducted using data from this clinical trial. A societal perspective will be employed as both direct health service utilization costs as well as indirect costs to families from URTI will be calculated for the 'high dose' vs. 'standard dose' groups. The time horizon of the analysis will be limited to the follow-up of this trial in order to leverage direct data. Given the short time horizon of this analysis, discounting of costs and outcomes will not be applied. This analysis will include the cost of 'high dose' vitamin D supplementation, physician visits, medications (antimicrobial and over the counter), hospital admissions, emergency department visits and laboratory testing abstracted from the child's medical record and costs associated with lost income from parental work absenteeism and time out of daycare obtained by monthly telephone call using previously described techniques.[45, 55, 86, 126, 138] Standard, publicly available costing sources will be used to cost resource utilization parameters. Specifically, we will use cost sources such as the Ontario Health Insurance Programs (OHIP), Ontario Case Costing Initiative (OCCI) and standard Ministry of Health (MOH) reimbursements for diagnostic tests. Sex-weighted hourly wage rate will be derived from Statistics Canada Data. For the cost-effectiveness analysis, the cost-effectiveness parameters will be the cost per laboratory-confirmed URTI avoided. The net benefit regression approach will be used to determine each patient's net benefit from treatment (NB_i) based upon the data collected on resource use and lost parental productivity.[139, 140] Graphs will be used to illustrate the incremental net benefit assuming varying willingness to pay. Each estimate of net benefit can be adjusted for potential confounders through regression. In its simplest form, net benefit regression involves fitting the following simple linear regression model: $NB_i = \beta_0 + \beta_{TX}TX_i + \varepsilon_i$ where TX_i is the i^{th} person's treatment indicator ($TX_i = 1$ for new treatment and 0 for usual care) and ε_i is a stochastic error term.[141] Confidence intervals for incremental net benefit will be compared to results of non-parametric bootstrapping to characterize statistical uncertainty in the economic analysis.

O. Compliance

Parents of participating children will receive a monthly telephone call reminding them to administer the study supplement daily and recording the number of missed days. In addition, compliance with supplementation will be measured by volume of solution left in vitamin D bottles at follow-up.[142]

P. Recruitment and retention

Strategies to achieve adequate recruitment will include approaching eligible subjects during a well child visit. Lists of children scheduled for a well child visit will be reviewed in advance; letters inviting participation will be mailed 2 to 4 weeks in advance of the visit. Subjects will be approached in person by the research assistant while registering for the clinic visit. At the initial visit, non-invasive measures (questionnaires, anthropometric measures) will be collected while the child and family are waiting for their appointment with baseline blood sampling occurring after the appointment. These methods have been successfully used in other TARGet Kids! studies.[2, 26, 27, 134, 143, 144]

Strategies for retention include a monthly telephone call to encourage collection of nasal swabs and completion of symptom checklists. Every reasonable attempt will be made to locate patients at follow-up. Topical anesthetic cream (EMLA or Ametop) will be offered to minimize discomfort from venipuncture. Blood will be drawn in the primary care physician's office negating the need to attend a

separate laboratory visit. Parents who have moved out of district will be offered to visit the Hospital for Sick Children for repeat laboratory testing. Furthermore, for children who do not attend their follow-up visit and refuse to visit the Hospital for Sick Children, a home visit for phlebotomy will be offered. This is expected to occur in less than 10% of subjects.

Q. Participant timeline:

Participants will be recruited September through November with baseline survey data, anthropometrics and baseline vitamin D serum level collected and followed until seen in follow-up in April or May to capture peak respiratory virus season. At the follow-up visit blood will be drawn for 25-hydroxyvitamin D level and a post intervention survey completed. **This will occur over four seasons with different participants recruited in each season as respiratory virus incidence, distribution and severity tend to vary from year to year.**)

SECTION 5: DATA COLLECTION AND MANAGEMENT

A. Data collection methods

Plans for the collection of baseline data and outcome assessment are described in Section 4B (Participants), 4F (Outcomes) and Section 4Q (Timeline). To ensure high quality data collection, research assistants will be trained in the accurate completion of questionnaires, anthropometric measurement and will be registered pediatric phlebotomists as per standard TARGet Kids! operating protocol. Weight will be measured using a precision digital scale (±0.025% SECA, Hamburg Germany) and standing height will be measured using a stadiometer (SECA, Hamburg Germany). Baseline questionnaires have been extensively pilot tested since September 2008, and numerous modifications have been made to ensure understandability and reduce incomplete responses. URTI symptom checklists have been thoroughly tested and extensively used.[125, 126] Collected data will be **electronically entered into the study database on a daily basis by research assistants at each site**. A secure web-based data portal has been established to electronically transfer data to a central database at the Applied Health Research Centre at St. Michael's Hospital.

B. Data management

The Applied Health Research Centre (AHRC) of the Keenan Research Centre in the Li Ka Shing Knowledge Institute of St. Michael's Hospital will be the data coordinating centre for this study. AHRC is a not-for profit academic research organization, dedicated to serving the needs of clinical investigators by providing the best research solutions. Medidata RAVE by Medidata Solutions Inc. is the electronic data capture technology and data repository for TARGet Kids! data collected during this study. Medidata RAVE is an industry-leading electronic data capture and clinical data management system, with user-configurable workflows, sophisticated case report form (CRF) design, complex edit checking, and customized security parameters. Medidata RAVE allows TARGet Kids! research assistants to enter data in real time to the TARGet Kids! central database from any of the practice sites. Medidata RAVE has extensive built-in reporting capabilities, and data can be exported to standard formats for data analysis (SAS, SPSS, and others). Laboratory tests from Mount Sinai Services will be directly uploaded to Medidata RAVE through a secure web portal. These features enable TARGet Kids! to be highly efficient and are ***vital to the success of this project.***

SECTION 6: ETHICAL CONSIDERATIONS AND SAFETY

A. Ethics

An information package will be mailed to each family 2 to 4 weeks prior to being formally recruited. This will allow parents time for consideration in advance and reduce pressure to participate. Parents will benefit by the provision of 4-8 months of vitamin D supplementation for their child free of charge. Children may directly benefit via the identification of viral etiology for URTI symptoms and through

identification of vitamin D deficiency. The child's pediatrician will receive viral test results as well as blood results and manage according to national clinical guidelines.[4] Parents of all study participants will have provided informed consent. The investigators will seek approval from the Hospital for Sick Children Research Ethics Board.

While *a placebo arm* may be ethically justified given the lack of evidence supporting improved health outcomes with supplementation, it is unlikely to be feasible given that current AAP vitamin D guidelines (2007) include a recommendation for routine vitamin D supplementation for children older than 1 year and the majority of families in our population are following this recommendation.[3, 27]

B. Safety

As vitamin D dosages in this study are within the Tolerable Upper Intake Level as recommended by Health Canada for children older than 1 year of age (2500 IU per day for children 1-3 years and 3000 IU/day for children 4-8 years), risk of vitamin D excess is low.[145] In addition, **other studies which have used vitamin D doses of up to 50,000 IU per week in children did not show evidence of vitamin D toxicity**.[146, 147] However, we will monitor for adverse events among study participants by having collaborating community physicians aware of signs and symptoms of vitamin D toxicity including nephrolithiasis and hypercalcemia. All adverse events will be reported to the Hospital for Sick Children's and St. Michael's Hospital's Research Ethics Board according to institutional adverse event reporting requirements. All serious, unexpected adverse drug reactions to the study medication will be reported to Health Canada within 15 calendar days or for death or life-threatening events, within 7 calendar days. In the latter case, a follow-up report will be filed within 8 calendar days. Additionally, each participant will be assessed for signs of vitamin D toxicity at follow-up through measurement of serum calcium, alkaline phosphatase and parathyroid hormone.[23, 148, 149]

A Data Safety and Monitoring Board (DSMB) will be established. The DSMB will be responsible for oversight of data and safety. The DSMB will be composed of experts in pediatric medicine, endocrinology, infectious disease and biostatistics and clinical trial methodology. The DSMB members will be independent of all those participating in the study. The DSMB will review data on adverse events, data quality, trial efficacy and subject recruitment between years 1 and 2. The DSMB will prepare meeting minutes and make recommendations about study conduct to the Principal Investigator.

SECTION 7: KNOWLEDGE TRANSLATION

TARGet Kids! is a unique collaboration between child health researchers at the St. Michael's Hospital, The Hospital for Sick Children and community health care providers. It provides a novel opportunity for truly *integrated knowledge translation*. TARGet Kids! primary care physician members have participated in the early stages of shaping the research process to reach the overarching TARGet Kids! goal: to promote health for all Canadian children. Other members of TARGet Kids! include policy makers in primary care.

Findings from this research will be disseminated directly to the physician participants and to their patients. A meeting of all the TARGet Kids! practices (physicians, nurses, office staff), research team (investigators, research assistants, students), and policy leaders (representatives from Section of Community Paediatrics, Family and Community Practice, Ontario Medical Association, and parent representatives) occurs annually. Downstream dissemination to primary care physicians will occur through formal and informal venues at local levels, such as City Wide Paediatric Rounds, HSC Paediatric Update and those held by local physician groups.

End of grant knowledge will be shared with the academic community through publication in relevant journals and presentations at national and international conferences, and locally through hospital rounds and presentations. Messages will be relevant to professionals working in the fields of

pediatrics, family medicine, endocrinology, infectious disease, nursing, dietetics, and public health. Information will be disseminated to professionals by the research team with colleagues at the Ontario Agency for Health Protection and Promotion, the Centre for Effective Practice, the Maternal Infant Child and Youth Research Network (MICYRN), the Ontario Medical Association, the Canadian Pediatrics Society and the American Academy of Pediatrics. Opportunities for coverage in lay media will be sought using an experienced knowledge broker (funded by current grants).

SECTION 10: SUMMARY

There is compelling evidence that young children in North America have vitamin D levels significantly lower than experts recommend. Basic science and epidemiological studies make the case that low wintertime vitamin D levels may be related to two common and costly child health outcomes: wintertime viral URTI and asthma exacerbations. **This study will be the first RCT to investigate vitamin D related health outcomes in preschoolers. The results of this trial will make an immediate contribution by defining clinical practice standards for vitamin D supplementation for young children and provide an evidence base for national vitamin D guidelines.**

Key strengths of this proposal are a **novel and important research question**, the outcome of which could affect nearly every young child in Canada, a **methodologically rigorous plan** and an implementation strategy that **leverages the efficiency of the only primary care research platform for children in Canada: TARGet Kids!.** Furthermore, the investigators form a solid team of highly qualified methodologists, content experts and primary care providers who have come together with the aim of **integrated knowledge translation** between policy makers, investigators, physicians and families.

REFERENCES

1. Holick, M.F., *Resurrection of vitamin D deficiency and rickets.* J Clin Invest, 2006. **116**(8): p. 2062-72.
2. Maguire, J.L., et al., *Prevalence and predictors of low vitamin D in urban Canadian toddlers: A TARGet Kids! Study.* Pediatr Child Health, 2011. **16**(2): p. e11-e15.
3. Wagner, C.L. and F.R. Greer, *Prevention of rickets and vitamin D deficiency in infants, children, and adolescents.* Pediatrics, 2008. **122**(5): p. 1142-52.
4. CPS, *Vitamin D supplementation: Recommmendations for Canadian mothers and infants.* Paediatrics & Child Health 2007. **12**(7): p. 593-589.
5. CIHI, *National Ambulatory Care Reporting System*. 2005, Ottawa: Canadian Institute for Health Information.
6. Holick, M.F., *Vitamin D deficiency.* N Engl J Med, 2007. **357**(3): p. 266-81.
7. Bouillon, R., *Vitamin D: From photosynthesis, metabolism, and action to clinical applications*, in *Endocrinology*, L.J. DeGroot and J.L. Jameson, Editors. 2001, W.B. Saunders: Philadelphia. p. 1009-28.
8. DeLuca H, F., *Overview of general physiologic features and functions of vitamin D.* Am J Clin Nutr, 2004. **80**(Suppl): p. 1689S-1696S.
9. MacLaughlin, J.A., R.R. Anderson, and M.F. Holick, *Spectral character of sunlight modulates photosynthesis of previtamin D3 and its photoisomers in human skin.* Science, 1982. **216**(4549): p. 1001-3.
10. Webb, A.R., L. Kline, and M.F. Holick, *Influence of season and latitude on the cutaneous synthesis of vitamin D3: exposure to winter sunlight in Boston and Edmonton will not promote vitamin D3 synthesis in human skin.* J Clin Endocrinol Metab, 1988. **67**(2): p. 373-8.
11. Behrman, R.K., R. Jenson, HB, *Nelson Textbook of Pediatrics*. Vol. 16th edition. 2000: W.B. Saunders Company.
12. Roth, D.E., et al., *Are national vitamin D guidelines sufficient to maintain adequate blood levels in children?* Can J Public Health, 2005. **96**(6): p. 443-9.
13. HealthCanada, *Do Canadian children meet their nutrient requirements through food intake alone?* http://www.hc-sc.gc.ca/fn-an/surveill/nutrition/commun/art-nutr-child-enf-eng.php#a33, 2007. **Cat. H164-112**.
14. Gordon, C.M., et al., *Prevalence of vitamin D deficiency among healthy infants and toddlers.* Arch Pediatr Adolesc Med, 2008. **162**(6): p. 505-12.
15. Mansbach, J.M., A.A. Ginde, and C.A. Camargo, Jr., *Serum 25-hydroxyvitamin D levels among US children aged 1 to 11 years: do children need more vitamin D?* Pediatrics, 2009. **124**(5): p. 1404-10.
16. Kumar, J., et al., *Prevalence and Associations of 25-Hydroxyvitamin D Deficiency in US Children: NHANES 2001-2004.* Pediatrics, 2009.
17. Stoian, C.A., et al., *Vitamin D concentrations among healthy children in Calgary, Alberta.* Paediatrics & Child health, 2011. **16**(2): p. 82-86.
18. Hammer, L.D., S. Bryson, and W.S. Agras, *Development of feeding practices during the first 5 years of life.* Arch Pediatr Adolesc Med, 1999. **153**(2): p. 189-94.
19. CPS. *Nutrition for Health Term Infants - Statement of the joint working group: Canadian Paediatric Society, Dieticians of Canada and Health Canada*. 2005 [cited 2009 January 13, 2008].

20. Northstone, K., I. Rogers, and P. Emmett, *Drinks consumed by 18-month-old children: are current recommendations being followed?* Eur J Clin Nutr, 2002. **56**(3): p. 236-44.
21. Korsch, M., K. Nelson, and J. Reinhart, *Feeding behaviors in infancy and ealry childhood*, in *Guidelines for Health Supervision III*. 1997, American Academy of Pediatrics: Elk Grove Village, Ill. p. 57.
22. Baker, R.D. and F.R. Greer, *Diagnosis and prevention of iron deficiency and iron-deficiency anemia in infants and young children (0-3 years of age).* Pediatrics. **126**(5): p. 1040-50.
23. Greer, F.R., *25-Hydroxyvitamin D: functional outcomes in infants and young children.* Am J Clin Nutr, 2008. **88**(2): p. 529S-533S.
24. Lips, P., *Vitamin D physiology.* Prog Biophys Mol Biol, 2006. **92**(1): p. 4-8.
25. Langlois, K., et al., *Vitamin D status of Canadians as measured in the 2007 to 2009 Canadian Health Measures Survey.* Health Reports (Statistics Canada), 2010. **21**(1): p. 47-55.
26. Maguire, J.L., et al., *How much cow's milk should preschoolers be drinking?: A TARGet Kids! study.* Pediatric Academic Societies Annual Meeting, 2011. **E-PAS 1385.5**.
27. Maguire, J.L., et al., *Milk volume, vitamin D supplementation, outdoor activity and season are important predictors of vitamin D serum level in preschoolers: A TARGet Kids! study.* E-PAS, 2011. **2155.8**.
28. Gessner, B.D., J. Plotnik, and P.T. Muth, *25-hydroxyvitamin D levels among healthy children in Alaska.* J Pediatr, 2003. **143**(4): p. 434-7.
29. Gordon, C.M., et al., *Prevalence of vitamin D deficiency among healthy adolescents.* Arch Pediatr Adolesc Med, 2004. **158**(6): p. 531-7.
30. Newhook, L.A., et al., *Vitamin D insufficiency common in newborns, children and pregnant women living in Newfoundland and Labrador, Canada.* Matern Child Nutr, 2009. **5**(2): p. 186-91.
31. Ward, L.M., et al., *Vitamin D-deficiency rickets among children in Canada.* CMAJ, 2007. **177**(2): p. 161-6.
32. Mallet, E., et al., *Vitamin D supplementation in pregnancy: a controlled trial of two methods.* Obstet Gynecol, 1986. **68**(3): p. 300-4.
33. Javaid, M.K., et al., *Maternal vitamin D status during pregnancy and childhood bone mass at age 9 years: a longitudinal study.* Lancet, 2006. **367**(9504): p. 36-43.
34. Markestad, T., et al., *25-Hydroxyvitamin D and 1,25-dihydroxyvitamin D of D2 and D3 origin in maternal and umbilical cord serum after vitamin D2 supplementation in human pregnancy.* Am J Clin Nutr, 1984. **40**(5): p. 1057-63.
35. Lee, J.M., et al., *Vitamin D deficiency in a healthy group of mothers and newborn infants.* Clin Pediatr (Phila), 2007. **46**(1): p. 42-4.
36. Hollis, B.W. and W.B. Pittard, 3rd, *Evaluation of the total fetomaternal vitamin D relationships at term: evidence for racial differences.* J Clin Endocrinol Metab, 1984. **59**(4): p. 652-7.
37. Bianchi, M.L., *Osteoporosis in children and adolescents.* Bone, 2007. **41**(4): p. 486-95.
38. Hypponen, E., et al., *Intake of vitamin D and risk of type 1 diabetes: a birth-cohort study.* Lancet, 2001. **358**(9292): p. 1500-3.
39. Zipitis, C.S. and A.K. Akobeng, *Vitamin D supplementation in early childhood and risk of type 1 diabetes: a systematic review and meta-analysis.* Arch Dis Child, 2008. **93**(6): p. 512-7.

40. Heikkinen, T. and A. Jarvinen, *The common cold.* Lancet, 2003. **361**(9351): p. 51-9.
41. Burt, C.W., L.F. McCaig, and E.A. Rechtsteiner, *Ambulatory medical care utilization estimates for 2005.* Adv Data, 2007(388): p. 1-15.
42. Vingilis, E., et al., *Evaluation of a cold/flu self-care public education campaign.* Health Educ Res, 1998. **13**(1): p. 33-46.
43. Lambert, S.B., et al., *Community epidemiology of human metapneumovirus, human coronavirus NL63, and other respiratory viruses in healthy preschool-aged children using parent-collected specimens.* Pediatrics, 2007. **120**(4): p. e929-37.
44. Monto, A.S. and F. Kioumehr, *The Tecumseh Study of Respiratory Illness. IX. Occurence of influenza in the community, 1966--1971.* Am J Epidemiol, 1975. **102**(6): p. 553-63.
45. Lambert, S.B., et al., *The cost of community-managed viral respiratory illnesses in a cohort of healthy preschool-aged children.* Respir Res, 2008. **9**: p. 11.
46. Hendley, J., *Epidemiology, Pathogenesis, and Treatment of the Common Cold.* Seminars in Pediatirc Infectious Diseases, 1998. **9**(1): p. 50-55.
47. Frank, A.L., et al., *Influenza B virus infections in the community and the family. The epidemics of 1976-1977 and 1979-1980 in Houston, Texas.* Am J Epidemiol, 1983. **118**(3): p. 313-25.
48. Collet, J.P., et al., *Type of day-care setting and risk of repeated infections.* Pediatrics, 1994. **94**(6 Pt 2): p. 997-9.
49. Schwartz, B., et al., *Respiratory infections in day care.* Pediatrics, 1994. **94**(6 Pt 2): p. 1018-20.
50. Benediktsdottir, B., *Upper airway infections in preschool children--frequency and risk factors.* Scand J Prim Health Care, 1993. **11**(3): p. 197-201.
51. Kvaerner, K.J., P. Nafstad, and J.J. Jaakkola, *Upper respiratory morbidity in preschool children: a cross-sectional study.* Arch Otolaryngol Head Neck Surg, 2000. **126**(10): p. 1201-6.
52. Roche, P., S. Lambert, and J. Spencer, *Surveillance of viral pathogens in Australia: respiratory syncytial virus.* Commun Dis Intell, 2003. **27**(1): p. 117-22.
53. Chonmaitree, T., et al., *Viral upper respiratory tract infection and otitis media complication in young children.* Clin Infect Dis, 2008. **46**(6): p. 815-23.
54. Carabin, H., et al., *Estimation of direct and indirect costs because of common infections in toddlers attending day care centers.* Pediatrics, 1999. **103**(3): p. 556-64.
55. Bell, D.M., et al., *Illness associated with child day care: a study of incidence and cost.* Am J Public Health, 1989. **79**(4): p. 479-84.
56. Nurmi, T., E. Salminen, and A. Ponka, *Infections and other illnesses of children in day-care centers in Helsinki. II: The economic losses.* Infection, 1991. **19**(5): p. 331-5.
57. Haskins, R., *Acute illness in day care: how much does it cost?* Bull N Y Acad Med, 1989. **65**(3): p. 319-43.
58. Hope-Simpson, R.E., *The Transmission of Epidemic Influenza*. 1992, Plenum Press: New York. p. 77.
59. Cannell, J.J., et al., *Epidemic influenza and vitamin D.* Epidemiol Infect, 2006. **134**(6): p. 1129-40.
60. Herr, C., R. Shaykhiev, and R. Bals, *The role of cathelicidin and defensins in pulmonary inflammatory diseases.* Expert Opin Biol Ther, 2007. **7**(9): p. 1449-61.
61. Bartley, J., *Vitamin D, innate immunity and upper respiratory tract infection.* J Laryngol Otol. **124**(5): p. 465-9.

62. Hansdottir, S., et al., *Respiratory epithelial cells convert inactive vitamin D to its active form: potential effects on host defense.* J Immunol, 2008. **181**(10): p. 7090-9.
63. Boucher, R.C., *Molecular insights into the physiology of the 'thin film' of airway surface liquid.* J Physiol, 1999. **516 (Pt 3)**: p. 631-8.
64. Diamond, G., D. Legarda, and L.K. Ryan, *The innate immune response of the respiratory epithelium.* Immunol Rev, 2000. **173**: p. 27-38.
65. Brogden, K.A., *Antimicrobial peptides: pore formers or metabolic inhibitors in bacteria?* Nat Rev Microbiol, 2005. **3**(3): p. 238-50.
66. Brogden, K.A., et al., *Antimicrobial peptides in animals and their role in host defences.* Int J Antimicrob Agents, 2003. **22**(5): p. 465-78.
67. Gudmundsson, G.H. and B. Agerberth, *Neutrophil antibacterial peptides, multifunctional effector molecules in the mammalian immune system.* J Immunol Methods, 1999. **232**(1-2): p. 45-54.
68. Liu, P.T., et al., *Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response.* Science, 2006. **311**(5768): p. 1770-3.
69. Gombart, A.F., N. Borregaard, and H.P. Koeffler, *Human cathelicidin antimicrobial peptide (CAMP) gene is a direct target of the vitamin D receptor and is strongly up-regulated in myeloid cells by 1,25-dihydroxyvitamin D3.* FASEB J, 2005. **19**(9): p. 1067-77.
70. Yim, S., et al., *Induction of cathelicidin in normal and CF bronchial epithelial cells by 1,25-dihydroxyvitamin D(3).* J Cyst Fibros, 2007. **6**(6): p. 403-10.
71. Adams, J.S., et al., *Vitamin d-directed rheostatic regulation of monocyte antibacterial responses.* J Immunol, 2009. **182**(7): p. 4289-95.
72. Aloia, J.F. and M. Li-Ng, *Re: epidemic influenza and vitamin D.* Epidemiol Infect, 2007. **135**(7): p. 1095-6; author reply 1097-8.
73. Aloia, J.F., et al., *A randomized controlled trial of vitamin D3 supplementation in African American women.* Arch Intern Med, 2005. **165**(14): p. 1618-23.
74. Ginde, A.A., J.M. Mansbach, and C.A. Camargo, Jr., *Association between serum 25-hydroxyvitamin D level and upper respiratory tract infection in the Third National Health and Nutrition Examination Survey.* Arch Intern Med, 2009. **169**(4): p. 384-90.
75. Laaksi, I., et al., *An association of serum vitamin D concentrations < 40 nmol/L with acute respiratory tract infection in young Finnish men.* Am J Clin Nutr, 2007. **86**(3): p. 714-7.
76. Sabetta, J.R., et al., *Serum 25-hydroxyvitamin d and the incidence of acute viral respiratory tract infections in healthy adults.* PLoS One. **5**(6): p. e11088.
77. Camargo, C.A., Jr., T. Ingham, and K. Wickens, *Cord blood 25-hydroxyvitamin D levels and risk of childhood wheeze in New Zealand [abstract].* American Journal of Respiratory Critical Care Medicine, 2008. **177(Suppl)**: p. A993.
78. Li-Ng, M., et al., *A randomized controlled trial of vitamin D3 supplementation for the prevention of symptomatic upper respiratory tract infections.* Epidemiol Infect, 2009. **137**(10): p. 1396-404.
79. Laaksi, I., et al., *Vitamin D supplementation for the prevention of acute respiratory tract infection: a randomized, double-blinded trial among young Finnish men.* J Infect Dis. **202**(5): p. 809-14.
80. Urashima, M., et al., *Randomized trial of vitamin D supplementation to prevent seasonal influenza A in schoolchildren.* Am J Clin Nutr. **91**(5): p. 1255-60.

81. HealthCanada. *Vitamin D: Recommendations and review status*. 2010 [cited 2010 January 5]; Available from: http://www.hc-sc.gc.ca/fn-an/nutrition/vitamin/vita-d-eng.php.
82. IOM. *Dietary Reference Intakes for Calcium and Vitamin D*. 2010 [cited 2010 January 5]; Available from: http://www.iom.edu/Reports/2010/Dietary-Reference-Intakes-for-Calcium-and-Vitamin-D/Report-Brief.aspx.
83. To, T., et al., *ICES Investigative Report: The Burden of Asthma in Ontario*. 2006, Toronto: Institute for Clinical Evaluative Sciences.
84. CIHI, *Respiratory disease in Canada*. 2001, Ottawa: Canadian Institute for Health Information: Canadian Lung Association, Statistics Canada.
85. ICES, *Research Atlas: Inpatient and day surgery use by children in Ontario*. 1998, Toronto: Institute for Clinical Evaluative Sciences.
86. Krahn, M.D., et al., *Direct and indirect costs of asthma in Canada, 1990.* CMAJ, 1996. **154**(6): p. 821-31.
87. Litonjua, A.A. and S.T. Weiss, *Is vitamin D deficiency to blame for the asthma epidemic?* J Allergy Clin Immunol, 2007. **120**(5): p. 1031-5.
88. Masoli, M., et al., *The global burden of asthma: executive summary of the GINA Dissemination Committee report.* Allergy, 2004. **59**(5): p. 469-78.
89. Bosse, Y., K. Maghni, and T.J. Hudson, *1alpha,25-dihydroxy-vitamin D3 stimulation of bronchial smooth muscle cells induces autocrine, contractility, and remodeling processes.* Physiol Genomics, 2007. **29**(2): p. 161-8.
90. Song, Y., H. Qi, and C. Wu, *Effect of 1,25-(OH)2D3 (a vitamin D analogue) on passively sensitized human airway smooth muscle cells.* Respirology, 2007. **12**(4): p. 486-94.
91. Wjst, M., et al., *Asthma families show transmission disequilibrium of gene variants in the vitamin D metabolism and signalling pathway.* Respir Res, 2006. **7**: p. 60.
92. Raby, B.A., et al., *Association of vitamin D receptor gene polymorphisms with childhood and adult asthma.* Am J Respir Crit Care Med, 2004. **170**(10): p. 1057-65.
93. Saadi, A., et al., *Association study between vitamin D receptor gene polymorphisms and asthma in the Chinese Han population: a case-control study.* BMC Med Genet, 2009. **10**: p. 71.
94. Vollmert, C., et al., *Single nucleotide polymorphism screening and association analysis--exclusion of integrin beta 7 and vitamin D receptor (chromosome 12q) as candidate genes for asthma.* Clin Exp Allergy, 2004. **34**(12): p. 1841-50.
95. Bosse, Y., et al., *Asthma and genes encoding components of the vitamin D pathway.* Respir Res, 2009. **10**: p. 98.
96. Black, P.N. and R. Scragg, *Relationship between serum 25-hydroxyvitamin d and pulmonary function in the third national health and nutrition examination survey.* Chest, 2005. **128**(6): p. 3792-8.
97. Brehm, J.M., et al., *Serum vitamin D levels and severe asthma exacerbations in the Childhood Asthma Management Program study.* J Allergy Clin Immunol. **126**(1): p. 52-8 e5.
98. Searing, D.A., et al., *Decreased serum vitamin D levels in children with asthma are associated with increased corticosteroid use.* J Allergy Clin Immunol. **125**(5): p. 995-1000.
99. Brehm, J.M., et al., *Serum vitamin D levels and markers of severity of childhood asthma in Costa Rica.* Am J Respir Crit Care Med, 2009. **179**(9): p. 765-71.

100. Greer, F.R., *Defining vitamin D deficiency in children: beyond 25-OH vitamin D serum concentrations.* Pediatrics, 2009. **124**(5): p. 1471-3.
101. Litonjua, A.A., *Childhood asthma may be a consequence of vitamin D deficiency.* Curr Opin Allergy Clin Immunol, 2009. **9**(3): p. 202-7.
102. Thorpe, K.E., et al., *A pragmatic-explanatory continuum indicator summary (PRECIS): a tool to help trial designers.* CMAJ, 2009. **180**(10): p. E47-57.
103. Chan, A.W., et al. *The Spirit Initiative: Defining Standard Protocol Items for Randomized Trials*. Enahancing the Quality and Transparency of Health Research 2010 [cited 2010 September 6]; Available from: http://www.equator-network.org/resource-centre/library-of-health-research-reporting/reporting-guidelines-under-development/reporting-guidelines-under-development/.
104. *Strengthening the credibility of clinical research.* Lancet. **375**(9722): p. 1225.
105. Zwarenstein, M., et al., *Improving the reporting of pragmatic trials: an extension of the CONSORT statement.* BMJ, 2008. **337**: p. a2390.
106. StatsCan. *Canadian Community Health Survey*. 2004 [cited 2009 April 1].
107. Gozdzik, A., et al., *Serum 25-hydroxyvitamin D concentrations fluctuate seasonally in young adults of diverse ancestry living in Toronto.* J Nutr. **140**(12): p. 2213-20.
108. Shriver, M.D. and E.J. Parra, *Comparison of narrow-band reflectance spectroscopy and tristimulus colorimetry for measurements of skin and hair color in persons of different biological ancestry.* Am J Phys Anthropol, 2000. **112**(1): p. 17-27.
109. Clarys, P., et al., *Skin color measurements: comparison between three instruments: the Chromameter(R), the DermaSpectrometer(R) and the Mexameter(R).* Skin Res Technol, 2000. **6**(4): p. 230-238.
110. Hunter, S., et al., *Assessment of elementary school students' sun protection behaviors.* Pediatr Dermatol. **27**(2): p. 182-8.
111. Byrdwell, W.C., et al., *Analyzing vitamin D in foods and supplements: methodologic challenges.* Am J Clin Nutr, 2008. **88**(2): p. 554S-557S.
112. Holden, J.M. and L.E. Lemar, *Assessing vitamin D contents in foods and supplements: challenges and needs.* Am J Clin Nutr, 2008. **88**(2): p. 551S-553S.
113. Barger-Lux, M.J., et al., *Vitamin D and its major metabolites: serum levels after graded oral dosing in healthy men.* Osteoporos Int, 1998. **8**(3): p. 222-30.
114. Aloia, J.F., et al., *Optimal vitamin D status and serum parathyroid hormone concentrations in African American women.* Am J Clin Nutr, 2006. **84**(3): p. 602-9.
115. PHAC. *Respiratory virus surveillance*. 2001 [cited 2010 August 22]; Available from: http://www.phac-aspc.gc.ca/publicat/ccdr-rmtc/01vol27/dr2711eb.html.
116. Call, S.A., et al., *Does this patient have influenza?* JAMA, 2005. **293**(8): p. 987-97.
117. King, J.C., Jr., et al., *Effectiveness of school-based influenza vaccination.* N Engl J Med, 2006. **355**(24): p. 2523-32.
118. Esposito, S., et al., *Collection by trained pediatricians or parents of mid-turbinate nasal flocked swabs for the detection of influenza viruses in childhood.* Virol J. **7**: p. 85.
119. Lambert, S.B., K.M. Allen, and T.M. Nolan, *Parent-collected respiratory specimens--a novel method for respiratory virus and vaccine efficacy research.* Vaccine, 2008. **26**(15): p. 1826-31.
120. Templeton, K.E., et al., *Rapid and sensitive method using multiplex real-time PCR for diagnosis of infections by influenza a and influenza B viruses, respiratory syncytial virus, and parainfluenza viruses 1, 2, 3, and 4.* J Clin Microbiol, 2004. **42**(4): p. 1564-9.

121. Habib-Bein, N.F., et al., *Comparison of SmartCycler real-time reverse transcription-PCR assay in a public health laboratory with direct immunofluorescence and cell culture assays in a medical center for detection of influenza A virus.* J Clin Microbiol, 2003. **41**(8): p. 3597-601.
122. Krunic, N., et al., *xTAG RVP assay: analytical and clinical performance.* J Clin Virol, 2007. **40 Suppl 1**: p. S39-46.
123. Mahony, J. and S. Chong, *Clinical evaluation of a new ID-Tag RVP assay for the detection of 20 respiratory viruses.* Clinical Microbiology and Infection, 2007(1732_224).
124. Merante, F., S. Yaghoubian, and R. Janeczko, *Principles of the xTAG respiratory viral panel assay (RVP Assay).* J Clin Virol, 2007. **40 Suppl 1**: p. S31-5.
125. Jacobs, B., et al., *Canadian Acute Respiratory Illness and Flu Scale (CARIFS): development of a valid measure for childhood respiratory infections.* J Clin Epidemiol, 2000. **53**(8): p. 793-9.
126. Loeb, M., et al., *Effect of influenza vaccination of children on infection rates in Hutterite communities: a randomized trial.* JAMA. **303**(10): p. 943-50.
127. Belshe, R., et al., *The efficacy of live attenuated, cold-adapted, trivalent, intranasal influenzavirus vaccine in children.* N Engl J Med, 1998. **338**(20): p. 1405-1412.
128. Erkkola, M., et al., *Maternal vitamin D intake during pregnancy is inversely associated with asthma and allergic rhinitis in 5-year-old children.* Clin Exp Allergy, 2009. **39**(6): p. 875-82.
129. Asher, M.I., et al., *International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods.* Eur Respir J, 1995. **8**(3): p. 483-91.
130. Maunsell, Z., D.J. Wright, and S.J. Rainbow, *Routine isotope-dilution liquid chromatography-tandem mass spectrometry assay for simultaneous measurement of the 25-hydroxy metabolites of vitamins D2 and D3.* Clin Chem, 2005. **51**(9): p. 1683-90.
131. Singh, R.J., et al., *C-3 epimers can account for a significant proportion of total circulating 25-hydroxyvitamin D in infants, complicating accurate measurement and interpretation of vitamin D status.* J Clin Endocrinol Metab, 2006. **91**(8): p. 3055-61.
132. DiaSorin. *The Diagnostic Specialist*. http://www.diasorin.com/en/productsandsystems/view/20 2007.
133. Carter, G.D., et al., *How accurate are assays for 25-hydroxyvitamin D? Data from the international vitamin D external quality assessment scheme.* Clin Chem, 2004. **50**(11): p. 2195-7.
134. Maguire, J., et al., *TARGet Kids! - Rationale, goals and first steps.* Canadian Pediatric Society Annual Meeting Published Abstract, 2010. **E-CPS2010: 126**.
135. Maguire, J.L., et al., *Office-Based Intervention to Reduce Bottle Use Among Toddlers: TARGet Kids! Pragmatic, Randomized Trial.* Pediatrics.
136. Fisher, L.D., et al., *Intention to treat in clinical trials*, in *Statistical issues in drug research and development*. 1990, Marcel Dekker: New York.
137. Andersen, P.K. and R.D. Gill, *Cox's regression model for counting processes: A large sample study.* Ann Statist, 1982. **10**: p. 1100-1120.
138. Fendrick, A.M., et al., *The economic burden of non-influenza-related viral respiratory tract infection in the United States.* Arch Intern Med, 2003. **163**(4): p. 487-94.

139. Hoch, J.S., *Improving efficiency and value in palliative care with net benefit regression: an introduction to a simple method for cost-effectiveness analysis with person-level data.* J Pain Symptom Manage, 2009. **38**(1): p. 54-61.
140. Hoch, J.S., M.A. Rockx, and A.D. Krahn, *Using the net benefit regression framework to construct cost-effectiveness acceptability curves: an example using data from a trial of external loop recorders versus Holter monitoring for ambulatory monitoring of "community acquired" syncope.* BMC Health Serv Res, 2006. **6**: p. 68.
141. Hoch, J.S., A.H. Briggs, and A.R. Willan, *Something old, something new, something borrowed, something blue: a framework for the marriage of health econometrics and cost-effectiveness analysis.* Health Econ, 2002. **11**(5): p. 415-30.
142. Haynes, R.B. and R. Dantes, *Patient compliance and the conduct and interpretation of therapeutic trials.* Control Clin Trials, 1987. **8**(1): p. 12-9.
143. Maguire, J.L., et al., *Office-based intervention to reduce bottle use among toddlers: TARGet Kids! Pragmatic, randomized trial.* Pediatrics. **126**(2): p. e343-50.
144. Maguire, J.L., et al., *Vitamin D and cardiometabolic risk factors in preschoolers: A TARGet Kids! study.* E-PAS, 2011. **1635.4**.
145. HealthCanada. *Vitamin D: Recommendations and review status*. 1997 [cited 2009 October 17]; Available from: http://www.hc-sc.gc.ca/fn-an/nutrition/vitamin/vita-d-eng.php.
146. Gordon, C.M., et al., *Treatment of hypovitaminosis D in infants and toddlers.* J Clin Endocrinol Metab, 2008. **93**(7): p. 2716-21.
147. Tau, C., et al., *Twice single doses of 100,000 IU of vitamin D in winter is adequate and safe for prevention of vitamin D deficiency in healthy children from Ushuaia, Tierra Del Fuego, Argentina.* J Steroid Biochem Mol Biol, 2007. **103**(3-5): p. 651-4.
148. Vieth, R., *Vitamin D toxicity, policy, and science.* J Bone Miner Res, 2007. **22 Suppl 2**: p. V64-8.
149. Vieth, R., *Vitamin D supplementation, 25-hydroxyvitamin D concentrations, and safety.* Am J Clin Nutr, 1999. **69**(5): p. 842-56.
150. Weng, F.L., et al., *Risk factors for low serum 25-hydroxyvitamin D concentrations in otherwise healthy children and adolescents.* Am J Clin Nutr, 2007. **86**(1): p. 150-8.

Table 1. North American studies of low vitamin D including children > 1 year of age

Author	Location	Year	Latitude	Age	Season	N	<50 nmol/L	<75 nmol/L
Kumar [16]	Nationally representative US sample (NHANES)	2001-2004	NA	1-21 years	NA	6275	9% *	70%
Mansbach [15]	Nationally representative US sample (NHANES)	2001-2006	NA	1-5 years	Winter for lower latitudes; summer for higher latitudes	1799	14%	63%
Weng[150]	Philadelphia, US	NA	40° N	6-21 years	All Seasons	382	25%	55%
Gordon [14]	Boston, US	2005-2007	42° N	8-24 months	All seasons	133	12%	40%
Gordon [29]	Boston, US	2001-2003	42° N	11-18 years	All seasons	307	42%	NA
Gessner [28]	Alaska, US	2001-2002	58-61° N	6-23 months	All seasons	133	11% *	31% †
Roth [12]	Edmonton, Canada	2003	52° N	2-8 years	Spring	35	17%*	N/A
Newhook [30]	St. John's, NL, Canada	2005-2006	47° N	0-14 years	Fall/Spring	48	35%	77%
Langlois [25]	Nationally representative Canadian (CHMS)	2007-2009	NA	6-11 years	All seasons	453	N/A	51%
Maguire [2]	Toronto, Canada	2007-2008	43° N	24-30 months	Winter/spring	91	32%	82%
Stoian[17]	Calgary, Canada	2006	51° N	2-13 years	All seasons	1442	N/A	39%

* < 37.5 nmol/L
† < 62.5 nmol/L
NA = not available

Figure 1: DO IT! Trial Schematic