

## INTERVENTIONS FOR THE PREVENTION OF RESPIRATORY INFECTIONS

INVESTIGATING INTERVENTIONS FOR THE PREVENTION OF UPPER RESPIRATORY  
TRACT INFECTIONS

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## ABSTRACT

Upper respiratory tract infection (URTI), which presents clinically as the common cold, is the most common form of respiratory illness experienced by humans and is a major cause of morbidity and hospitalizations. Currently, URTI prevention focuses on hand hygiene with some consideration given to other lifestyle factors such as sleep, nutrition, and exercise. Identifying additional interventions for the prevention of URTI could reduce the burden of this disease.

In this thesis, I examine the role of vitamin D3 supplementation and tap water gargling for the prevention of URTI. I employ experimental and observational study designs to assess the effect of these interventions on the risk of URTI in the context of a randomized controlled trial of 600 participants, and a longitudinal cohort of 416 participants. Further, I investigate the association between modifiable lifestyle factors and risk of URTI using data from the longitudinal cohort. Data from this study is also used to explore statistical methods for the analysis of repeated events.

When evaluating self-reported, clinical URTI, all analyses supported the use of vitamin D3 supplementation to reduce the risk of URTI. However, this finding was only statistically significant in the analysis of the longitudinal cohort study; results from the RCT indicated that vitamin D3 supplementation statistically significantly reduced the risk of laboratory confirmed infections but had a non-significant benefit for clinical infections. Gargling did not reduce the risk of clinical or laboratory confirmed infections.

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## LIST OF ABBREVIATIONS

25(OH)D	25-hydroxyvitamin D
AG	Andersen-Gill model
ARI	Acute respiratory infection
CI	Confidence interval
CIHR	Canadian Institutes of Health Research
COPD	Chronic obstructive pulmonary disease
df	Degrees of freedom
DFA	Direct fluorescent antibody
GEE	Generalized estimating equations
HR	Hazard ratio
IU	International units
IQR	Interquartile range
MI	Multiple imputation
NAAT	Nucleic acid amplification test
NPS	Nasopharyngeal swabs
NS	Nasal swabs
ONBOIDS	Ontario burden of infectious disease study
OR	Odds ratio
PCR	Polymerase chain reaction
PWP	Prentice, Williams and Peterson model
QICC	Quasi-likelihood under independence model criterion
RCT	Randomized controlled trial

RR	Relative risk
RSV	Respiratory syncytial viruses
RT-PCR	Real-time polymerase chain reaction
URTI	Upper respiratory tract infection
WLW	Wei, Lin and Weissfeld model
WURSS	Wisconsin upper respiratory symptom survey

## PREFACE

This PhD thesis is based on the development, execution and analysis of a factorial randomized controlled trial (RCT). The McFlu2 COLD<sub>3</sub> Prevention study was designed to assess vitamin D3 versus placebo, and gargling versus no gargling for the prevention of upper respiratory tract infection in university students. The doctoral work is presented as a “sandwich thesis” which consists of three chapters written as manuscripts, along with introductory and concluding chapters and two appendices. Details of my contributions at each stage of the study and to each manuscript are provided below.

The study protocol, included as an appendix, details the methods and rationale behind the factorial randomized controlled trial. The concept of exploring vitamin D3 supplementation and gargling for the prevention of upper respiratory tract infections was first suggested by my supervisor, Dr. Marek Smieja, however, I assumed the lead role in designing the trial and writing the protocol. Throughout this process, I benefited from consultation with Marek, as well as two of my committee members: Dr. Eleanor Pullenayegum and Dr. Mark Loeb. In addition to writing the protocol, I was responsible for successfully completing the research ethics board approval process and developing related trial documents such as consent forms, enrollment and randomization logs, and study questionnaires. I also developed the recruitment plan, and coordinated and managed the execution of the trial. While I oversaw the daily trial activities, I received help from four colleagues, Andrea Granados, Lisa Banh, Siddhi Mathur, and Sue Carruthers, during the recruitment and collection phases of the study to ensure sufficient study personnel were available to support the participants.

There were four major areas of the study that were executed by other individuals. Rita Chan was hired to prepare the Health Canada Clinical Trial Application. During this process, I provided the relevant information about the study protocol but Rita wrote and compiled the document for approval from the Health Products and Food Branch. Gita Sobhi, the Hamilton Health Sciences Research pharmacist, was hired to produce aesthetically matched placebo and active vitamin D3 capsules. Ms. Sobhi was also responsible for packaging the appropriate capsules according to the randomization scheme and for the appropriate destruction of unused capsules upon the study completion. The randomization scheme was prepared by Dr. Marek Smieja using Microsoft Excel. Finally, the laboratory work, including specimen preparation and molecular diagnostic testing of collected nasal swabs, was performed by Kathy Luinstra and Andrea Granados at St. Joseph's Healthcare in Hamilton, Ontario.

For all submitted manuscripts I was first author. Chapter 2 presents the main findings from the McFlu2 COLD<sub>3</sub> Prevention study. As outlined above, I had a leading role in the design and execution of this trial. Furthermore, I planned and conducted the statistical analysis of the data and wrote the manuscript. I benefitted greatly from initial statistical consultation with Dr. Eleanor Pullenayegum and from further input from Dr. Marek Smieja, Dr. Brenda Coleman and Dr. Mark Loeb. I presented the trial results at the 2012 annual Association of Medical Microbiology and Infectious Disease Canada conference, and at the 2012 Canadian Institutes of Health Research (CIHR) Young Investigators Forum, supported by an Institute Community Support Travel Award.

The third and fourth chapters are subgroup studies of the original RCT. At the end of the RCT, interested participants were invited to complete an additional two months of surveillance in the absence of interventions. This group of participants formed a longitudinal cohort. Data was collected for up to 15 weeks per participant and the data set had the capacity to capture multiple outcomes per subject. I maintained the lead role in running this study extension, submitting an amendment to the Research Ethics Board, re-consenting participants and collecting data.

Chapter 3 is an exploration and comparison of statistical methods for the analysis of repeated events data. For this paper, I conceptualized the study, reviewed the literature, and prepared and analyzed the data. I proposed four statistical methods for comparison and received invaluable consultation from Dr. Eleanor Pullenayegum. I received extensive help from Eleanor to overcome a flawed multiple imputation (MI) model by writing the code to execute a more sophisticated and accurate MI model in the statistical software package R. Eleanor also wrote the code in R to execute the Andersen-Gill statistical model. I wrote the manuscript and interpreted the results with invaluable feedback and insight from my co-authors.

Chapter 4 is an analysis of the subgroup described above for the purpose of identifying lifestyle and behaviour factors associated with the risk of upper respiratory tract infections. This study was conceived in consultation with Dr. Marek Smieja soon after the conceptualization of the McFlu2 COLd3 Prevention RCT. I collected and analyzed the data, interpreted the results and wrote the manuscript. Dr. Marek Smieja

wisely suggested an additional analysis to strengthen the paper and present the results in a novel manner.

All co-authors helped to improve the interpretation and discussion of the results. All manuscripts required some introduction to the burden of disease associated with URTI and as such, the reader should expect some overlap in the “Background” sections of each paper. Further, the reader should expect overlap in the description of the study design for Chapter 3 and Chapter 4.

A fourth manuscript is presented in Appendix 2. This systematic review and meta-analysis of vitamin D3 supplementation for the prevention of respiratory tract infections was completed as coursework in the Systematic Review and Meta-Analysis course offered in the Health Research Methodology program. As first author, I designed and executed the study, and wrote the manuscript. I received invaluable counsel from Dr. Marek Smieja regarding clinical and methodological considerations. Dr. Michelle Science acted as the second reviewer for the evaluation of eligible studies and data extraction, and helped with the interpretation of results. All co-authors offered feedback to improve and strengthen the paper.

The introduction and concluding chapters were written without co-authors and will not be submitted for publication elsewhere as manuscripts.

## CHAPTER 1

### **Introduction**

#### **The Burden of Upper Respiratory Tract Infections**

The phrase “common cold” is a colloquial term for upper respiratory tract infection (URTI).[1, 2] This conventional name accurately reflects the ubiquitous nature of the illness: globally, URTI is the most common form of illness experienced by humans.[3-5] Annually, it is estimated that children will experience six to ten colds while adults are experience between two and four episodes of URTI.[2, 5]

Although the term common cold might imply a single cause for this syndrome, multiple respiratory viruses are known to cause episodes of illness of variable severity but similar symptoms. These viral agents include rhinoviruses, enteroviruses, adenoviruses, coronaviruses, influenza viruses, parainfluenza viruses and respiratory syncytial viruses (RSV).[4, 6] Consistently, rhinovirus has been identified as the most common cause of the common cold and is believed to be the cause of 30% to 50% of all colds.[1, 2, 5, 7] During the autumn months in the northern hemisphere, this proportion has been reported to be as high as 80%.[8, 9] While the majority of infections are relatively mild and self-limiting, respiratory viral infections can exacerbate existing medical conditions and can cause severe illness which may result in hospitalization or death.[3] Among the most frequent complications associated with URTI, and rhinovirus infection in particular, are



acute otitis media, sinusitis, pneumonia and exacerbations of asthma and chronic obstructive pulmonary disease (COPD).[1, 2, 5, 10]

Notably, URTI represents a major burden for the economy and health care systems worldwide. In the United States of America, after excluding influenza infection, a potentially more serious illness, URTI related expenses are estimated to cost \$40 billion dollars in the United States.[11] These costs are primarily due to the huge number of primary care office visits, estimated to be greater than 116 million per year, and the hundreds of millions days lost from work and school.[11, 12] Recently, the Ontario Burden of Infectious Disease Study (ONBOIDS) identified rhinovirus, the most common viral agent implicated in URTI, as one of the top ten most burdensome infectious agents.[13] The ONBOIDS report estimated that rhinovirus infections were responsible for more than 1.6 million health care utilization episodes in the province of Ontario annually.[13] The true impact of URTI is likely to be substantially greater since many individuals with URTI do not seek medical attention, and URTI is neither a reportable disease, nor one that is routinely confirmed with laboratory diagnosis in clinical practice.

Although the common cold is often considered a trivial illness, it is becoming clearer that the viral agents involved in URTI are responsible for tremendous morbidity worldwide. As the identification of these viruses becomes more routine, and the importance of their role in human disease is better understood, the demand and capacity for improved prevention will follow. New preventive interventions that are associated with even a modest reduction in the number of common colds in a population will have the potential to have a substantial impact on the burden associated with URTI.

## **Defining Upper Respiratory Tract Infections**

As has been written, “The diagnosis of a cold is not difficult – you know it when you see it.” [2] Despite the familiarity of the common cold among the general population, health care providers and medical researchers, it is difficult to define. In fact, there is currently no standardized definition for a common cold. Often, a cold is defined as the acute onset of an infection of the upper respiratory tract accompanied by one or more of the following symptoms: sore throat, nasal congestion, runny nose, sneezing, and cough.[1, 2, 14] Fatigue, wheeze, headache, malaise and fever (usually  $< 37.8^{\circ}\text{C}$ ) are other symptoms that are sometimes included in the definition of a common cold.[1, 2, 14] Research that is focused on the prevention or treatment of the common cold requires a systematic approach, robust against manipulation by researchers, to determine if participants have or have not experienced a cold during the study. In the absence of a standardized definition, many researchers create a definition of their own that relies on the participant’s perception of a cold and the presence of one or more symptoms. In the literature, only two tools appear repeatedly for measuring the presence of a cold and the severity of the illness in adults. The Jackson scale was developed in 1958 in the context of research using viral challenge for the induction of experimental URTI.[15] Eight symptoms (sneezing, nasal obstruction, nasal discharge, sore throat, cough, headache, chilliness, and malaise) are rated on a scale ranging from zero to three representing the absence of a symptom, or mild, moderate or severe presence of a symptom respectively. The score is simply the sum of each symptom rating. A Jackson verified cold must meet two criteria: 1) the subject must perceive himself to have a cold, and 2) a symptom score

of 14 or more must be reported.[15] If only the first criterion is met, a participant is still deemed to have a cold if nasal discharge is increased on three or more days after the viral challenge.[15] The use of the Jackson scale as designed seldom appears in the literature however, several researchers report using a modified Jackson score for the purposes of classifying and evaluating the severity of a cold.[16-20] Among these users are Barrett and colleagues who sought to develop and validate an improved tool, the Wisconsin Upper Respiratory Symptom Survey (WURSS), for the evaluation of URTI.[17, 21] The WURSS-21, a 21 item questionnaire, has been designed to measure the symptomatic and functional impact of URTI, according to patient important constructs.[17, 21] The tool is sensitive to change over time and is consequently useful for researchers interested in evaluating the effect of an intervention thought to decrease the burden of URTI. [17, 21] Although the tool does not directly address the issue of defining the symptoms of a common cold, the authors offer the following three criteria: 1) the participant must think that he has a cold or is coming down with a cold, 2) the participant must report at least one of the following four symptoms: nasal discharge, nasal obstruction, sneezing, or sore (or scratchy) throat, and 3) the participant must score a minimum of two points on the Jackson scale.[22] As the WURSS continues to gain acceptance by researchers in this field, the definition they provide may approximate a standardized definition for use in future studies.

Given the subjective nature of clinical diagnoses and definitions of the common cold, interest in, and use of laboratory methods for the objective assessment of the common cold, by identification of a respiratory virus, is increasing. Clinically, it is nearly

impossible to differentiate between illnesses caused by diverse viruses because of the high degree in symptom overlap. As advances in laboratory methods available to detect various respiratory viruses have improved, the clinical impact of such viral agents has become better understood.[23, 24] Viruses previously thought to only cause benign common cold syndromes, such as rhinovirus, have now been identified as the cause of more serious illness and sometimes fatality.[23, 24] Significant advances in methods for identification of respiratory viruses have been made in the past twenty years.[23-25] Traditionally, respiratory virus detection has relied on techniques like cell culturing and serology.[23-25] However, these methods are too slow to provide a result quickly enough to influence clinical care.[23, 25] Direct fluorescent antibody (DFA) staining of cells was a substantial improvement and quickly became a standard method for diagnosis of common respiratory viruses such as RSV because of its ability to provide a rapid result, in the range of three hours.[26] However, nucleic acid amplification tests (NAATs) have since revolutionized respiratory virus laboratory diagnostics. Techniques like polymerase chain reaction (PCR), and real-time PCR (RT-PCR) are highly sensitive, and can be performed rapidly enough to provide timely results to inform clinical therapy, and potentially infection control protocols.[23] These techniques are also able to identify viruses otherwise undetectable by conventional culture methods, and since NAATs do not require a viable sample, specimens can be transported across greater distances, however further research is needed to optimize transport media for optimal specimen stability and safety.[23, 26] Further progress in the field of molecular diagnostics has been achieved with the development of multiplex assays that are capable of detecting up to 19 different

viruses in one test and several multiplex assays are commercially available such as xTAG Respiratory Viral Panel from Luminex Molecular Diagnostics, RespiFinder-19 by PathoFinder, and ResPlex II by Qiagen, however these tests remain relatively expensive (\$100 or more) and are usually reserved for inpatients and others at higher risk for severe outcomes and for research.[23, 26] Simultaneous advances have been made in the area of specimen collection. The development of new flocced nylon nasopharyngeal and nasal swabs has enhanced the collection of epithelial cells, used for diagnostics, compared to traditional rayon swabs.[27] Furthermore, when considering epithelial cell yield as a measure of sample adequacy, self-collected flocced mid-turbinate nasal swabs have been shown to be equivalent to staff-collected swabs and superior to traditional rayon nasopharyngeal swabs.[28] Self-collected nasal swabs are less invasive than nasopharyngeal swabs or nasal aspirates and since they can be used in the absence of professional help, these novel swabs make serial sampling for research into viral load and viral shedding across episodes of URTI more feasible. The combination of improved specimen collection and molecular diagnostics will allow more researchers in the field of viral respiratory infections to consider using a definition of the common cold, or URTI, that includes laboratory confirmation. This, in turn may provide clearer results about which interventions do or do not modify the incidence, severity and duration of symptomatic URTI.

### **Prevention of URTI**

The common cold has brought misery to humans for centuries and has been subject to many bizarre practices believed to hold the key to prevention or treatment.[29]

Thomas Jefferson believed that soaking one's feet in cold water every morning would prevent infection while Pliny the Elder, a Roman scholar, believed that rubbing the hairy muzzle of a mouse on one's nose was an effective treatment.[29] Although there is no shortage of beliefs pertaining to the prevention and treatment of the common cold, few interventions have proven universally effective. Vaccination has been an effective approach against many pathogens including the respiratory virus influenza. However, due to the number of different viruses that elicit symptoms of the common cold and the significant antigenic variation, creating a vaccine that will provide comprehensive protection is very difficult.[14] Research is ongoing to create vaccines that target specific viruses such as rhinovirus, adenovirus, or RSV but current evidence doesn't support the use of these vaccines.[14]

Numerous nutritional supplements have been investigated for the prevention of URTI. *Echinacea*, ginseng, zinc, and vitamin C are among the most studied supplements but vitamin D has received increasing attention in the past decade.

Various species and parts of the *Echinacea* plant have been used to make preparations marketed for the prevention and treatment of the common cold.[30-32] Several biochemical compounds have been identified in *Echinacea* extracts and some, such as glycoproteins, are believed to contribute to improved macrophage and natural killer cell activity, while others, such as alkamides, are believed to display anti-inflammatory properties.[31, 32] The benefit of *Echinacea* is unclear. A Cochrane systematic review and meta-analysis of *Echinacea* for the prevention of the common cold did not find a clear effect associated with the prophylactic use of *Echinacea* extracts.[32]

However, a more recent randomized controlled trial of *Echinacea purpurea*, involving 755 healthy individuals followed for four months, reported a significant reduction in the total number of URTI in the treatment group compared to the placebo group (149 episodes vs. 188 respectively) among other benefits.[30] More research is needed to elucidate the true effect of *Echinacea* for the prevention of colds.

The root of the Panax plant is commonly known as ginseng and two species, *Panax ginseng* and *Panax quinquefolius*, have been used as herbal medication for the prevention and treatment of the common cold.[31, 33] A proprietary extract isolated from *Panax quinquefolius* is marketed under the name COLD-fX and has quickly gained popularity among the general public.[20, 33] A recent systematic review and meta-analysis of five RCTs reported that ginseng, compared to placebo, was associated with a non-significant reduction in the risk of having at least one acute respiratory infection (RR: 0.70, 95%CI:0.48, 1.02).[33] Ultimately, the authors concluded that there was insufficient evidence to conclude that ginseng reduces the incidence of the common cold.[33]

Zinc, an essential mineral, has also been investigated as a potential intervention for the prevention and treatment of the common cold. To date, nearly all studies have been interested in zinc exclusively as a treatment.[31, 34, 35] However, a recent Cochrane review analyzed the results of two trials in which zinc was administered for the prevention of URTI and reported that zinc significantly reduced the incidence of the common cold.[35] The authors concluded that despite the favourable results for zinc, more research was needed before recommendations for clinical practice could be made.[35]

Vitamin C, or ascorbic acid, has long been considered an effective supplement to prevent and treat the common cold. A major source of this belief was Nobel laureate Linus Pauling's work in the 1970s and his book "Vitamin C and the Common Cold." [36] Dozens of clinical trials have been executed to examine this relationship. A recent Cochrane meta-analysis of 32 vitamin C supplementation arms in placebo controlled trials representing more than eleven thousand participants reported that prophylactic use of vitamin C did not reduce the incidence of colds in the general public. [36] Although the meta-analysis did report a statistically significant result (RR: 0.95, 95% CI: 0.92, 0.98) supporting a biological effect of vitamin C, the authors concluded that there would be no clinically relevant effect across populations due to such a narrow confidence interval. [36] However, the supplementation did appear to significantly reduce the risk of infection among individuals exposed to intense physical exercise (RR: 0.48, 95% CI: 0.35, 0.64). Prophylactic vitamin C supplementation was also associated with significantly shorter URTI duration in adults and children. [36]

Vitamin D is a fat-soluble secosteroid and is predominantly obtained through exposure to the sun and, to a lesser extent, through dietary intake. The term 'vitamin D' refers collectively to two forms of the secosteroid: vitamin D2 and vitamin D3, also known as ergocalciferol and cholecalciferol respectively. [37] Vitamin D3 is the preferred formulation for supplementation due to its superior potency and greater bioactivity, and circulating levels of vitamin D are measured as serum 25-hydroxyvitamin D (25(OH)D) concentrations. [37, 38] Several observational studies have demonstrated an association between low 25(OH)D levels and more frequent and more severe respiratory infections in



pediatric and adult populations.[39-46]. In recent years, vitamin D3, has become the focus of a growing number of experimental studies assessing its effect on respiratory health outcomes. At the outset of this doctoral degree, only four interventional studies had investigated the effect of vitamin D3 supplementation on respiratory outcomes.[47-50] Only one of these studies, by Li-Ng *et al.*[49], was specifically designed to measure the incidence of upper respiratory tract infections, and the remaining three studies were secondary analyses of studies designed primarily for other outcomes such as osteoporotic fractures[48], postmenopausal bone density[47], and any infections in children with sub-clinical rickets.[50] Collectively the results did not provide conclusive evidence for or against vitamin D3 supplementation for the prevention of URTI. These results did, however, emphasize the need for a large, methodologically sound randomized controlled trial to investigate the effects of vitamin D3 supplementation for the prevention of URTI. This was the rationale behind the development and execution of the McFlu2 COLD3 Prevention trial that forms the basis of this thesis.

Since that time, many more RCTs have investigated the role of vitamin D3 in respiratory infections. However, the topic remains heavily debated due to conflicting results from individual trials. Three systematic reviews and meta-analyses have been published and a fourth unpublished meta-analysis is presented as an appendix in this thesis. These studies estimate relative risk and odds reductions associated with vitamin D3 supplementation in the range of 2% to 42 %, however not all studies have achieved statistical significance and there remains no consensus about the true effect of vitamin D3 for the prevention of URTI.

Apart from nutritional supplements, personal hygiene practices and lifestyle factors have also been studied for the prevention of URTI. Hand washing is one of the best known methods to prevent URTI and studies have demonstrated that frequent hand washing can reduce the risk of infection by 25% to 45%. [51, 52] A meta-analysis of numerous hand hygiene interventions in community settings reported 21% reduction in the rate of respiratory illnesses in the intervention groups. [53] Not smoking, and ensuring adequate sleep and moderate exercise have also been identified as healthy habits that could help prevent URTI. [54-56] An additional personal hygiene habit that may help to reduce susceptibility to URTI is gargling. In Japan, gargling is a common hygiene practice and is strongly recommended as a preventive measure against URTI. [57] Japanese observational studies have reported reduced risk of respiratory infections associated with gargling two or three times daily with various solutions including povidine- iodine solutions, black tea extracts and tap water. [57-60] Evidence from a Japanese RCT involving 387 healthy adults reported a 36% reduction in incident URTI among those randomized to gargle three times daily with plain water compared to the control group (incident rate ratio: 0.64, 95% CI: 0.38, 0.93). [57] Compared to the control group, the incident URTI ratio was lower in the povidine-iodine group but this was not statistically significant (incident rate ratio: 0.88, 95% CI: 0.58, 1.34). [57] Tap water gargling may represent a safe and effective intervention, however this has not been assessed in another population and more evidence is required.

Although it is unlikely that a single intervention will successfully prevent URTI, identifying a few effective methods to reduce the risk of these infections has the potential to reduce the burden of this disease.

## **Summary**

Globally, URTI are a significant cause of morbidity. While it is common to think of URTI simply as a nuisance, these illnesses should not be considered exclusively benign. Attempts to quantify the burden of these diseases have demonstrated the immense impact that these illnesses have on disease exacerbation, health care utilization, work and school absences, and the associated financial costs. There is a large body of literature examining numerous measures for the prevention of URTI. Nevertheless, little evidence exists to support the efficacy of many of these interventions and there remains a need to improve URTI prevention. Investigating novel approaches for the prevention of URTI is necessary, as even modest improvements could help reduce the impact of these ubiquitous infections.

In the remainder of this thesis, I explore vitamin D3 and gargling for the prevention of URTI. I use experimental and observational study designs to investigate the impact of these two potential interventions. Additionally, I compare statistical methods for the analysis of repeated events data relevant to research in many fields, but particularly applicable to studies of URTI. Finally, I use observational methods to examine the association of modifiable lifestyle factors and the risk of URTI.

## CHAPTER 2

**A Factorial Randomized Controlled Trial of Vitamin D3 and Gargling for the Prevention of Respiratory Infections**

This chapter presents the main findings from the McFlu2 COLD3 Prevention Study. We report a potentially clinically important but non-significant reduction in risk of self-reported, clinical upper respiratory tract infections associated with vitamin D3 supplementation. We also report a statistically significant reduction in the risk of laboratory confirmed infections associated with vitamin D3. Gargling did not reduce the risk of infection for clinical or laboratory confirmed illness.

This manuscript was submitted to BMC Infectious Diseases September 2013 after rejection by Canadian Medical Association Journal and Clinical Infectious Diseases. The full citation is:

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I presented the results, in part, as a poster session at the Association of Medical Microbiology and Infectious Disease Canada annual conference, May 3-5, 2012 in Vancouver BC and as a second poster presentation at the Canadian Institutes of Health Research (CIHR) Young Investigators Forum, June 4-6, 2012, in Montreal QC.

**Vitamin D<sub>3</sub> and Gargling for the Prevention of Upper Respiratory Tract Infections:  
A Randomized Controlled Trial**

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**Running title:** Vitamin D, gargling for cold prevention

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**Summary:** Weekly vitamin D3 supplementation demonstrated a non-significant relative risk reduction (RRR, 20%) in symptomatic upper respiratory tract infections (URTI) and a statistically significant 46% RRR in laboratory confirmed URTI. Daily gargling was not associated with a reduction in URTI.

**Abstract:** 253 words

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**Figures:** 1

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## Abstract

**Background:** We undertook a 2X2 factorial, randomized controlled trial (RCT) to assess whether vitamin D<sub>3</sub> supplementation versus placebo and gargling versus no gargling could prevent clinical upper respiratory tract infection (URTI) in university students.

**Methods:** We randomized 600 students into 4 treatment arms: 1) vitamin D<sub>3</sub> and gargling, 2) placebo and gargling, 3) vitamin D<sub>3</sub> and no gargling, and 4) placebo and no gargling. Students completed weekly electronic surveys and submitted self-collected mid-turbinate nasal flocked swabs during September and October in 2010 or 2011.

Symptomatic students also completed an electronic symptom diary. **Results:** Of 600 participants, 471 (78.5%) completed all surveys while 43 (7.2%) completed none); 150 (25.0%) reported clinical URTI. Seventy participants (23.3%) randomized to vitamin D<sub>3</sub> reported clinical URTI compared to 80 (26.7%) randomized to placebo (RR:0.79, CI<sub>95</sub>:0.61-1.03, p=0.09). Eighty-five participants (28.3%) randomized to gargling reported clinical URTI compared to 65 participants (21.7%) randomized to the no gargling arm (RR:1.3, CI<sub>95</sub>:0.92-1.57, p=0.19). Laboratory testing identified 70 infections (46.7 per 100 URTIs). Vitamin D<sub>3</sub> treatment was associated with a significantly lower risk for laboratory confirmed URTI (RR: 0.54, CI<sub>95</sub>:0.34-0.84, p=0.007) and with a significantly lower mean log viral load (mean difference: -0.89, CI<sub>95</sub>: -1.7, -0.06, p=0.04). Fewer students assigned to gargling experienced laboratory confirmed URTI, however this was not statistically significant (RR:0.82, CI<sub>95</sub>:0.53-1.26, p=0.36). **Conclusions:** These results suggest that vitamin D<sub>3</sub> is a promising intervention for the prevention of URTI. Vitamin

D<sub>3</sub> significantly reduced the risk of laboratory confirmed URTI and may reduce the risk of clinical infections. **Clinical Trials Registration:** NCT01158560.

**Key words:** Rhinovirus, vitamin D<sub>3</sub>, viral load, gargling, randomized controlled trial, upper respiratory tract infection

## **Background**

Upper respiratory tract infection (URTI), which presents clinically as the common cold, is the most common human illness.[1, 2] While the majority of infections are mild and self-limiting, URTI can exacerbate existing medical conditions and can cause severe illness which may result in hospitalization or death.[1-3]

Observational studies have consistently demonstrated an association between low vitamin D levels and greater frequency and severity of URTI in children and adults.[4-10] Results from several trials of vitamin D<sub>3</sub> supplementation report reduced risk of infection, but only two studies have reported statistically significant findings.[11-18] Many of these trials were post-hoc analyses of trials with non-respiratory outcomes while others were limited by small sample size and relatively low dose of vitamin D<sub>3</sub> supplementation.[11-17] More rigorously-designed clinical trials are needed to investigate the effect of vitamin D<sub>3</sub> on URTI.

In Japan, daily gargling with water is recommended as a preventive measure against URTI. Results from a Japanese trial reported a 36% reduction in incident URTI amongst



participants randomized to gargle with water three times daily compared to the control group.[19]

The current study was designed to assess the effectiveness of vitamin D<sub>3</sub> supplementation versus placebo, and of gargling versus no-gargling, for the prevention of URTI in university students.

## **Methods**

### *Study Design*

We conducted a 2x2 factorial RCT of vitamin D<sub>3</sub> versus placebo and gargling versus no-gargling with students at McMaster University, Hamilton, Ontario. Participants were enrolled during the first two weeks of September 2010 or 2011 and were followed to the end of October 2010 or 2011, respectively. Individuals were eligible for the study if they were enrolled at McMaster University, were  $\geq 17$  years, and lived with at least one student housemate. Participants with contra-indicated medical conditions (hypercalcemia, parathyroid disorder, chronic kidney disease, use of anticonvulsants, malabsorption syndromes, sarcoidosis), who were currently or planning to become pregnant, who were taking  $\geq 1000$  international units (IU)/day vitamin D<sub>3</sub>, or who were unable to swallow capsules were excluded. All participants provided written consent. The study protocol was approved by the Hamilton Health Sciences / Faculty of Health Sciences Research Ethics Board and was registered at [clinicaltrials.gov](http://clinicaltrials.gov) (NCT01158560).

Participants completed a baseline questionnaire that collected demographic, health and lifestyle information and submitted a self-collected mid-turbinate flocked nasal swab (Copan Italia, Brescia Italy).[20] Participants were then randomized to one of four allocation arms: 1) vitamin D<sub>3</sub> and gargling, 2) vitamin D<sub>3</sub> and no gargling, 3) placebo and gargling, or 4) placebo and no gargling. The study sample was stratified based on housing (in residence versus off-campus) and block randomization occurred within each stratum using a 1:1:1:1 allocation ratio. Only the study pharmacist knew the randomization scheme. The study was double-blind with respect to the vitamin D<sub>3</sub>/placebo intervention. Due to the nature of the gargling intervention, participants randomized to the gargling were not blinded. All other participants and study personnel remained blinded.

### Interventions

Participants were randomized to receive a container with eight capsules of either 10,000 IU of active vitamin D<sub>3</sub> or identical placebo. All participants were instructed to take one pill weekly and received a weekly email reminder. Individuals randomized to the gargling intervention were asked to gargle with approximately 30 mL of tap water for 30 seconds twice daily. All participants received general lifestyle and health advice about the benefits of appropriate sleep, nutrition, hand hygiene, and exercise. Intervention allocation occurred via serially numbered pill containers and opaque envelopes, containing gargling allocation only, given directly to participants.

### Assessments

Participants were asked to complete weekly online surveys which screened for URTI symptoms and to submit one self-collected nasal swab weekly. Participants with URTI were asked to complete a symptom survey for seven consecutive days following symptom onset and a follow-up survey 14 days after symptom onset. They were also asked to collect seven consecutive daily nasal swabs starting from symptom onset. Swabs were stored at room temperature in CyMol™ transport medium (Copan Italia, Brescia Italy), an alcohol-based medium which inactivates respiratory viruses on contact.[21] Only swabs submitted from symptomatic participants were tested for respiratory viruses. All other swabs were stored for separate studies, including investigations into asymptomatic illnesses.

### Outcome Measures

The primary outcome was the incidence of clinical URTI, defined as the participant's perception of a "cold" in conjunction with two or more symptoms (runny/stuffy nose, congestion, cough, sneezing, sore throat, muscle aches, or fever). Students were asked to immediately electronically report the onset of a "cold" and the weekly survey asked participants if they considered themselves to be sick. Adjudication by two clinicians was applied when participants reported symptoms but were uncertain if they were ill. Self-reported and adjudicated episodes of clinical URTI were considered 'events' if the onset occurred at least seven days after the participant's randomization date.

Secondary outcomes included laboratory confirmed illness, viral load, and symptom duration and severity. Laboratory confirmed illness was determined by testing nasal

swabs using an in-house enterovirus/rhinovirus polymerase chain reaction (PCR) and, if negative, a commercial multiplex PCR able to detect 16 respiratory viruses and viral subtypes (xTAG RVP FAST, Luminex, Austin TX). Viral load was determined for rhinovirus infections using quantitative PCR.[22] Symptom severity and duration were measured using the 21-item Wisconsin Upper Respiratory Symptom Survey.[23] Symptom severity was calculated as the sum of seven consecutive daily severity scores. Symptom duration was defined as the total number of days from symptom onset until the participant responded “I do not feel sick today”.

At enrolment, participants were asked about the frequency of hand washing before meals, average weekly hours of exercise, average hours of sleep per night, current vitamin supplement use and gargling habits, as well as asthma and smoking status to provide information for potential confounders, mediators, or moderators.

### Statistical Analysis

We aimed to recruit 600 unique participants to ensure greater than 80% power to detect a 25% reduction (from 50% to 37.5%) in the proportion of students with URTI, with 10% over-recruitment to adjust for attrition. This study was powered to detect main effects and was underpowered to definitively investigate interactions.

Poisson regression with robust standard errors was used to assess our primary question of whether vitamin D<sub>3</sub> or gargling could reduce the number of clinical URTIs experienced in those groups. This analysis was chosen in place of logistic regression since odds ratios

overestimate treatment effects when incorrectly interpreted as risk ratios.[24] Robust standard errors were calculated in place of model based standard errors which are typically too large.[24] Multiple imputation, using the Markov chain Monte Carlo method, was conducted to address missing data. Information collected at baseline and through weekly surveys was used to predict missing values for independent and dependent variables.[25] The pooled imputed data was used to conduct an intention-to-treat analysis adjusted for randomization strata: housing, trial year, vitamin D<sub>3</sub> and gargling allocation. Interaction between vitamin D<sub>3</sub> use and gargling was investigated using a cross-product term. A complete case analysis, adjusted for the same variables, was performed as a sensitivity analysis. An identical complete-case analysis was conducted to assess the secondary outcome of laboratory confirmed infections. Symptom severity and viral load were compared by *t*-test. Cox regression was used to assess time to symptom resolution adjusted for the variables listed above.

Results were considered statistically significant with  $p < 0.05$ . Statistical analyses were conducted using IBM SPSS statistical software version 20.0 (IBM SPSS Inc., Chicago, IL, USA).

## **Results**

### *Participants*

Six hundred ninety-eight prospective participants provided written consent. Of these, 600 completed the baseline survey, provided a nasal swab and were randomized into the

study. Four hundred seventy one (78.5%) completed all weekly surveys, 86 (14.3%) completed at least one but not all, and 43 (7.2%) completed none (Figure 1). The median age of the participants was 19 years (interquartile range 18-20), 60% were first or second year undergraduate students, and 64% were female. Baseline characteristics were similar across the intervention arms (Table 1).

### Outcomes

#### Incidence of Clinical and Laboratory Confirmed URTI

Clinical URTI and laboratory confirmation data was available for 492 (82%) and 489 (81.5%) participants, respectively. A total of 150 individuals experienced symptomatic (clinical) URTI with 106 episodes self-reported and 44 episodes established through adjudication. Laboratory testing identified viral pathogens in 70 infections, with rhinovirus being the predominant viral pathogen (61 cases), six cases of enterovirus and one case of coronavirus NL63. The distinction between rhinovirus and enterovirus was not established for two samples.

As shown in Table 2, fewer participants randomized to receive vitamin D<sub>3</sub> (70 or 27%) reported a clinical URTI than those randomized to receive placebo (80 or 34%) (imputed analysis RR:0.80, CI<sub>95</sub>:0.63,1.02, p=0.08; complete case analysis RR:0.79, CI<sub>95</sub>:0.61,1.03, p=0.09). Laboratory confirmed events were significantly lower in participants receiving vitamin D<sub>3</sub> supplementation (26 or 10.2%) compared with those receiving placebo (44 or 18.9%) (RR:0.54, CI<sub>95</sub>:0.34,0.84, p=0.007) (Table 2).

Eighty-five (33.2%) participants assigned to gargle reported clinical URTI compared to 65 (27.5%) participants not assigned to gargle (imputed analysis RR: 1.1, CI<sub>95</sub>: 0.78,1.44, p=0.69; complete case analysis RR: 1.3, CI<sub>95</sub>: 0.92,1.57, p=0.19) (Table 3). Thirty-three (13.0%) participants assigned to gargle had a laboratory confirmed URTI compared to 37 (18.7%) participants randomized to the control arm; however this was not a statistically significant difference (Table 3) (RR: 0.82, CI<sub>95</sub>:0.53,1.26, p=0.36).

### Viral Load

Viral load was established for 59 of 61 rhinovirus infections. The mean viral load in the vitamin D<sub>3</sub> group was lower compared to those in the placebo group, with 5.51 log<sub>10</sub> viral copies/mL versus 6.40 log<sub>10</sub> viral copies/mL, respectively (mean difference: -0.89, CI<sub>95</sub>: -1.7, -0.06, p=0.04) (Table 2). The mean viral load in those assigned to the gargle group was 6.24 log<sub>10</sub> viral copies/mL compared to 5.95 log<sub>10</sub> viral copies/mL in the control group (mean difference: 0.37, CI<sub>95</sub>: -0.44, 1.02, p=0.43) (Table 3).

### Symptom Duration and Severity of Clinical and Laboratory Confirmed URTI

The mean duration of clinical and laboratory confirmed URTI was non-significantly lower in the vitamin D<sub>3</sub> group compared to the placebo group (6.0 versus 6.2 days and 5.8 versus 6.2 days, respectively). Time to symptom resolution was not significantly different between the groups (clinical URTI HR: 1.3, CI<sub>95</sub>: 0.59, 2.90, p=0.49; laboratory confirmed URTI HR: 1.3, CI<sub>95</sub>: 0.49,3.48, p=0.59) (Table 2).

Gargling did not reduce the mean duration of symptoms or improve time to symptom resolution. Results were similar for clinical URTIs (HR: 0.85, CI<sub>95</sub>: 0.38,1.89, p=0.69) and laboratory confirmed URTIs (HR:1.5, CI<sub>95</sub>:0.56,4.0, p=0.43) (Table 3).

Mean symptom severity appeared to be greater in the vitamin D<sub>3</sub> group for clinical and laboratory confirmed URTI (218.6 versus 199.8 and 229.7 versus 181.5, respectively) (Table 2). However, the difference was not statistically significant. Symptom severity also appeared to be greater in the gargling group for clinical and laboratory confirmed URTI, however this was not statistically significant (225.3 versus 191.8 and 210.5 versus 191.8, respectively) (Table 3).

## **Discussion**

Weekly supplementation with 10,000 IU of vitamin D<sub>3</sub> in university students during September and October was associated with a non-significant, but potentially clinically important 20% risk reduction of clinical URTI. Importantly, vitamin D supplementation was associated with a statistically significant 46% risk reduction of laboratory confirmed URTI.

Results from previous trials of vitamin D<sub>3</sub> supplementation for the prevention of URTI have been conflicting. Two placebo controlled RCTs in pediatric populations have demonstrated that vitamin D<sub>3</sub> significantly reduced the risk of clinical and lab-confirmed URTI.[16, 17] However, previous trials in adult populations have not yielded statistically



significant results. The effect estimates from our study are similar to those reported in pediatric trials. Urashima *et al.* reported that children, ages 6-15, receiving daily supplementation with 1200 IU vitamin D<sub>3</sub> had a 42% lower risk of influenza A infection.[16] Camargo *et al.*, reported a 50% risk reduction in parent-reported URTI among children, ages 9-11, receiving daily supplementation with 300 IU of vitamin D<sub>3</sub>. [17]

Consistent with previous studies in adults, our primary analysis did not show that vitamin D<sub>3</sub> significantly reduced the risk of clinical URTI; however the 20% relative risk reduction may be clinically relevant. Li-Ng *et al.* randomized 162 adults to 2000 IU vitamin D<sub>3</sub> or placebo daily from December to March and reported no difference in self-reported URTI.[14] Laaksi and colleagues randomized 164 men to 400 IU vitamin D<sub>3</sub> or placebo daily from October through March but did not detect a significant difference in the number of days absent from duty due to URTI.[15] However, the proportion of men who did not experience a URTI was significantly greater in the intervention group (51.3%) than in the control group (35.7%).[15] Murdoch *et al.* followed 322 adults randomized to monthly doses of 100,000 IU vitamin D<sub>3</sub> or placebo.[18] After 18 months, the study recorded 593 and 611 URTI episodes in the vitamin D<sub>3</sub> and control groups respectively, however this was not a significant difference.[18] Notably, our *a priori* secondary outcome which assessed laboratory confirmed URTI demonstrated a significant 46% relative risk reduction associated with vitamin D<sub>3</sub> supplementation. This result is consistent with two recent meta-analyses by Charan *et al* and Bergman *et al* which reported that vitamin D<sub>3</sub> supplementation significantly decreased the odds of

respiratory infections (pooled OR: 0.58, CI<sub>95</sub>: 0.42-0.81, p=0.001 and OR: 0.64, CI<sub>95</sub>: 0.49-0.84, p=0.0014, respectively).[26, 27] Although these results support the use of vitamin D<sub>3</sub> supplementation, further meta-analysis including the results from more recent trials and an exploration of heterogeneity between trials should be conducted.

Research has demonstrated that vitamin D plays a role in the innate immune response by stimulating the production of antimicrobial peptides, such as defensins and cathelicidins, and by enhancing the microbicidal action of monocytes and macrophages.[28, 29] For this reason we hypothesized that vitamin D supplementation might reduce the amount of virus in infected persons. Our data support this hypothesis: mean log viral load was significantly lower in the vitamin D<sub>3</sub> group compared to the placebo group. No statistically significant differences in the duration or severity of URTI were identified. However, it may be clinically relevant that participants in the vitamin D<sub>3</sub> group appeared to experience more severe symptoms. This may be a result of vitamin D<sub>3</sub> completely preventing milder infections but not those that were more severe. Alternatively, vitamin D<sub>3</sub> may have enhanced intracellular killing which could be associated with increased inflammatory responses and greater symptom severity. Further research is needed to explore this hypothesis.

Our study differed in several potentially important ways from previous studies. It is well established that vitamin D levels fluctuate seasonally.[30] Studies of Canadians have demonstrated a marked drop in vitamin D levels in the fall from their peak levels in the summer, and a high prevalence of low wintertime vitamin D levels.[31, 32] To capture

peak rates of rhinovirus infection in students, we initiated our intervention in early September, rather than during late autumn and winter months. Thus, serum vitamin D levels did not have the same opportunity to decline as they naturally would during the autumn. Rather than having to overcome depletion in vitamin D levels, our study may have maintained and potentially enhanced vitamin D levels. However, in the absence of blood tests this cannot be proven. Additional differences include the frequency and quantity of vitamin D<sub>3</sub> supplementation. Our study used a relatively high dose of 10,000 IU of vitamin D<sub>3</sub> per week ingested as a single dose, an average of approximately 1400 IU/day. Although the optimal dosing regimen remains uncertain, it has been acknowledged that adherence with daily supplements is often suboptimal and larger, less frequent doses may be an effective alternative.[33, 34] A final methodological difference was our use of self-collected nasal swabs and laboratory confirmation of URTI. Our definition of clinical URTI may have been excessively broad and insufficiently specific which may have led to incorrectly classified events. It is possible that this definition captured episodes attributable to allergies or other causes, creating error in the statistical model and pushing the results towards a null effect.

Ultimately, our study was underpowered since the observed event rate was lower than predicted. This would contribute to larger variance in the estimates and uncertainty surrounding our results. This study was conducted over a relatively short period of time and while it captured peak rhinovirus activity, it was unable to capture URTI caused by other viruses. Consequently, it is uncertain whether vitamin D<sub>3</sub> supplementation is beneficial for the prevention of non-rhinovirus URTI.

Gargling did not appear to reduce the risk of URTI in our study population, in contrast to previous reports.[19] Although our collection period captured peak rhinovirus activity, gargling may be more effective for pathogens which predominantly colonize the oropharynx. Additionally, we were unable to observe whether, or how often, gargling was practiced by participants; gargling may need to be carried out more frequently than twice daily to be beneficial.

## **Conclusions**

Our findings support the growing body of literature that proposes vitamin D<sub>3</sub> as a promising intervention for the prevention of URTI in young adults. This study demonstrated that vitamin D<sub>3</sub> can prevent laboratory confirmed acute, viral respiratory infections. While vitamin D supplementation may represent an effective, accessible and safe intervention for the prevention of URTI, many questions which might guide clinical practice remain unanswered. A rigorous systematic review and meta-analysis of current studies would be instrumental to the design of future trials in this field which may need to include a larger sample size, longer period of follow-up or different dosing regimens.

## **List of Abbreviations**

CI<sub>95</sub> 95% Confidence interval

IU International units

HR Hazard ratio

PCR Polymerase chain reaction

RCT Randomized controlled trial

RR Relative risk

RRR Relative risk reduction

URTI Upper respiratory tract infection

### **Competing Interests**

All authors have reported no competing interests.

### **Author Contributions**

EG had full access to all study data and takes responsibility for the integrity of the data and the accuracy of the data analysis. EG, MS, EP and ML participated in the study design and development. Study conduct and supervision were conducted by EG and MS. EG, AG and KL were responsible for the acquisition of data. AG and KL conducted all laboratory testing. The analysis and interpretation of data was conducted by EG, EP, MS, ML and BC. EG drafted the manuscript and all authors critically reviewed the manuscript for intellectual content.

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## References

1. Proud D: **Upper airway viral infections.** *Pulmonary pharmacology & therapeutics* 2008, **21**(3):468-473.
2. Monto AS: **Epidemiology of viral respiratory infections.** *The American journal of medicine* 2002, **112 Suppl 6A**:4S-12S.
3. Kwong JC, Crowcroft NS, Campitelli MA, Ratnasingham S, Daneman N, Deeks SL, Manuel DG: **Ontario Burden of Infectious Disease Study (ONBOIDS): An OAHPP/ICES Report.** In. Toronto: Ontario Agency for Health Protection and Promotion, Institute for Clinical and Evaluative Sciences; 2010.
4. Ginde AA, Mansbach JM, Camargo CA, 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):384-390.
5. Karatekin G, Kaya A, Salihoglu O, Balci H, Nuhoglu A: **Association of subclinical vitamin D deficiency in newborns with acute lower respiratory infection and their mothers.** *Eur J Clin Nutr* 2009, **63**(4):473-477.
6. Laaksi I, Ruohola JP, Tuohimaa P, Auvinen A, Haataja R, Pihlajamaki H, Ylikomi T: **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):714-717.

7. McNally JD, Leis K, Matheson LA, Karuananyake C, Sankaran K, Rosenberg AM: **Vitamin D deficiency in young children with severe acute lower respiratory infection.** *Pediatric pulmonology* 2009, **44**(10):981-988.
8. Roth DE, Shah R, Black RE, Baqui AH: **Vitamin D status and acute lower respiratory infection in early childhood in Sylhet, Bangladesh.** *Acta paediatrica (Oslo, Norway : 1992)* 2010, **99**(3):389-393.
9. Sabetta JR, DePetrillo P, Cipriani RJ, Smardin J, Burns LA, Landry ML: **Serum 25-hydroxyvitamin d and the incidence of acute viral respiratory tract infections in healthy adults.** *PLoS One* 2010, **5**(6):e11088.
10. Wayse V, Yousafzai A, Mogale K, Filteau S: **Association of subclinical vitamin D deficiency with severe acute lower respiratory infection in Indian children under 5 y.** *Eur J Clin Nutr* 2004, **58**(4):563-567.
11. Rehman PK: **Sub-clinical rickets and recurrent infection.** *Journal of tropical pediatrics* 1994, **40**(1):58.
12. Avenell AC, J. A.; Maclellan, G. S.; Macpherson, G. C.: **Vitamin D supplementation to prevent infections: a sub-study of a randomised placebo-controlled trial in older people (RECORD trial, ISRCTN 51647438).** *Age and ageing* 2007, **36**(5):574-577.
13. Aloia JFL-N, M.: **Re: epidemic influenza and vitamin D.** *Epidemiology and Infection* 2007, **135**(7):1095-1096.
14. Li-Ng MA, J. F.; Pollack, S.; Cunha, B. A.; Mikhail, M.; Yeh, J.; Berbari, N.: **A randomized controlled trial of vitamin D3 supplementation for the**



- prevention of symptomatic upper respiratory tract infections. *Epidemiol Infect* 2009, **137**(10):1396-1404.**
15. Laaksi IR, Juha-Petri; Mattila, Ville; Auvinen, Anssi; Ylikomi, Timo; Pihlajamaki, Harri: **Vitamin D supplementation for the prevention of acute respiratory tract infection: a randomized, double-blinded trial among young Finnish men.** *J Infect Dis* 2010, **202**(5):809-814.
  16. Urashima MS, Takaaki; Okazaki, Minoru; Kurihara, Mana; Wada, Yasuyuki; Ida, Hiroyuki: **Randomized trial of vitamin D supplementation to prevent seasonal influenza A in schoolchildren.** *American Journal of Clinical Nutrition* 2010, **91**(5):1255-1260.
  17. Camargo CA, Jr.; Ganmaa, Davaasambuu; Frazier, A. Lindsay; Kirchberg, Franca F.; Stuart, Jennifer J.; Kleinman, Ken; Sumberzul, Nyamjav; Rich-Edwards, Janet W.: **Randomized trial of vitamin D supplementation and risk of acute respiratory infection in Mongolia.** *Pediatrics* 2012, **130**(3):e561-567.
  18. Murdoch DRS, Sandy; Chambers, Stephen T.; Jennings, Lance C.; Stewart, Alistair W.; Priest, Patricia C.; Florkowski, Christopher M.; Livesey, John H.; Camargo, Carlos A.; Scragg, Robert: **Effect of vitamin D3 supplementation on upper respiratory tract infections in healthy adults: the VIDARIS randomized controlled trial.** *Jama* 2012, **308**(13):1333-1339.
  19. Satomura K, Kitamura T, Kawamura T, Shimbo T, Watanabe M, Kamei M, Takano Y, Tamakoshi A: **Prevention of upper respiratory tract infections by**

- gargling: a randomized trial.** *American journal of preventive medicine* 2005, **29**(4):302-307.
20. Smieja M, Castriciano S, Carruthers S, So G, Chong S, Luinstra K, Mahony JB, Petrich A, Chernesky M, Savarese M *et al*: **Development and evaluation of a flocked nasal midturbinate swab for self-collection in respiratory virus infection diagnostic testing.** *Journal of clinical microbiology* 2010, **48**(9):3340-3342.
21. Luinstra K, Petrich A, Castriciano S, Ackerman M, Chong S, Carruthers S, Ammons B, Mahony JB, Smieja M: **Evaluation and clinical validation of an alcohol-based transport medium for preservation and inactivation of respiratory viruses.** *Journal of clinical microbiology* 2011, **49**(6):2138-2142.
22. Granados A, Luinstra K, Chong S, Goodall E, Banh L, Mubareka S, Smieja M, Mahony J: **Use of an improved quantitative polymerase chain reaction assay to determine differences in human rhinovirus viral loads in different populations.** *Diagnostic microbiology and infectious disease* 2012, **74**(4):384-387.
23. Barrett B, Brown RL, Mundt MP, Thomas GR, Barlow SK, Highstrom AD, Bahrainian M: **Validation of a short form Wisconsin Upper Respiratory Symptom Survey (WURSS-21).** *Health and quality of life outcomes* 2009, **7**:76.
24. Knol MJ, Le Cessie S, Algra A, Vandenbroucke JP, Groenwold RH: **Overestimation of risk ratios by odds ratios in trials and cohort studies:**

- alternatives to logistic regression.** *CMAJ : Canadian Medical Association journal = journal de l'Association medicale canadienne* 2012, **184**(8):895-899.
25. Groenwold RH, Donders AR, Roes KC, Harrell FE, Jr., Moons KG: **Dealing with missing outcome data in randomized trials and observational studies.** *Am J Epidemiol* 2012, **175**(3):210-217.
26. Charan J, Goyal JP, Saxena D, Yadav P: **Vitamin D for prevention of respiratory tract infections: A systematic review and meta-analysis.** *Journal of pharmacology & pharmacotherapeutics* 2012, **3**(4):300-303.
27. Bergman P, Lindh ÅU, Björkhem-Bergman L, Lindh JD: **Vitamin D and Respiratory Tract Infections: A Systematic Review and Meta-Analysis of Randomized Controlled Trials.** *PLoS ONE* 2013, **8**(6):e65835.
28. Wang TT, Nestel FP, Bourdeau V, Nagai Y, Wang Q, Liao J, Tavera-Mendoza L, Lin R, Hanrahan JW, Mader S *et al*: **Cutting edge: 1,25-dihydroxyvitamin D3 is a direct inducer of antimicrobial peptide gene expression.** *Journal of immunology (Baltimore, Md : 1950)* 2004, **173**(5):2909-2912.
29. Liu PT, Stenger S, Li H, Wenzel L, Tan BH, Krutzik SR, Ochoa MT, Schaubert J, Wu K, Meinken C *et al*: **Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response.** *Science* 2006, **311**(5768):1770-1773.
30. Webb AR, Kline L, Holick MF: **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.** *The Journal of clinical endocrinology and metabolism* 1988, **67**(2):373-378.

31. Rucker D, Allan JA, Fick GH, Hanley DA: **Vitamin D insufficiency in a population of healthy western Canadians.** *CMAJ : Canadian Medical Association journal = journal de l'Association medicale canadienne* 2002, **166**(12):1517-1524.
32. Gozdzik A, Barta JL, Wu H, Wagner D, Cole DE, Vieth R, Whiting S, Parra EJ: **Low wintertime vitamin D levels in a sample of healthy young adults of diverse ancestry living in the Toronto area: associations with vitamin D intake and skin pigmentation.** *BMC public health* 2008, **8**:336.
33. Stoll D, Dudler J, Lamy O, Hans D, Krieg MA, Aubry-Rozier B: **Can one or two high doses of oral vitamin D3 correct insufficiency in a non-supplemented rheumatologic population?** *Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA* 2013, **24**(2):495-500.
34. Tripkovic L, Lambert H, Hart K, Smith CP, Bucca G, Penson S, Chope G, Hypponen E, Berry J, Vieth R *et al*: **Comparison of vitamin D2 and vitamin D3 supplementation in raising serum 25-hydroxyvitamin D status: a systematic review and meta-analysis.** *Am J Clin Nutr* 2012, **95**(6):1357-1364.

**Table 2-1.** Baseline characteristics of student participants across study arms, Hamilton, Canada, 2010 and 2011.

	<b>Vitamin D &amp; Gargling (N=150)</b>	<b>Vitamin D &amp; No Gargling (N=150)</b>	<b>Placebo &amp; Gargling (N=150)</b>	<b>Placebo &amp; No Gargling (N=150)</b>
<b>Age, median years (IQR)</b>	19 (18-20)	19 (18-21)	19 (18-20)	19 (18-21)
<b>Female, No. (%)</b>	96 (64.0)	97 (64.7)	100 (66.7)	89 (59.3)
<b>Living in Residence, No. (%)</b>	48 (32.0)	50 (33.3)	50 (33.3)	49 (32.7)
<b>Asthma, No. (%)</b>	14 (9.3)	16 (10.7)	16 (10.7)	12 (8.0)
<b>≥ 7 Hours Sleep, No. (%)</b>	114 (76.0)	102 (68.0)	111 (74.0)	116 (77.3)
<b>Exercise, mean hours (SD)</b>	5.1 (4.2)	4.9 (3.5)	4.8 (4.9)	4.8 (4.0)
<b>Hand washing before meals, No. (%)</b>				
<b>Never</b>	34 (22.7)	30 (20.0)	36 (24.0)	42 (28.0)
<b>Usually</b>	75 (50.0)	71 (47.3)	68 (45.3)	70 (46.7)
<b>Always</b>	40 (26.7)	45 (30.0)	42 (28.0)	35 (23.3)
<b>Smoking, No. (%)</b>				
<b>Occasionally</b>	6 (4.0)	5 (3.3)	6 (4.0)	6 (4.0)
<b>Daily</b>	0	1 (0.7)	2 (1.3)	4 (2.7)
<b>*Vitamin Use, No. (%)</b>	30 (20.0)	40 (26.7)	34 (22.7)	21 (14.0)
<b>Daily Gargling, No. (%)</b>	15 (10.0)	19 (12.7)	18 (12.0)	8 (5.3)

SD: Standard deviation, No: Number

**Table 2-2.** Frequency, severity, duration and viral load associated with URTI according to vitamin D allocation.

Outcome Measure	Clinical URTI				Laboratory Confirmed URTI			
	Vitamin D (n=258)	Placebo (n=234)	Effect Measure (95% CI)	P Value	Vitamin D (n=256)	Placebo (n=233)	Effect Measure (95% CI)	P Value
Imputed URTI Episodes, No. (%)	91 (30.3)	114 (38.0)	*RR:0.80 (0.63-1.02)	0.08				
Complete Case URTI Episodes, No. (%)	70 (27.1)	80 (34.2)	*RR:0.79 (0.61-1.03)	0.09	26 (10.2)	44 (18.9)	*RR:0.54 (0.34-0.84)	0.007
Viral Load, Mean (SD)					5.51 (1.6)	6.40 (1.2)		0.036
†Symptom Duration, Mean days, (SD)	6.0 (1.6)	6.2 (1.3)	*HR: 1.32 (0.59-2.90)	0.49	5.8 (1.5)	6.2 (1.2)	*HR: 1.30 (0.49-3.48)	0.59
†Symptom Severity, Mean (SD)	218.6 (124.0)	199.8 (108.1)		0.41	229.7 (110.3)	181.5 (91.9)		0.09

RR: relative risk, HR: Hazard ratio

\*The Poisson and Cox regression models were adjusted exclusively for randomization strata: vitamin D allocation, gargling allocation, year of participation and type of housing.

†Symptom duration and severity was measured only in participants who self-reported illness (ie. not for adjudicated events), n=106. For clinical URTI, n=53 in each of the vitamin D and placebo groups. For laboratory confirmed URTI n=22 and n=34 in the vitamin D and placebo groups respectively.

**Table 2-3.** Frequency, severity, duration and viral load associated with URTI according to gargling allocation.

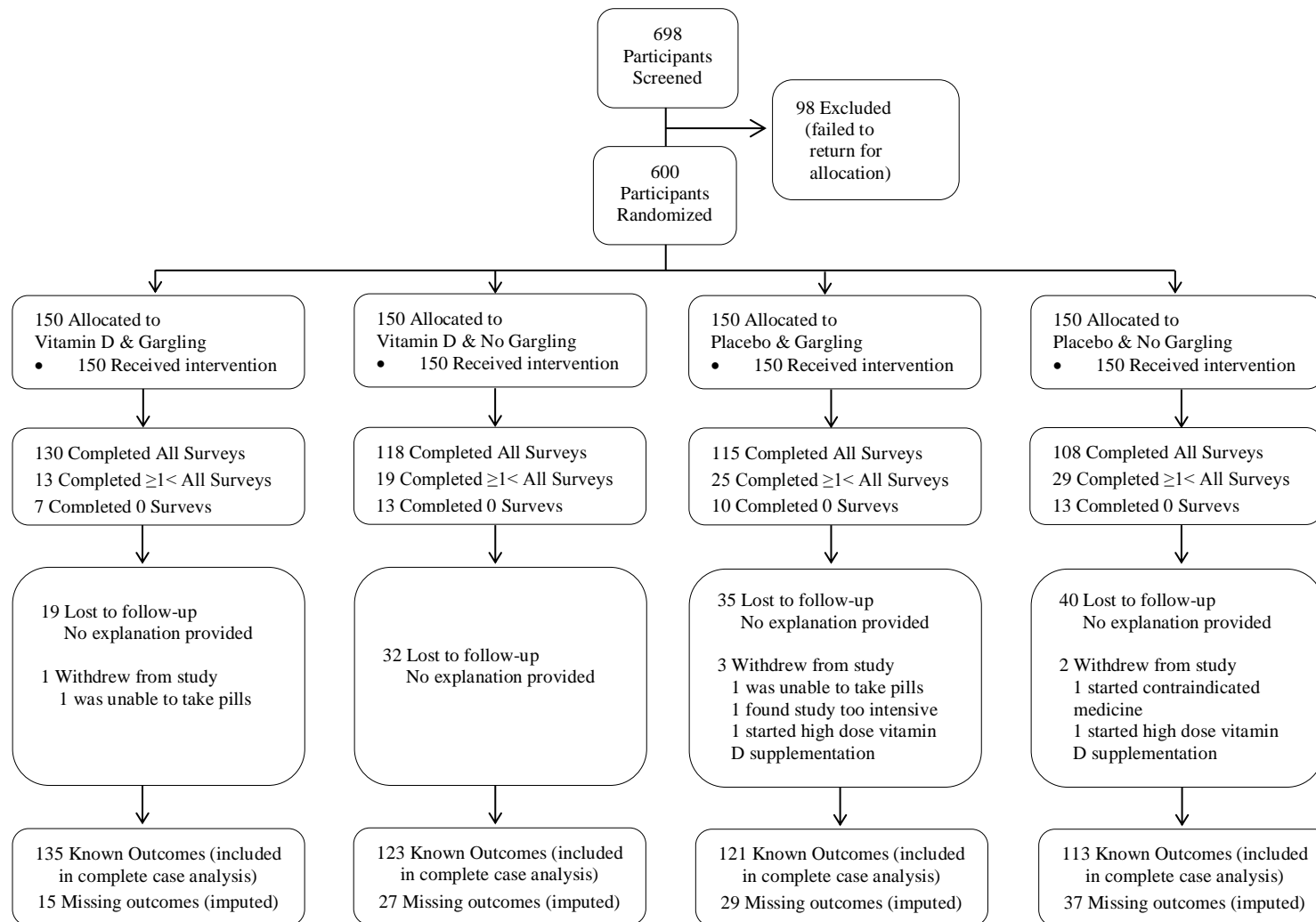
Outcome Measure	Clinical URTI				Laboratory Confirmed URTI			
	Gargling (n=256)	Control (n=236)	Effect Measure (95% CI)	P Value	Gargling (n=254)	Control (n=235)	Effect Measure (95% CI)	P Value
Imputed URTI Episodes, No. (%)	106 (35.3)	100 (33.3)	*RR: 1.1 (0.78-1.44)	0.69				
Complete Case URTI Episodes, No. (%)	85 (33.2)	65 (27.5)	*RR: 1.2 (0.92-1.57)	0.19	33 (13.0)	37 (18.7)	*RR:0.82 (0.53-1.26)	0.36
Viral Load, Mean (SD)					6.24 (1.3)	5.95 (1.5)		0.43
†Symptom Duration, Mean days (SD)	6.2 (1.3)	6.0 (1.6)	*HR: 0.85 (0.38-1.89)	0.69	5.9 (1.3)	6.1 (1.4)	*HR: 0.43 (0.56-4.0)	0.43
†Symptom Severity, Mean (SD)	225.3 (123.9)	191.8 (105.1)		0.13	210.5 (105.3)	191.8 (98.8)		0.49

RR: relative risk, HR: Hazard ratio

\*The Poisson and Cox regression models were adjusted exclusively for randomization strata: vitamin D allocation, gargling allocation, year of participation and type of housing.

†Symptom duration and severity was measured only in participants who self-reported illness (ie. not for adjudicated events), n=106. For clinical URTI, n=55 and n=51 in the gargling and control groups respectively. For laboratory confirmed URTI n=26 and n=30 in the gargling and control groups respectively.

**Figure 2-1.** Flow diagram of participant randomization and follow-up during the McFLu2 COLd3 Prevention Trial, Hamilton, Canada; 2010 and 2011.





## CHAPTER 3

**A Comparison of Four Statistical Methods for the Analysis of Repeated Events Data**

In Chapter 3, I contrast four statistical methods for the analysis of repeated events data. In contrast to the published literature, this investigation is conducted using a data set with few repeated events. The results suggest that even when there are few repeated events, using first event statistical methods which ignore repeated events reduces the analytic power and wastes valuable available data.

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**Applied analysis of repeated events with low rates of recurrent events: a comparison of first event and repeated events methods**

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**Abstract**

**Background:** Epidemiological and medical research investigators are often interested in questions of treatment efficacy for recurrent conditions. Analyses of these data often ignore the correlation between repeated events in a given individual, or employ a limited analysis using only a first event to avoid the more complex statistical methods required for repeated events analysis. Previously published papers have demonstrated that the former approach can lead to invalid inferences by ignoring the correlation between repeated events analysis in data sets with high rates of recurrent events, and the latter approach leads to reduced power. This issue has previously been explored in the context of data where repeated events are frequent. When there are few participants with repeated events, it may be tempting to assume that repeated events analysis is not different from first event analysis, however this has not been demonstrated. In this paper, we explore the impact of first event versus repeated events analysis for data with low rates of recurrent events. We simultaneously ask the clinical question “Does vitamin D supplementation in early autumn prevent upper respiratory tract infections in university students during the first school semester?” **Methods:** We apply first event and repeated events analyses for Poisson regression and Cox regression to a data set with low rates of recurrent events. We *a priori* designated generalized estimating equations with a Poisson loglinear link for the evaluation of our clinical question. **Results:** The parameter estimates were somewhat similar between first event and repeated events analysis however, first event analyses produced more conservative estimates with non-significant effects. Repeated events analyses consistently demonstrated a statistically significant risk reduction in upper

respiratory tract infections associated with vitamin D supplementation (RR: 0.75, 95% CI: 0.58-0.96,  $p=0.021$ ). **Conclusion:** Using repeated events analyses incorporates all available data and may enhance the power to detect a statistically significant effect. Even in data sets with low rates of repeated events, using all events rather than just first events may improve power.

## 1.0 Introduction

Epidemiological and medical research investigators are frequently interested in the effect of a treatment on the incidence of recurrent outcomes such as asthma attacks, epileptic seizures, hospitalizations, and infections. A major methodological consideration for the analysis of such longitudinal data is properly accounting for the correlation between repeated events within a participant. Failure to account for correlation leads to biased estimates of coefficients and standard error.(1, 2) Overlooking positive correlation between events will result in underestimating the true variability which leads to confidence intervals that are too narrow and p values that are too small. (1) Furthermore, measuring multiple events per participant is more efficient than a limited analysis restricted to a single, or first, event when the outcome of interest is a commonly recurrent health condition. (3) Indeed, several papers have highlighted various approaches for the analysis of repeated events data and consistently conclude that first event analysis is inadequate.(1, 3-11) However, these studies have been conducted in the context of commonly recurrent events such as falls,(12, 13) hospitalization in the elderly, (7) emergency department visits of pediatric firearm victims,(9) and episodes of diarrhea and respiratory illness in young children in the developing world (1) when at least 20% of the

study population experiences repeated events. Longitudinal statistical methods with correlated outcomes remain unfamiliar to many researchers and implementation of these methods is more complex relative to first event models. When very few recurrent events are captured in a data set, it may be particularly tempting to choose a first event analysis. Although Twisk et al. suggest that simple, first event analysis can be justified in certain conditions, the condition of infrequent repeated events has not been considered.<sup>(10)</sup> We are unaware of any papers that have examined the benefit of repeated events analysis when there are few participants with recurrent events.

In this paper, we analyze data with low rates of repeated events from a randomized controlled trial assessing vitamin D3 supplementation for the prevention of upper respiratory tract infections (URTI). Furthermore, we contrast the implementation of first event and repeated events analysis using two familiar and accessible methods: (1) regression and (2) time-to-event analysis.

## **2.0 Methods**

### *2.1 Clinical Study*

The data used in this paper are from a longitudinal cohort of a subset of participants enrolled in a randomized controlled trial and then followed for an additional period of time. The McFlu2 COLD<sub>3</sub> Prevention study was a factorial randomized controlled trial (RCT) designed to assess whether vitamin D3 supplementation versus placebo, and gargling versus no gargling could prevent clinical upper respiratory tract infections (URTI) in university students. Complete details of the study and the results have been

previously reported.(14) Briefly, participants completed a baseline questionnaire that collected demographic, health and lifestyle information and thereafter were asked to complete weekly online surveys which screened for URTI symptoms. At the end of the RCT , students were invited to continue completing the weekly online surveys through to the end of December in an extended surveillance portion of the study. The vitamin D3 and gargling interventions were not continued during this portion of the study. The primary outcome was the incidence of clinical URTI, defined as the participant's perception of a "cold" in conjunction with two or more symptoms (runny/stuffy nose, congestion, cough, sneezing, sore throat, muscle aches, fever). Adjudication by two clinicians was applied when participants reported symptoms but were uncertain if they were ill. Self-reported and adjudicated episodes of clinical URTI were considered 'events' if the onset occurred at least seven days after the participant's randomization date. Recurrent episodes of URTI were considered events if the onset occurred at least one week after the resolution of a previous event.

## 2.2 Statistical Analysis

Differences in baseline characteristics by treatment group were compared using two-sided chi-square tests for categorical variables, and t-test or Mann-Whitney U test for normally and non-normally distributed continuous variables respectively. All P values and 95% confidence intervals were calculated with two-tailed tests and differences with  $P < 0.05$  were considered significant.

We analyzed the results using two common approaches: Poisson regression and time-to-event analysis (Cox regression). For each of these, we first considered a first event, or simple, analysis which ignored repeated events in the same participant. The outcome for these analyses reflected only whether or not the participant reported any URTI during the study. We then conducted longitudinal repeated events analyses which allowed for recurrent events to be considered. Generalized estimating equations (GEE) using a Poisson loglinear link and the Andersen-Gill (AG) model for Cox regression of recurrent events were used as parallel extensions of the respective first event analyses.

All models were adjusted for the randomization strata used in the RCT: vitamin D3 allocation, gargling allocation, year of participation and type of housing. For this paper, we *a priori* assigned the GEE model as our primary analysis for assessing the effect of vitamin D3 supplementation for the prevention of URTI.

### *2.2.1 First Event Poisson Regression and GEE with a Poisson Loglinear Link*

First event Poisson regression was used to analyze the proportion of participants who experienced at least one URTI in each treatment group during the study period. Although logistic regression could have been used, the resulting odds ratios are known to overestimate treatment effects when interpreted as relative risk.(10, 15) Poisson regression with robust standard errors has been demonstrated as an appropriate alternative to logistic regression.(15)

To account for repeated events within the same person during the weeks they were at risk of developing a new infection, we used GEE with a Poisson loglinear link and a first-



order autoregressive covariance matrix. GEE analysis accounts for correlation between observations in the way the model is fitted, and the model incorporates a working correlation matrix.(2, 11, 16, 17) This matrix represents the average correlation between different observations within the same person and the matrix is used to compute the robust estimator of the variance.(2, 3, 11) Several correlation matrix structures are available to choose from and although the researcher must select which covariance structure to use without actually knowing the true correlation, GEE is robust against misspecification of this covariance matrix (18-20). For a more detailed discussion of GEE analysis, the reader is referred elsewhere.(2, 16, 20)

### *2.2.2 Cox Regression Analysis and Andersen-Gill Repeated Events Regression*

We performed a standard Cox regression using the time to the first event to compare the effect of vitamin D3 supplementation on survival time. Participants who did not report an event were censored after their last completed survey. The assumption of proportional hazards was tested using multiplicative interaction variables of the natural logarithm of time crossed with each covariate.(21)

Several different extensions to the Cox regression have been developed to accommodate repeated events data.(6, 9, 11) Among the most commonly discussed extensions are the Andersen-Gill (AG) model, the Prentice, Williams and Peterson model (PWP) and the Wei, Lin and Weissfeld (WLW) model.(6, 9, 11) These models differ in the definition of the risk interval which defines when a participant is at risk of having an event, the treatment of the baseline hazard, and the definition of the risk set which varies with time

and depends on the risk interval.(6) We chose to use the AG model which treats intra-individual correlation as a nuisance parameter, assumes that a participant's recurrent event is unaffected by preceding events, and that the baseline hazard is constant across events but can vary across time.(1, 6, 7, 9, 11) The risk interval starts at the study baseline or at the end of an earlier event and ends at the start of a recurrent event or at the end of the study.(7) Each participant can experience any number of events of varying duration, and the duration of each event is excluded from the risk set. The AG model is an appropriate choice when the treatment effect is constant across events, the hazard of an event is not affected by having had a previous event, and the researcher is interested in the overall treatment effect rather than the treatment effect specific to each higher-order event.(6, 9, 11) Although the structure of the AG model accommodates recurrent events, it is the use of robust variance estimators that accounts for the correlation between multiple events within participants.(1, 6, 7, 9, 11)

### *2.2.3 Missing data*

Multiple imputation (MI), using the Markov chain Monte Carlo method and predictive mean matching, was conducted to address missing data. No variables had more than six per cent missing data. Information collected at baseline and through weekly surveys was used to model missing values for independent and dependent variables. (22) The duration of illness could span multiple weekly surveys and the raw data structure did not distinguish between new and ongoing events. Consequently, we created interaction variables between independent variables and the preceding week's illness status to improve the distinction between new and ongoing events in the MI model. Pooled

imputed data was used as a sensitivity analysis for the first event and repeated events (GEE) Poisson regression analyses. The first event and repeated events Cox regression analyses were not conducted with the pooled imputed data since a natural advantage of time-to-event analysis methods is the ability to use censored data.

The multiple imputation and AG models were created in R: A language and Environment for Statistical Computing version 2.8 (R Foundation for Statistical Computing, Vienna, Austria). All other statistical analyses were conducted using SPSS statistical software version 20.0 (IBM SPSS Inc., Chicago, IL, USA). A discussion of appropriate data structures for first event and repeated events analyses is presented in Appendix 1.

### **3.0 Results**

#### ***3.1 Study Population***

A total of 416 students consented to additional surveillance following their participation in a randomized controlled trial and formed the longitudinal cohort analyzed here (Figure 1). The median age of the participants was 19 years (interquartile range 18-20), 60% were first or second year undergraduate students, and 68% were female. Baseline characteristics were similar across the intervention arms (Table 1).

The maximum number of weeks of participation was 14 and 15 in 2010 and 2011 respectively, and the median number of completed surveys per participant was 13. Participation rates were high and 5499/5653 surveys (97.3%) were returned. Outcome

information was available for 391 (94%) individuals: 213 (51.2%) reported zero URTI, 146 (35.1%) reported one URTI and 32 (7.7%) reported two URTIs (Table 2).

### 3.2 First Event Poisson regression and Generalized Estimating Equations with a Poisson Loglinear Link

Results from the first event Poisson regression and GEE Poisson regression analyses are presented in (Table 3). Both analyses favored vitamin D3 supplementation for the prevention of URTI, however results differed. The first event analysis suggested a more conservative, non-statistically significant effect of vitamin D3 supplementation (RR: 0.82, 95% CI: 0.66-1.02,  $p=0.074$ ) while the longitudinal GEE analysis resulted in a statistically significant risk reduction associated with vitamin D3 supplementation, with a lower relative risk and nominally wider confidence band (RR: 0.75, 95% CI: 0.58-0.96,  $p=0.021$ ). Analysis of the pooled imputed data did not change the results (Table 3).

### 3.3 Cox regression analysis and Andersen-Gill Repeated Events Regression

Results from the first event Cox regression and AG repeated events survival analyses are presented in Table 4. Both analyses suggested that vitamin D3 supplementation reduced the risk of URTI however as above, results from the first event analysis were not statistically significant (HR: 0.77, 95% CI: 0.57-1.03,  $p=0.078$ ) while the AG model for repeated events demonstrated a statistically significant risk reduction associated with vitamin D3 supplementation, with a lower hazard ratio but narrowed confidence band (HR: 0.74, 95% CI: 0.57-0.95,  $p=0.020$ ).

## Discussion

In this paper, we contrasted first- and repeated events statistical methods for the analysis of recurrent events data when few participants (<10%) experienced repeated events. We applied these analyses to data from a longitudinal cohort study looking at vitamin D3 for the prevention of upper respiratory tract infections. These results demonstrated that weekly oral supplementation with 10,000 international units of vitamin D3 throughout September and October significantly reduced the average risk of having an upper respiratory tract infection from September to December by 25% (RR: 0.75, 95% CI: 0.58-0.96,  $p=0.021$ ). Results from this analysis suggest that vitamin D3 supplementation during the early fall may have prevented the natural seasonal depletion in vitamin D levels associated with the onset of autumn, thus enhancing the vitamin D modulated innate immune function.(23-28) Furthermore, the dosing regimen used here may have built sufficient stores of vitamin D3 to provide benefit beyond the period of active supplementation. However, in the absence of blood tests, this cannot be proven.

The relative simplicity and familiarity of first event statistical methods, those which ignore repeated events, make them appealing to many researchers for ease of analysis and communication to the broader community. However, this is a justifiable approach when interested in a specific short term (e.g., first recurrence) or long term (e.g., 5 year survival) results.(10) These first event methods may be particularly tempting when there are few repeated events in a data set. However, our analysis demonstrates that even when less than ten percent of the study population experiences repeated events, first event analyses suffer from the reduced power associated with ignoring subsequent events. This

is consistent with the literature examining the analysis of more frequent (>20%) repeated events. In our data set, the first event analyses considered only 178 of the 210 events observed among 416 participants. Although the effect estimates from both first event analyses suggested that vitamin D3 was beneficial for the prevention of URTI, neither reached statistical significance. While the effect estimates produced by the first event analyses were similar to those from the longitudinal repeated events analyses, the first event estimates were more conservative, as has been previously observed.(11)

Nonetheless it is important to recognize that the use of first event versus repeated events analyses allows slightly different questions to be answered. For example, the first event Poisson regression asks “To what extent does vitamin D3 supplementation reduce the probability of having a URTI at any point during the study?” In contrast, the repeated events GEE Poisson regression really asks “To what extent does vitamin D3 supplementation reduce the incidence for any new infection?” The differences in the question and related analyses may lead to slightly different estimates and standard errors.

In contrast to the first event analyses, results from both repeated events analyses demonstrated a statistically significant risk reduction associated with vitamin D3 supplementation. Consistent with the literature, the effect estimates from the GEE Poisson analysis and AG extended Cox analysis were nearly identical and suggested an approximate risk reduction of 25% in the intervention group.(11) The effect estimate for both repeated events analyses was marginally larger than the estimates from the first events analyses. It could be that vitamin D3 was more effective for later events because it took several weeks for serum vitamin D3 levels to be increased. Alternatively, vitamin

D3 might have heterogeneous effectiveness and may be more effective in those people who also tend to have more events. The two longitudinal methods used here are considered ‘population average’ procedures. In each case, the model can be interpreted as the overall effect of the intervention on the outcome since the start of the intervention, irrespective of the timing or number of previous events.(6, 9, 11) That is to say, on average, individuals who received vitamin D3 supplementation had a 25% lower risk of URTI compared to individuals who received placebo.

While both the GEE Poisson and AG models would be appropriate for dealing with repeated events data when research questions are concerned with an overall treatment effect, some practical considerations of the data may influence the choice of one model over the other. When repeated events are recorded on a continuous time scale rather than at fixed measurement intervals, creating an appropriate data structure for the AG model may be less demanding. In this case, one would create one record per participant, per event, taking care to respect the discontinuous risk intervals and excluding the duration of each event.

In this paper we have demonstrated that first event data analyses, which ignore correlated repeated events, produce different estimates and outcomes compared with repeated events analyses, even in the context of infrequent repeated events. We present two valid and accessible approaches to analyzing repeated events data and encourage researchers to consider these analyses, which make use of all available data in the context of studies of repeated events, when the primary interest is assessing an average effect of an intervention in a population.

## References

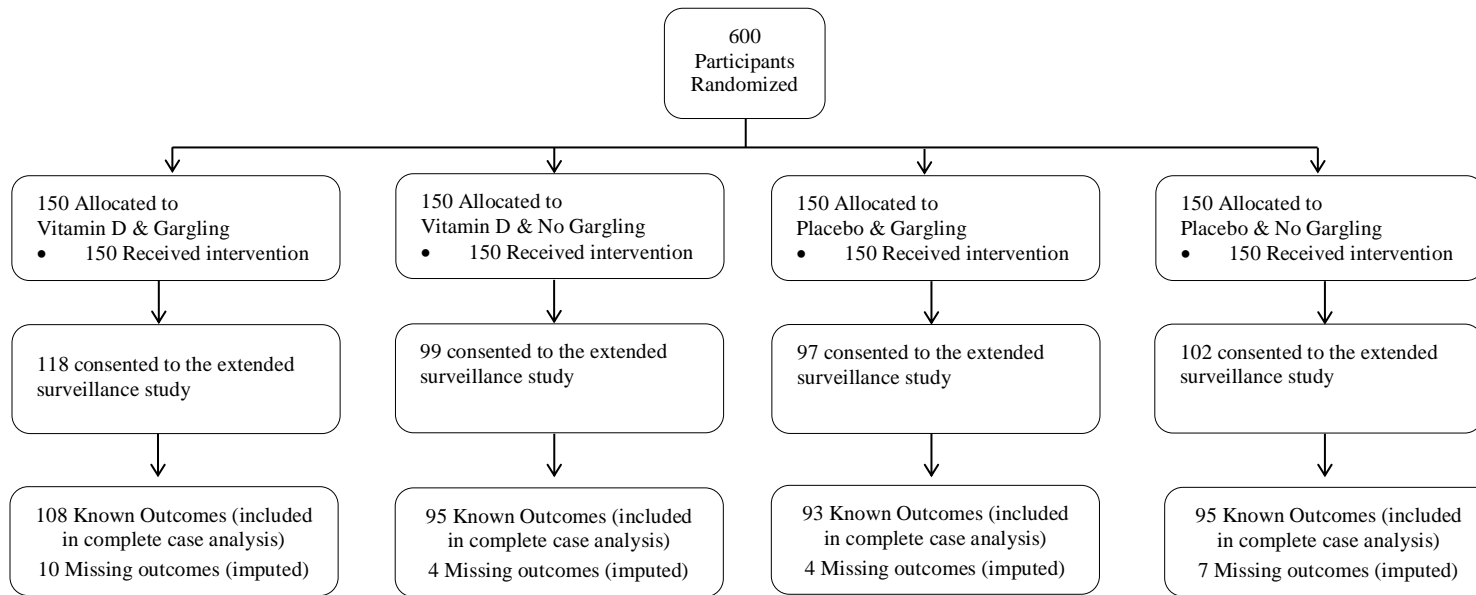
1. Moulton LH, Dibley MJ: **Multivariate time-to-event models for studies of recurrent childhood diseases.** *Int J Epidemiol* 1997, **26**(6):1334-1339.
2. Ballinger G: **Using Generalized Estimating Equations for Longitudinal Data Analysis.** *Organizational Research Methods* 2004, **7**(12):127-150.
3. Eisen EA: **Methodology for analyzing episodic events.** *Scandinavian journal of work, environment & health* 1999, **25 Suppl 4**:36-42.
4. Clayton D: **Some approaches to the analysis of recurrent event data.** *Stat Methods Med Res* 1994, **3**(3):244-262.
5. Wei LJ, Glidden DV: **An overview of statistical methods for multiple failure time data in clinical trials.** *Statistics in medicine* 1997, **16**(8):833-839; discussion 841-851.
6. Kelly PJ, Lim LL: **Survival analysis for recurrent event data: an application to childhood infectious diseases.** *Statistics in medicine* 2000, **19**(1):13-33.
7. Sturmer T, Glynn RJ, Kliebsch U, Brenner H: **Analytic strategies for recurrent events in epidemiologic studies: background and application to hospitalization risk in the elderly.** *Journal of clinical epidemiology* 2000, **53**(1):57-64.
8. Cook RJ, Lawless JF: **Analysis of repeated events.** *Stat Methods Med Res* 2002, **11**(2):141-166.
9. Lim HJ, Liu J, Melzer-Lange M: **Comparison of methods for analyzing recurrent events data: application to the Emergency Department Visits of**



- Pediatric Firearm Victims.** *Accident; analysis and prevention* 2007, **39**(2):290-299.
10. Twisk JW, Smidt N, de Vente W: **Applied analysis of recurrent events: a practical overview.** *Journal of epidemiology and community health* 2005, **59**(8):706-710.
  11. Guo Z, Gill TM, Allore HG: **Modeling repeated time-to-event health conditions with discontinuous risk intervals. An example of a longitudinal study of functional disability among older persons.** *Methods of information in medicine* 2008, **47**(2):107-116.
  12. Donaldson MG, Sobolev B, Cook WL, Janssen PA, Khan KM: **Analysis of recurrent events: a systematic review of randomised controlled trials of interventions to prevent falls.** *Age and ageing* 2009, **38**(2):151-155.
  13. Kuramoto L, Sobolev BG, Donaldson MG: **On reporting results from randomized controlled trials with recurrent events.** *BMC medical research methodology* 2008, **8**:35.
  14. Goodall E, Granados A, Luinstra K, Pulleneyagum E, Coleman B, Loeb M, Smieja M: **Vitamin D3 and gargling for the prevention of upper respiratory tract infections: a randomized controlled trial.** In. Review; 2013.
  15. Knol MJ, Le Cessie S, Algra A, Vandenbroucke JP, Groenwold RH: **Overestimation of risk ratios by odds ratios in trials and cohort studies: alternatives to logistic regression.** *CMAJ : Canadian Medical Association journal = journal de l'Association medicale canadienne* 2012, **184**(8):895-899.

16. Hanley JA, Negassa A, Edwardes MD, Forrester JE: **Statistical analysis of correlated data using generalized estimating equations: an orientation.** *Am J Epidemiol* 2003, **157**(4):364-375.
17. Twisk JW: **Longitudinal data analysis. A comparison between generalized estimating equations and random coefficient analysis.** *European journal of epidemiology* 2004, **19**(8):769-776.
18. Freedman DA: **On the So-Called "Huber Sandwich Estimator" and "Robust Standard Errors".** *The American Statistician* 2006, **60**(4):299-302.
19. Hubbard AE, Ahern J, Fleischer NL, Laan MVd, Satariano SA, Jewell N, Bruckner T, Satariano WA: **To GEE or Not to GEE: Comparing Population Average and Mixed Models for Estimating the Associations Between Neighborhood Risk Factors and Health.** *Epidemiology* 2010, **21**(4):467-474.
20. Ziegler A, Vens M: **Generalized estimating equations. Notes on the choice of the working correlation matrix.** *Methods of information in medicine* 2010, **49**(5):421-425; discussion 426-432.
21. Tabachnick BG, Fidell LS: **Using Multivariate Statistics**, 6 edn: Pearson; 2013.
22. Groenwold RH, Donders AR, Roes KC, Harrell FE, Jr., Moons KG: **Dealing with missing outcome data in randomized trials and observational studies.** *Am J Epidemiol* 2012, **175**(3):210-217.
23. Liu PT, Stenger S, Li H, Wenzel L, Tan BH, Krutzik SR, Ochoa MT, Schaubert J, Wu K, Meinken C *et al*: **Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response.** *Science* 2006, **311**(5768):1770-1773.

24. Wang TT, Nestel FP, Bourdeau V, Nagai Y, Wang Q, Liao J, Tavera-Mendoza L, Lin R, Hanrahan JW, Mader S *et al*: **Cutting edge: 1,25-dihydroxyvitamin D3 is a direct inducer of antimicrobial peptide gene expression.** *Journal of immunology (Baltimore, Md : 1950)* 2004, **173**(5):2909-2912.
25. Gozdzik A, Barta JL, Weir A, Cole DE, Vieth R, Whiting SJ, Parra EJ: **Serum 25-hydroxyvitamin D concentrations fluctuate seasonally in young adults of diverse ancestry living in Toronto.** *The Journal of nutrition* 2010, **140**(12):2213-2220.
26. Gozdzik A, Barta JL, Wu H, Wagner D, Cole DE, Vieth R, Whiting S, Parra EJ: **Low wintertime vitamin D levels in a sample of healthy young adults of diverse ancestry living in the Toronto area: associations with vitamin D intake and skin pigmentation.** *BMC public health* 2008, **8**:336.
27. Rucker D, Allan JA, Fick GH, Hanley DA: **Vitamin D insufficiency in a population of healthy western Canadians.** *CMAJ : Canadian Medical Association journal = journal de l'Association medicale canadienne* 2002, **166**(12):1517-1524.
28. Webb AR, Kline L, Holick MF: **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.** *The Journal of clinical endocrinology and metabolism* 1988, **67**(2):373-378.



**Figure 3-1.** Flow diagram of participant randomization and follow-up during the McFLu2 COLD3 Prevention Trial and extended surveillance study, Hamilton, Canada; 2010 and 2011

**Table 3-1.** Baseline Characteristics According to Intervention

	<b>Vitamin D (N=217)</b>	<b>Placebo (N=199)</b>	<b>p Value</b>	<b>Gargling (N=215)</b>	<b>No Gargling (N=201)</b>	<b>p Value</b>
<b>Age, median years (IQR)</b>	19 (18-21)	19 (18-20)	0.09	19(18 20)	19 (18-21)	0.74
<b>Female, No. (%)</b>	151 (69.6)	131 (66.2)	0.46	149(69.6)	133 (66.2)	0.45
<b>Living in Residence, No. (%)</b>	68 (31.3)	68 (34.2)	0.54	68 (31.6)	68 (33.8)	0.63
<b>Asthma, No. (%)</b>						
<b>Yes</b>	20 (9.4)	14 (7.1)		16 (8.0)	18 (8.5)	
<b>No</b>	186 (87.3)	175 (88.8)		177 (89.0)	184 (87.2)	
<b>Unsure</b>	7 (3.3)	8 (4.1)	0.66	6 (3.0)	9 (4.3)	0.78
<b>Hours Sleep, No. (%)</b>						
<b>≥7</b>	162 (75.3)	156 (78.8)		165(77.5)	153 (76.5)	
<b>≤6</b>	53 (24.7)	42 (21.2)	0.42	48 (22.5)	47 (23.5)	0.82
<b>Exercise, mean hours (SD)</b>	4.9 (3.7)	4.7 (3.5)	0.52	4.8 (3.6)	4.8 (3.5)	0.90
<b>Hand washing before meals, No. (%)</b>						
<b>Never</b>	45 (21.0)	47 (23.7)		55 (25.8)	54 (27.1)	
<b>Usually</b>	110 (51.4)	101 (51.0)		109 (51.2)	102 (51.3)	
<b>Always</b>	59 (27.7)	50 (25.3)	0.55	49 (23.0)	43 (21.6)	0.92
<b>Smoking, No. (%)</b>						
<b>Never</b>	207 (96.7)	190 (96.0)		1 (0.5)	1 (0.5)	
<b>Occasionally</b>	7 (3.3)	6 (3.0)		7 (3.3)	7 (3.5)	
<b>Daily</b>	0 (0)	2 (1.0)	0.34	205(96.2)	192 (96.5)	0.32
<b>Vitamin Use, No. (%)</b>						
<b>Yes</b>	54(25.4%)	38(19.4%)		43 (20.5)	49 (24.6)	
<b>No</b>	148 (69.5)	146 (74.5)		151 (71.9)	143( 71.9)	
<b>Unsure</b>	11 (5.2)	12 (6.1)	0.34	16 (7.6)	7 (3.5)	0.15
<b>Gargling, No. (%)</b>						
<b>Daily</b>	24 (11.2)	16 (8.1)		25 (11.8)	15 (7.5)	
<b>Occasionally</b>	79 (36.9)	80 (40.4)		83 (39.2)	76 (38.0)	
<b>Never</b>	111 (51.9)	102 (51.5)	0.30	104(49.0)	109 (54.5)	0.32

**Table 3-2.** Distribution of events across interventions

	Overall (n=416)	Vitamin D3 (n=217)	Placebo (n=199)
0 Events	213	119	94
1 Event	146	73	73
2 Event	32	11	21
Missing	25	14	11

**Table 3-3.** First event Poisson Regression and GEE Poisson Analysis

	First Event Poisson Regression				GEE Poisson Analysis			
	<i>Complete Case</i>		<i>Multiple Imputation</i>		<i>Complete Case</i>		<i>Multiple Imputation</i>	
	RR (95% CI)	p Value	RR (95% CI)	p Value	RR (95% CI)	p Value	RR (95% CI)	p Value
Vitamin D	0.82 (0.66-1.02)	0.074	0.81 (0.65-1.01)	0.066	0.75 (0.58-0.96)	0.021	0.74 (0.58-0.95)	0.016
Gargling	1.01 (0.82-1.26)	0.907	1.00 (0.80-1.25)	0.999	1.05 (0.82-1.34)	0.694	1.03 (0.80-1.32)	0.813
2011 vs. 2010	1.17 (0.94-1.45)	0.153	1.18 (0.95-1.48)	0.140	1.11 (0.87-1.43)	0.395	1.11 (0.86-1.42)	0.430
Living in Residence	0.77 (0.59-0.99)	0.042	0.73 (0.56-0.94)	0.017	0.67 (0.50-0.89)	0.006	0.66 (0.49-0.88)	0.005

**Table 3-4.** Cox Regression and Andersen-Gill Repeated-Events Analysis

	<b>Cox Regression</b>		<b>Andersen-Gill Repeated Events Analysis</b>	
	HR (95% CI)	p Value	HR (95% CI)	p Value
Vitamin D	0.77 (0.57-1.03)	0.078	0.74 (0.57-0.95)	0.020
Gargling	1.05 (0.78-1.41)	0.736	1.05 (0.82-1.35)	0.696
2011 vs. 2010	1.17 (0.87-1.57)	0.308	1.14 (0.89-1.47)	0.304
Living in Residence	0.70 (0.50-0.98)	0.038	0.66 (0.49-0.88)	0.006



**Appendix 1.** Data structures for first event and repeated events analyses

Both first event analyses required the data to be structured with one record per participant (Supplementary Table 1). For the first event Poisson regression, a binary variable indicating whether or not the participant had an event was required. This variable was coded as ‘missing’ for those participants who had incomplete follow-up and did not report an event prior to attrition. For the first event Cox regression, the data structure required a binary variable indicating whether the participant had an event, or either did not have an event, or was censored before the end of the study. Consequently, no data is coded as ‘missing’ in contrast to the first event Poisson regression. Cox regression also requires a variable representing the period of observation contributed by each participant before experiencing an event, attrition or the end of follow-up was necessary.

In contrast, the repeated events analyses required the data to be structured with one record per participant, per measurement based on a counting process structure (Supplementary Table 2). The variables “Start” and “Stop” define each interval which corresponds to a measurement. The variable “At\_Risk” defines whether or not the participant was at risk of a new event during that interval. Finally, the variable “Event” indicates whether or not the participant had an event during that interval. The onset of a new event would be represented by the combination of a participant being at risk of an event and reporting an event. The health condition under investigation here is one with discontinuous risk intervals. That is, a participant is not at risk of a subsequent event until the first event has resolved. Studies of outcomes with discontinuous risk intervals require the exclusion of the duration of each event from the total time at risk in order to derive valid effect

estimates. (11) This is achieved simply by including only those records where the participant is at risk of a new event in the analysis. When a participant experienced an event that lasted several weeks, she was only considered to be at risk during the first week of the illness and was not at risk during subsequent weeks of the same illness. An alternative data structure would be to create one record per participant per event instead of per measurement (Supplementary Table 3).(11)

**Supplementary Table 3-S1.** Example data structure for first event analyses

This table demonstrates the appropriate data structure for first event Poisson and Cox regression analysis based on fictional participants. Participant 1 had an event at week 12, participant 2 did not have an event and provided complete follow-up, and participant three provided follow-up for seven weeks and was then censored without knowledge of an event. The variable “Ever\_Event” is the necessary outcome variable for naïve Poisson regression and demonstrates the missing data for participant 3. The variables “Censored” and “Duration” are necessary for naïve Cox regression.

<b>Study_ID</b>	<b>Vitamin D</b>	<b>Censored</b>	<b>Duration</b>	<b>Ever_Event</b>
1	1	1	12	1
2	1	0	15	0
3	0	0	7	-9

Variable coding: Censored, 1= event, 0 = no event/censored; Ever\_Event, 1= event, 0= no event, -9=missing; Vitamin D, 1= vitamin D3, 0=placebo

**Supplementary Table 3-S2.** Example Data Structure for repeated event analyses

This table demonstrates the appropriate data structure for repeated events GEE Poisson regression and Cox regression using the AG model. Data is presented for the first seven weeks of participation for three fictional participants. Participant 1 had an event at week five and was well again at week six. Participant 2 had an event at week two and was well again at week four, and then had a second event at week six which resolved by week seven. Participant 3 had an event at week four and was then lost-to-follow-up.

Study_ID	Vitamin D	Start	Stop	At_Risk	Event
1	1	0	1	1	0
1	1	1	2	1	0
1	1	2	3	1	0
1	1	3	4	1	0
1	1	4	5	1	1
1	1	5	6	1	0
1	1	6	7	1	0
2	0	0	1	1	0
2	0	1	2	1	1
2	0	2	3	0	1
2	0	3	4	1	0
2	0	4	5	1	0
2	0	5	6	1	1
2	0	6	7	1	0
3	1	0	1	1	0
3	1	1	2	1	0
3	1	2	3	1	0
3	1	3	4	1	1
3	1	4	5	0	0
3	1	5	6	0	0
3	1	6	7	0	0

Variable coding: At\_Risk, 1= at risk, 0 = not at risk; Event, 1= event, 0= no event;  
Vitamin D, 1= vitamin D3, 0=placebo

**Supplementary Table 3-S3.** Example data structure for repeated events recorded on a continuous time scale

Data is presented for fifteen weeks of participation for four fictional participants. Participant 1 had an event at week five and was well again at week six. Participant 2 had an event at week two and was well again at week four, and then had a second event at week six which resolved by week eight. Participant 3 had an event at week four and was then lost-to-follow-up. Participant 4 remained healthy for the entire study.

<b>Study_ID</b>	<b>Vitamin D</b>	<b>Start</b>	<b>Stop</b>	<b>Event</b>
1	1	0	5	1
1	1	6	15	0
2	0	0	2	1
2	0	4	6	1
2	0	8	15	0
3	1	0	4	1
4	0	0	15	0

## CHAPTER 4

**Lifestyle Factors Associated with the Risk of Respiratory Infections**

In Chapter 4, my co-authors and I sought to identify lifestyle factors that are associated with the risk of upper respiratory tract infections. We identified six lifestyle factors that were statistically significantly associated with the risk of infection. Being female, every additional year of age, and living with a housemate who reported an illness were all associated with increased risk of upper respiratory tract infection. Frequent hand washing, adequate sleep, and vitamin D3 supplementation were all associated with reduced risk of infection. The latter three behaviours are modifiable and may represent potential means for improved infection prevention. We show that the combination of multiple protective behaviors was associated with a larger risk reduction.

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**Association of lifestyle and behavior factors with risk of upper respiratory tract infections**

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## Abstract

**Background:** Upper respiratory tract infections (URTI) are responsible for significant health care usage and considerable lost time from work and school. We sought to identify lifestyle choices that act as risk factors for or protection against URTI. **Methods:** We designed a longitudinal cohort study by extending the surveillance of a subgroup (416/600) of Canadian university students originally enrolled in a randomized controlled trial of vitamin D3 and gargling for the prevention of URTI. Participants completed an electronic survey capturing lifestyle choices at baseline and weekly thereafter. Participants reported the onset of URTI throughout the study using standardized definitions. **Results:** Outcomes were available for 391 (94%) participants who reported 210 episodes of URTI. Weekly vitamin D3 supplementation with 10,000 IU significantly reduced the risk of reported URTI the following week by 26% (RR: 0.74, CI<sub>95%</sub>: 0.58,0.95, p=0.016). Hand washing more than six times daily, or four to five times daily was associated with a significant risk reduction compared to hand washing zero to three times daily (RR: 0.65, CI<sub>95%</sub>: 0.47,0.89, p=0.008 and RR: 0.75, CI<sub>95%</sub>: 0.57,0.99, p=0.044 respectively). Compared to participants reporting one or more nights of four or fewer hours of sleep, those reporting seven hours or more, or reporting five to six hours of sleep had a lower risk of URTI of 47% and 29% respectively (RR: 0.53, CI<sub>95%</sub>: 0.35, 0.81, p=0.004 and RR: 0.71, CI<sub>95%</sub>: 0.54,0.95, p=0.020). **Conclusions:** Further studies should examine whether improving specific lifestyle factors may be an effective and safe way to decrease the risk of URTI.

## **Introduction**

Upper respiratory tract infections (URTI), generally known as the common cold, are one of the most frequent human infections.[1, 2] Viral pathogens that cause the symptoms of a common cold, such as rhinoviruses, coronaviruses, and respiratory syncytial virus, are ubiquitous and are associated with high attack rates.[2, 3] Typically children will experience six to ten infections each year and adults will suffer two to four infections.[1, 2] While these infections are usually self-limited and non-fatal, they are the cause of millions of health care visits, substantial workplace and school absenteeism, and exacerbations of underlying medical conditions such as asthma and chronic obstructive pulmonary disease.[1, 3, 4] Currently, effective prevention of these infections is poor but even a small improvement could lead to substantial benefit for the general public. Simple lifestyle factors such as hand washing, adequate sleep, exercise and nutritional supplementations may contribute to prevention.[5-9] In this paper, we assess the role of several lifestyle factors as predictors of URTI in university students.

## **Methods**

### *Clinical Study*

The data were derived from a longitudinal cohort study. These data are from a large subset of participants originally enrolled in a factorial randomized controlled trial who completed additional observational surveillance in the absence of interventions. The original trial of 600 students was designed to assess whether vitamin D3 supplementation (10,000 international units (IU) per week) versus placebo, and gargling versus no gargling

could prevent clinical upper respiratory tract infections in university students.[10]

Individuals were eligible for the study if they were enrolled at McMaster University, were  $\geq 17$  years, and lived with at least one student housemate. Participants with contraindicated medical conditions (hypercalcemia, parathyroid disorder, chronic kidney disease, use of anticonvulsants, malabsorption syndromes, sarcoidosis), who were currently or planning to become pregnant, or who were unable to swallow capsules were excluded. Participants were enrolled during the first two weeks of September 2010 or 2011 and were followed to the end of October 2010 or 2011, respectively. Participants were randomized to one of four treatment arms: 1) vitamin D3 and gargling, 2) vitamin D3 and no gargling, 3) placebo and gargling, or 4) placebo and no gargling. Participants completed a baseline questionnaire that collected demographic, health and lifestyle information. Thereafter, participants were asked to complete weekly online surveys which screened for URTI symptoms and collected information about lifestyle factors in the week preceding the survey. Students also submitted weekly self-collected nasal swabs. At the end of October, students were invited to continue completing the weekly online surveys through to the end of December as participants in an extended surveillance portion of the study. Interested students signed a new consent form for this observational study. The vitamin D3 and gargling interventions and weekly self-collected nasal swabs were not continued during this portion of the study. The study protocol was approved by the Hamilton Health Sciences / Faculty of Health Sciences Research Ethics Board. Complete details of the trial study design and results from the core randomized controlled trial have been published elsewhere. [10]

### Measures of Lifestyle Factors

Participants were asked to complete a baseline survey and weekly online questionnaires that screened for symptoms of URTI and included questions on specific lifestyle factors. Smoking status, age, sex, asthma status, use of vitamins, alcohol consumption and type of housing were assessed once at baseline. Measures of sleep, exercise, gargling, hand washing, and the presence of a sick housemate or roommate were assessed at baseline and weekly for the duration of the study. Sleep was assessed by asking each participant to report the average number of hours of sleep they had, and the least amount of sleep they had in one night the preceding week. Participants were asked to report the average frequency of daily hand washing, and also whether they always, sometimes or never washed their hands before having a meal in the preceding week. Exercise was measured as the total number of hours of moderate exercise completed in the previous 7 days. Finally, participants were asked how frequently they gargled in the preceding week. The complete details of the survey questions, response options and variable coding are available in Supplementary Table 1.

### Outcome Measure

The primary outcome was the incidence of clinical URTI, defined as the participant's perception of a cold in conjunction with two or more symptoms (runny/stuffy nose, congestion, cough, sneezing, sore throat, muscle aches, fever). Adjudication by two clinicians was applied when participants reported symptoms but were uncertain if they were ill. Self-reported and adjudicated episodes of clinical URTI were considered

‘events’ if the onset occurred at least seven days after the participant’s randomization date. Recurrent episodes of URTI were considered events if the onset occurred at least one week after the resolution of a previous event.

### Statistical Analysis

Baseline characteristics are presented as mean and standard deviation, and median and interquartile range for normally and non-normally distributed continuous variables respectively. Categorical variables are presented as counts and percentages.

We sought to assess the relationship between lifestyle factors and the risk of having a respiratory infection. To account for the correlation associated with repeated responses per participant, we used generalized estimating equations with a loglinear link and robust standard errors and a first-order autoregressive covariance matrix. We chose to use the loglinear link instead of a binary logit link since the odds ratios resulting from the latter are known to overestimate the treatment effect when incorrectly interpreted as risk ratios. [11] The data set consisted of all baseline and weekly survey information for participants who were at risk of having an event. Weeks representing persistent illness were omitted. Multiple imputation, using the Markov chain Monte Carlo method and predictive mean matching, was conducted to address all missing data. No variables had more than six per cent missing data. Information collected at baseline and through weekly surveys was used to model missing values. *A priori*, we designated the analysis using the pooled imputed data as our primary analysis and performed a sensitivity analysis using the complete case data.

### Model Variables and Development

We identified independent variables and covariates for inclusion in the model based on the literature. We then conducted a preliminary univariable analysis of each variable with the number of events. Variables were considered for inclusion in the multivariable analysis if  $p < 0.25$  at the univariable stage.[12] When data was sparse in categories of ordinal variables, we collapsed these variables into approximate tertiles representing low, moderate and high frequency of a given lifestyle factor. After identifying the initial multivariable model, variables that were not statistically significant at  $p < 0.05$  were excluded. We then systematically re-introduced variables, one by one, that were initially excluded to investigate important effects of groups of variables. Plausible interactions were assessed using the same process. Model fit was assessed using the corrected quasi-likelihood under independence model criterion (QICC).

As a sensitivity analysis, we *a priori* decided to include the original stratification variables from the randomized controlled trial regardless of the association on univariable analysis. These variables were: vitamin D supplementation status, type of housing, year of participation in the study and daily gargling. For the multivariable models we entered all predictors simultaneously as we did not hypothesize an order of importance among the predictor variables.[13]

We sought to investigate whether the concurrent practice of multiple modifiable protective behaviors would lead to a greater associated risk reduction compared to single behaviors. We first used cross product interaction terms to test for effect modification

between variables. We reasoned that in the absence of effect modification, it would be appropriate to expect each variable to contribute independently to a predictive score. We sought to develop a 'protective behavior score' that reflected the combined effect of vitamin D supplementation, frequency of baseline daily hand washing and the least amount of reported weekly sleep. The data was divided by trial year (ie. 2010 vs. 2011) into two data sets to allow the score to be derived and internally validated using separate data. We derived the protective behavior score based on the size of individual regression coefficients estimated from a multivariable analysis. The protective behavior score ranged from zero to four points. One point was awarded for each of: i) vitamin D supplementation, ii) baseline daily hand washing more than four times daily, and iii) reporting the least amount of sleep in the previous week as five to six hours, with one extra point awarded if the least amount of sleep on any given night was seven hours or longer. The effect of concurrently practicing multiple protective behaviors was assessed by including the score as an ordinal variable in a final multivariable model adjusted for sex, age, and sick housemates.

## **Results**

A total of 416/600 (69%) participants originally randomized were followed in the longitudinal cohort from September to the end of December in 2010 and 2011. Of these, 68% were female, the median age was 19 years (interquartile range 18-20), and 60% were first or second year undergraduate students. Only 15 participants (4%) reported smoking cigarettes on a daily or occasional basis, and 34 participants (8%) reported having asthma.

Participation rates were high; 5499/5653 surveys (97.3%) were returned and outcome information was available for 391 (94%) individuals. A total of 210 episodes of URTI were recorded: 32 (7.7%) individuals reported two URTIs, 146 (35.1%) individuals reported one URTI, and 213 (51.2%) individuals reported zero URTI.

The univariable analysis identified 15 variables for inclusion in the initial multivariable model (Table 1). After the multivariable model building process, six lifestyle factors remained statistically significant in the multivariable model (Table 2). Weekly supplementation with 10,000 IU of vitamin D3 was associated with a statistically significant 26% risk reduction in URTI (RR: 0.74, CI<sub>95%</sub>: 0.58, 0.95, p=0.016). Frequent ( $\geq 6$  times/day) daily hand washing at baseline was associated with a statistically significant 35% risk reduction in URTI compared to those who reported hand washing zero to three times per day (RR: 0.65, CI<sub>95%</sub>: 0.47, 0.89, p=0.008). Moderate daily hand washing (4-5 times/day) was also associated with significantly reduced the risk of URTI by 25% (RR: 0.75, CI<sub>95%</sub>: 0.57, 0.99, p=0.044) compared to those with the least frequent hand washing. The least amount of sleep in one night during the preceding week was significantly associated with the risk of URTI. Compared to participants who reported four hours or less, those who reported five to six hours of sleep had a lower risk of URTI of 29% (RR: 0.71, CI<sub>95%</sub>: 0.54, 0.95, p=0.020). Individuals who reported a minimum of seven hours in the past week were associated with a reduced risk of URTI of 47% (RR: 0.53, CI<sub>95%</sub>: 0.35, 0.81, p=0.004). Being female was associated with significantly greater risk of URTI (RR: 1.34, CI<sub>95%</sub>: 1.02, 1.75, p=0.035) as was being older (RR: 1.10 per year, CI<sub>95%</sub>: 1.06, 1.14, p< 0.001). Reporting a sick housemate or roommate more than



doubled a participant's risk of URTI the following week (RR: 2.29, CI<sub>95%</sub>: 1.73, 3.03, p< 0.001). Analysis of the complete case data was similar (Table 3) however moderate (4-5 times/day) hand washing at baseline was associated with non-significant, rather than significant, risk reduction compared to individuals who reported low (0-3 times/day) daily hand washing (RR: 0.78, CI<sub>95%</sub>: 0.58, 1.03, p=0.076).

The sensitivity analysis including the type of housing, year of participation in the study and frequency of gargling with tap water did not substantially change the parameter estimates (Table 3). However, being female was no longer associated with a statistically significant increased risk of URTI. There was no statistically significant association between risk of URTI and: living in residence compared to off-campus, participating in 2011 compared to 2010, or any frequency of gargling with tap water.

Analysis of the protective behavior score suggested cumulative benefit associated with practicing multiple protective behaviors (test of model effects, p=0.02), however not all levels of the score were associated with statistically significant risk reductions. This may reflect the reduced sample size used for the internal model validation. The benefit associated with the protective score did appear to demonstrate a dose response; the higher the protective score, the greater the associated relative risk reduction. After adjusting for sex, age, and presence of sick housemates, individuals who scored four points (by receiving vitamin D supplementation, washing their hands four or more times daily, and reporting a minimum of seven hours of sleep per night in the previous week) were associated with a non-significant relative risk reduction of 67% compared to those who reported none of these protective behaviors (RR: 0.37, CI<sub>95%</sub>: 0.12, 1.15). Individuals who

received a score of three, two or one for protective behaviors also associated with relative risk reductions compared to those who reported no protective lifestyle factors by 58%, 46%, and 16% respectively (RR: 0.42, CI<sub>95%</sub>: 0.19, 0.94 and RR: 0.54, CI<sub>95%</sub>: 0.26, 1.11 and RR: 0.84, CI<sub>95%</sub>: 0.39, 1.80).

## **Discussion**

Weekly surveillance of 416 university students during the months of September through December 2010 and 2011 identified several modifiable lifestyle factors associated with the risk of URTI: vitamin D supplementation, and frequent hand-washing, adequate sleep. Being female, age, and staying with symptomatic roommates were also significantly associated with the risk of URTI, however these variables are not easily modified.

Supplementation in early autumn with 10,000 IU of vitamin D3 per week was associated with a statistically significant 26% risk reduction for URTI, in this extension of a randomized clinical trial of supplementation that showed similar, albeit non-statistically significant, results.[10] Although results from individual randomized controlled trials of vitamin D3 have been conflicting, our study is in agreement with a recent meta-analysis of 11 randomized controlled trials which reported a significant 36% reduction in the odds of any respiratory tract infection.[14] Vitamin D3 is an inexpensive, well tolerated intervention and evidence is mounting that it is beneficial for the prevention of respiratory tract infections [14, 15]. However, before comprehensive recommendations can be made, more research is required to better understand the necessary dose and the dose frequency for achieving optimal respiratory health for people from different demographics.

Hand hygiene has long been considered one of the most important interventions for the prevention of disease transmission.[16] Measures of frequency of hand washing relative to food consumption were not associated with risk of URTI. However, our study demonstrated that the frequency of total daily hand washing, as reported at the start of the study, was significantly and negatively associated with the risk of URTI. Furthermore, the benefit appeared to be graded according to frequency. Compared to the least frequent hand washers (0-3 times/day), hand washing more than six times daily was associated with a 35% risk reduction while washing four to five times was associated with a 25% risk reduction. Similar to our results, a study of military naval recruits instructed to wash their hands at least five times daily demonstrated a 45% reduction in outpatient visits for respiratory illness compared to rates from the year preceding the intervention.[17] White and colleagues reported that university students who received free hand sanitizer and were exposed to an educational campaign promoting hand hygiene reported significantly better hand hygiene and 26% fewer illnesses than students in the control group.[18] A meta-analysis of multiple hand hygiene interventions in community settings reported an overall 21% reduction in the rate of respiratory illness in the intervention group.[5] A separate meta-analysis of six case-control studies of methods to prevent the spread of severe acute respiratory syndrome (SARS) reported a significant, 55% reduction in odds of illness among people who washed their hands more than ten times daily.[6] Frequent daily hand cleansing is clearly important for the prevention of URTI and greater frequency appears to be associated with greater benefit. Our results should, however, be interpreted with some caution. The weekly measure of daily hand washing frequency was

not significantly associated with URTI risk. It could be that the weekly measures were not accurately capturing the behavior due to respondent bias, or that the baseline measure was actually a marker of another protective behavior.

Although research has identified an important link between sleep and immune function, few studies have directly examined the association between sleep and risk of URTI.[19] Results from our study did not reveal a significant association between the average nightly sleep duration and risk of illness. However, our results did indicate a significant relationship between the weekly measure of the least amount of sleep in a given night and risk of URTI, with a biological gradient in risk reduction. Compared to those who reported four hours sleep or less in a single night, the risk of URTI was reduced by 29% among those whose minimum nightly sleep was five to six hours. Among those whose minimum nightly sleep in the prior week was seven hours or more, the risk of URTI was reduced by 47%. Cohen and colleagues reported that adult participants who described an average nightly sleep duration of less than seven hours were 2.9 times more likely to develop a cold after experimental exposure to rhinovirus compared to those whose nightly average sleep time was more than eight hours.[7] However, when entered into the same predictive model with sleep efficiency, duration was no longer a significant predictor.[7]

Consistent with the influenza and influenza-like-illness literature, living with a sick person was a risk factor for URTI.[20] In our study, the risk of URTI was 2.3 times greater when a housemate or roommate was sick during the previous week. It is widely recognized that viruses that cause symptoms of URTI are transmitted through aerosol droplets and hand-to-hand contact with an infected person or contaminated object.[1]

Consequently, it is not surprising that sharing living space with an infected individual is associated with significantly increased risk of illness. It may be of interest for future studies to explore potential methods of minimizing transmission from one housemate to another to modify this risk.

Consistent with previous reports, our data demonstrated that the risk of URTI was 1.3 times greater in females than in males. However, this result must be interpreted with caution as it was not statistically significant in all models. Although it has been reported that URTI occurs more frequently in young boys, the inverse has been reported in adults.[1, 21-23] The reason for this pattern is unknown but may be related to biological differences in immune function, or to increased contact with young children and consequently increased exposure to viral agents.[21]

In contrast to previous research, our data did not identify smoking as a significant risk factor for URTI.[24, 25] However, very few participants (n=15, 3.6%) self-identified as smokers and this likely contributed to the null effect. Based on current literature, we also anticipated that a moderate duration of weekly exercise would be protective against URTI.[8, 26-28] There is growing evidence to support a “J” shaped curve associated with the benefit of exercise. Compared to inactive adults, moderate activity is beneficial, whereas prolonged, intense exercise may be harmful.[27, 28] Our data did not identify any level of exercise as a statistically significant protective factor, however, univariable analysis did appear to be consistent with the “J” shaped curve hypothesis. Effect estimates for students who reported three to four hours of exercise weekly suggested benefit (RR: 0.72, CI<sub>95%</sub>: 0.48,1.09) compared to students who reported no exercise.

Little benefit appeared to be gained by one to two hours of exercise or more than five hours of weekly exercise.

Our study is one of the first to report a cumulative benefit associated with the concurrent use of multiple protective behaviors. While individual behaviors are associated with important risk reduction, we show that the combined effect is larger; integrating three simple lifestyle habits is associated with a statistically significant, 58% risk reduction in URTI. However, the benefit associated with multiple concurrent protective behaviours should continue to be investigated in future studies.

Like all observational studies, our study is at risk of uncontrolled confounding. We did not record body mass, ethnic background or stress levels and were consequently unable to control for any potential associated confounding effects. Our study was conducted with university students during months with peak rhinovirus activity and we therefore cannot be certain about the generalizability of these results to illnesses caused by other viral agents or during other seasons or the generalizability to other populations. However, behaviors that prevent the spread of viral particles, such as hand washing, or those that impact the immune system, such as sleep, should be effective against many viral agents and for other demographics. Our study had several strengths. We measured behaviors at baseline and weekly thereafter which reduced the likelihood of recall bias, and allowed for weekly differences in lifestyle factors to be reflected in the analysis. Our study also benefitted from high response rates and very little missing data.

Our analysis identified several lifestyle factors as risks for, and protective behaviors against, self-reported URTI in university students. Furthermore, we demonstrated that practicing several protective behaviors together was associated with superior risk reduction. Although further study would increase our understanding of how such behaviors, and combinations thereof, could decrease URTI, our results support the ongoing promotion of frequent hand washing and consistent sleep. Moreover, our results support the growing body of literature that identifies vitamin D3 supplementation as a safe and effective intervention for the prevention of URTI. Given the frequency of URTI in the general public and the substantial associated costs, promoting simple and effective techniques for the prevention of URTI has the potential to greatly reduce the burden of disease and improve public health.

## References

1. Lorber B: **The common cold.** *J Gen Intern Med* 1996, **11**(4):229-236.
2. Proud D: **Upper airway viral infections.** *Pulmonary pharmacology & therapeutics* 2008, **21**(3):468-473.
3. Fendrick AM, Monto AS, Nightengale B, Sarnes M: **The economic burden of non-influenza-related viral respiratory tract infection in the United States.** *Arch Intern Med* 2003, **163**(4):487-494.
4. Kwong JC, Crowcroft NS, Campitelli MA, Ratnasingham S, Daneman N, Deeks SL, Manuel DG: **Ontario Burden of Infectious Disease Study (ONBOIDS): An OAHPP/ICES Report.** In. Toronto: Ontario Agency for Health Protection and Promotion, Institute for Clinical and Evaluative Sciences; 2010.
5. Aiello AE, Coulborn RM, Perez V, Larson EL: **Effect of hand hygiene on infectious disease risk in the community setting: a meta-analysis.** *American journal of public health* 2008, **98**(8):1372-1381.
6. Jefferson T, Foxlee R, Del Mar C, Dooley L, Ferroni E, Hewak B, Prabhala A, Nair S, Rivetti A: **Physical interventions to interrupt or reduce the spread of respiratory viruses: systematic review.** *BMJ* 2008, **336**(7635):77-80.
7. Cohen S, Doyle WJ, Alper CM, Janicki-Deverts D, Turner RB: **Sleep habits and susceptibility to the common cold.** *Arch Intern Med* 2009, **169**(1):62-67.
8. Nieman DC, Henson DA, Austin MD, Sha W: **Upper respiratory tract infection is reduced in physically fit and active adults.** *British journal of sports medicine* 2011, **45**(12):987-992.



9. Bartley J: **Vitamin D, innate immunity and upper respiratory tract infection.** *The Journal of laryngology and otology* 2010, **124**(5):465-469.
10. Goodall E, Granados A, Luinstra K, Pulleneyagum E, Coleman B, Loeb M, Smieja M: **Vitamin D3 and gargling for the prevention of upper respiratory tract infections: a randomized controlled trial..** In Review; 2013.
11. Knol MJ, Le Cessie S, Algra A, Vandenbroucke JP, Groenwold RH: **Overestimation of risk ratios by odds ratios in trials and cohort studies: alternatives to logistic regression.** *CMAJ : Canadian Medical Association journal = journal de l'Association medicale canadienne* 2012, **184**(8):895-899.
12. Hosmer DW: **Applied logistic regression**, 2nd ed. edn. Wiley: New York ,bToronto; 2000.
13. Tabachnick BG, Fidell LS: **Using Multivariate Statistics**, 6 edn: Pearson; 2013.
14. Bergman P, Lindh ÅU, Björkhem-Bergman L, Lindh JD: **Vitamin D and Respiratory Tract Infections: A Systematic Review and Meta-Analysis of Randomized Controlled Trials.** *PLoS ONE* 2013, **8**(6):e65835.
15. Hathcock JN, Shao A, Vieth R, Heaney R: **Risk assessment for vitamin D.** *Am J Clin Nutr* 2007, **85**(1):6-18.
16. Boyce JM, Pittet D: **Guideline for Hand Hygiene in Health-Care Settings: recommendations of the Healthcare Infection Control Practices Advisory Committee and the HICPAC/SHEA/APIC/IDSA Hand Hygiene Task Force.** *Infection control and hospital epidemiology : the official journal of the Society of Hospital Epidemiologists of America* 2002, **23**(12 Suppl):S3-40.

17. Ryan MA, Christian RS, Wohlrabe J: **Handwashing and respiratory illness among young adults in military training.** *American journal of preventive medicine* 2001, **21**(2):79-83.
18. White C, Kolble R, Carlson R, Lipson N: **The impact of a health campaign on hand hygiene and upper respiratory illness among college students living in residence halls.**[Erratum appears in *J Am Coll Health*. 2005 Jul-Aug;**54**(1):64]. *J Am Coll Health* 2005, **53**(4):175-181.
19. Gamaldo CE, Shaikh AK, McArthur JC: **The sleep-immunity relationship.** *Neurol Clin* 2012, **30**(4):1313-1343.
20. Guh A, Reed C, Gould LH, Kutty P, Iuliano D, Mitchell T, Dee D, Desai M, Siebold J, Silverman P *et al*: **Transmission of 2009 pandemic influenza A (H1N1) at a Public University--Delaware, April-May 2009.** *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2011, **52 Suppl 1**:S131-137.
21. Graham NM: **The epidemiology of acute respiratory infections in children and adults: a global perspective.** *Epidemiologic reviews* 1990, **12**:149-178.
22. Monto AS, Sullivan KM: **Acute respiratory illness in the community. Frequency of illness and the agents involved.** *Epidemiol Infect* 1993, **110**(1):145-160.
23. J Y, R B: **Why the common cold and flu matter.** In. Tamarind Health Care Communications: The Centre for Studies in Primary Care; 2011.

24. Cohen S, Tyrrell DA, Russell MA, Jarvis MJ, Smith AP: **Smoking, alcohol consumption, and susceptibility to the common cold.** *American journal of public health* 1993, **83**(9):1277-1283.
25. Murin S, Bilello KS: **Respiratory tract infections: another reason not to smoke.** *Cleve Clin J Med* 2005, **72**(10):916-920.
26. Chubak J, McTiernan A, Sorensen B, Wener MH, Yasui Y, Velasquez M, Wood B, Rajan KB, Wetmore CM, Potter JD *et al*: **Moderate-intensity exercise reduces the incidence of colds among postmenopausal women.** *The American journal of medicine* 2006, **119**(11):937-942.
27. Matthews CE, Ockene IS, Freedson PS, Rosal MC, Merriam PA, Hebert JR: **Moderate to vigorous physical activity and risk of upper-respiratory tract infection.** *Medicine and science in sports and exercise* 2002, **34**(8):1242-1248.
28. Moreira A, Delgado L, Moreira P, Haahtela T: **Does exercise increase the risk of upper respiratory tract infections?** *British medical bulletin* 2009, **90**:111-131.

**Table 4-1.** Results from univariable GEE analysis of baseline and weekly lifestyle factors.

Variable	<i>Pooled Imputed Data</i>			<i>Complete Case Data</i>		
	RR (95% CI)	p Value	Test of model, p	RR (95% CI)	p Value	Test of model, p
<b>Baseline Variables</b>						
Vitamin D allocation <sup>1</sup>	0.76 (0.59,0.97)	0.028		0.76 (0.59,0.97)	0.028	
Female	1.27 (0.97,1.67)	0.079		1.26 (0.96,1.65)	0.096	
Age	1.09 (1.05,1.13)	<0.001		1.09 (1.05,1.13)	<0.001	
Residence	No missing data	-----		0.67 (0.50,0.90)	0.007	
Participated in 2011	No missing data	-----		1.13 (0.88,1.45)	0.358	
Asthma	1.43 (0.98,2.09)	0.061		1.35 (0.90,2.02)	0.144	
Smoking	0.88 (0.44,1.77)	0.716		1.00 (0.49,2.07)	0.994	
Baseline vitamins	1.05 (0.78,1.41)	0.751		1.04 (0.77,1.41)	0.793	
Baseline mean sleep*	0.96 (0.82,1.13)	0.611		0.94 (0.80,1.11)	0.468	
Baseline daily hand washing*	0.89 (0.79,1.02)	0.091		0.89 (0.78,1.01)	0.079	
Baseline daily hand washing, tertiles						
≥6 times	0.75 (0.55,1.04)	0.088	0.179	0.74 (0.54,1.03)	0.071	0.145
4-5 times	0.81 (0.61,1.08)	0.157		0.80 (0.60,1.07)	0.127	
0-3 times	Referent	-----		Referent	-----	
Baseline exercise	0.99 (0.96,1.03)	0.703		0.99 (0.96,1.03)	0.789	
<b>Weekly Variables</b>						
Sick housemate	2.37 (1.80,3.12)	<0.000		2.47 (1.87,3.26)	<0.001	
Hand wash before meals						
Always	0.82 (0.55,1.23)	0.348	0.385	0.85 (0.57,1.28)	0.438	0.430
Usually	1.00 (0.70,1.44)	0.938		1.04 (0.73,1.49)	0.818	
Never	Referent	-----		Referent	-----	
Hand wash, daily avg.*	0.96 (0.84,1.10)	0.592		0.97 (0.85,1.10)	0.616	
Hand wash daily avg.						
≥6 times	0.95 (0.69,1.31)	0.754	0.930	0.98 (0.70,1.37)	0.911	0.880
4-5 times	1.02 (0.73,1.41)	0.914		1.06 (0.76,1.48)	0.729	
0-3 times	Referent	-----		Referent	-----	
Weekly gargle						
Twice daily	0.74 (0.60,1.15)	0.182	0.595	0.74 (0.48,1.16)	0.188	0.583
Daily	0.91 (0.60,1.38)	0.657		0.92 (0.60,1.40)	0.690	
Occasionally	0.89 (0.65,1.23)	0.487		0.89 (0.65,1.22)	0.462	
Never	Referent	-----		Referent	-----	
Weekly Exercise						
≥ 5 hours	0.91 (0.56,1.48)	0.710	0.549	0.94 (0.58,1.53)	0.807	0.582
3-4 hours	0.77 (0.51,1.15)	0.199		0.78 (0.52,1.18)	0.239	
1-2 hours	0.98 (0.71,1.37)	0.924		1.01 (0.73,1.39)	0.968	
0	Referent	-----		Referent	-----	

Weekly mean sleep/night*	0.93 (0.80,1.07)	0.285		0.91 (0.79,1.05)	0.194	
Weekly mean sleep/night, tertiles						
≥ 8 hours	0.82 (0.58,1.18)	0.287	0.533	0.78 (0.55,1.12)	0.186	0.410
7 hours	0.90 (0.67,1.21)	0.496		0.90 (0.67,1.21)	0.488	
≤ 6 hours	Referent	-----		Referent	-----	
Weekly least sleep/night *	0.86 (0.78,0.95)	0.002		0.86 (0.78,0.95)	0.002	
Weekly least sleep/night, tertiles						
≥7 hours	0.56 (0.36,0.88)	0.011	0.021	0.55 (0.35,0.88)	0.011	0.020
5-6 hours	0.75 (0.57,1.00)	0.053		0.77 (0.58,1.02)	0.071	
≤4 hours	Referent	-----		Referent	-----	
Frequency of alcohol per week	1.13 (0.99,1.28)	0.051		1.14 (1.01,1.29)	0.036	
Number of alcoholic drinks per occasion	1.05 (1.01,1.10)	0.021		1.05 (1.01,1.10)	0.023	
Number of alcoholic drinks per occasion, tertiles						
≥ 3	1.42 (1.05,1.91)	0.023	0.048	1.41 (1.04,1.91)	0.026	0.066
1-2	1.36 (0.98,1.89)	0.067		1.35 (0.97,1.88)	0.079	
0	Referent	-----		Referent	-----	

<sup>1</sup> Randomized to 10,000 IU/week or placebo

\*Treated as a continuous variable from lowest value to highest value

**Table 4-2.** Final multivariable GEE analysis of association between number of URTI and baseline and weekly lifestyle factors of university students (Sept. to Dec., 2010 and 2011)

Variable	<i>Pooled Imputed Data</i>		<i>Complete Case Data</i>	
	<b>RR (95% CI)</b>	<b>p Value</b>	<b>RR (95% CI)</b>	<b>p Value</b>
Vitamin D allocation <sup>1</sup>	0.74 (0.58,0.95)	0.016	0.72 (0.56,0.93)	0.010
Sex (Female)	1.34 (1.02,1.75)	0.035	1.32 (1.01,1.72)	0.043
Age	1.10 (1.06,1.14)	<0.001	1.10 (1.06,1.14)	<0.001
Baseline daily hand washing				
≥6 times	0.65 (0.47,0.89)	0.008	0.68 (0.49,0.94)	0.020
4-5 times	0.75 (0.57,0.99)	0.044	0.78 (0.58,1.03)	0.076
0-3 times	Referent	-----	Referent	-----
Sick housemate	2.29 (1.73,3.03)	<0.001	2.40 (1.82,3.17)	<0.001
Weekly least sleep				
≥7 hours	0.53 (0.35,0.81)	0.004	0.52 (0.34,0.82)	0.004
5-6 hours	0.71 (0.54,0.95)	0.020	0.74 (0.56,0.98)	0.034
≤4 hours	Referent	-----	Referent	-----

<sup>1</sup> Randomized to 10,000 IU/week or placebo

**Table 4-3.** Sensitivity analysis of the multivariable model adjusted for type of housing, year of participation and frequency of daily tap water gargling.

Variable (Referent)	<i>Pooled Imputed Data</i>		<i>Complete Case Data</i>	
	RR (95% CI)	p Value	RR (95% CI)	p Value
Vitamin D allocation <sup>1</sup>	0.753 (0.59,0.96)	0.024	0.735(0.57,0.94)	0.016
Sex (Female)	1.300 (0.99,1.70)	0.057	1.280 (0.98,1.67)	0.072
Age	1.094 (1.05,1.14)	<0.001	1.095 (1.05,1.14)	<0.001
Housing (Residence)	0.871 (0.64,1.18)	0.373	0.856 (0.63,1.16)	0.312
Trial year (2010)	1.130 (0.89,1.44)	0.325	1.109 (0.87,1.41)	0.400
Baseline daily hand washing				
≥6 times	0.652 (0.48,0.89)	0.008	0.686 (0.50,0.94)	0.021
4-5 times	0.758 (0.57,0.99)	0.049	0.782 (0.59,1.03)	0.084
0-3 times	Referent	-----	Referent	-----
Sick housemate	2.29 (1.72,3.03)	<0.001	2.411 (1.82,3.20)	<0.001
Weekly gargle				
Twice daily	0.769 (0.51,1.17)	0.220	0.741 (0.49,1.13)	0.164
Daily	1.017 (0.67,1.55)	0.938	1.045 (0.70,1.59)	0.832
Occasionally	0.927 (0.67,1.29)	0.649	0.906 (0.66,1.25)	0.547
Never	Referent	-----	Referent	-----
Weekly least sleep				
≥7 hours	0.518 (0.34,0.80)	0.003	0.509 (0.33,0.79)	0.003
5-6 hours	0.694 (0.52,0.92)	0.012	0.717 (0.54,0.95)	0.020
≤4 hours	Referent	-----	Referent	-----

<sup>1</sup> Randomized to 10,000 IU/week or placebo

**Supplementary Table 4-S1:** Description of the baseline and weekly survey questions and response coding

<b>Independent Variable Characteristics</b>		
<b>Variable (Survey Question)</b>	<b>Data type</b>	<b>Coding</b>
<b>Baseline Questions</b>		
Vitamin D allocation <sup>1</sup>	Binary	0=placebo 1=vitamin D
Gender	Binary	0=male 1=female
Age	Continuous	Years
Type of housing (Do you live in residence or off-campus?)	Binary	0= off-campus 1=residence
Year of participation	Binary	0=2010 1=2011
Asthma (Do you have asthma?)	Binary	0=no 1=yes
Smoking (Do you currently smoke?)	Binary	0=no 1=yes
Baseline vitamins (Do you use vitamins regularly?)	Binary	0=no 1=yes
*Baseline mean sleep (How much sleep do you get on an average night?)	Ordinal	1= ≤ 5 hours 2= 6 hours 3= 7 hours 4= 8 hours 5= ≥ 9 hours
*Baseline daily hand washing (How many times do you wash your hands (or use an alcohol-based hand cleanser) in an average day?)	Ordinal	0= 0 times 1=1 time 2= 2-3 times 3=4-5 times 4= ≥6 times
Baseline exercise (On average, how many hours of exercise do you get in a week?)	Continuous	hours
<b>Weekly Questions</b>		
Sick housemate (Have any of your roommates or housemates been ill in the past week?)	Binary	0=no 1=yes
Hand wash before meals (In the past week, did you wash your hands (or use an alcohol-based cleanser) before each meal?)	Ordinal	0=no 1=usually 2=always
*Weekly daily hand washing (In the past week, how many times did you wash your	Ordinal	0= 0 times 1=1 time



<i>hands (or use and alcohol-based hand cleanser) in an average day?)</i>		2= 2-3 times 3=4-5 times 4= $\geq 6$ times
Weekly gargle <i>(In the past week, did you gargle as part of your daily oral hygiene?)</i>	Ordinal	1=no 2=occasionally 3=daily 4= twice daily
Weekly Exercise <i>(In the past week, how many hours of moderate exercise (you can speak with brief sentences, are sweating and cannot sing a song) did you get over the entire week?)</i>	Ordinal	1= 0 hours 2= 1-2 hours 3= 3-4 hours 4= $\geq 5$ hours
*Weekly Mean Sleep <i>(In the past week, how much sleep did you get on an average night?)</i>	Ordinal	1= $\leq 5$ hours 2= 6 hours 3=7 hours 4= 8 hours 5= $\geq 9$ hours
*Weekly Least sleep <i>(In the past week, what is the least amount of sleep that you had in 1 night?)</i>	Ordinal	1 = $\leq 3$ hours 2= 4 hours 3= 5 hours 4= 6 hours 5= 7 hours 6= 8 hours 7= $\geq 9$ hours

<sup>1</sup> Randomized to 10,000 IU/week or placebo

\*This variable was also collapsed into tertiles for analysis

## CHAPTER 5

### **Conclusions**

In this chapter, I summarize the work presented in the preceding chapters and discuss the current context of research in this field. I then expand on the evidence for vitamin D3 as a preventive measure against respiratory infections. Finally, I suggest several ideas for future studies that could contribute to a better understanding of the effect of some of the interventions explored within this thesis, and potentially aid in the development of clinical guidelines.

The introductory chapter presents a brief overview of the burden of disease associated with upper respiratory tract infections. These illnesses have truly earned their colloquial name, the “common cold”, due to the extreme frequency with which they appear in the human population. No age, race, or gender is exempt from the discomfort and disruption caused by URTI. Although generally considered to be only a nuisance, more recent research has sought to quantify the financial impact, the stress exerted on health care systems, and the role in more severe disease and medical complications that is associated with URTI. Results from these studies suggest that URTI are not entirely benign and that improving the prevention of such illnesses could reduce morbidity, relieve some demand on healthcare systems, and reduce the financial and productivity losses associated with URTI. Currently, few interventions and practices have been demonstrated to successfully reduce the risk of URTI. The work presented in this thesis sought to investigate new approaches to URTI prevention and to identify modifiable risk factors that could be the focus of further investigation for infection prevention.

Chapter 2 describes a factorial, randomized controlled trial of vitamin D3 supplementation versus placebo, and tap water gargling versus no gargling for the prevention of URTI in university students. The study was conducted over the course of two consecutive autumn seasons to capture peak rhinovirus activity and included 600 participants, making it the largest RCT to assess vitamin D3 supplementation in this context. We considered a primary and secondary outcome which reflected different definitions of illness. The primary outcome considered clinical URTI defined as the participant's perception of a "cold" in conjunction with at least two symptoms. The secondary outcome considered only episodes where clinical illnesses were confirmed through laboratory testing and identification of one of 16 respiratory viral agents. The complete study protocol and design are provided in Appendix 1.

Despite rigorous methods and a large sample size, our trial did not yield definitive results. As a result of overestimating the number of expected URTI, the trial was underpowered. Analysis of our primary outcome indicated that vitamin D3 supplementation may reduce the risk of URTI, however this was a non-significant result. We did, however, find a significant risk reduction for laboratory confirmed viral respiratory infections. In this population, gargling did not reduce the risk of either clinical or laboratory confirmed infections.

The goal of Chapter 3 was twofold: 1) the paper sought to compare first event and repeated event statistical analyses in the context of a data set where few participants had more than one event, and 2) the paper continued to assess vitamin D3 for URTI

prevention by considering the effect of early autumn supplementation on clinical URTI over a four month period.

The benefit of repeated event analyses compared to first event analyses had been previously discussed in the context of data sets where many (>20%) participants experienced multiple events. Repeated events analyses are often more complicated to implement and less accessible to researchers and readers, so there remains a temptation to use first event analyses. Consequently, I questioned whether the benefit of repeated events analyses persisted in data sets where few participants (<10%) experienced repeated events. Using data from 416 participants who were followed from September to December, first event and repeated event variations of Poisson regression and time-to-event analysis were compared. Consistently, the repeated events analyses which incorporated all available data sufficiently enhanced the power to detect associations that were non-significant using first event analyses. It would be enlightening if future studies evaluated whether this conclusion remained true at decreasing frequencies of repeated events, or if there is a threshold at which employing repeated events analyses is no more powerful than first event analyses.

Applying repeated events analysis, GEE, to the data demonstrated that vitamin D3 supplementation in September and October was associated with a statistically significant 25% risk reduction in clinical URTI over a four month period. Although the primary analysis of the RCT data presented in Chapter 2 was non-significant, I acknowledged that the study was underpowered due to a lack of events. The repeated events analysis presented in Chapter 3 supports the premise that the primary RCT result, an estimated

20% RRR, reflects a true relationship between vitamin D3 supplementation and risk of clinical URTI, but that too few events were captured to provide sufficient power to detect a definitive result. This discrepancy in statistical significance emphasizes the need for researchers to consider entire bodies of literature and evidence rather than relying on single studies, to draw conclusions about the effect of interventions. It also supports the use of repeated events analyses rather than relying on first events, especially in light of underpowered studies.

Chapter 4 expands on the theme of infection prevention by presenting the results from a longitudinal cohort study designed to identify lifestyle factors associated with the risk of URTI. We demonstrated that several lifestyle factors and behaviours appeared to be associated with the risk of URTI. Being female, every additional year of age (albeit, within a limited age range), and living with a sick roommate or housemate were associated with increased risk of infection. Three modifiable behaviours were associated with protective effects. These included the frequency of hand washing, vitamin D3 supplementation, and quantity of nightly sleep. Individually, these factors were associated with relative risk reductions in the range of 26% to 47%. These behaviours are not mutually exclusive and we reasoned that the concurrent use of multiple protective factors should lead to greater relative risk reduction. We investigated this hypothesis by deriving a protective behaviour score in 50% of the data and then testing it in the remaining 50%. Including the protective behaviour score in a GEE model adjusted for sex, age and sick housemates demonstrated that practicing multiple protective behaviours concurrently was associated with significant relative risk reductions of as much as 67%. Combinations of

protective behaviours were associated with larger relative risk reductions than individual behaviours. Rather than focusing on a single intervention, greater benefit may be achieved by promoting several easy, safe and effect practices. Currently, few researchers have investigated these behaviours as risk factors for URTI. It will be important for future research to verify these relationships, and explore whether or not there is a causal link between these factors and URTI.

### **Vitamin D3 and Upper Respiratory Tract Infections**

The overarching theme of this thesis has been the prevention of upper respiratory tract infections. However, a major focus of this work has been dedicated to answering the question “Does vitamin D3 supplementation prevent URTI?” This goal has been shared by many researchers worldwide and it is important to consider the data presented in this thesis within the context of the current body of evidence.

Practitioners of evidence based medicine often refer to a “hierarchy of evidence” which ranks studies according to methodological designs, giving greater weight to those believed to provide valid results with fewer potential biases.[61] Systematic reviews and meta-analyses are at the top of the evidence hierarchy, followed by RCTs with definitive results, RCTs with non-definitive results, cohort studies and finally other observational studies culminating with case reports.[61, 62] However, relying on this hierarchy of evidence does not lead to a definitive answer to the question posed above. To date, three published meta-analyses and my own unpublished meta-analysis (Appendix 2) have attempted to resolve whether or not vitamin D3 supplementation prevents respiratory

infections. Conflicting results persist. The first meta-analysis published included five trials and reported a significant reduction in odds in the vitamin D3 group (OR: 0.58, CI<sub>95%</sub>: 0.42, 0.81).[63] Bergman and colleagues also reported a significant reduction in odds of infection in the vitamin D3 group after pooling results from 11 trials (OR: 0.64, CI<sub>95%</sub>: 0.49,0.84).[64] Mao and colleagues combined data from seven trials and reported no benefit in the vitamin D3 group (RR:0.98, CI<sub>95%</sub>:0.93,1.03).[65] Similarly, Goodall *et al.* combined data from seven trials, including the McFlu2 COLD3 Prevention Study presented in Chapter 2, and reported no statistically significant relative risk reduction in the vitamin D3 group (RR: 0.69, CI<sub>95%</sub>: 0.45,1.05).[66] Differences in methods such as the definition of the outcome, study inclusion criteria, and choice of summary statistic may account for some of the differences in the results of these four meta-analyses. Although these meta-analyses still do not provide a conclusive answer to the question of vitamin D3 supplementation for URTI prevention, they do consistently suggest that vitamin D3 supplementation is beneficial.

To date, no single methodologically rigorous RCT has produced definitive results to answer the question. Of eight RCTs designed to measure the effect of vitamin D supplementation for the prevention of acute respiratory viral outcomes, three reported statistically significant relative risk reductions associated with the intervention [47, 64, 67], and four reported non-significant point estimates that favoured vitamin D3 supplementation [49, 68-70], while one trial reported a null effect.[71]

This scenario is repeated among observational studies of various respiratory outcomes. Joliffe *et al.* summarized the results of four cross-sectional, eight case-control

and thirteen cohort studies that investigated the association between vitamin D intake, circulating vitamin D, or clinical presentation of vitamin D deficiency on the risk of acute respiratory infection (ARI), or acute exacerbation of COPD or asthma.[72] Among these studies, seven supported the inverse association between vitamin D levels and risk of respiratory infections or exacerbation outcomes.[72] The authors concluded that this body of literature represented “consistent evidence of an association between vitamin D status and susceptibility to” ARI across diverse populations.[72] The GEE analyses of the longitudinal cohort presented in Chapter 2 and Chapter 3 are further evidence of an association between increased vitamin D levels and decreased risk of URTI.

The hierarchy of evidence exists as a guideline to help identify studies that, in theory, should yield more valid results. However, study design alone should not be used to determine quality of evidence.[73] Furthermore, interpreting individual studies in isolation is rarely the best approach. Rather, cumulative evidence from numerous studies with various designs should be considered for a balanced assessment of new interventions.[73] Applying this approach to the body of literature presented above, I would argue that the evidence is in favour of vitamin D3 supplementation as a protective factor against viral respiratory infections. Nevertheless, significant uncertainties remain regarding the best estimate of true effect, the method of supplementation, and the populations best served by this intervention.



## **Future Studies**

Nearly every publication discussed above concluded that large, methodologically rigorous RCTs are warranted to further investigate a causal relationship between vitamin D3 supplementation and the risk of acute respiratory infections. Indeed, larger, definitive RCTs that reflect the current understanding of this relationship should be carefully designed and executed. However it is unlikely that a single new RCT will resolve the many uncertainties in this field. Meta-analyses have questioned whether the efficacy of vitamin D3 supplementation is influenced by age, baseline vitamin D status, and the size and frequency of the vitamin D3 dose. I propose a series of studies that could contribute to the body of evidence of the issues above.

Uncertainty surrounds what serum levels of vitamin D need to be achieved for optimal respiratory health and immune function. Current literature suggests that the minimum desirable 25(OH)D concentrations should be 30 ng/mL (75 nmol/L), although no formal consensus has been adopted.[74-76] This recommendation, which is higher than that of the Institutes of Medicine, reflects research of various health outcomes, but not specifically respiratory outcomes. Observational studies that have measured the association between 25(OH)D concentrations and respiratory infections suggest that optimal serum levels may be even higher, in the range of 40 ng/mL or more.[39, 41, 44] A systematic review and meta-analysis of observational studies that present data on the association between 25(OH)D concentrations and acute respiratory infection would be useful to estimate the target serum vitamin D level for optimal respiratory health.

Building on the best estimate of an optimal 25(OH)D concentration, studies comparing different dosing regimens would be beneficial to understand the best way to achieve that status. Published RCTs have used doses ranging from 800 IU/day to 100,000 IU/month. Unfortunately, few studies have simultaneously monitored serum 25(OH)D concentrations and the question of how much those doses influenced serum levels remains unanswered. It would be beneficial to evaluate the effect of the same total amount of supplementation administered at different frequencies. For example, over a period of eight weeks, what is the effect of 2000 IU/day vitamin D3 versus 14,000 IU once weekly, versus 60,000 IU once monthly on 25(OH)D concentrations in Canadian adults? A multi-arm, RCT could inform the best way to administer vitamin D3 supplementation to successfully raise serum vitamin D levels to the therapeutic range.

Ultimately, large, definitive RCTs will be needed to confirm a causal relationship between vitamin D3 supplementation and the risk of acute respiratory infections. My experiences to date have taught me that designing such a study is an iterative process; methods can only be improved by considering the strengths and weaknesses of previous studies, and by considering the totality of available evidence. Results from the hypothetical studies suggested above, in combination with those from relevant published RCTs and meta-analyses should lead to the design and execution of trials that are properly powered to detect realistic treatment effects and subgroup effects.

Future studies should continue to use molecular diagnostics to investigate the relationship between interventions and respiratory infections caused by various pathogens. While the McFlu2 COLD3 Prevention study did incorporate molecular

diagnostics, the dominant pathogen was rhinovirus. There were too few infections caused by other viral agents to investigate whether the effect of vitamin D3 was similar for other pathogens. Similarly, the effect of modifiable lifestyle factors on the prevention of infections caused by viruses other than rhinovirus should be investigated. If these practices are also associated with relative risk reductions for other viral respiratory infections, particularly influenza, they may represent safe, inexpensive and easy methods to reduce the burden of respiratory infections and improve public health.

Over the course of the McFlu2 COLD3 Prevention Study, thousands of self-collected nasal swabs were accumulated and preserved for future studies. Swabs were collected from individuals who perceived themselves to be healthy and those who perceived themselves to be sick with a common cold. It would be illuminating to investigate if the rates of asymptomatic illness were higher in the vitamin D3 group compared to the placebo group. It is possible that adequate 25(OH)D concentrations may prevent some infections from becoming symptomatic which would also be beneficial to individuals.

## **Conclusions**

To conclude, experimental and observational study designs were used to assess the relationship between potential protective factors and the risk of URTI.

The primary focus of this work was evaluating vitamin D3 supplementation for the prevention of URTI. Results from RCT and longitudinal cohort studies favoured vitamin D3 and demonstrated a statistically non-significant 20% relative risk reduction

and a statistically significant 26% relative risk reduction, respectively, for clinical URTI. The RCT demonstrated a statistically significant 46% relative risk reduction for laboratory confirmed URTI in the vitamin D3 group.

Several other behaviours were investigated as interventions and risk factors. Results from the RCT suggest that gargling with tap water twice daily did not reduce the risk of clinical or laboratory confirmed URTI in our population. Modifiable lifestyle habits including the quantity of sleep and frequency of daily hand washing were independently and significantly associated with the risk of clinical URTI. Further investigation into combinations of interventions and health habits may lead to significant improvements in respiratory infection prevention.

## REFERENCES

1. Heikkinen T, Jarvinen A: **The common cold.** *Lancet* 2003, **361**(9351):51-59.
2. Lorber B: **The common cold.** *J Gen Intern Med* 1996, **11**(4):229-236.
3. Graham NM: **The epidemiology of acute respiratory infections in children and adults: a global perspective.** *Epidemiologic reviews* 1990, **12**:149-178.
4. Monto AS: **Epidemiology of viral respiratory infections.** *The American journal of medicine* 2002, **112 Suppl 6A**:4S-12S.
5. Proud D: **Upper airway viral infections.** *Pulmonary pharmacology & therapeutics* 2008, **21**(3):468-473.
6. Lessler J, Reich NG, Brookmeyer R, Perl TM, Nelson KE, Cummings DA: **Incubation periods of acute respiratory viral infections: a systematic review.** *The Lancet infectious diseases* 2009, **9**(5):291-300.
7. Gwaltney JM: **Clinical significance and pathogenesis of viral respiratory infections.** *The American journal of medicine* 2002, **112 Suppl 6A**:13S-18S.
8. Arruda E, Pitkaranta A, Witek TJ, Jr., Doyle CA, Hayden FG: **Frequency and natural history of rhinovirus infections in adults during autumn.** *Journal of clinical microbiology* 1997, **35**(11):2864-2868.
9. Fendrick AM: **Viral respiratory infections due to rhinoviruses: current knowledge, new developments.** *American journal of therapeutics* 2003, **10**(3):193-202.
10. Peltola V, Waris M, Osterback R, Susi P, Hyypia T, Ruuskanen O: **Clinical effects of rhinovirus infections.** *Journal of clinical virology : the official*

- publication of the Pan American Society for Clinical Virology* 2008, **43**(4):411-414.
11. Fendrick AM, Monto AS, Nightengale B, Sarnes M: **The economic burden of non-influenza-related viral respiratory tract infection in the United States.** *Arch Intern Med* 2003, **163**(4):487-494.
  12. Palmer LA, Rousculp MD, Johnston SS, Mahadevia PJ, Nichol KL: **Effect of influenza-like illness and other wintertime respiratory illnesses on worker productivity: The child and household influenza-illness and employee function (CHIEF) study.** *Vaccine* 2010, **28**(31):5049-5056.
  13. Kwong JC, Crowcroft NS, Campitelli MA, Ratnasingham S, Daneman N, Deeks SL, Manuel DG: **Ontario Burden of Infectious Disease Study (ONBOIDS): An OAHPP/ICES Report.** In. Toronto: Ontario Agency for Health Protection and Promotion, Institute for Clinical and Evaluative Sciences; 2010.
  14. Simancas-Racines D, Guerra CV, Hidalgo R: **Vaccines for the common cold.** *The Cochrane database of systematic reviews* 2013, **6**:CD002190.
  15. Jackson GG, Dowling HF, Spiesman IG, Boand AV: **Transmission of the common cold to volunteers under controlled conditions. I. The common cold as a clinical entity.** *AMA archives of internal medicine* 1958, **101**(2):267-278.
  16. Avila PC, Abisheganaden JA, Wong H, Liu J, Yagi S, Schnurr D, Kishiyama JL, Boushey HA: **Effects of allergic inflammation of the nasal mucosa on the severity of rhinovirus 16 cold.** *J Allergy Clin Immunol* 2000, **105**(5):923-932.

17. Barrett B, Brown R, Mundt M, Safdar N, Dye L, Maberry R, Alt J: **The Wisconsin Upper Respiratory Symptom Survey is responsive, reliable, and valid.** *Journal of clinical epidemiology* 2005, **58**(6):609-617.
18. Fleming HE, Little FF, Schnurr D, Avila PC, Wong H, Liu J, Yagi S, Boushey HA: **Rhinovirus-16 colds in healthy and in asthmatic subjects: similar changes in upper and lower airways.** *Am J Respir Crit Care Med* 1999, **160**(1):100-108.
19. Schulten B, Bulitta M, Ballering-Bruhl B, Koster U, Schafer M: **Efficacy of Echinacea purpurea in patients with a common cold. A placebo-controlled, randomised, double-blind clinical trial.** *Arzneimittel-Forschung* 2001, **51**(7):563-568.
20. Predy GN, Goel V, Lovlin R, Donner A, Stitt L, Basu TK: **Efficacy of an extract of North American ginseng containing poly-furanosyl-pyranosyl-saccharides for preventing upper respiratory tract infections: a randomized controlled trial.** *CMAJ : Canadian Medical Association journal = journal de l'Association medicale canadienne* 2005, **173**(9):1043-1048.
21. Barrett B, Brown RL, Mundt MP, Thomas GR, Barlow SK, Highstrom AD, Bahrainian M: **Validation of a short form Wisconsin Upper Respiratory Symptom Survey (WURSS-21).** *Health and quality of life outcomes* 2009, **7**:76.
22. **Wisconsin Upper Respiratory Symptom Survey (WURSS)**  
[<http://www.fammed.wisc.edu/research/external-funded/wurss>]

23. Buller RS: **Molecular detection of respiratory viruses.** *Clin Lab Med* 2013, **33**(3):439-460.
24. Mahony JB: **Detection of respiratory viruses by molecular methods.** *Clinical microbiology reviews* 2008, **21**(4):716-747.
25. Loeffelholz M, Chonmaitree T: **Advances in diagnosis of respiratory virus infections.** *International journal of microbiology* 2010, **2010**:126049.
26. Mahony JB, Petrich A, Smieja M: **Molecular diagnosis of respiratory virus infections.** *Critical reviews in clinical laboratory sciences* 2011, **48**(5-6):217-249.
27. Daley P, Castriciano S, Chernesky M, Smieja M: **Comparison of flocked and rayon swabs for collection of respiratory epithelial cells from uninfected volunteers and symptomatic patients.** *Journal of clinical microbiology* 2006, **44**(6):2265-2267.
28. Smieja M, Castriciano S, Carruthers S, So G, Chong S, Luinstra K, Mahony JB, Petrich A, Chernesky M, Savarese M *et al*: **Development and evaluation of a flocked nasal midturbinate swab for self-collection in respiratory virus infection diagnostic testing.** *Journal of clinical microbiology* 2010, **48**(9):3340-3342.
29. Gwaltney JM: **Viral respiratory infection therapy: historical perspectives and current trials.** *The American journal of medicine* 2002, **112 Suppl 6A**:33S-41S.
30. Jawad M, Schoop R, Suter A, Klein P, Eccles R: **Safety and Efficacy Profile of Echinacea purpurea to Prevent Common Cold Episodes: A Randomized,**



- Double-Blind, Placebo-Controlled Trial.** *Evidence-based complementary and alternative medicine : eCAM* 2012, **2012**:841315.
31. Nahas R, Balla A: **Complementary and alternative medicine for prevention and treatment of the common cold.** *Canadian family physician Medecin de famille canadien* 2011, **57**(1):31-36.
32. Linde K, Barrett B, Bauer R, Melchart D, Wolkart K: **Echinacea for preventing and treating the common cold.** *The Cochrane database of systematic reviews* 2009(1):CD000530.
33. Seida JK, Durec T, Kuhle S: **North American (Panax quinquefolius) and Asian Ginseng (Panax ginseng) Preparations for Prevention of the Common Cold in Healthy Adults: A Systematic Review.** *Evidence-based complementary and alternative medicine : eCAM* 2011, **2011**:282151.
34. Science M, Johnstone J, Roth DE, Guyatt G, Loeb M: **Zinc for the treatment of the common cold: a systematic review and meta-analysis of randomized controlled trials.** *CMAJ : Canadian Medical Association journal = journal de l'Association medicale canadienne* 2012, **184**(10):E551-561.
35. Singh M, Das RR: **Zinc for the common cold.** *The Cochrane database of systematic reviews* 2013, **6**:CD001364.
36. Hemila H, Chalker E: **Vitamin C for preventing and treating the common cold.** *The Cochrane database of systematic reviews* 2013, **1**:CD000980.
37. Cannell JJ, Hollis BW: **Use of vitamin D in clinical practice.** *Alternative medicine review : a journal of clinical therapeutic* 2008, **13**(1):6-20.

38. Vieth R, Bischoff-Ferrari H, Boucher BJ, Dawson-Hughes B, Garland CF, Heaney RP, Holick MF, Hollis BW, Lamberg-Allardt C, McGrath JJ *et al*: **The urgent need to recommend an intake of vitamin D that is effective.** *Am J Clin Nutr* 2007, **85**(3):649-650.
39. Ginde AA, Mansbach JM, Camargo CA, 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):384-390.
40. Karatekin G, Kaya A, Salihoglu O, Balci H, Nuhoglu A: **Association of subclinical vitamin D deficiency in newborns with acute lower respiratory infection and their mothers.** *Eur J Clin Nutr* 2009, **63**(4):473-477.
41. Laaksi I, Ruohola JP, Tuohimaa P, Auvinen A, Haataja R, Pihlajamaki H, Ylikomi T: **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):714-717.
42. McNally JD, Leis K, Matheson LA, Karuananyake C, Sankaran K, Rosenberg AM: **Vitamin D deficiency in young children with severe acute lower respiratory infection.** *Pediatric pulmonology* 2009, **44**(10):981-988.
43. Roth DE, Shah R, Black RE, Baqui AH: **Vitamin D status and acute lower respiratory infection in early childhood in Sylhet, Bangladesh.** *Acta paediatrica (Oslo, Norway : 1992)* 2010, **99**(3):389-393.

44. Sabetta JR, DePetrillo P, Cipriani RJ, Smardin J, Burns LA, Landry ML: **Serum 25-hydroxyvitamin d and the incidence of acute viral respiratory tract infections in healthy adults.** *PLoS One* 2010, **5**(6):e11088.
45. Science M, Maguire JL, Russell ML, Smieja M, Walter SD, Loeb M: **Low serum 25-hydroxyvitamin d level and risk of upper respiratory tract infection in children and adolescents.** *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2013, **57**(3):392-397.
46. Wayse V, Yousafzai A, Mogale K, Filteau S: **Association of subclinical vitamin D deficiency with severe acute lower respiratory infection in Indian children under 5 y.** *Eur J Clin Nutr* 2004, **58**(4):563-567.
47. Aloia JFL-N, M.: **Re: epidemic influenza and vitamin D.** *Epidemiology and Infection* 2007, **135**(7):1095-1096.
48. Avenell AC, J. A.; MacLennan, G. S.; Macpherson, G. C.: **Vitamin D supplementation to prevent infections: a sub-study of a randomised placebo-controlled trial in older people (RECORD trial, ISRCTN 51647438).** *Age and ageing* 2007, **36**(5):574-577.
49. Li-Ng MA, J. F.; Pollack, S.; Cunha, B. A.; Mikhail, M.; Yeh, J.; Berbari, N.: **A randomized controlled trial of vitamin D3 supplementation for the prevention of symptomatic upper respiratory tract infections.** *Epidemiol Infect* 2009, **137**(10):1396-1404.
50. Rehman PK: **Sub-clinical rickets and recurrent infection.** *Journal of tropical pediatrics* 1994, **40**(1):58.

51. Ryan MA, Christian RS, Wohlrabe J: **Handwashing and respiratory illness among young adults in military training.** *American journal of preventive medicine* 2001, **21**(2):79-83.
52. White C, Kolble R, Carlson R, Lipson N: **The impact of a health campaign on hand hygiene and upper respiratory illness among college students living in residence halls.**[Erratum appears in *J Am Coll Health*. 2005 Jul-Aug;**54**(1):64]. *J Am Coll Health* 2005, **53**(4):175-181.
53. Aiello AE, Coulborn RM, Perez V, Larson EL: **Effect of hand hygiene on infectious disease risk in the community setting: a meta-analysis.** *American journal of public health* 2008, **98**(8):1372-1381.
54. Cohen S, Doyle WJ, Alper CM, Janicki-Deverts D, Turner RB: **Sleep habits and susceptibility to the common cold.** *Arch Intern Med* 2009, **169**(1):62-67.
55. Nieman DC, Henson DA, Austin MD, Sha W: **Upper respiratory tract infection is reduced in physically fit and active adults.** *British journal of sports medicine* 2011, **45**(12):987-992.
56. Cohen S, Tyrrell DA, Russell MA, Jarvis MJ, Smith AP: **Smoking, alcohol consumption, and susceptibility to the common cold.** *American journal of public health* 1993, **83**(9):1277-1283.
57. Satomura K, Kitamura T, Kawamura T, Shimbo T, Watanabe M, Kamei M, Takano Y, Tamakoshi A: **Prevention of upper respiratory tract infections by gargling: a randomized trial.** *American journal of preventive medicine* 2005, **29**(4):302-307.

58. Iwata M, Toda M, Nakayama M, Tsujiyama H, Endo W, Takahashi O, Hara Y, Shimamura T: **[Prophylactic effect of black tea extract as gargle against influenza]**. *Kansenshogaku zasshi The Journal of the Japanese Association for Infectious Diseases* 1997, **71**(6):487-494.
59. Nagatake T, Ahmed K, Oishi K: **Prevention of respiratory infections by povidone-iodine gargle**. *Dermatology* 2002, **204** Suppl 1:32-36.
60. Yamada H, Takuma N, Daimon T, Hara Y: **Gargling with tea catechin extracts for the prevention of influenza infection in elderly nursing home residents: a prospective clinical study**. *Journal of alternative and complementary medicine (New York, NY)* 2006, **12**(7):669-672.
61. Petticrew M, Roberts H: **Evidence, hierarchies, and typologies: horses for courses**. *Journal of epidemiology and community health* 2003, **57**(7):527-529.
62. Barton S: **Which clinical studies provide the best evidence? The best RCT still trumps the best observational study**. *BMJ* 2000, **321**(7256):255-256.
63. Charan J, Goyal JP, Saxena D, Yadav P: **Vitamin D for prevention of respiratory tract infections: A systematic review and meta-analysis**. *Journal of pharmacology & pharmacotherapeutics* 2012, **3**(4):300-303.
64. Bergman P, Lindh ÅU, Björkhem-Bergman L, Lindh JD: **Vitamin D and Respiratory Tract Infections: A Systematic Review and Meta-Analysis of Randomized Controlled Trials**. *PLoS ONE* 2013, **8**(6):e65835.

65. Mao S, Huang S: **Vitamin D supplementation and risk of respiratory tract infections: A meta-analysis of randomized controlled trials.** *Scand J Infect Dis* 2013.
66. Goodall E, Science M, Asner S, Smieja M: **Vitamin D3 for the prevention of acute respiratory tract infections: a systematic review and meta-analysis of randomized controlled trials.** In.
67. Camargo CA, Jr.; Ganmaa, Davaasambuu; Frazier, A. Lindsay; Kirchberg, Franca F.; Stuart, Jennifer J.; Kleinman, Ken; Sumberzul, Nyamjav; Rich-Edwards, Janet W.: **Randomized trial of vitamin D supplementation and risk of acute respiratory infection in Mongolia.** *Pediatrics* 2012, **130**(3):e561-567.
68. Goodall E, Granados A, Luinstra K, Pulleneyagum E, Coleman B, Loeb M, Smieja M: **Vitamin D3 and gargling for the prevention of upper respiratory tract infections: a randomized controlled trial.** In. In Review; 2013.
69. Laaksi IR, Juha-Petri; Mattila, Ville; Auvinen, Anssi; Ylikomi, Timo; Pihlajamaki, Harri: **Vitamin D supplementation for the prevention of acute respiratory tract infection: a randomized, double-blinded trial among young Finnish men.** *J Infect Dis* 2010, **202**(5):809-814.
70. Urashima MS, Takaaki; Okazaki, Minoru; Kurihara, Mana; Wada, Yasuyuki; Ida, Hiroyuki: **Randomized trial of vitamin D supplementation to prevent seasonal influenza A in schoolchildren.** *American Journal of Clinical Nutrition* 2010, **91**(5):1255-1260.

71. Murdoch DRS, Sandy; Chambers, Stephen T.; Jennings, Lance C.; Stewart, Alistair W.; Priest, Patricia C.; Florkowski, Christopher M.; Livesey, John H.; Camargo, Carlos A.; Scragg, Robert: **Effect of vitamin D3 supplementation on upper respiratory tract infections in healthy adults: the VIDARIS randomized controlled trial.** *Jama* 2012, **308**(13):1333-1339.
72. Jolliffe DA, Griffiths CJ, Martineau AR: **Vitamin D in the prevention of acute respiratory infection: Systematic review of clinical studies.** *The Journal of steroid biochemistry and molecular biology* 2012.
73. Glasziou P, Vandenbroucke JP, Chalmers I: **Assessing the quality of research.** *BMJ* 2004, **328**(7430):39-41.
74. Bischoff-Ferrari HA, Giovannucci E, Willett WC, Dietrich T, Dawson-Hughes B: **Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes.** *Am J Clin Nutr* 2006, **84**(1):18-28.
75. Heaney RP: **Health is better at serum 25(OH)D above 30ng/mL.** *The Journal of steroid biochemistry and molecular biology* 2013, **136**:224-228.
76. Vieth R: **Why the minimum desirable serum 25-hydroxyvitamin D level should be 75 nmol/L (30 ng/ml).** *Best practice & research Clinical endocrinology & metabolism* 2011, **25**(4):681-691.

## APPENDIX 1

This appendix contains the complete study protocol for the McFlu2 COLD3 Prevention study as it was submitted to the Hamilton Health Sciences Research Ethics Board. Following the protocol is a copy of the consent form and electronic surveys administered.



**Protocol** - McFlu2 COLD<sub>3</sub> Prevention: A randomized, placebo-controlled, double blind trial of vitamin D and health advice for the prevention of upper respiratory tract infections in McMaster University students

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**Sponsor:** Father Sean O'Sullivan Research Centre, Canadian Institute of Health Research, Copan Diagnostics

## **I. Executive Summary**

Upper respiratory tract infections (URTI) and influenza like illnesses (ILI) affect many McMaster students annually. In previous surveys, we found that 25% of McMaster students reported ILI over a 4-month period, and 85% reported at least one URTI. These illnesses resulted in sleep disruptions, missed classes and reduced productivity. In the McFlu Study--a cohort study of McMaster University students living in residence conducted between September 2009 and April 2010--we found that rhinovirus accounted for most respiratory infections and was most common in September and October 2009.

In the current study, we propose investigating the roles of vitamin D supplementation and of regular gargling to prevent URTI/ILI. Vitamin D may be an important factor in respiratory health. Observational studies have associated low serum 25(OH)D levels with more frequent and more severe respiratory infections. However, evidence is lacking that

replacement of vitamin D decreases respiratory infections. Four interventional studies of vitamin D supplementation have noted a reduction in respiratory infections, with an estimated 5-25% reduction, but results were not statistically significant.

In Japanese populations, gargling has been found to significantly reduce the incidence of upper respiratory tract infections by approximately 35% .(1) This intervention has not been studied in different populations but may be a useful personal practice to reduce URTI.

We propose a 2X2 factorial, randomized, placebo-controlled trial of vitamin D/placebo and gargling/no gargling to study the effects of vitamin D supplementation and gargling on upper respiratory tract infections in McMaster students. This study will be conducted over two years, during September and October of each study year, to capture the natural peak in rhinovirus activity. The primary outcomes of interest will be the number of individuals who report an URTI in each of the intervention and control groups. Secondary outcomes will be the duration and severity of symptoms in each of the intervention and control groups.

Consenting students will be randomized to one of four intervention arms: (1) vitamin D<sub>3</sub> supplementation and gargling, (2) vitamin D<sub>3</sub> supplementation and no gargling, (3) vitamin D<sub>3</sub> placebo and gargling or (4) vitamin D<sub>3</sub> placebo and no gargling. At enrolment, a baseline questionnaire and self-collected nasal swab will be obtained from each participant. Students will be invited to complete brief, weekly electronic surveys and submit weekly self-collected nasal swabs. During symptomatic illness, students will be asked to complete a series of 7 consecutive daily swabs and symptom surveys. Swabs will be stored in an alcohol-based medium which inactivates respiratory viruses on contact, to ensure biosafety and will be collected daily at the McMaster Campus Health Centre by study personnel.

## **II. Background and Significance**

Upper respiratory tract infection (URTI), which presents clinically as the common cold, is the most common acute respiratory ailment suffered by humans.(2, 3) Human rhinoviruses (HRV) are the predominant viral agent and annually, the incidence of HRV infection peaks during the autumn.(2, 3) Respiratory viruses such as rhinovirus affect many McMaster university students every year. In our previous surveys at McMaster University, 85% of students reported at least one upper respiratory tract infection over a 4-month period. These illnesses resulted in sleep disruptions, missed classes and reduced productivity. In the fall of 2009, 46/122 (38%) students in the McFlu cohort study had a

positive HRV diagnosis. Although morbidity is low amongst healthy individuals who experience a common cold, the illness can result in days lost from work or school, can cause discomfort and sleeplessness and can exacerbate existing medical conditions such as asthma.(3) Consequently, preventing URTI is desirable, would be a benefit to public health and may be aided by gargling and vitamin D<sub>3</sub> supplementation.

Gargling is a common personal hygiene practice in Japan and is strongly recommended as a preventative measure against URTI.(1) Various gargling solutions including black tea, green tea, and providine-iodine solutions, and water, have been studied and found to be effective preventative measures against URTI.(1, 4-6) Interestingly, the effectiveness of gargling with water was supported by a Japanese randomized controlled trial which found a 36% reduction in incident URTI amongst participants randomized to gargle three times daily with water when compared to the control group.(1) To date, this intervention has not been assessed in another population.

Vitamin D is a fat-soluble secosteroid and is predominantly obtained through exposure to the sun, and to a lesser extent through dietary intake. Vitamin D obtained from these sources is biologically inert and must be hydroxylated twice to achieve the biologically active metabolite, 1,25-dihydroxyvitamin D [1,25(OH)<sub>2</sub>D]. The term ‘vitamin D’ refers collectively to two forms of the secosteroid: vitamin D<sub>2</sub> and vitamin D<sub>3</sub>, also known as ergocalciferol and cholecalciferol respectively.(7) Studies have found that vitamin D<sub>2</sub> is significantly less potent, has less bioactivity and has a shorter duration of action than vitamin D<sub>3</sub>.(8, 9) Consequently, vitamin D<sub>3</sub> is the preferred form for vitamin D intake and supplementation.(7)

While much attention has been paid to the significance of vitamin D<sub>3</sub> in bone health, it is increasingly being investigated as an important factor in respiratory health. Indeed, while this idea is garnering new attention it is not the first time vitamin D has been linked to infectious diseases. During the 1920s, sun exposure was recognized as an effective treatment for pulmonary tuberculosis and in 1926 D.F. Smiley published the observation that upper respiratory tract infections were inversely associated with sun exposure. (Cited in (10)) He hypothesized that lack of sun exposure during the winter months affected vitamin metabolism which might be responsible for the seasonal fluctuation in respiratory tract infections.(Cited in (10)) Contemporary observational studies have also identified a link between vitamin D deficiency and respiratory infections. A retrospective study of Dutch children demonstrated an association between higher exposure to sunlight and lower occurrence of respiratory symptoms.(11) Furthermore, several studies have demonstrated that children with rickets, a disease caused by severe vitamin D deficiency, have an increased risk of acute respiratory infections.(11-14) Similarly, case-control

studies have described an association between acute lower respiratory infections (ALRI) in young children and newborns with subclinical vitamin D deficiency (vitamin D deficiency without clinical signs of rickets).(15-17) In these three studies, all cases were admitted to hospital for treatment of their respiratory illness and the mean serum 25(OH)D in the case groups was less than 30 nmol/L.(15-17) An additional case-control study found that vitamin D levels were associated with the severity of ALRI.(18) Although no difference was found between the entire ALRI mean 25(OH)D serum levels compared to the control group, the serum levels of children with ALRI admitted to the paediatric intensive care unit were significantly lower than both the health control group and children with ALRI admitted to the general paediatric ward.(18)

Evidence of an association between vitamin D levels and respiratory infections has also been documented in studies with an adult population. A study of Finnish army recruits demonstrated a significant negative association between serum 25(OH)D levels and the number of days missed from work due to a physician diagnosed respiratory tract infection. (19) Men who had a 25(OH)D serum level of less than 40 nmol/L were at greater risk of being absent from duty because of a respiratory tract infection.(19) Additionally, analysis of data collected in the Third National Health and Nutrition Examination Survey (NHANES III) in the United States demonstrated an independent, inverse association between serum 25(OH)D levels and self-reported upper respiratory tract infection.(20) This association was found to be stronger in participants with asthma compared to those without asthma.(20) The authors hypothesize that an association between asthma and vitamin D insufficiency may be mediated through the risk of respiratory infection.(20)

The results from observational studies suggest a link between vitamin D deficiency and upper and lower respiratory tract infections. To date, only four interventional studies have investigated the effect of vitamin D supplementation on upper respiratory tract infection outcomes and they have been summarized in a recent systematic review.(21) Several of the studies were designed for the purposes of investigating the effect of vitamin D supplementation on bone density or related issues, and the investigation of URTI outcomes was done as follow-up. Avenell *et al.*, investigated daily supplementation with 800 IU of vitamin D versus placebo, for two years, in 3444 elderly ( $\geq 70$  years) adults in England and Scotland with a primary outcome of osteoporotic fractures.(22) The authors reported a minimal reduction in self-reported URTI and self-reported use of antibiotics in the vitamin D group, however this was not a statistically significant finding.(22) A study, with bone density as a primary outcome, of 208 health postmenopausal African American women administered 800 IU of vitamin D or placebo daily for two years, and then gave 2000 IU of vitamin D or placebo daily for one year.(23) The authors reported a

statistically significant lower rate of self-reported URTI in the vitamin D group compared to the placebo group, and reported that this effect was greater when the vitamin D dose was increased from 800 IU to 2000 IU daily. One randomized controlled trial studied the effect of 2000 IU vitamin D daily versus placebo supplementation for three months on the incidence and severity of self-reported URTI in 162 ambulatory adults in New York.(24) The authors reported that vitamin D supplementation did not result in a statistically significant decrease in URTI incidence or symptom severity, however there was a statistical trend which favoured the vitamin D group.(24) These studies relied entirely on self-reported URTI outcomes and did not include any laboratory confirmation. Collectively, these studies do not provide a consensus about the potential benefit of vitamin D supplementation and respiratory tract infections (See Appendix A). Consequently, there is a need for additional, larger randomized controlled studies designed with respiratory tract infections as a primary outcome to investigate the potential benefit of vitamin D supplementation.

We propose a two year study that will use a factorial, randomized, placebo-controlled trial design to study the effects of gargling and vitamin D<sub>3</sub> supplementation on upper respiratory tract infections, specifically HRV infections, in the McMaster student population. In addition to the randomly assigned interventions, this study will offer a weekly survey to participating students to monitor symptoms consistent with URTI and the opportunity to participate in nasal swabbing for specific viral diagnosis.

### **III. Study Aims**

#### **Purpose:**

The specific objectives of this investigation are to assess the effectiveness of daily gargling and vitamin D supplementation as preventative measures against incident upper respiratory tract infection (URTI) in students attending McMaster University. The primary outcome of interest will be the number of individuals with self-reported URTI in each of the intervention and control groups. Secondary outcomes will be the duration and severity of symptoms in each of the intervention and control groups.

#### **Hypotheses:**

1. Vitamin D<sub>3</sub> supplementation will decrease the incidence of symptomatic upper respiratory tract infections in university students
2. Gargling will decrease the incidence of symptomatic upper respiratory tract infections in university students

#### **IV. Administrative Organization**

##### **Participating Sites**

1. McMaster University, Campus Health Center (CHC)
2. McMaster University, Commons Market Place

#### **V. Study Design and Methodology**

**Design:** Factorial randomized controlled trial with placebo control for the vitamin D<sub>3</sub> supplementation. No placebo will be used for the gargling intervention.

**Study Period:** September and October in 2010 and 2011

**Study Population:** The study population will include adult (age  $\geq 17$ ) students at McMaster University, living in residence or living off-campus with at least one housemate.

##### **Inclusion Criteria:**

1. Current full or part-time student at McMaster University
2. 17 years of age or older
3. Currently living

In residence or,

Off-campus with at least one student housemate

4. Willing and able to sign an informed consent

##### **Exclusion Criteria:**

Students who do not meet the inclusion criteria or have the conditions listed below will be excluded:

Currently living at home with parents

History of or diagnosis of hypercalcemia (high calcium)

Diagnosis of parathyroid disorder (hyper or hypo)

Diagnosis of chronic kidney disease

Use of anticonvulsants (Phenytoin, Phenobarbital)

Malabsorption syndromes (use of Cholestyramine, Colestipol, Mineral Oil)

Diagnosis of sarcoidosis

Currently pregnant or planning a pregnancy

Inability to swallow capsules

**Sample Size:** The total sample size for the study is  $n=544$  over two years. This sample size gives us 80% power to detect a reduction in the proportion of students with URTI from 50% to 37.5% (a 25% reduction), using a 2-sided 5% significance level. An additional 10% was added to account for participant attrition.

**Randomization Scheme:** Randomization will occur within each sample stratum and will use randomization blocks, of multiples of 4 or 8, to ensure equal distribution in each of the four intervention arms. Once a participant has signed a consent form, an individual sealed opaque envelope will be opened and the participant will be assigned to one of the following groups: vitamin D<sub>3</sub> supplementation and gargling, vitamin D<sub>3</sub> supplementation and no gargling, vitamin D<sub>3</sub> placebo and gargling or vitamin D<sub>3</sub> placebo and no gargling. Participants will be asked not to reveal their assignment to the study personnel. To prevent participants from exchanging group assignments, study personnel will record the participant's study ID number and envelope number.

**Blinding:** This study will be conducted using maximum blinding procedures. Study participants, personnel, lab assessors and statistical analysts will be blind regarding the vitamin D intervention. However, due to the nature of the gargling intervention it will be impossible to blind the subjects. Consequently, this interventional arm will be single-blind. All other personnel and analysts involved in the study will remain blind to the gargling intervention. The study pharmacist will not be blind to either intervention.

**Interventions: Vitamin D<sub>3</sub> Supplementation and Gargling**

All participants will be provided with general lifestyle and health advice including information about the benefits of appropriate sleep, nutrition, hand washing hygiene, and exercise. Beyond this, each participant will be randomized to receive vitamin D<sub>3</sub> or placebo, and to gargle twice daily or receive no advice about gargling.

**Vitamin D<sub>3</sub> Supplementation and Placebo**

This study will use vitamin D<sub>3</sub> for supplementation as it is more effective, and consequently more clinically relevant, than vitamin D<sub>2</sub>.<sup>(7)</sup> Currently, there is a lack of consensus regarding recommended daily intake levels for vitamin D and Health Canada is working with US federal agencies to review the current recommendations. Although the current tolerable upper limit (UL) published by Health Canada is 2000 IU daily, research suggests that this is too conservative and is not supported by current evidence.<sup>(25, 26)</sup> Sun exposure can provide a significant amount of vitamin D, in the range equivalent to a daily oral dose 10,000 IU, and is not associated with toxicity.<sup>(26)</sup> When Hathcock *et al.*, applied the risk assessment methodology used by the Food and Nutrition Board to determine an updated UL, their work suggested a UL of 10,000 IU of vitamin D<sub>3</sub>.<sup>(25)</sup> This work was based on well-designed, human clinical trials of vitamin D.<sup>(25)</sup> An additional benefit-risk assessment of vitamin D supplementation suggested that mean serum 25(OH)D levels for optimal health benefits are best achieved through daily oral supplementation with doses between 1800 and 4000 IU.<sup>(27)</sup> Similarly, additional literature concerning the safety of vitamin D indicates that daily supplementation of 1000 to 2000 IU is safe and that toxicity is associated with excessive supplementation, in the range of 20,000 to 50,000 IU daily.<sup>(28)</sup> Relevant Canadian research and recommendations also indicate that doses above the current Health Canada recommendations are safe and beneficial. The Canadian Cancer Society has issued a recommendation that Canadian adults consider a daily vitamin D supplement of 1000 IU. Recently, a Canadian research group presented results at an international paediatrics meeting indicating that daily supplementation of 4000 IU during pregnancy was well tolerated and contributes to several beneficial outcomes (Canadian Press, 2010).<sup>(29)</sup> Furthermore, a randomized controlled trial comparing sustained daily doses of 600 IU and 4000 IU in adults living in Toronto found that the higher dose was safe, well tolerated and associated with an increased sense of well-being.<sup>(30)</sup> Interestingly, daily vitamin D supplementation for eight weeks at a dose of 400 IU was associated with only a modest increase in serum 25(OH)D levels, with an increase of approximately 11 nmol/L.<sup>(31)</sup> Authors of this work predicted that a daily intake of nearly 2000 IU would be needed to



raise serum 25(OH)D to a beneficial level.(31) The evidence of the safety and benefit of vitamin D doses greater than 400 IU daily has already been incorporated into newly designed and approved clinical trials. A brief summary of some ongoing trials in healthy adults and the respective vitamin D doses is available in Appendix B. Based on the current safety and efficacy information available in the literature, we propose to use a dose of 10,000 IU/week.

Participants will be randomized to an active vitamin D<sub>3</sub> supplementation group, at a dose of 10,000 IU weekly, or a placebo control group. Vitamin D<sub>3</sub> will be provided as a capsule for oral ingestion and an aesthetically matched placebo will be available. Each active pill will contain a 10,000 IU dose and students in the active and placebo groups will be instructed to take one pill, once weekly and a reminder email will be sent to the participants. All participants will receive the total number of pills (8) required for the complete study at the time of randomization. A weekly electronic questionnaire will ask participants about their weekly pill consumption to estimate compliance. Additionally, the participants will be asked to return all unused pills at the end of the study. The returned pills will be counted as an additional measure of compliance, and will be disposed of safely and appropriately.

### **Gargling**

Participants randomized to the gargling intervention will be asked to gargle with approximately 30 mL of tap water for 30 seconds twice daily. Participants will be provided with and asked to complete a gargling diary daily. No placebo will be available for the participants randomized to the non-gargling group. These participants will be asked to continue with their personal hygiene routine with no modifications and gargling will not be discussed with them, however a general question about gargling will be included in the weekly survey administered to all participants.

**Recruitment Procedures:** Recruitment will start on September 7, 2010 and will occur on McMaster University campus. Advertising for the study will include a campus-wide poster campaign, as well as an electronic poster posted on LearnLink, an online interface used by Health Sciences, Midwifery and other students. Information about the study will also be available online at our blog ([www.mcflu.blogspot.com](http://www.mcflu.blogspot.com)) and our website ([www.mcflustudy.csu.mcmaster.ca](http://www.mcflustudy.csu.mcmaster.ca)).

Study personnel will be present in The Commons Marketplace cafeteria for several hours each day to provide information about the study and invite students to join the study. Similarly, study personnel will attend “Clubsfest” and will be present at the student “Sidewalk Sale” to recruit students. Study information may be advertised through publications mailed to new students by the “First Year Experience” group, or publications in the McMaster newspaper “The Silhouette” and online media. During the first weeks of school, short presentations (2-3 minutes) will be made in a variety of classes, when permitted by the professors, and information will be passed out to the attending students. Interested students will be directed to visit study personnel outside of the Campus Health clinic where they will be able to ask detailed questions about the study. Written consent will be obtained by research staff or by participating graduate students.

**Methodology:** Students who are interested in participating in the study will provide written-consent. The consent form will record the student’s full name and email address for communication purposes throughout the study. At this time, a unique study ID number will be assigned to the student for use throughout the study. Upon the completion of the informed consent, students will be given an opaque sealed envelope containing their intervention assignment, the appropriate pills (vitamin D<sub>3</sub> or placebo), and general information about lifestyle factors for maximum health and wellness. Students who are randomized to the gargling intervention will also receive information explaining how to, and at what frequency they should gargle with tap water. Students who are not randomized to the gargling intervention will not receive any information about gargling.

At the time of enrolment, students will be asked to complete a baseline questionnaire which will collect basic demographic, health and lifestyle information. Students will also be asked to submit a self-collected nasal swab and will be provided with appropriate written instructions. We have previously validated the use of self-collected nasal swabs in this population and have found that they are effective, safe and well tolerated.(32)

Participants will receive a weekly email encouraging them to access a secure, web-based survey with screening questions for upper respiratory tract infection and influenza-like illness. Students will identify themselves only by their study ID on the website, and SSL encryption will be used to ensure privacy of data transmission. No personal identifying data will be entered on the website. Students with symptomatic respiratory tract infection will be encouraged to fill out a more detailed survey of symptoms, outcomes, and risk

factors. These students will be asked to fill out a brief symptom survey for seven consecutive days following symptom onset, and a follow-up symptom survey 14 days after symptom onset. Participants will be asked to submit one self-collected nasal swab weekly. On collection of the swab, they will be provided with three more nasal swabs, and when symptomatic, they will be asked to self-sample daily for a total of seven days (days 1 through 7 of illness) with nasal swabs using written instructions. Swabs are stored at room temperature in Cymol, an alcohol-based medium which inactivates influenza and other respiratory viruses on contact, to ensure biosafety. Swabs will be collected daily at the McMaster Campus Health Centre by study personnel, and symptomatic students will be given four more swabs to sample on days 4 through 7 of illness. For students who report an URTI, a follow-up survey comprised of the WURSS-21 symptom survey and additional questions will be administered electronically 14 days after their initial symptom report.

Symptomatic students will also be asked about their symptoms, time to resolution, sleep habits, school missed, and how much their illness interfered with their studies.

Participating students will receive a \$10.00 gift certificate for the campus book store for the initial nasal swab and completion of the baseline questionnaire, \$5 gift certificated weekly upon subsequent collection of routine samples, and a \$10 gift certificate at the end of the study. No additional compensation will be given for the series of seven swabs submitted during an illness.

In summary, the following will be obtained from participants:

1. Informed, written consent
2. Baseline electronic questionnaire at time of enrolment
3. Baseline self-collected nasal swab at time of enrolment
4. Weekly web-based screening questionnaire for respiratory symptoms
5. Weekly self-collected nasal swab from all participants
6. A series of seven self-collected nasal swabs (collected on days 1 through 7 of illness) from students who identify themselves as being sick with a cold
7. Daily symptom surveys for seven consecutive days for students who report a self-identified upper respiratory tract infection or influenza-like-illness
8. A follow-up symptom survey 14 days after a self-reported URTI

**Measures:** The duration and severity of symptoms will be measured using the Wisconsin Upper Respiratory Symptom Survey -21 (WURSS-21), a validated tool.(33) This scale was developed to evaluate symptoms and functional impairment associated with a cold, over time and will be administered daily for seven days upon symptom onset. Additional questions will be added to collect information about symptoms of influenza-like-illness but will not disrupt the integrity of the WURSS-21 and will be evaluated separately. Laboratory confirmation of URTI will be made from the self-collected nasal swabs.

**Laboratory Methods:** An in-house PCR assay will be used to detect rhinovirus and will be performed in the research laboratories at St. Joseph's Hospital. A separate in-house influenza PCR will be performed if there is evidence of influenza activity in the area. All influenza A results will be verified by a separate, validated in-house influenza A PCR, and identified as H1N1 (swine), H1 (seasonal) or H3 (seasonal) influenza. Assays will be run in batches, and individual swab results will not be made available to clinicians or to study participants in time to affect clinical decisions. Interested participants who provide an ID number can determine their lab results approximately 2 to 4 weeks after submission of samples.

**Outcomes:** Participants must be in the study for seven days from the time of enrolment before a reported URTI will count as an outcome. An episode of a symptomatic upper respiratory tract infection will be defined as: The subject's perception of a cold in conjunction with the presence of two or more URTI symptoms (runny/stuffy nose, congestion, cough, sneezing, sore throat, muscle aches, fever).

Our primary outcome of interest is the number of individuals in each intervention group who report a symptomatic URTI.

Secondary outcomes of interest include:

- i- The number of lab-confirmed (symptomatic and asymptomatic) URTI reported in each intervention group
- ii- The duration of symptoms reported in each intervention group
- iii- The severity of symptoms reported in each intervention group

## **VI. Risks and Monitoring Plan**

**Risks:** This is a minimal risk study. Students may experience slight discomfort and may be at risk for developing a minor nosebleed when using the nasal swabs. The dose of vitamin D<sub>3</sub> used in this study (10,000 IU/week) is within the tolerable limit presented in current literature (10,000 IU/day) and the potential to develop hypervitaminosis D is very low. Studies have shown that toxicity did not develop after daily intake of 50,000 IU for eight weeks.(34)

**Monitoring Plan:** The study coordinator will ensure that the participants are properly instructed on obtaining self-collected nasal swabs. She will also ensure that all procedures for participant confidentiality are followed according to protocol. Students will be informed that they should seek medical attention from Campus Health at their discretion, during episodes of illness. If they require medical attention, students will be advised to inform their physician of their involvement in the study. If a suspected severe adverse effect is reported, whether or not related to vitamin D<sub>3</sub>, then a case report will be completed by the primary care giver involved and reviewed by Dr. Fiona Smaill who will act as our Data and Safety Monitoring Board. All students will be asked weekly via the electronic survey if they have experienced any adverse effects.

We will not be monitoring female participants for pregnancy during the trial. If we learn that a participant has become pregnant since enrolling in the study we will recommend that she discuss her participation with a physician and decide if she would like to continue with or withdraw from the trial.

Dr. Marek Smieja, the Principal Investigator, will monitor the conduct of the trial.

## **VII. Plan for Analysis**

The two interventions used in this trial are not expected to interact and we expect that gargling will be equally effective regardless of vitamin D<sub>3</sub> or placebo intake, and vice-versa. Appropriate tests for interactions will be carried out at the interim analysis and with the full data set at the end of the two-year study.

An interim analysis will be completed after the first year of the study to estimate an effect size and potentially revise sample size and data capturing techniques for the second year of the study. For this analysis, blinding will be revealed however the results will not be published. The study will not be stopped after the interim analysis. At the end of the two year study, data will be analyzed on an “intention-to-treat” basis. A “per protocol”

analysis will also be performed and reported. Blinding will be broken after the analysis is complete. The primary outcome will be analyzed by multivariable logistic regression where presence or absence of self-reported URTI over the course of the study is the outcome, and vitamin D<sub>3</sub> and gargling are the predictors. The interaction between the two will also be entered into the model and will be removed if not significant. Odds ratios and 95% confidence intervals will be calculated. The effect of the two interventions will be analyzed separately with a significance cut-off of  $p=0.05$ .

Missing data will be minimized by offering weekly incentives to complete the brief electronic survey and self-collected nasal swabs for the duration of the 8 week study. Reminder emails will be sent to all participants encourage adherence. In the event that students do not complete a weekly survey, they will be contacted to inquire if their participation was inhibited by illness. This will allow us to capture all self-reported illnesses. Any data that remains missing after these efforts to recover it will be handled through multiple imputation.

ANOVA analysis will be used to compare mean symptom severity scores and duration in each of the intervention and control groups.

## References:

1. Satomura K, Kitamura T, Kawamura T, Shimbo T, Watanabe M, Kamei M, Takano Y, Tamakoshi A: **Prevention of upper respiratory tract infections by gargling: a randomized trial.** *American journal of preventive medicine* 2005, **29**(4):302-307.
2. Arruda E, Pitkaranta A, Witek TJ, Jr., Doyle CA, Hayden FG: **Frequency and natural history of rhinovirus infections in adults during autumn.** *Journal of clinical microbiology* 1997, **35**(11):2864-2868.
3. Proud D: **Upper airway viral infections.** *Pulmonary pharmacology & therapeutics* 2008, **21**(3):468-473.
4. Iwata M, Toda M, Nakayama M, Tsujiyama H, Endo W, Takahashi O, Hara Y, Shimamura T: **[Prophylactic effect of black tea extract as gargle against influenza].** *Kansenshogaku zasshi The Journal of the Japanese Association for Infectious Diseases* 1997, **71**(6):487-494.
5. Nagatake T, Ahmed K, Oishi K: **Prevention of respiratory infections by povidone-iodine gargle.** *Dermatology* 2002, **204** Suppl 1:32-36.
6. Yamada H, Takuma N, Daimon T, Hara Y: **Gargling with tea catechin extracts for the prevention of influenza infection in elderly nursing home residents: a prospective clinical study.** *Journal of alternative and complementary medicine (New York, NY)* 2006, **12**(7):669-672.
7. Cannell JJ, Hollis BW: **Use of vitamin D in clinical practice.** *Alternative medicine review : a journal of clinical therapeutic* 2008, **13**(1):6-20.

8. Armas LA, Hollis BW, Heaney RP: **Vitamin D2 is much less effective than vitamin D3 in humans.** *The Journal of clinical endocrinology and metabolism* 2004, **89**(11):5387-5391.
9. Trang HM, Cole DE, Rubin LA, Pierratos A, Siu S, Vieth R: **Evidence that vitamin D3 increases serum 25-hydroxyvitamin D more efficiently than does vitamin D2.** *Am J Clin Nutr* 1998, **68**(4):854-858.
10. Bartley J: **Vitamin D, innate immunity and upper respiratory tract infection.** *The Journal of laryngology and otology* 2010, **124**(5):465-469.
11. Termorshuizen F, Wijga A, Garssen J, Den Outer PN, Slaper H, Van Loveren H: **Exposure to solar ultraviolet radiation in young Dutch children: assessment by means of a 6-week retrospective questionnaire.** *Journal of exposure analysis and environmental epidemiology* 2002, **12**(3):204-213.
12. Banajeh SM, al-Sunbali NN, al-Sanahani SH: **Clinical characteristics and outcome of children aged under 5 years hospitalized with severe pneumonia in Yemen.** *Ann Trop Paediatr* 1997, **17**(4):321-326.
13. Muhe L, Lulseged S, Mason KE, Simoes EA: **Case-control study of the role of nutritional rickets in the risk of developing pneumonia in Ethiopian children.** *Lancet* 1997, **349**(9068):1801-1804.
14. Najada AS, Habashneh MS, Khader M: **The frequency of nutritional rickets among hospitalized infants and its relation to respiratory diseases.** *Journal of tropical pediatrics* 2004, **50**(6):364-368.



15. Karatekin G, Kaya A, Salihoglu O, Balci H, Nuhoglu A: **Association of subclinical vitamin D deficiency in newborns with acute lower respiratory infection and their mothers.** *Eur J Clin Nutr* 2009, **63**(4):473-477.
16. Roth DE, Shah R, Black RE, Baqui AH: **Vitamin D status and acute lower respiratory infection in early childhood in Sylhet, Bangladesh.** *Acta paediatrica (Oslo, Norway : 1992)* 2010, **99**(3):389-393.
17. Wayse V, Yousafzai A, Mogale K, Filteau S: **Association of subclinical vitamin D deficiency with severe acute lower respiratory infection in Indian children under 5 y.** *Eur J Clin Nutr* 2004, **58**(4):563-567.
18. McNally JD, Leis K, Matheson LA, Karuananyake C, Sankaran K, Rosenberg AM: **Vitamin D deficiency in young children with severe acute lower respiratory infection.** *Pediatric pulmonology* 2009, **44**(10):981-988.
19. Laaksi I, Ruohola JP, Tuohimaa P, Auvinen A, Haataja R, Pihlajamaki H, Ylikomi T: **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):714-717.
20. Ginde AA, Mansbach JM, Camargo CA, 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):384-390.
21. Yamshchikov AV, Desai NS, Blumberg HM, Ziegler TR, Tangpricha V: **Vitamin D for treatment and prevention of infectious diseases: a systematic review of**

- randomized controlled trials.** *Endocrine practice : official journal of the American College of Endocrinology and the American Association of Clinical Endocrinologists* 2009, **15**(5):438-449.
22. Avenell AC, J. A.; MacLennan, G. S.; Macpherson, G. C.: **Vitamin D supplementation to prevent infections: a sub-study of a randomised placebo-controlled trial in older people (RECORD trial, ISRCTN 51647438).** *Age and ageing* 2007, **36**(5):574-577.
23. Aloia JFL-N, M.: **Re: epidemic influenza and vitamin D.** *Epidemiology and Infection* 2007, **135**(7):1095-1096.
24. Li-Ng MA, J. F.; Pollack, S.; Cunha, B. A.; Mikhail, M.; Yeh, J.; Berbari, N.: **A randomized controlled trial of vitamin D3 supplementation for the prevention of symptomatic upper respiratory tract infections.** *Epidemiol Infect* 2009, **137**(10):1396-1404.
25. Hathcock JN, Shao A, Vieth R, Heaney R: **Risk assessment for vitamin D.** *Am J Clin Nutr* 2007, **85**(1):6-18.
26. Vieth R: **Vitamin D toxicity, policy, and science.** *Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research* 2007, **22 Suppl 2**:V64-68.
27. Bischoff-Ferrari HA, Shao A, Dawson-Hughes B, Hathcock J, Giovannucci E, Willett WC: **Benefit-risk assessment of vitamin D supplementation.** *Osteoporosis international : a journal established as result of cooperation*

- between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA* 2010, **21**(7):1121-1132.
28. Heaney RP: **Vitamin D: criteria for safety and efficacy**. *Nutr Rev* 2008, **66**(10 Suppl 2):S178-181.
29. Press C: **Studies: high-dose vitamin D safe during pregnancy**. In: *Canadian Press*. 2010.
30. Vieth R, Kimball S, Hu A, Walfish PG: **Randomized comparison of the effects of the vitamin D3 adequate intake versus 100 mcg (4000 IU) per day on biochemical responses and the wellbeing of patients**. *Nutrition journal* 2004, **3**:8.
31. Barger-Lux MJ, Heaney RP, Dowell S, Chen TC, Holick MF: **Vitamin D and its major metabolites: serum levels after graded oral dosing in healthy men**. *Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA* 1998, **8**(3):222-230.
32. Goodall E, Banh L, Granados A, Luinstra K, Chong S, Carruthers S, Mahony J, Petrich A, Smieja M: **Respiratory virus diagnosis using self-collected nasal swabs in undergraduate students**. In: *AMMI Canada - CACMID Annual Conferece*. Edmonton, Alberta; 2010: SP38.
33. Barrett B, Brown RL, Mundt MP, Thomas GR, Barlow SK, Highstrom AD, Bahrainian M: **Validation of a short form Wisconsin Upper Respiratory Symptom Survey (WURSS-21)**. *Health and quality of life outcomes* 2009, **7**:76.

34. National Institutes of Health. Office of Dietary Supplements. **Dietary Supplements Fact Sheet: Vitamin D Health Professional Fact Sheet.**  
<http://ods.od.nih.gov/factsheets/VitaminD-HealthProfessional/>.

**Appendix A:** Summary of published vitamin D interventional trials

<b>Reference, location, study type</b>	<b>Population</b>	<b>Intervention</b>	<b>Outcomes [OR (95% CI)]</b>
Rehman PK., 1994 India Controlled trial	27 children (3-12y) with $\geq 6$ respiratory or antibiotic-requiring illnesses in previous 6 months (test group)  20 children (3-12y) with $\leq 1$ respiratory or antibiotic-requiring illnesses in previous 6 months (control group)  Participants were age & sex matched	Test group received 60,000 IU/week for 6 weeks and 650 mg calcium/day in three doses for 6 weeks  Subsequent observation of all participants for 6 months to monitor frequency of infections	Fall in serum alkaline phosphatase to normal levels (from $299 \pm 41$ to $197 \pm 7$ )  No difference in frequency of infections in test and control groups of children (no numbers reported)  Suggests that children in test group had subclinical rickets and benefitted from vitamin D supplementation
Avenell et al. 2007 England and Scotland Double blind, placebo controlled, randomized controlled trial	3444 elderly ( $\geq 70$ y) participants in the RECORD trial who responded to a follow-up questionnaire about infections and antibiotics	800 IU/d for 24-62 months versus placebo	Odds ratio (95% CI) for:  Reported infection: 0.90 (0.76-1.07) p=0.23  Reported antibiotics use: 0.84 (0.54-1.09) p=0.18  <i>Per protocol analysis</i> Reported infection: 0.80 (0.64-1.01) p=0.06  Reported antibiotics use: 0.74 (0.52-1.06) p=0.10

<p>Aloia JF, Li-Ng M, 2007</p> <p>United States</p> <p>Double blind, placebo controlled, randomized controlled trial</p>	<p>208 healthy post-menopausal, African-American women n1=104 n2=104</p>	<p>800 IU/d for 24 months followed by 2000 IU/d for 12 months versus placebo for 36 months</p>	<p>Self reported colds after 3 years: 8 vitamin D patients vs. 26 placebo patients (p=0.002)</p> <p>OR: 0.25 (0.12-0.58)</p>
<p>Li-Ng et al., 2009</p> <p>United States</p> <p>Double blind, placebo controlled, randomized controlled trial</p>	<p>162 healthy adult (18-80 y) outpatients</p>	<p>2000 IU/d for 12 weeks versus placebo</p>	<p>Reported URI: 48/388 reports in active group vs. 50/363 reports in placebo group <math>\Delta</math> in favour of vD: 1.4% (-2.4 to 3.4) p=0.56 OR: 0.88 (0.58-1.35)</p> <p>URI incidence: 28/78 (36%) patients in active group vs. 29/70 (41%) in placebo group <math>\Delta</math> in favour of vD: 5% (-12 to 20) p=0.74 OR: 0.79 (0.41-1.54)</p>

**Appendix B:** A sample of current clinical trials using vitamin D in healthy adults

<b>Title</b>	<b>Objective</b>	<b>Study design &amp; Location</b>	<b>Participants</b>	<b>Vitamin D<sub>3</sub> Dose</b>	<b>Primary Outcome(s)</b>
Randomized Trial: Maternal Vitamin D Supplementation to Prevent Childhood Asthma (VDAART) (NCT00920621)	Vitamin D supplementation given to pregnant women will prevent asthma in their offspring and children	Double blind placebo controlled randomized controlled trial  USA	Pregnant females 18-35, non-smoking, without chronic med. Conditions, single pregnancy, not already taking vitamin D >2000 IU/d	4000 IU/d D <sub>3</sub> x 4 years	25(OH)D levels in maternal plasma, cord blood, and children's blood at 1 and 3 yrs of age.
Pilot Pharmacokinetic Study of Daily Versus Monthly High-Dose Cholecalciferol Supplementation (NCT01079923)	To compare total Vitamin D levels resulting from daily Vitamin D supplementation of 5,000 international units of cholecalciferol (Vitamin D <sub>3</sub> ) orally for 28 days vs. 150,000 international units of cholecalciferol	Randomized trial, no placebo  USA	Women 18-40, not pregnant/lactating, no chronic conditions, no indoor tanning, no chronic use of steroids, anti-convulsants, or barbiturates	5000 IU/d x 28 days (D <sub>3</sub> )  Or 1 dose 150,000 IU D <sub>3</sub>	Pharmacokinetics

	orally once in healthy women				
A Clinical Trial to Study the Effect of the Addition of Vitamin D to Conventional Treatment in New Pulmonary Tuberculosis Patients (NCT00366470)	People receiving Vitamin D with anti TB therapy will be compared against people receiving anti TB therapy alone to see if vitamin D contributes to a quicker recovery	Double blind placebo controlled randomized controlled trial  India	Adults 18-75, new TB dx, no liver/renal disease, no baseline hypercalcemia(>10.5mg/dl), no pregnant women	100,000 IU/every 2 weeks for 2 months	Time to sputum culture conversion
Vitamin D Inadequacy in Rural Populations, Evaluation of Correction by Food Supplementation(NCT00690417)	to describe vitamin D levels and bone status in a rural Wisconsin population	Double blind placebo controlled randomized controlled trial  USA	Healthy, community-dwelling ambulatory women. Ages: 20-30, 55-65 or >75 Baseline serum 25OHD concentration > 10 ng/ml and < 60 ng/ml Exclusions for medical conditions, tanning and use of >50,000IU D3 weekly	2,500 IU/d for 4 months	Proportion of postmenopausal women with D inadequacy defined by current consensus as a serum 25(OH)D < 30 ng/ml
Vitamin D Supplement Study for Adolescents (VIP) (NCT00909454)	To determine if 14-19 year old African American	Single blind randomized controlled	Healthy African-American adolescents, age 14-19	400 IU/d or 2000 IU/d for 4 months	Plasma 25-OH D level



	adolescents are able to take a daily vitamin D supplement daily for about 4 months and how well a daily dose of 400 IU or 2000 IU vitamin D supplement raises their vitamin D blood level	trial, dose comparison USA			
Efficacy of Daily Vitamin D3 Supplementation in Normal Weight Adolescents(NCT01058720)	To evaluate the increment of serum 25 (OH) vitamin D levels in normal weight adolescents	Open label study USA	ages 12 and 18 years with BMI between the 5th and the 85th percentile for age and gender, exclude if 25 (OH)- D levels >80 ng/mL ;Serum calcium >10.8 mg/dL ;Serum phosphorus > 5.5 mg/dl, exclude chronic illness and pregnancy	2000 IU/d for 12 weeks	Increment in 25(OH)vitamin D level
Effect of Supplemental Vitamin D on Skeletal Muscle Function in Chronic Obstructive Pulmonary Disease (COPD) Patients (NCT00914810)	To determine whether or not vitamin D supplementation can improve physical performance in	Double blind placebo controlled randomized controlled trial	Adults >40 yrs, with COPD and Forced expiratory volume in one second (FEV1) < or = 50% of predicted ; Smoking history of at least 10 pack-years.	2000 IU/d for 6 weeks	Short Physical Performance Battery (SPPB) score and Blood level of vitamin D (25-hydroxyvitamin

	persons with severe chronic obstructive pulmonary disease (COPD).	USA	Exclusions for > D3 400 IU/d and medical conditions		D)
Vitamin D Needs of Early Adolescent Children(NCT00931580)	To determine the effects of varying doses of vitamin D supplementation over 12 weeks on blood indicators of health in white and black children, aged 9 to 13 years, from both the northern and southern US	Double blind placebo controlled randomized controlled trial  USA	Healthy adolescents(Male, 10-13 years of age or female 9-11 years of age), excluding Hispanic children, girls who reached menarche, known bone disease or use of meds affect bone metabolism	400 IU/d vs 1,000 IU/d vs 4,000 IU/d vs 2,000 IU/d vs placebo for 12-weeks	serum 25(OH)D PTH 1,25(OH)2D fractional calcium absorption biochemical markers of bone turnover
Vitamin D Supplementation for the Prevention of Acute Respiratory Tract Infections(NCT00973583)	To determine whether vitamin D supplementation may decrease the incidence of acute respiratory tract infections.	Double blind placebo controlled randomized controlled trial  Finland	164 male conscripts at a military base in Finland	400 IU/day for 6 months	Number of days absent from duty due to respiratory tract infection
Effects of Vitamin D on Lipids	To examine	Double	Health adults (>18 yrs)	1000 IU	LDL-cholesterol

(NCT00723385)	whether oral vitamin D supplementation will lower LDL-cholesterol and total cholesterol concentrations	blind placebo controlled randomized controlled trial  USA	able to swallow pills Exclusions for chronic medical conditions	D <sub>2</sub> /day or 1000 IU D <sub>3</sub> /day or placebo x 12 weeks	
Vitamin D Deficiency in Patients With Hypertension (NCT00974922)	To evaluate the effects of Vitamin D replacement and the effects of an approved medication for hypertension, aliskiren (Tekturna), in patients with high blood pressure who have low levels of vitamin D in their blood	Double blind placebo controlled randomized controlled trial  USA	Adults $\geq 21$ with hx of stage 1 or 2 hypertension and 25-OH-D $< 30$ ng/ml and $> 12$ ng/ml.  Exclusions for high bp, cvd, etc.	aliskiren (Tekturna) 150 mg once daily for 2 weeks, then titrated to 300 mg once daily for 4 weeks For everyone  3000 IU D <sub>3</sub> /day x 6 weeks	Change in the baseline blood pressure measurement in 24-hour mean diastolic blood pressure
Vitamin D for the Treatment of Severe Asthma(NCT00712205)	To test if vitamin D <sub>3</sub> improves pulmonary function and quality of life	Double blind placebo controlled randomized controlled	Healthy adults $\geq 18$ years old with asthma	40 IU/day for 28 days	

	in patients with asthma relatively resistant to glucocorticoids	trial, crossover design Switzerland			
Vitamin D for the Prevention of Diabetes Type 2 (NCT00685594)	Prevention of Type 2 Diabetes With Vitamin D Supplementation in Subjects With Reduced Glucose Tolerance Detected in the Tromso Study	Double blind placebo controlled randomized controlled trial Norway	Adults 21-80 years old with impaired glucose tolerance.  Exclusions for serious heart disease, Renal stone disease, Hypercalcemia, Sarcoidosis	20,000 IU/week for 5 years versus placebo	development of diabetes type 2
The Influence of Vitamin D on Mineral Metabolism, Blood Pressure and Pulse Wave Analysis in Healthy Individuals (NCT00952562)	To evaluate the effect of the recently recommended high doses of cholecalciferol (3000 IU/day) on mineral metabolism, blood pressure and pulse wave analysis in healthy individuals	Double blind placebo controlled randomized controlled trial Denmark	Healthy adults $\geq 18$ years old with 25(OH)D $< 50$ nmol/L  Exclusions for pregnancy/lactation, sarcoidosis, renal failure/stones, malignancy, etc	3000 IU/day for 16 weeks versus placebo	urinary calcium excretion

Vitamin D, Blood Pressure, Lipids, Infection and Depression (NCT00960232)	To test of vitamin D supplementation is a benefit to blood pressure, lipid profile, infection incidence and depression	Double blind placebo controlled randomized controlled trial  Norway	Healthy adults 30-75 years old, low serum 25(OH)D Exclusions for: diabetes other serious diseases hypercalcemia	40,000 IU cholecalciferol per week for 6 months versus placebo	Depression score, blood pressure, lipid profile, frequency of infections
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**INFORMATION AND CONSENT FORM (Participant)**

**Title:** McFlu2 COLD<sub>3</sub> Prevention: A randomized, placebo-controlled, double blind trial of vitamin D and health advice for the prevention of upper respiratory tract infections in McMaster University students

**Local Principal Investigator:** Dr. Marek Smieja, Pathology and Molecular Medicine, McMaster University, St. Joseph's Healthcare, 50 Charlton Ave. E., L-424, St. Luke's Wing, Hamilton, ON L8N 4A6

**Student Principal Investigator:** Emma Goodall, MSc student, Health Research Methodology, Department of Clinical Epidemiology and Biostatistics, McMaster University, 1280 Main Street West, Hamilton ON, L8S 4L8

**Co-Investigators:** Dr. Mark Loeb, Pathology and Molecular Medicine, McMaster University, 1200 Main St. W., MDCL-3203, Michael Degroote Centre for Learning, Hamilton ON L8N 3Z5

Dr. Eleanor Pullenayegum, Clinical Epidemiology and Biostatistics, McMaster University, St. Joseph's Healthcare,

50 Charlton Ave. E., H-325, Martha Wing, Hamilton, ON L8N 4A6

Dr. Jan Young-Baker, Campus Health, McMaster University, 1280 Main St. W., McMaster University Student Centre, B101 and B106, Hamilton ON, L8S 4L8

**Sponsor:** Father Sean O'Sullivan Research Centre, Canadian Institute of Health Research, Copan Diagnostics

**Invitation to participate:**

You are being invited to participate in a research study because you are a student at McMaster University and you may experience symptoms of a respiratory virus during the current school year. Participation in this study is entirely voluntary.

Choosing not to participate in this study will not have any negative consequences for you.

**Why is the study being done?**

Upper respiratory tract infection (URTI), and influenza like illnesses (ILI) affect many McMaster students annually. In our previous studies we found that 25% of McMaster students reported ILI over a 4 month period and 85% reported at least one URTI. These illnesses resulted in sleep disruptions, missed classes and reduced productivity. In a study of McMaster university students living in residence conducted between September 2009 and April 2010, we found that rhinovirus accounted for most respiratory infections and was most common in September and October 2009.

Observational studies have associated low serum D levels with more frequent and more severe respiratory infections. Personal hygiene practices and lifestyle factors including hand washing, smoking, gargling, nutrition, sleep and exercise have also been shown to influence a person's susceptibility to respiratory infections.

In this study we would like to investigate the effects of vitamin D supplementation and of health advice regarding hand washing, smoking, gargling, nutrition, sleep and exercise on upper respiratory tract infections in McMaster University students.

**How many participants will be in this study?**

The study will be conducted over 2 years, during September and October of each study year, to capture the natural peak in rhinovirus activity. Five hundred and forty-four students will take part in this study at McMaster University and will represent students who live in residence and students who live off-campus with at least one housemate.

**What will happen to participants this study?**

The research visits will take place at the Campus Health Centre.

On enrolment:

The participants will provide informed written consent with email address

Participants will then be provided with an individual sealed opaque envelope that assigns at random, that is, by a method of chance (like the flip of a coin) to either vitamin D3 supplementation or placebo, and one of two versions of health advice.

A placebo is an inactive substance that has no medicinal value. Participants will have a 50% chance of receiving vitamin D<sub>3</sub>, 10,000 IU/week for 8 weeks and a 50% chance of receiving an identical placebo. Neither the participants nor the study personnel will know if they are taking vitamin D<sub>3</sub> or placebo, until completion of the study. Each individual envelope will provide the participant with his or her instructions for their study arm assignment.

Participants will be asked not to reveal their assignment to the study personnel.

Participants will then receive the total number of pills (vitamin D<sub>3</sub> or placebo) required for the complete study. They will also receive general information about health advice for maximum health and wellness.

Participants will be asked to complete a baseline questionnaire which will collect basic demographic, health and lifestyle information.

With written instructions, participants will be asked to submit a self-collected nasal swab. This swab will be tested for respiratory viruses.

This enrolment visit will occupy about 15 minutes of your time.

Participants will receive a weekly email encouraging them to access a secure, web-based survey with screening questions for upper respiratory tract infection and influenza like illness. They will also be asked if they have experienced any adverse events (change in health). They will identify themselves only by their Study ID.

Participants with respiratory symptoms will be asked to fill out a more detailed survey of their symptoms. They will also be asked to fill out brief symptom surveys for seven consecutive days following symptom onset.

These students will be asked to fill out a follow-up symptom survey 14 days after their initial symptom report.

Each survey should occupy about 2 - 5 minutes of their time.

Participants will be asked to submit one self-collected nasal swab weekly. This swab will be collected daily at the Campus Health Centre by study personnel.

On collection of this swab participants will be provided with 3 more nasal swabs. If they begin to have respiratory symptoms, they will be asked to self-sample, with the nasal swabs using written instructions, daily for a total of 7 days (days 1 through 7 of illness). Swabs will be collected daily at the Campus Health Centre by study personnel daily and symptomatic participants will be given 4 more swabs to sample on days 4 through 7 of illness. These swabs will be tested for a respiratory viral infection.

This visit will occupy about 5 minutes of your time.



Testing will be done in batches and individual swab results will not be made available to clinicians or to study participants in time to affect clinical decisions. Interested participants, who provide their Study ID number, can determine their lab results approximately 2 to 4 weeks after submission of swabs.

The samples will be used for research and such use may result in inventions or discoveries that create new products or diagnostic agents. In some instances, these inventions and discoveries may be of potential commercial value and may be patented and licensed by the researcher. You will not receive any money or other benefits derived from any commercial or other products that may be developed from use of the specimens.

**Are there any risks?**

A nasal swab collection may cause slight discomfort and the possibility for a nosebleed. The dose of vitamin D used in this study (10,000 IU/week for 8 weeks) is within the tolerable limit presented the current literature (10,000 IU/day). Studies have shown that vitamin D toxicity did not develop after intake of 50,000 IU/daily for eight weeks.

**Are there any benefits?**

We cannot promise any personal benefits to you from your participation in this study. However, possible benefits could include the prevention or the reduction in severity of a respiratory tract infection.

**Will I be paid to participate in this study?**

Participants will receive a \$10.00 gift certificate for the campus book store for the initial nasal swab and completion of the baseline questionnaire, \$5.00 gift certificate weekly upon subsequent collection of routine samples, and a \$10.00 gift certificate at the end of the study. No additional compensation will be given for the series of 7 swabs submitted during an illness.

**What will happen to my personal information?**

Your information will not be shared with anyone except with your consent or as required by law. All personal information such as your name and email address will be removed from the data and will be replaced with a unique study number. A list linking the number with your name will be

kept in a secure place, separate from your files. Only study personal will have access to your information. All identifying information will be stored separately in a locked office and on password protected computer files. If the results of the study are published, your name will not be used and no information that discloses your identity will be released or published without your specific consent to the disclosure. The data for this research study will be kept for 25 years. At the end of 25 years, CRF's will be shredded and data files will be irreversibly anonymized (the key identifying the link between data and the individual's identity is deleted).

**Can participation end early?**

Your decision to participate in this study is entirely voluntary, and will not affect your current or future medical care or treatment. You may withdraw at any time during the study. You have the option of removing your data from the study. You may also refuse to answer any questions you don't want to answer and still remain in the study. You will be informed of any new information which might affect your willingness to continue in this study.

**If I have any questions about this study, who should I call?**

If you have questions or comments regarding the study, you may contact Dr. Marek Smieja, St. Joseph's Healthcare, at 905 521 6143

**CONSENT****Participant:**

I have read the preceding information thoroughly. I have had an opportunity to ask questions and all of my questions have been answered to my satisfaction. I agree to participate in this study. I understand that I will receive a signed copy of this form.

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Participants Name (please Print)

Signature

Date

Participants email address: \_\_\_\_\_

This study has been reviewed by the Hamilton Health Sciences/McMaster Faculty of Health Sciences Research Ethics Board (HHS/FHS REB). The REB is responsible for ensuring that participants are informed of the risks associated with the research, and that participants are free to decide if participation is right for them. If you have any questions about your rights as a research participant, please call The Office of the Chair, HHS/FHS REB at 905.521.2100 x 42013

**McFlu2: COL<sub>3</sub> Prevention**

**Baseline Survey**

1. Please enter your 4-digit study ID number
2. Gender : male  female
3. Age: \_\_\_\_\_
4. Year of Study: 1<sup>st</sup>  2<sup>nd</sup>  3<sup>rd</sup>  4<sup>th</sup>  5<sup>th</sup>  MSc  PhD  Other \_\_\_\_\_
5. Do you live: Off-campus  In residence  (which one \_\_\_\_\_)
6. How many roommates do you have? \_\_\_\_\_
7. How many housemates do you have? \_\_\_\_\_
8. Are you living with any new housemates compared to previous years? ? Yes  No
9. Do you have asthma? Yes  No  Not Sure  I don't want to say
10. In general, how would you describe the severity of your asthma?

No asthma	Very mild 1	2	Mild 3	4	Moderate 5	6	Severe 7

11. Do you currently have any of the allergy symptoms below? (circle all that apply)
  - a. Clear nasal discharge
  - b. Watery eyes
  - c. Itchy nose
  - d. No symptoms
12. Do you ever have allergy symptoms?
  - a. Yes, every year
  - b. Sometimes
  - c. Never
  - d. Not sure
13. Do you use vitamins regularly?
  - a. Yes
  - b. No
  - c. Not sure
  - d. Specify \_\_\_\_\_
14. Do you drink milk, soy, or rice milk?
  - a. Yes, daily
  - b. Yes, occasionally
  - c. Only in my coffee/tea
  - d. Never
  - e. Specify \_\_\_\_\_
15. Do you eat breakfast?
  - a. Yes, daily
  - b. Yes, occasionally

- c. No
16. Do you eat cold cereal ?
- a. Yes, daily
  - b. Yes, 3-6 times week
  - c. Yes, 1-2 times a week
  - d. No
17. Do you eat fish?
- a. Yes, at least twice per week
  - b. Yes, once per week
  - c. Occasionally
  - d. Never
18. How many servings of fruits and vegetables do you have daily?
- a. None
  - b. One
  - c. Two
  - d. Three
  - e. Four
  - f. Five or More
19. Do you wash your hands (or use an alcohol-based hand cleanser) before each meal?
- a. Yes, always
  - b. Usually
  - c. No
  - d. Comments\_\_\_\_\_
20. How many times do you wash your hands (or use an alcohol-based hand cleanser) in an average day?
- a. 0
  - b. 1
  - c. 2-3
  - d. 4-5
  - e. 6 or more
21. How many hours of exercise do you get in a week?\_\_\_\_\_
22. How many hours/week do you get at each of the intensities listed below?
- a. Low (you can carry on a full conversation or even sing a song with ease and you are not sweating)\_\_\_\_\_
  - b. Moderate (you can speak with brief sentences, are sweating, and cannot sing a song)\_\_\_\_\_
  - c. Vigorous\_\_\_\_\_ (you can only speak a few sentences in between heavy breaths)
23. Do you currently smoke?
- a. No
  - b. Yes, occasionally

- c. Yes, daily
24. On average, how many days per week do you have one or more alcoholic drinks? \_\_\_\_\_
25. On average, how many alcoholic drinks do you have on one occasion? \_\_\_\_\_
26. Do you gargle as part of your daily oral hygiene?
- a. No
  - b. Only when I have a sore throat
  - c. Yes, occasionally
  - d. Yes, daily
27. Do you use non-medicated (saline) nasal spray?
- a. Yes, daily
  - b. Yes, occasionally if I have congestion or an itchy nose
  - c. No
28. How much sleep do you get on an average night?
- a. Less than 5 hours
  - b. 6 hours
  - c. 7 hours
  - d. 8 hours
  - e. 9 hours or more
29. In the past week, what is the least amount of sleep that you had in one night?
- a. Less than 3 hours
  - b. 4 hours
  - c. 5 hours
  - d. 6 hours
  - e. 7 hours
  - f. 8 hours
  - g. 9 hours or more
  - h. Comments \_\_\_\_\_

**McFlu2: COL<sub>D</sub>3 Prevention****Weekly Survey**

30. Please enter your 6-digit study ID number
31. Have you been ill in the past 7 days?
- Yes
  - No
  - Not Sure
  - Comments \_\_\_\_\_
32. If yes, did you start the daily symptom survey?
- Yes
  - No
33. Have any of your roommates or housemates been ill in the past week?
- Yes
  - No
  - Not applicable
34. Did you take your study pill last week?
- Yes
  - No
35. In the past week, did you wash your hands (or use an alcohol-based hand cleanser) before each meal?
- Yes, always
  - Usually
  - No
  - Comments \_\_\_\_\_
36. In the past week, how many times did you wash your hands (or use an alcohol-based hand cleanser) in an average day?
- 0
  - 1
  - 2-3
  - 4-5
  - 6 or more
37. In the past week, did you gargle as part of your daily oral hygiene?
- No
  - Yes, occasionally
  - Yes, daily
  - Yes, twice daily
38. In the past week, how many hours of moderate exercise (you can speak with brief sentences, are sweating, and cannot sing a song) did you get over the entire week?
- 0 hours
  - 1-2 hours

- c. 3-4 hours
  - d. 5 hours or more
39. How much sleep do you get on an average night?
- a. Less than 5 hours
  - b. 6 hours
  - c. 7 hours
  - d. 8 hours
  - e. 9 hours or more
40. In the past week, what is the least amount of sleep that you had in one night?
- a. Less than 3 hours
  - b. 4 hours
  - c. 5 hours
  - d. 6 hours
  - e. 7 hours
  - f. 8 hours
  - g. 9 hours or more
  - h. Comments\_\_\_\_\_
41. Please indicate if you have experienced any of the following symptoms in the past week:
- a. Fatigue
  - b. Sleep disturbance
  - c. Headache
  - d. Fever
  - e. Stuffed nose
  - f. Cough
  - g. Wheezing
  - h. Sore throat
  - i. Muscle soreness
  - j. Nausea
  - k. Vomiting
  - l. Diarrhea
  - m. None of the above
  - n. Other\_\_\_\_\_
42. In the past week, did you miss any school for any reason?
- a. No
  - b. Yes. Specify\_\_\_\_\_
43. Have you experienced any adverse effects in the last week (any change in health other than a cold)?
- a. No
  - b. Not sure
  - c. Yes; Please describe your experience:\_\_\_\_\_



**McFlu2 COLD<sub>3</sub> Prevention: Daily Symptom Survey**

Please fill out this survey daily for 7 consecutive days following the beginning of your illness. If you want to do this on paper, you will need to print 7 copies of this. The survey is also available electronically, please email [mcflustudy@gmail.com](mailto:mcflustudy@gmail.com) for a link. You can consider 'Day 1' to be the first day that you noticed symptoms. Please also remember to collect a nasal swab daily for 7 days. If you need more nasal swabs, please visit us in front of Campus Health. Thanks!

**Study ID:** \_\_\_\_\_

To help you remember when you started this series of 7 consecutive surveys, please fill in the date of your first entry (day 1). Date: \_\_\_\_\_

Today's Date: \_\_\_\_\_

Please fill in one box for each of the following								
	Not Sick	Very mildly		Mildly		Moderately		Severely
	0	1	2	3	4	5	6	
How sick do you feel today?								
Please rate the average severity of your cold symptoms over the last 24 hours for each symptom:								
	Do not have this symptom	Very mild		Mild		Moderate		Severe
	0	1	2	3	4	5	6	7
Runny nose								
Plugged nose								
Sneezing								
Sore throat								
Scratchy throat								
Cough								
Hoarseness								
Head congestion								
Chest congestion								
Feeling tired								
Over the last 24 hours, how much has your cold interfered with your ability to:								
	Not at all	Very mildly		Mildly		Moderately		Severely
	0	1	2	3	4	5	6	7
Think clearly								
Sleep well								
Breathe easily								

Walk, climb stairs, exercise								
Accomplish daily activities								
Work outside the home								
Work inside the home								
Interact with others								
Live your personal life								
Compared to yesterday, I feel that my cold is...								
Very much better	Somewhat better	A little better	The same	A little worse	Somewhat worse	Very much worse		

**Additional Questions**

1. In the past 24 hours, did you have any of these symptoms? (select all that apply)
  - a. Fever
  - b. Diarrhea
  - c. Nausea
  - d. Vomiting
  
2. Did you seek medical attention for your symptoms in the last 24 hours?
  - a. No
  - b. Yes, at Campus Health
  - c. Yes, at another medical facility
  - d. Other \_\_\_\_\_
  
3. If you have asthma, has it changed since before you got sick?
  - a. Not applicable
  - b. No
  - c. Yes, it has gotten worse
  - d. Yes, it has gotten better
  - e. I don't know
  
4. Please rate the severity of your asthma over the last 24 hours

No asthma	Very mild 1	2	Mild 3	4	Moderate 5	6	Severe 7

5. In the last 24 hours, did you miss any school because of your illness?
  - a. No
  - b. Yes, I missed \_\_\_\_ classes

6. In the last 24 hours, has your illness affected your ability to study or do school work at home?
  - a. Not at all
  - b. A little bit
  - c. Quite a bit
  - d. A lot

**McFlu2 COLD<sub>3</sub> Prevention: Day 14 Follow-Up Survey**

44. Please enter your 6-digit study ID number

Please fill in one box for each of the following								
	Not Sick	Very mildly		Mildly		Moderately		Severely
	0	1	2	3	4	5	6	
How sick do you feel today?								
Please rate the average severity of your cold symptoms over the last 24 hours for each symptom:								
	Do not have this symptom	Very mild		Mild		Moderate		Severe
	0	1	2	3	4	5	6	7
Runny nose								
Plugged nose								
Sneezing								
Sore throat								
Scratchy throat								
Cough								
Hoarseness								
Head congestion								
Chest congestion								
Feeling tired								
Over the last 24 hours, how much has your cold interfered with your ability to:								
	Not at all	Very mildly		Mildly		Moderately		Severely
	0	1	2	3	4	5	6	7
Think clearly								
Sleep well								
Breathe easily								
Walk, climb stairs, exercise								
Accomplish daily activities								
Work outside the home								
Work inside the home								
Interact with others								
Live your								

personal life							
Compared to yesterday, I feel that my cold is...							
Very much better	Somewhat better	A little better	The same	A little worse	Somewhat worse	Very much worse	

45. During your illness, what symptoms did you experience? (select all that apply even if they were mild or did not last long)

- a. Fatigue
- b. Sleep disturbance
- c. Headache
- d. Fever
- e. Stuffed nose
- f. Cough
- g. Wheezing
- h. Sore throat
- i. Muscle soreness
- j. Nausea
- k. Vomiting
- l. Diarrhea
- m. Other\_\_\_\_\_

46. For how many days did your major symptoms last? \_\_\_\_\_

47. If you reported a fever, did you use a thermometer to measure this?

- a. Not applicable
- b. Yes
- c. No

48. Did you seek medical attention for your symptoms?

- a. No
- b. Yes, at Campus Health
- c. Yes, at another medical facility
- d. Other\_\_\_\_\_

49. Did you take any medication or use any remedies for your symptoms? (select all that apply)

- a. Prescription medication
- b. Over the counter medication (eg. Cold and sinus medication, medicated nasal spray, medicated throat lozenges)
- c. Herbal remedies
- d. Vitamins
- e. Other\_\_\_\_\_

50. For how many days did you use any medication or remedies?\_\_\_\_\_

51. Approximately how much money did you spend on medications or remedies for your illness? \_\_\_\_\_

52. Did you spend any days sick in bed or entirely at home?

- a. Yes,\_\_\_\_\_days

- b. No
53. During your illness, how many days of the following did you miss due to illness?
- Days of class\_\_\_\_\_
  - Days of work\_\_\_\_\_
  - Days with physical impairment\_\_\_\_\_
  - Days of social activity\_\_\_\_\_
  - Days of extra-curricular activities\_\_\_\_\_
54. If you are still experiencing symptoms, do you still have to miss classes or other activities?
- Not applicable
  - No, I am no longer missing any classes or activities
  - Yes, I am still missing all of my classes
  - Yes, I am still missing some of my classes
  - Yes, I am still missing all of my regular activities (sports, extra-curricular, social)
  - Yes, I am still missing some of my regular activities (sports, extra-curricular, social)
55. While you were ill, do you think your academic performance was negatively affected?
- Not at all
  - A little bit
  - Quite a lot
  - A lot
56. Do you think that your illness negatively affected your academic performance for the semester?
- Not at all
  - A little bit
  - Quite a bit
  - A lot
57. If you have asthma, was it affected by your illness?
- Not applicable
  - Yes, it was worse when I was sick
  - No, it did not change when I was sick
  - I don't know
58. If you have a chronic medical condition, was it affected by your illness?
- Not applicable
  - Yes, it was worse when I was sick
  - No, it did not change when I was sick
  - I don't know
  - I don't want to say
  - Specify \_\_\_\_\_
59. Is there anything else that you would like to tell us about how your life was impacted by your cold?
-

**McFlu2: COLD<sub>3</sub> Prevention****Extension Study Weekly Survey**

60. Please enter your 6-digit study ID number
61. Have you been ill in the past 7 days?
- Yes
  - No
  - Not Sure
  - Specify \_\_\_\_\_
62. Have any of your roommates or housemates been ill in the past week?
- Yes
  - No
  - Not applicable
63. In the past week, did you wash your hands (or use an alcohol-based hand cleanser) before each meal?
- Yes, always
  - Usually
  - No
  - Comments \_\_\_\_\_
64. In the past week, how many times did you wash your hands (or use an alcohol-based hand cleanser) in an average day?
- 0
  - 1
  - 2-3
  - 4-5
  - 6 or more
65. In the past week, did you gargle as part of your daily oral hygiene?
- No
  - Yes, occasionally
  - Yes, daily
  - Yes, twice daily
66. In the past week, how many hours of moderate exercise (you can speak with brief sentences, are sweating, and cannot sing a song) did you get over the entire week?
- 0 hours
  - 1-2 hours
  - 3-4 hours
  - 5 hours or more
67. How much sleep do you get on an average night?
- Less than 5 hours
  - 6 hours
  - 7 hours

- d. 8 hours
  - e. 9 hours or more
68. In the past week, what is the least amount of sleep that you had in one night?
- a. Less than 3 hours
  - b. 4 hours
  - c. 5 hours
  - d. 6 hours
  - e. 7 hours
  - f. 8 hours
  - g. 9 hours or more
  - h. Comments \_\_\_\_\_
69. Please indicate if you have experienced any of the following symptoms in the past week:
- a. No symptoms
  - b. Fatigue
  - c. Sleep disturbance
  - d. Headache
  - e. Fever
  - f. Stuffed nose
  - g. Cough
  - h. Wheezing
  - i. Sore throat
  - j. Muscle soreness
  - k. Nausea
  - l. Vomiting
  - m. Diarrhea
  - n. Other \_\_\_\_\_
70. In the past week, did you miss any school for any reason?
- a. No
  - b. Yes. Specify \_\_\_\_\_
71. Have you experienced any adverse effects in the last week (any change in health other than a cold)?
- a. No
  - b. Not sure
  - c. Yes; Please describe your experience: \_\_\_\_\_
72. Have you received your seasonal flu vaccination for this year?
- a. Yes
  - b. Not yet but I intend to
  - c. No, I am not sure if I will get vaccinated
  - d. No, I will not get vaccinated
73. Last year, did you receive either or both of the seasonal and H1N1 flu vaccinations?
- a. I received both



- b. I received only the H1N1 flu vaccination
- c. I received only the seasonal flu vaccination
- d. I received neither flu vaccination
- e. I don't remember

## APPENDIX 2

This appendix contains a manuscript presenting a systematic review and meta-analysis of randomized controlled trials evaluating vitamin D3 supplementation for the prevention of acute respiratory infection. This manuscript was developed from the final project submitted to the HRM 743 “Systematic Review Methods”. The manuscript is intended for submission for peer review and publication.

**Vitamin D for prevention of acute respiratory infections: a systematic review and meta-analysis of randomized controlled trials**

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**Abstract:** 267 words

**Text only:** 2833

**Figures:**5

**Tables:** 5

**Appendices:** 7

**Abstract**

**Background:** Vitamin D3 supplementation is hypothesized to prevent acute respiratory infections (ARI), however results from randomized controlled trials (RCTs) are conflicting. We conducted a systematic review and meta-analysis to evaluate the efficacy and safety of vitamin D3 for this purpose. **Methods:** We searched electronic databases and other sources for studies through February 13, 2013. We included all RCTs comparing orally administered vitamin D3 to placebo or no treatment. Two reviewers independently assessed studies for inclusion, performed data extraction and assessed risk-of-bias. Meta-analyses were conducted using random-effects models. Our primary outcome was the number of participants who had an ARI. Secondary outcomes included the number of participants with laboratory confirmed ARI, symptom duration and severity, and adverse events. **Results:** Eight trials (N=2273) were included in the systematic review and seven trials (N=2133) could be combined in the meta-analysis. Vitamin D3 reduced the risk of having ARI, however this was not statistically significant (RR: 0.69; CI<sub>95%</sub>: 0.45, 1.05; p=0.08) and heterogeneity was high (I<sup>2</sup>=94%). Similarly, vitamin D3 supplementation appeared to reduce the risk of having a laboratory confirmed ARI, however this was not statistically significant (RR: 0.72; CI<sub>95%</sub>: 0.40, 1.27; p=0.25), and heterogeneity was high (I<sup>2</sup>=86%). Vitamin D3 did not affect symptom severity (standardized mean difference: 0.04; CI<sub>95%</sub>: -0.28, 0.35; p=0.81), illness duration (mean difference: -0.18; CI<sub>95%</sub>: -0.71, 0.35; p=0.50), or occurrence of adverse events (RR: 1.05; CI<sub>95%</sub>: 0.79, 1.40; p=0.72). **Interpretation:** Results from our meta-analysis suggest that vitamin D3 may reduce the risk of clinical and laboratory confirmed ARI. Due to significant heterogeneity, large, methodologically rigorous trials are needed to inform clinical practice.

## Background

Acute respiratory infections (ARI), which often present clinically as the common cold, are among the most common human illness.[1, 2] Multiple respiratory viruses are known to cause symptomatic episodes of illness of variable severity.[2-4] Although the majority of infections are mild, ARI can exacerbate existing medical conditions and can lead to significant morbidity and workplace absenteeism.[3, 5-7] Furthermore, ARI exert a tremendous burden on the healthcare system and are associated with millions of visits to primary caregivers.[7] These data suggest that ARI should not be considered a benign health condition and that more should be done to improve the treatment and prevention of these infections.

Currently, ARI prevention focuses on hand hygiene with some consideration given to lifestyle factors such as exercise, sleep and smoking habits. Vitamin D3 has been the recent focus of many observational and experimental studies investigating its role in respiratory health. [8-24] Although the exact mechanism is unclear, vitamin D has been shown to play a role in the innate immune response by stimulating the production of antimicrobial peptides and by enhancing microbicidal action of monocytes and macrophages.[25, 26]

A recent meta-analysis of randomized controlled trials concluded that vitamin D3 was effective at reducing the occurrence of ARI. [27] Since then, there have been several new trials with conflicting results, highlighting the need for an updated systematic review and meta-analysis. Consequently, we conducted a systematic review and meta-analysis to evaluate the efficacy and safety of vitamin D3 for the prevention of ARI. Our study improves upon the published meta-analysis [27] by comprehensively searching multiple databases, identifying new trials, including information about adverse events, using *a priori* subgroup analyses to explore heterogeneity and using the GRADE guidelines to evaluate the quality of evidence.

## **Methods**

### Eligibility Criteria

We included all randomized controlled trials in humans comparing oral vitamin D3 supplementation to placebo or no treatment for the prevention of ARIs. Studies were excluded if they used vitamin D3 as an adjunct treatment rather than prophylaxis, if vitamin D3 was part of a compound preparation (e.g. multivitamin), if ultraviolet light exposure was the source of vitamin D3 or if the study used oral supplementation with vitamin D2. ARI was defined according to the study authors. Studies were excluded if they evaluated lower respiratory tract infections (e.g. pneumonia), tuberculosis or other chronic respiratory infections. No limitations were placed on dose, frequency of dose, or duration of supplementation. The primary outcome was the number of participants who experienced an ARI in each trial arm. Secondary outcomes included the number of participants who experienced a laboratory confirmed ARI (symptoms with an identified viral etiology) in each trial arm, symptom severity, symptom duration and the occurrence of any adverse events.

### Literature Search

We searched MEDLINE (1946 to February 13, 2012), Embase (1947 to Week 6, 2013), CINAHL (1983 to February 13, 2013), AMED (1985 to February 2013) and the Cochrane Central Register of Controlled Trials (until January 2013) and CAB (Commonwealth Agricultural Bureaux Abstracts, 1973 to Week 5, 2013). Conference proceedings, clinical trials registries ([www.clinicaltrials.gov](http://www.clinicaltrials.gov), [www.anzctr.org.au/](http://www.anzctr.org.au/), [www.clinicaltrialsregister.eu](http://www.clinicaltrialsregister.eu), [www.controlled-trials.com](http://www.controlled-trials.com)) and reference lists from key articles were also reviewed for relevant entries. No restrictions were placed on language or year of publication and databases were searched for

records indexed by February 13, 2013. Details of the final search strategies are available in Appendix 1.

#### Study Selection and Data Extraction

Two reviewers (E.G., M.S.) independently screened the titles and abstracts of all unique articles. All potentially relevant articles were independently reviewed in full. A third reviewer was available in the event that any discrepancies could not be resolved by consensus. Agreement between reviewers was calculated using Cohen's Kappa coefficient, based on results from the full text review. Data extraction was completed in duplicate for eligible studies. When necessary, study authors were contacted to clarify or provide information. (Appendix 2)

#### Assessment of Bias

The Cochrane risk-of-bias tool was used by reviewers to independently assess all included trials.[28] Disagreement was resolved by consensus. The GRADE approach (Grading of Recommendations Assessment, Development and Evaluation) was used to assess the risk of bias for each outcome.[29] Reviewers first considered the included studies independently, and then assigned a measure of overall quality of evidence for each outcome after discussion.

#### Statistical Analyses

For continuous outcomes, means and standard deviations were recorded. When studies reported data as other values (e.g. medians and interquartile range [IQR]), and authors were unable or unwilling to provide additional information, methods outlined in the literature and in the Cochrane handbook were used to estimate mean and standard deviation values.[28, 30]

Statistical analyses were performed using Review Manager software (RevMan, version 5.2.3).[31]

Data were pooled using a random-effects model due to anticipated heterogeneity. Pooled dichotomous data were summarized with risk ratios. Continuous data were pooled using mean differences when the measurement scale was the same across studies (duration of symptoms).

When the scale varied, continuous data were pooled using standardized mean differences.

A statistical assessment of heterogeneity was performed using a  $X^2$  test (defining statistical significance if  $p < 0.10$ ) and the  $I^2$  statistic was used to quantify the effect of heterogeneity on the variability in an effect estimate.[28] *A priori* subgroup analyses were implemented to explore substantial heterogeneity ( $I^2 > 40\%$ ). Significance of subgroup effects was tested with a  $X^2$  test, and subgroup validity was assessed according to the eleven criteria presented by Sun *et al.*[32] (Appendix 3). *A priori* subgroups were proposed to explore age (children  $< 17$  years vs. adults  $\geq 17$  years), dosing regimen (daily versus non-daily), and risk of bias in the trial (high versus low), and type of infection (naturally acquired versus experimentally induced). Subgroup analyses were only conducted if there were at least two studies in each subgroup.

Meta-regression was planned *a priori* to further explore heterogeneity, using Stata Statistical Software release 12 (StataCorp. 2011. College Station, TX: StataCorp LP), to assess the effect of continuous variables including vitamin D3 dose, duration of supplementation, and baseline serum vitamin D levels.[33]

A sensitivity analysis was planned *a priori* to assess the potential risk of bias resulting from missing data.

## Results



We screened the titles and abstracts of 453 unique records identified through our search. Thirty-four were identified for full text review. Of those, eight were included in the systematic review, and seven were included in the meta-analysis (Figure 1). Cohen's kappa coefficient for agreement between the reviewers was 1.0.

### Study Characteristics

Eight studies (n= 2273) met the inclusion criteria for our systematic review however, one trial did not report data in a form which could be used in any part of our meta-analysis (Table 1). [34] Data from seven trials involving 2133 participants, ranging in age from 6 to 80 years, were included in our meta-analysis.[15, 17-19, 21, 22, 24] Two studies were conducted in pediatric populations and no study included both children and adults.[17, 24] Two studies were post-hoc analyses of trials using vitamin D3 supplementation for primary outcomes other than the prevention of respiratory infections.[15, 17] Six studies reported data on the prevention of any acute respiratory infection [15, 17-19, 21, 22], one reported an annual composite infection score [34], and one reported specifically on the prevention of influenza [24]. All studies assessed naturally acquired infections. Dosing regimens ranged from daily supplementation to monthly doses and the duration of supplementation and dose varied across trials from 7 weeks to 36 months.

The risk of bias assessment of each study is presented in Table 2 and an overall summary of risk of bias is presented in Appendix 4. All trials reported appropriate methods of randomization, and blinding of the participants and study personnel with aesthetically matched placebos. Five studies reported using coded medication containers for concealment however three did not explicitly address concealment. Many studies did not report methods for dealing with missing data and the proportion of missing data ranged from 1 - 36.6%. When possible, trial protocols were compared to published results to assess the potential risk of selective reporting. The risk of bias for each

outcome is presented in Table 3 and a detailed GRADE Evidence Profile is available in Appendix 5.

#### Incidence of ARI

Data was pooled for seven trials (n=2133) that reported the number of participants who experienced at least one ARI (Figure 2).[15, 17-19, 22, 24] For two trials, these data were calculated from the reported number of participants who remained healthy over the course of the study.[19, 22] The pooled estimate favored the vitamin D3 supplementation, however, the results were not statistically significant (RR: 0.69; 95% CI: 0.45, 1.05; p=0.08). The very high degree of heterogeneity ( $I^2=94%$ ,  $p<0.001$ ) and the high risk of bias in some trials, resulted in a low quality of evidence rating for this outcome (Table 3, Appendix 5).

The *a priori* subgroup analyses did not substantially explain the observed heterogeneity and no subgroup effects were statistically significant. Application of subgroup criteria developed by Sun et al.[32], suggested that the credibility of the subgroups tested was low (Appendix 3). However, the benefit of vitamin D3 supplementation appeared to be greater in children (RR: 0.59, 95% CI: 0.45, 0.77) compared to adults (RR: 0.74, 95% CI: 0.48-1.14), and when given as daily doses (RR: 0.65, 95% CI: 0.50, 0.83) compared to weekly or monthly doses (RR: 0.90, 95% CI: 0.57,1.42). Complete results from these analyses are summarized in Table 4 and presented in Appendix 6. All infections were naturally acquired and therefore the planned subgroup analysis was not possible to compare these infections with those that are experimentally induced.

#### Incidence of laboratory confirmed ARI

Three trials [18, 22, 24] involving 1352 participants sought laboratory confirmation for ARIs. Among these trials, vitamin D3 supplementation non-significantly reduced the relative risk of ARI (RR: 0.72; 95% CI: 0.40, 1.27; p=0.25) (Figure 3). Heterogeneity was high ( $I^2= 86\%$ , p=0.001) and the quality of evidence was judged to be low (Table 3, Appendix 5).

#### Symptom Severity

Symptom severity was measured in three trials (n=1084) and data could be combined from two trials involving 762 participants (Figure 4). The excluded trial reported median severity for all recorded episodes and it was not possible to account for the effect of repeated episodes per participant.[22] Symptom severity was not statistically significantly different between the vitamin D3 and control groups (standardized mean difference: 0.04; 95%CI:-0.28, 0.35; p=0.81). Heterogeneity was low ( $I^2= 6\%$ , p=0.30) but results from the two available studies were associated with substantial imprecision represented by wide confidence intervals and the overall quality of evidence for this outcome was judged to be moderate (Table 3, Appendix 5).

#### Symptom Duration

Symptom duration was measured in three trials (n=1083) and data could be combined from only two trials (n=762) (Figure 5). There was no statistically significant difference in symptom duration between the vitamin D3 and control groups (mean difference: -0.18; 95%CI: -0.71, 0.35; p=0.50). Heterogeneity was low ( $I^2= 0\%$ , p=0.79) and the overall quality of evidence for this outcome was moderate (Table 3, Appendix 5).

#### Adverse Events

Four trials (n=1248) reported data on adverse events, however little or no distinction was made between mild and severe adverse events, or those which lead to discontinuation of the intervention. The pooled estimate demonstrated that there was no statistically significant difference in the relative risk of having any adverse event between the intervention and control groups (RR: 1.05; 95% CI: 0.79, 1.40; p=0.72). Heterogeneity was low ( $I^2=0\%$ , p=0.76) and the overall quality of evidence for this outcome was judged to be moderate (Table 3, Appendix 5) because of imprecision in the summary estimate and the risk of bias in the included trials. Subgroup analyses were not conducted due to the minimal heterogeneity.

#### Sensitivity analyses for missing data

The proportion of missing data in each trial ranged from approximately 1 to 37 % and the approaches to dealing with missing data also differed (Table 2). Reasons for drop-out or loss-to-follow-up were infrequently reported and no study commented on the timing of drop-out or loss-to-follow-up relative to whether or not a participant had reported an infection. We chose to run sensitivity analyses to assess the potential impact of missing data on the effect estimate. This analysis reflected varying amounts of potential benefit associated with the intervention based on assumptions from the incidence in the published trials (Appendix 7).[35]

Applying these various assumptions to the original data, the primary outcome did not demonstrate any statistically significant results. The pooled estimate of effect always favored vitamin D3, even when assuming that the rate of events among participants lost to follow-up in the intervention arm was the same as that in the control arm (Table 5).

#### Meta-regression

Results from meta-regression analyses are at significant risk of being underpowered and unlikely to be useful when fewer than ten studies are included. [36] Since fewer than ten studies were available for the proposed meta-regression, it was not conducted.

## **Discussion**

We found that oral vitamin D3 supplementation may reduce the risk of having an acute respiratory infection, however this was not statistically significant (RR: 0.69; 95% CI: 0.45, 1.05). These findings must be interpreted with caution as there was considerable heterogeneity ( $I^2=94\%$ ) and low quality of evidence for this outcome. Three *a priori* subgroup analyses were conducted, however heterogeneity remained significant in at least one subgroup of each analysis, no subgroup effects were statistically significant, and subgroup credibility was low. These subgroup analyses may, however, be helpful for hypothesis generation for future studies. The benefit of vitamin D3 supplementation appeared to be greater in children (RR: 0.59, 95% CI: 0.45, 0.77) than in adults (RR: 0.74, 95% CI: 0.48, 1.14). Similarly, the benefit of daily supplementation appeared to be greater than weekly or monthly supplementation (RR: 0.65, 95%CI: 0.50, 0.83 versus RR: 0.90, 95% CI: 0.57,1.42 respectively). Future trials may benefit from considering the impact of these factors on the effect of vitamin D3 for the prevention of ARI. Analysis of laboratory confirmed ARI also suggested a protective benefit associated with vitamin D3 supplementation, however this was also not statistically significant.

There were no significant differences in symptom duration or severity however these outcomes were infrequently reported and further investigation in future trials and meta-analyses should be undertaken. Similarly, there was no significant difference in the number of adverse events reported in the vitamin D3 group compared to the control group. This is not surprising given the very low risk profile associated with vitamin D3 supplementation.[37]

To date, only two meta-analyses on the same topic have been published.[27, 38] Charan *et al.* combined data from five available studies and reported that vitamin D3 significantly reduced the odds of ARI by 42% (OR: 0.52, 95% CI: 0.42, 0.81).[27] However, the authors did not account for correlated, repeated events within individuals reported in one study and this may have affected the overall tests of statistical significance. Bergman *et al.* included 11 studies of children and adults which considered upper and lower respiratory tract infections.[38] The authors reported that vitamin D3 use was associated with a statistically significant 36% reduction in the odds of infection (OR: 0.64, 95% CI: 0.49, 0.84,  $I^2=72\%$ ).[38] Our analysis differed by using more restrictive inclusion criteria. To this end, we excluded studies that reported composite endpoints which allegedly reflected respiratory infections, studies that reported exacerbations of respiratory conditions but did not directly record evidence of respiratory infection, studies that included data from incomplete trials, and studies of pneumonia. Unlike the previous meta-analyses, our analysis of seven trials yielded a non-significant result, however the effect estimate did favor vitamin D3 supplementation. Two reasons may explain this discrepancy. First, our results may reflect the influence of a single, large trial which demonstrated no benefit associated with vitamin D3 supplementation for the prevention of ARI in healthy adults.[22] This trial was the only study to use large bolus doses of 100,000 IU monthly and it has previously been hypothesized that daily doses of vitamin D3 may be more physiologically relevant than large bolus doses.[39] Indeed, a post hoc analysis excluding this trial did indicate a statistically significant 32% reduction in the risk of infection associated with vitamin D3 (OR: 0.68, 95% CI: 0.56, 0.84,  $I^2=42\%$ ) Exclusion of this study also remarkably reduced heterogeneity between studies. Similarly, the benefit of vitamin D3 was statistically significant for the prevention of laboratory confirmed infections (OR: 0.56, 95% CI: 0.39, 0.78,  $I^2=0\%$ ). A second reason for the differences in statistical significance between meta-analyses may be related to the choice of summary statistic. Charan *et al.*, and

Bergman *et al.*, both reported odds ratios which are often interpreted as risk ratios.[40] In these cases, the odds ratio always overestimates the true risk ratio, and this is made worse with frequently occurring outcomes.[40] Notably, rerunning our meta-analysis and presenting the pooled data as an odds ratio instead of a risk ratio also identified a statistically significant effect. In this case, vitamin D3 supplementation was associated with a 44% reduction in the odds of infection (OR: 0.56, 95% CI: 0.42, 0.73,  $I^2=25\%$ ).

Our study has several limitations. The definition of an acute respiratory infection was different in each trial and this may have contributed to the considerable heterogeneity detected. It is possible that many different pathogens were the cause of ARI in different trials and prophylactic vitamin D3 supplementation may impact disparate pathogens differently. Too few studies reported viral etiology to allow investigation of this hypothesis. Furthermore, the variation in the definition of ARI may have affected the magnitude of the effect since less precise definitions may have allowed for incorrectly classified events. Indeed, analysis of a subset of trials reporting laboratory confirmed ARI, a more precise measure, did reveal a significant protective benefit associated with vitamin D3. Studies included in this meta-analysis used a wide range of doses and varying frequency which may have contributed to the high degree of heterogeneity. Unfortunately, meta-regression could not be conducted to assess the impact of dose due to the small number of trials. Similarly, too few studies were available to investigate the reported baseline serum vitamin D levels and meta-regression exploring the impact of this variable on the overall effect could not be conducted. It could be that vitamin D3 supplementation might be the most beneficial in those individuals with the greatest deficiencies.

Our findings support the growing body of literature that proposes vitamin D3 as a potential intervention for the prevention of ARI. Prophylactic supplementation with vitamin D3 appeared

beneficial, though not statistically significant, for the prevention of clinical and laboratory confirmed ARI. Given the high incidence, significant morbidity, burden on the healthcare system, and economic burden associated with ARI[2, 6, 7], even a small improvement in prevention could have a substantial benefit. Future studies should strive to be sufficiently large to provide definitive results and should be designed to evaluate various dosing strategies and the potential differences in effect in various patient populations.



## References

1. Proud D: **Upper airway viral infections.** *Pulmonary pharmacology & therapeutics* 2008, **21**(3):468-473.
2. Monto AS: **Epidemiology of viral respiratory infections.** *The American journal of medicine* 2002, **112 Suppl 6A**:4S-12S.
3. Graham NM: **The epidemiology of acute respiratory infections in children and adults: a global perspective.** *Epidemiologic reviews* 1990, **12**:149-178.
4. Lessler J, Reich NG, Brookmeyer R, Perl TM, Nelson KE, Cummings DA: **Incubation periods of acute respiratory viral infections: a systematic review.** *The Lancet infectious diseases* 2009, **9**(5):291-300.
5. Bramley TJ, Lerner D, Sames M: **Productivity losses related to the common cold.** *Journal of occupational and environmental medicine / American College of Occupational and Environmental Medicine* 2002, **44**(9):822-829.
6. Fendrick AM, Monto AS, Nightengale B, Sarnes M: **The economic burden of non-influenza-related viral respiratory tract infection in the United States.** *Arch Intern Med* 2003, **163**(4):487-494.
7. Kwong JC, Crowcroft NS, Campitelli MA, Ratnasingham S, Daneman N, Deeks SL, Manuel DG: **Ontario Burden of Infectious Disease Study (ONBOIDS): An OAHPP/ICES Report.** In. Toronto: Ontario Agency for Health Protection and Promotion, Institute for Clinical and Evaluative Sciences; 2010.

8. Wayse V, Yousafzai A, Mogale K, Filteau S: **Association of subclinical vitamin D deficiency with severe acute lower respiratory infection in Indian children under 5 y.** *Eur J Clin Nutr* 2004, **58**(4):563-567.
9. Laaksi I, Ruohola JP, Tuohimaa P, Auvinen A, Haataja R, Pihlajamaki H, Ylikomi T: **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):714-717.
10. Karatekin G, Kaya A, Salihoglu O, Balci H, Nuhoglu A: **Association of subclinical vitamin D deficiency in newborns with acute lower respiratory infection and their mothers.** *Eur J Clin Nutr* 2009, **63**(4):473-477.
11. McNally JD, Leis K, Matheson LA, Karuananyake C, Sankaran K, Rosenberg AM: **Vitamin D deficiency in young children with severe acute lower respiratory infection.** *Pediatric pulmonology* 2009, **44**(10):981-988.
12. Ginde AA, Mansbach JM, Camargo CA, 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):384-390.
13. Roth DE, Shah R, Black RE, Baqui AH: **Vitamin D status and acute lower respiratory infection in early childhood in Sylhet, Bangladesh.** *Acta paediatrica (Oslo, Norway : 1992)* 2010, **99**(3):389-393.

14. Sabetta JR, DePetrillo P, Cipriani RJ, Smardin J, Burns LA, Landry ML: **Serum 25-hydroxyvitamin d and the incidence of acute viral respiratory tract infections in healthy adults.** *PLoS One* 2010, **5**(6):e11088.
15. Aloia JFL-N, M.: **Re: epidemic influenza and vitamin D.** *Epidemiology and Infection* 2007, **135**(7):1095-1096.
16. Avenell AC, J. A.; MacLennan, G. S.; Macpherson, G. C.: **Vitamin D supplementation to prevent infections: a sub-study of a randomised placebo-controlled trial in older people (RECORD trial, ISRCTN 51647438).** *Age and ageing* 2007, **36**(5):574-577.
17. Camargo CA, Jr.; Ganmaa, Davaasambuu; Frazier, A. Lindsay; Kirchberg, Franca F.; Stuart, Jennifer J.; Kleinman, Ken; Sumberzul, Nyamjav; Rich-Edwards, Janet W.: **Randomized trial of vitamin D supplementation and risk of acute respiratory infection in Mongolia.** *Pediatrics* 2012, **130**(3):e561-567.
18. Goodall E, Granados A, Luinstra K, Pulleneyagum E, Coleman B, Loeb M, Smieja M: **Vitamin D3 and gargling for the prevention of upper respiratory tract infections: a randomized controlled trial.** In. In Review; 2013.
19. Laaksi IR, Juha-Petri; Mattila, Ville; Auvinen, Anssi; Ylikomi, Timo; Pihlajamaki, Harri: **Vitamin D supplementation for the prevention of acute respiratory tract infection: a randomized, double-blinded trial among young Finnish men.** *J Infect Dis* 2010, **202**(5):809-814.
20. Linday LAS, Richard D.; Tapia-Mendoza, Juan; Dolitsky, Jay N.: **Effect of daily cod liver oil and a multivitamin-mineral supplement with selenium on upper**

- respiratory tract pediatric visits by young, inner-city, Latino children: randomized pediatric sites.** *Ann Otol Rhinol Laryngol* 2004, **113**(11):891-901.
21. Li-Ng MA, J. F.; Pollack, S.; Cunha, B. A.; Mikhail, M.; Yeh, J.; Berbari, N.: **A randomized controlled trial of vitamin D3 supplementation for the prevention of symptomatic upper respiratory tract infections.** *Epidemiol Infect* 2009, **137**(10):1396-1404.
22. Murdoch DRS, Sandy; Chambers, Stephen T.; Jennings, Lance C.; Stewart, Alistair W.; Priest, Patricia C.; Florkowski, Christopher M.; Livesey, John H.; Camargo, Carlos A.; Scragg, Robert: **Effect of vitamin D3 supplementation on upper respiratory tract infections in healthy adults: the VIDARIS randomized controlled trial.** *Jama* 2012, **308**(13):1333-1339.
23. Rehman PK: **Sub-clinical rickets and recurrent infection.** *Journal of tropical pediatrics* 1994, **40**(1):58.
24. Urashima MS, Takaaki; Okazaki, Minoru; Kurihara, Mana; Wada, Yasuyuki; Ida, Hiroyuki: **Randomized trial of vitamin D supplementation to prevent seasonal influenza A in schoolchildren.** *American Journal of Clinical Nutrition* 2010, **91**(5):1255-1260.
25. Wang TT, Nestel FP, Bourdeau V, Nagai Y, Wang Q, Liao J, Tavera-Mendoza L, Lin R, Hanrahan JW, Mader S *et al*: **Cutting edge: 1,25-dihydroxyvitamin D3 is a direct inducer of antimicrobial peptide gene expression.** *Journal of immunology (Baltimore, Md : 1950)* 2004, **173**(5):2909-2912.

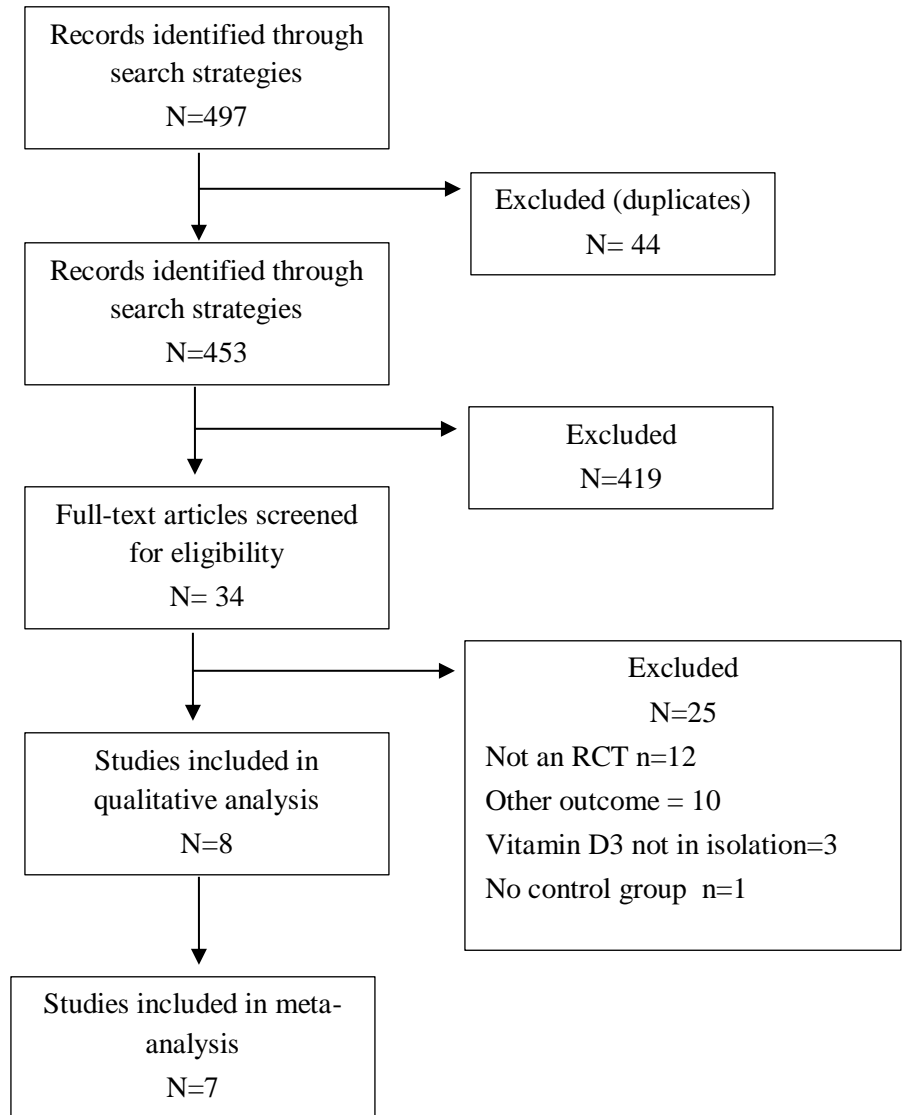
26. Liu PT, Stenger S, Li H, Wenzel L, Tan BH, Krutzik SR, Ochoa MT, Schaubert J, Wu K, Meinken C *et al*: **Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response**. *Science* 2006, **311**(5768):1770-1773.
27. Charan J, Goyal JP, Saxena D, Yadav P: **Vitamin D for prevention of respiratory tract infections: A systematic review and meta-analysis**. *Journal of pharmacology & pharmacotherapeutics* 2012, **3**(4):300-303.
28. Collaboration TC (ed.): **Cochrane Handbook for Systematic Reviews of Interventions**; 2011.
29. Guyatt GH, Oxman AD, Vist GE, Kunz R, Falck-Ytter Y, Alonso-Coello P, Schunemann HJ: **GRADE: an emerging consensus on rating quality of evidence and strength of recommendations**. *BMJ* 2008, **336**(7650):924-926.
30. Hozo SP, Djulbegovic B, Hozo I: **Estimating the mean and variance from the median, range, and the size of a sample**. *BMC medical research methodology* 2005, **5**:13.
31. **Review Manager (RevMan)**. In., 5.2.3 edn. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration; 2011.
32. Sun X, Briel M, Walter SD, Guyatt GH: **Is a subgroup effect believable? Updating criteria to evaluate the credibility of subgroup analyses**. *BMJ* 2010, **340**:c117.
33. StataCorp: **Stata Statistical Software: Release 12**. In. College Station, TX: StataCorp LP; 2011.

34. Bergman PN, A. C.; Hansen, S.; Rekha, R. S.; Agerberth, B.; Bjorkhem-Bergman, L.; Ekstrom, L.; Lindh, J. D.; Andersson, J.: **Vitamin D3 supplementation in patients with frequent respiratory tract infections: a randomised and double-blind intervention study.** *BMJ open* 2012, **2**(6).
35. Akl EA, Johnston BC, Alonso-Coello P, Neumann I, Ebrahim S, Briel M, Cook DJ, Guyatt GH: **Addressing dichotomous data for participants excluded from trial analysis: a guide for systematic reviewers.** *PLoS One* 2013, **8**(2):e57132.
36. Baker WL, White CM, Cappelleri JC, Kluger J, Coleman CI: **Understanding heterogeneity in meta-analysis: the role of meta-regression.** *International journal of clinical practice* 2009, **63**(10):1426-1434.
37. Hathcock JN, Shao A, Vieth R, Heaney R: **Risk assessment for vitamin D.** *Am J Clin Nutr* 2007, **85**(1):6-18.
38. Bergman P, Lindh ÅU, Björkhem-Bergman L, Lindh JD: **Vitamin D and Respiratory Tract Infections: A Systematic Review and Meta-Analysis of Randomized Controlled Trials.** *PLoS ONE* 2013, **8**(6):e65835.
39. Stoll D, Dudler J, Lamy O, Hans D, Krieg MA, Aubry-Rozier B: **Can one or two high doses of oral vitamin D3 correct insufficiency in a non-supplemented rheumatologic population?** *Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA* 2013, **24**(2):495-500.
40. Knol MJ, Le Cessie S, Algra A, Vandenbroucke JP, Groenwold RH: **Overestimation of risk ratios by odds ratios in trials and cohort studies:**

**alternatives to logistic regression.** *CMAJ : Canadian Medical Association*

*journal = journal de l'Association medicale canadienne* 2012, **184**(8):895-899.

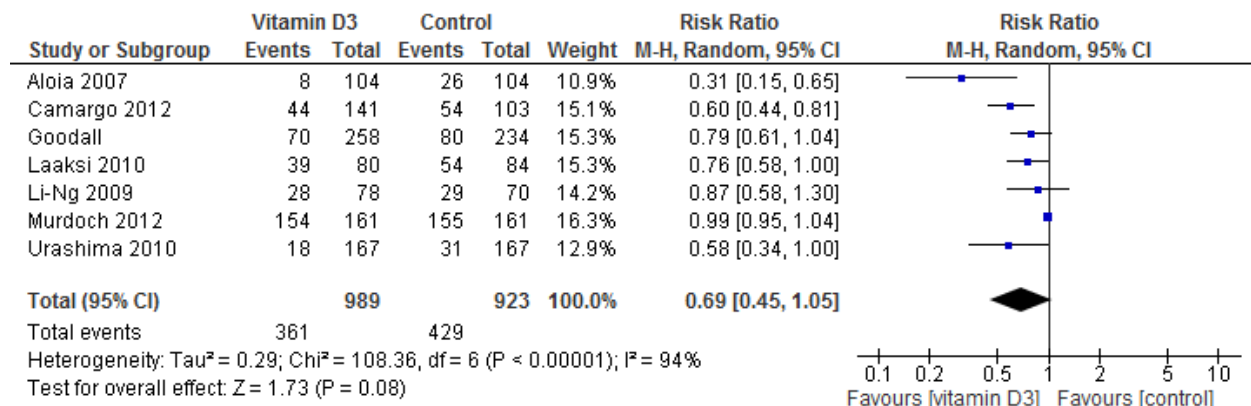
41. Aloia JF, Talwar SA, Pollack S, Yeh J: **A randomized controlled trial of vitamin D3 supplementation in African American women.** *Arch Intern Med* 2005, **165**(14):1618-1623.
42. Rich-Edwards JW, Ganmaa D, Kleinman K, Sumberzul N, Holick MF, Lkhagvasuren T, Dulguun B, Burke A, Frazier AL: **Randomized trial of fortified milk and supplements to raise 25-hydroxyvitamin D concentrations in schoolchildren in Mongolia.** *Am J Clin Nutr* 2011, **94**(2):578-584.

**FIGURES**

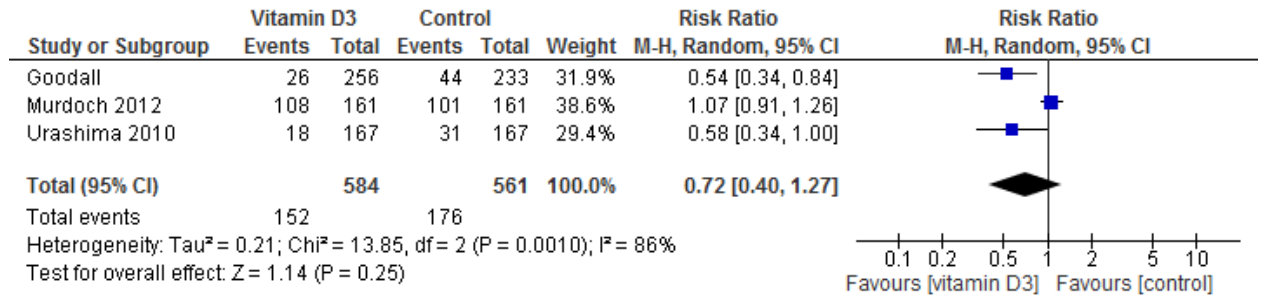
**Figure 1.** Study selection process for inclusion in the qualitative and quantitative analysis.

RCT: randomized controlled trial

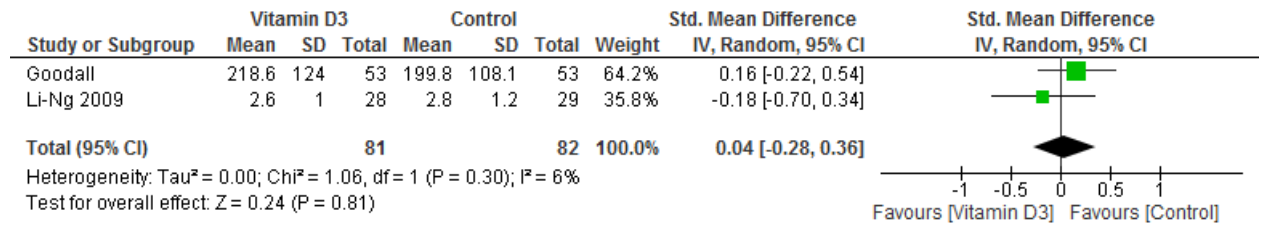




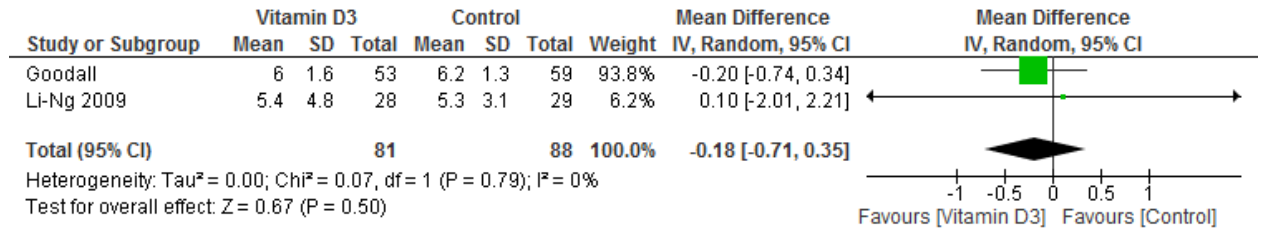
**Figure 2.** Meta-analysis of the number of individuals with at least one infection in randomized controlled trials of oral vitamin D3 supplementation for the prevention of acute respiratory infection. A pooled relative risk of less than one represents benefit from vitamin D3 supplementation. RR= relative risk, CI= confidence interval, df= degrees of freedom



**Figure 3.** Meta-analysis of the number of individuals with at least one laboratory confirmed infection in randomized controlled trials of oral vitamin D3 supplementation for the prevention of acute respiratory infection. A pooled relative risk of less than one represents benefit from vitamin D3 supplementation. RR= relative risk, CI= confidence interval, df= degrees of freedom



**Figure 4.** Meta-analysis of the severity of symptoms in randomized controlled trials of oral vitamin D3 supplementation for the prevention of acute respiratory infection. A pooled standardized mean difference less than zero represents benefit from vitamin D3 supplementation. CI= confidence interval, df= degrees of freedom



**Figure 5.** Meta-analysis of symptom duration in randomized controlled trials of oral vitamin D3 supplementation for the prevention of acute respiratory infection. A pooled mean difference less than zero represents benefit from vitamin D3 supplementation. CI= confidence interval, df= degrees of freedom

**Table 1.** Study characteristics of randomized controlled trials included in the qualitative analysis

Study	Country	Study Period	Population	Sample Size (# completed)	Infection Outcome	Definition of Event	Vitamin D3 administration, dose (mean dose/day)	Duration of Treatment & Follow-Up*	Post-Hoc Analysis of another Trial	Funding
<b>Studies included in the meta-analysis</b>										
Aloia & Li-Ng, 2007[15]	United States	NR	Postmenopausal African American women	208 (148)	Any self-reported ARI	Participant's perception of having had a cold or flu	Tablets; 800 IU/day x 24 months, 2000 IU/day x 12 months (1200 IU/day)	36 months	Yes (Aloia et al., 2005)[41]	Non-industry
Camargo et al., 2012 [17]	Mongolia	January–March 2009	Children, age 9-11 years	247 (244)	Parent reported ARI or chest infection	Parental perception of child experiencing a chest infection of cold with symptoms persisting $\geq 24$ hours	Fortified milk, 300 IU/day (~280 IU/day) †	7 weeks	Yes (Rich-Edwards et al., 2011)[42]	Non-industry, and in-kind industry support, and anonymous foundation funding
Goodall et al.,	Canada	September-	University students, age	600	Any self-	Participant's	Capsules, 10,000	8 weeks	No	Non-

unpublished [18]		October 2010 & 2011	≥17 years	(492)	reported ARI & laboratory confirmed ARI	perception of a cold & ≥2 symptoms <sup>‡</sup>	IU/week (1430 IU/day)			industry
Laaksi et al., 2010 [19]	Finland	October 2005 -	Male military recruits, age 18-28 years	164 (104)	Medically diagnosed ARI <sup>‡</sup>	Medically diagnosed ARI <sup>‡</sup>	Capsules, 400 IU/day (400 IU/day)	6 months	No	Non-industry
Li-Ng et al., 2009 [21]	United States	December 2006- June 2007	Adults, age 18-80 years	162 (148)	Any self-reported ARI	Participant's perception & ≥1 symptom <sup>€</sup>	Tablets, 2000 IU/day (2000 IU/day)	12 weeks	No	Non-industry
Murdoch et al., 2012 [22]	New Zealand	February 2010 – November 2011	Adults, age ≥ 18 years	322 (304)	Any ARI	Sudden onset of ≥1 symptom <sup>€</sup>	Tablets, 200,000 IU/month x 2 months, then 100,000 IU/month x 16 months (3649 IU/day)	18 months	No	Non-industry
Urashima et al., 2010 [24]	Japan	December 2008- March 2009	Children, age 6-15 years	430 (334)	Laboratory confirmed influenza	Laboratory confirmed influenza A	Tablet, 600 IU/ twice daily (1200	4 months	No	Non-industry

					a A infection	infection	IU/day)			
<b>Studies excluded from the meta-analysis</b>										
Bergman et al., 2012 [34]	Sweden	March 2010 – June 2011	Adults, age 18- 75 years receiving care from the Immunodeficiency Unit	140 (124)	Annual composite infectious score	Composite infectious score <sup>o</sup>	Oil, 4000 IU/day  (4000 IU/day)	12 months	No	Non- Industry and in- kind industry support

NR= not reported; ARI= acute respiratory infection; NA= not applicable

\*Within each study, duration of treatment and follow-up were the same

<sup>o</sup> Composite score accounted for presence of symptoms (respiratory tract, ears and sinuses, malaise), use of antibiotics and incidence of pneumonia

† Introduction of fortified and control milk was introduced gradually over the course of the first five days

£ Runny/stuffy nose, congestion, cough, sneezing, sore throat, muscle aches, fever

\*\*Cough, sore throat, limb/joint pain, runny nose, headache, vomiting, diarrhea

‡ Retrieved from medical records. ARI included: sinusitis, tonsillitis, otitis, bronchitis, pneumonia, pharyngitis, laryngitis

€ Runny/stuffy nose, sore throat, cough, sneeze, coloured discharge

Ⓞ Runny nose, nasal congestion, sore throat, cough; all symptoms must be unrelated to allergies

**Table 2.** Risk of bias assessment of individual studies

Study	Random sequence generation	Allocation concealment	Blinding	Incomplete outcome data (% missing)	Selective reporting	Other bias (post-hoc analysis, recall bias)
Aloia & Li-Ng, 2007[15]	Low	Unclear	Low	High (28.9%)	Low	High*
Bergman et al., 2012 [34]	Low	Low	Low	Low (11.4)	Low	Low
Camargo et al., 2012 [17]	Low	Low	Low	Low (1.2)	Low	High*
Goodall et al., [18]	Low	Low	Low	Low** (18)	Low	Low
Laaksi et al, 2010 [19]	Low	Unclear	Low	High (36.6)	Low	Unclear†
Li-Ng et al., 2009 [21]	Low	Low	Low	Low (8.4)	Low	Low
Murdoch et al., 2012 [22]	Low	Unclear	Low	Low (5.6)	Low <sup>o</sup>	Low
Urashima et al., 2010 [24]	Low	Low	Low	High (22.3)	High‡	Low

\*Results derived from post-hoc analyses of trials not originally designed to assess ARI; high risk of bias for potential recall bias ( $\geq 3$  months recall period) and imprecise measure of outcome

\*\*Mechanism of missingness was missing at random

† Mild illness may not have been reported in medical records, but should be similar between groups

<sup>o</sup>Did not report all outcomes listed in protocol however unreported outcomes were irrelevant for the systematic review

‡ Registered protocol did not make a distinction between influenza A and influenza B as outcomes; suspect it was a post-hoc choice to report strains separately without biological rationale



**Table 3.** GRADE Summary of Findings

<b>Vitamin D3 versus placebo or no treatment for the prevention of acute respiratory infections</b>						
<b>Outcomes</b>	<b>Illustrative comparative risks* (95% CI)</b>		<b>Relative effect (95% CI)</b>	<b>No of Participants (studies)</b>	<b>Quality of the evidence (GRADE)</b>	<b>Comments</b>
	<b>Assumed risk</b>	<b>Corresponding risk</b>				
	<b>Control</b>	<b>Vitamin D3 versus placebo or no treatment</b>				
<b>Number of participants with 1 or more events</b>	<b>Study population</b>		<b>RR 0.69</b> (0.45 to 1.05) (7 studies)	1908 (7 studies)	⊕⊕⊕⊖ <b>low</b> <sup>1,2,3</sup>	
	<b>462 per 1000</b>	<b>319 per 1000</b> (208 to 486)				
	<b>Moderate</b>					
<b>Number of participants with 1 or more laboratory confirmed event</b>	<b>Study population</b>		<b>RR 0.67</b> (0.51 to 0.86) (3 studies)	983 (3 studies)	⊕⊕⊕⊖ <b>low</b> <sup>4,5</sup>	
	<b>260 per 1000</b>	<b>174 per 1000</b> (133 to 224)				
	<b>Moderate</b>					
<b>Symptom Severity</b>	The mean symptom severity in the intervention groups was <b>0.04 standard deviations higher</b> (0.28 lower to 0.36 higher)			163 (2 studies)	⊕⊕⊕⊖ <b>moderate</b> <sup>6</sup>	SMD 0.04 (-0.28 to 0.36)
	<b>Duration</b>			169 (2 studies)	⊕⊕⊕⊖ <b>moderate</b> <sup>6</sup>	
<b>Occurrence of any adverse events</b>	<b>Study population</b>		<b>RR 0</b> (0.79 to 1.4) (4 studies)	1122 (4 studies)	⊕⊕⊕⊖ <b>moderate</b> <sup>7,8</sup>	
	<b>101 per 1000</b>	<b>0 per 1000</b> (80 to 141)				
	<b>Moderate</b>					
	<b>65 per 1000</b>	<b>0 per 1000</b> (51 to 91)				

\*The basis for the **assumed risk** (e.g. the median control group risk across studies) is provided in footnotes. The **corresponding risk** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).

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**CI:** Confidence interval; **RR:** Risk ratio;

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GRADE Working Group grades of evidence

**High quality:** Further research is very unlikely to change our confidence in the estimate of effect.

**Moderate quality:** Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.

**Low quality:** Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.

**Very low quality:** We are very uncertain about the estimate.

---

<sup>1</sup> Two trials were at risk of recall and memory bias as a result of being post-hoc analyses of earlier trials. One trial was at risk of selective outcome reporting bias. Three trials were at risk of bias due to missing data.

<sup>2</sup> Considerable heterogeneity was detected (I<sup>2</sup>=94%).

<sup>3</sup> The random effects pooled estimate included 'no effect' and 'appreciable benefit' (RRR >25%).

<sup>4</sup> One trial was at risk of bias due to selective outcome reporting.

<sup>5</sup> Considerable heterogeneity was detected (I<sup>2</sup>=86%).

<sup>6</sup> The random effects pooled estimate included 'no effect' and 'appreciable benefit' (upper or lower arm of 95% CI crosses an effect size of 0.5 in either direction).

<sup>7</sup> One trial was at high risk of bias due to missing data.

<sup>8</sup> The random effects model showed 'no benefit' and 'appreciable harm' (RRI>25%).

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**Table 4.** Summary of subgroup analyses assessing the number of participants with at least one ARI

Outcome	Trials (patients)	RR (95% CI)	P value	I <sup>2</sup> (%)	Subgroup Difference (p value)
<b>Children vs. Adults</b>					
Children	2 (677)	0.59 (0.45, 0.77)	0.0001	0	0.39
Adults	5 (1456)	0.74 (0.48, 1.14)	0.17	93	
<b>Frequency of Dosing</b>					
Daily	5 (1211)	0.65 (0.50,0.84)	0.0007	48	0.22
Any other dose	2 (922)	0.90 (0.57, 1.42)	0.64	91	
<b>Trial Risk-of-Bias</b>					
High*	4 (1049)	0.60 (0.45,0.80)	0.0005	49	0.09
Low	3 (1084)	0.89 (0.63, 1.26)	0.51	85	
<b>Naturally acquired vs. Experimentally induced</b>					
Not enough trials for subgroup analysis					

\*High risk of bias studies were defined as any study where at least one item on the Cochrane risk-of-bias tool was assessed as high risk. Low risk studies were defined as studies where five of six items on the risk-of-bias tool were assessed as low risk and no items were assessed as high risk

**Table 5.** Results from sensitivity analyses performed under various assumptions of missing data considering the primary outcome, the number of participants with at least one ARI

<b>Assumptions applied to missing data</b>	<b>RR</b>	<b>95% CI</b>	<b>p</b>
Most extreme benefit of intervention	0.48	0.14-1.66	0.25
Neutral scenario	0.65	0.18-2.36	0.51
No benefit of intervention	0.74	0.33-1.65	0.46

RR = relative risk, CI= confidence interval

## APPENDICES

## Appendix 1. Detailed literature search strategy

Set	MEDLINE (1946 to February 13, 2013)	Results	Comments
1	vitamin d/ or cholecalciferol/ or hydroxycholecalciferols/ or calcifediol/ or dihydroxycholecalciferols/ or calcitriol/ or 24,25-dihydroxyvitamin d 3/ or Cod Liver Oil/ or Vitamin D Deficiency/ or (cholecalciferol* or "vitamin d3" or "vitamin d" or "vitamin d 3" or dihydroxycholecalciferol* or (cod adj2 liver adj2 oil*) or "dihydroxyvitamin d 3" or "dihydroxyvitamin d3" or colecalciferol* or "hydroxyvitamin D" or arachitol or mulsin or tracetten or vicotrat or devaron or duphafra or dupharinterfran or irradiia or irradian or "vedose vi-de 3" or vigantol or vigorsan).mp. or (Calcidiol or Calcitriol or "25-hydroxycholecalciferol" or "25-hydroxyvitamin D" or "25(OH)D" or "sunshine vitamin*").mp.	57043	Vitamin D Terms
2	respiratory tract infections/ or bronchitis/ or bronchiolitis/ or bronchiolitis, viral/ or common cold/ or influenza, human/ or laryngitis/ or pharyngitis/ or nasopharyngitis/ or pneumonia/ or bronchopneumonia/ or pleuropneumonia/ or pneumonia, bacterial/ or chlamydial pneumonia/ or pneumonia, mycoplasma/ or pneumonia, pneumococcal/ or pneumonia, rickettsial/ or pneumonia, staphylococcal/ or pneumonia, viral/ or severe acute respiratory syndrome/ or sinusitis/ or ethmoid sinusitis/ or frontal sinusitis/ or maxillary sinusitis/ or sphenoid sinusitis/ or Orthomyxoviridae/ or Viral Envelope Proteins/ or Adenoviridae/ or enterovirus/ or enterovirus a, human/ or exp enterovirus b, human/ or enterovirus c, human/ or enterovirus d, human/ or rhinovirus/ or Paramyxoviridae Infections/ or Adenoviridae/ or metapneumovirus/ or respiratory syncytial viruses/ or respiratory syncytial virus, human/ or (coronavirus* or (corona adj2 virus*)).mp. or (parainfluenz* or adenovirus*).mp. or ((Respiratory adj2 Syncytial adj2 Virus*) or rsv).mp.	246657	Respiratory Tract infections Terms
3	1 and 2	242	Base clinical Set
4	controlled clinical trial.pt. or controlled clinical trials as topic/ or meta-analysis.pt. or meta-analysis as topic/ or multicenter study.pt. or multicenter studies as topic/ or randomized controlled trial.pt. or randomized controlled trials as topic/ or random allocation/ or double-blind method/ or single-blind	702951	Study design Methodology terms

	method/		
5	3 and 4	29	FINAL Results
<b>Set</b>	<b>EMBASE Classic+Embase &lt;1947 to 2013 Week 06&gt;</b>	<b>Results</b>	<b>Comments</b>
1	vitamin d/ or 24,25 dihydroxyvitamin d/ or calcifediol/ or calcitriol/ or exp colecalciferol derivative/ or vitamin d derivative/ or colecalciferol/ or colecalciferol derivative/ or colecalciferol/ or colecalciferol derivative/ or dihydroxycolecalciferol/ or cod liver oil/ or vitamin d/ or cholecalciferol/ or dihydroxycholecalciferols/ or 24,25-dihydroxyvitamin d 3/ or Cod Liver Oil/ or (cholecalciferol* or "vitamin d3" or "vitamin d" or "vitamin d 3" or dihydroxycholecalciferol* or (cod adj2 liver adj2 oil*) or "dihydroxyvitamin d 3" or "dihydroxyvitamin d3" or colecalciferol* or "hydroxyvitamin D" or arachitol or mulsin or tracetten or vicotrat or devaron or duphafra or dupharinterfran or irradi or irradiation or "vedose vi-de 3" or vigantol or vigersan).mp. or vitamin D deficiency/ or (Calcidiol or Calcitriol or "25-hydroxycholecalciferol" or "25-hydroxyvitamin D" or "25(OH)D" or "sunshine vitamin*").mp.	98397	Vitamin D Terms
2	respiratory tract infection/ or influenza/ or exp influenza a/ or influenza b/ or influenza c/ or pandemic influenza/ or seasonal influenza/ or lower respiratory tract infection/ or exp infectious pneumonia/ or viral bronchiolitis/ or parainfluenza virus infection/ or respiratory syncytial virus infection/ or upper respiratory tract infection/ or viral upper respiratory tract infection/ or viral respiratory tract infection/ or common cold/ or pneumonia/ or acute sinusitis/ or viral sinusitis/ or exp laryngitis/ or orthomyxovirus/ or exp influenza virus/ or adenovirus/ or adenovirus 12/ or adenovirus 2/ or adenovirus 5/ or adenovirus 7/ or enterovirus/ or enterovirus infection/ or human rhinovirus/ or paramyxovirus infection/ or exp parainfluenza virus infection/ or exp pneumovirus infection/ or (coronavirus* or (corona adj2 virus*)).mp. or (parainfluenz* or adenovirus*).mp. or ((Respiratory adj2 Syncytial adj2 Virus*) or rsv).mp	387706	Respiratory Tract infections Terms
3	1 and 2	1289	Base clinical Set
4	4 (randomized controlled trial or controlled clinical trial or multicenter study).pt. or ct.fs. or controlled clinical trial/ or multicenter study/ or meta analysis/ or randomized controlled	849375	Study design Methodology

	trial/ or crossover procedure/ or double blind procedure/ or triple blind procedure/		terms
5	3 and 4	430	FINAL Results
<b>Set</b>	<b>Cochrane Central Register of Controlled Trials</b>	<b>Results</b>	<b>Comments</b>
1	vitamin d/ or 24,25 dihydroxyvitamin d/ or calcifediol/ or calcitriol/ or exp colecalciferol derivative/ or vitamin d derivative/ or colecalciferol/ or colecalciferol derivative/ or colecalciferol/ or colecalciferol derivative/ or dihydroxycolecalciferol/ or cod liver oil/ or cholecalciferol/ or dihydroxycholecalciferols/ or 24,25-dihydroxyvitamin d 3/ or cholecalciferol/ or hydroxycholecalciferols/ or dihydroxycholecalciferols/ or Vitamin D Deficiency/ or (cholecalciferol* or "vitamin d3" or "vitamin d" or "vitamin d 3" or dihydroxycholecalciferol* or (cod adj2 liver adj2 oil*) or "dihydroxyvitamin d 3" or "dihydroxyvitamin d3" or colecalciferol* or "hydroxyvitamin D" or arachitol or mulsin or tracetten or vicotrat or devaron or duphafraal or dupharinterfran or irradiia or irradian or "vedose vi-de 3" or vigantol or vigorsan).mp. or (Calcidiol or Calcitriol or "25-hydroxycholecalciferol" or "25-hydroxyvitamin D" or "25(OH)D" or "sunshine vitamin*").mp.	3011	Vitamin D Terms
2	respiratory tract infection/ or influenza/ or exp influenza a/ or influenza b/ or influenza c/ or pandemic influenza/ or seasonal influenza/ or lower respiratory tract infection/ or exp infectious pneumonia/ or viral bronchiolitis/ or parainfluenza virus infection/ or respiratory syncytial virus infection/ or upper respiratory tract infection/ or viral upper respiratory tract infection/ or viral respiratory tract infection/ or common cold/ or pneumonia/ or acute sinusitis/ or viral sinusitis/ or exp laryngitis/ or orthomyxovirus/ or exp influenza virus/ or adenovirus/ or adenovirus 12/ or adenovirus 2/ or adenovirus 5/ or adenovirus 7/ or enterovirus/ or enterovirus infection/ or human rhinovirus/ or paramyxovirus infection/ or exp parainfluenza virus infection/ or exp pneumovirus infection/ or respiratory tract infections/ or bronchitis/ or bronchiolitis/ or bronchiolitis, viral/ or influenza, human/ or laryngitis/ or pharyngitis/ or nasopharyngitis/ or pneumonia/ or bronchopneumonia/ or pleuropneumonia/ or pneumonia, bacterial/ or chlamydial pneumonia/ or pneumonia, mycoplasma/ or pneumonia, pneumococcal/ or pneumonia, rickettsial/ or pneumonia, staphylococcal/ or pneumonia, viral/ or severe acute respiratory syndrome/ or sinusitis/ or ethmoid	6842	Respiratory Tract infections Terms

	sinusitis/ or frontal sinusitis/ or maxillary sinusitis/ or sphenoid sinusitis/ or Orthomyxoviridae/ or Viral Envelope Proteins/ or Adenoviridae/ or enterovirus a, human/ or exp enterovirus b, human/ or enterovirus c, human/ or enterovirus d, human/ or rhinovirus/ or Paramyxoviridae Infections/ or Adenoviridae/ or metapneumovirus/ or respiratory syncytial viruses/ or respiratory syncytial virus, human/ or (coronavirus* or (corona adj2 virus*)).mp. or (parainfluenz* or adenovirus*).mp. or ((Respiratory adj2 Syncytial adj2 Virus*) or rsv).mp. [****Respiratory Tract infections****]		
3	1 and 2	14	Base clinical Set and FINAL Results
<b>Set</b>	<b>AMED (1985 to February 2013)</b>	<b>Results</b>	<b>Comments</b>
1	vitamin d/ or cholecalciferols/ or calcitriol/ or (cholecalciferol* or "vitamin d3" or "vitamin d" or "vitamin d 3" or dihydroxycholecalciferol* or (cod adj2 liver adj2 oil*) or "dihydroxyvitamin d 3" or "dihydroxyvitamin d3" or colecalciferol* or "hydroxyvitamin D" or arachitol or mulsin or tracetten or vicotrat or devaron or duphafraal or dupharinterfran or irradiia or irradian or "uedose vi-de 3" or vigantol or vigorsan).mp. or (Calcidiol or Calcitriol or "25-hydroxycholecalciferol" or "25-hydroxyvitamin D" or "25(OH)D" or "sunshine vitamin*").mp.	340	Vitamin D Terms
2	respiratory tract infections/ or bronchitis/ or common cold/ or influenza/ or pharyngitis/ or pneumonia/ or severe acute respiratory syndrome/ or sinusitis/ or paramyxovirus infections/ or severe acute respiratory syndrome/ or sars/ or (Orthomyxovirus* or Orthomyxovirid* or bronchiolitis or laryngitis or coronavirus* or (corona adj2 virus*) or parainfluenz* or adenovirus* or enterovirus* or (Respiratory adj2 Syncytial adj2 Virus*) or rsv).mp. [****Respiratory Tract infections****]	801	Respiratory Tract infections Terms
3	1 and 2	4	Base clinical Set & Final Results
4	randomized controlled trials/ or double blind method/ or meta analysis/ or random allocation/ or (random* or (multicent* adj2	13727	Study design Methodology



	stud*)).mp. [****Study design Methodology terms ****]		terms
5	3 and 4	3	FINAL Results
<b>Set</b>	<b>CAB Abstracts 1973 to 2013 Week 05</b>	<b>Results</b>	<b>Comments</b>
1	(cholecalciferol* or "vitamin d3" or "vitamin d" or "vitamin d 3" or dihydroxycholecalciferol* or (cod adj2 liver adj2 oil*) or "dihydroxyvitamin d 3" or "dihydroxyvitamin d3" or colecalciferol* or "hydroxyvitamin D" or arachitol or mulsin or tracetten or vicotrat or devaron or duphafra or dupharinterfran or irradi or irradi or "vedose vi-de 3" or vigantol or vigorsan).mp. or (Calcidiol or Calcitriol or "25-hydroxycholecalciferol" or "25-hydroxyvitamin D" or "25(OH)D" or "sunshine vitamin*").mp.	19656	Vitamin D Terms
2	bronchiolitis/ or bronchitis/ or laryngitis/ or lower respiratory tract infections/ or pneumonia/ or sinusitis/ or upper respiratory tract infections/ or paramyxovirinae/ or human paramyxoviruses/ or parainfluenza viruses/ or coronavirus/ or human coronavirus 229e/ or human coronavirus oc43/ or human coronaviruses/ or human enteric coronavirus/ or infectious bronchitis virus/ or severe acute respiratory syndrome coronavirusidae/ or coronavirus/ or parainfluenza viruses/ or parainfluenza/ or human parainfluenza virus 1/ or human parainfluenza virus 2/ or human parainfluenza virus 3/ or human parainfluenza virus 4/ or human adenovirus/ or human adenovirus 3/ or human adenovirus 40/ or human adenovirus 41/ or human adenovirus 7/ or enterovirus/ or human enterovirus a/ or human enterovirus b/ or human enterovirus c/ or human enterovirus d/ or human enterovirus e/ or human enteroviruses/ or pneumovirus/ or human respiratory syncytial virus/ or (Orthomyxovirus* or Orthomyxovirid* or bronchiolitis or laryngitis or coronavirus* or (corona adj2 virus*) or parainfluenz* or adenovirus* or enterovirus* or (Respiratory adj2 Syncytial adj2 Virus*) or rsv).mp.	55998	Respiratory Tract infections Terms
3	1 and 2	57	Base clinical Set
4	randomized controlled trials/ or meta-analysis/ or systematic reviews/ or random sampling/ or (random* or (multicent* adj2 stud*)).mp.	202047	Study design Methodology terms

5	3 and 4	9	FINAL Results
<b>Set</b>	<b>CINAHL (1983 to February 13, 2013)</b>	<b>Results</b>	<b>Comments</b>
S1	(MH "Vitamin D") OR (MH "Calcitriol") OR (MH "Cholecalciferol") OR (MH "Vitamin D Deficiency") OR (TX cholecalciferol* OR "vitamin d3" OR "vitamin d" OR "vitamin d 3" OR dihydroxycholecalciferol* OR "dihydroxyvitamin d 3" OR "dihydroxyvitamin d3" OR colecalciferol* OR "hydroxyvitamin D" OR arachitol OR mulsin OR tracetten OR vicotrat OR devaron OR duphafra OR dupharinterfran OR irradi OR irradi OR "uedose vi-de 3" OR vigantol OR vigorsan OR Calcidiol OR Calcitriol OR "25-hydroxycholecalciferol" OR "25-hydroxyvitamin D" OR "25(OH)D") OR (TX cod N2 liver N2 oil*) OR (TX "sunshine vitamin*")	7172	Vitamin D Terms
S2	(MH "Respiratory Tract Diseases") OR (MH "Bronchitis") OR (MH "Bronchiolitis") OR (MH "Bronchitis, Acute") OR (MH "Bronchopneumonia") OR (MH "Laryngitis") OR (MH "Sinusitis") OR (MH "Rhinosinusitis") OR (MH "Influenza") OR (MH "Influenza, Human") OR (MH "Influenza A H5N1") OR (MH "Influenza, Seasonal") OR (MH "Common Cold") OR (MH "Pneumonia") OR (MH "Community-Acquired Pneumonia") OR (MH "Pneumonia, Viral") OR (MH "Paramyxovirus Infections") OR (MH "Respiratory Syncytial Virus Infections") OR (MH "Orthomyxoviridae") OR (MH "Influenzavirus A") OR (MH "Influenza A Virus") OR (MH "Influenza A Virus, H1N1 Subtype") OR (MH "Influenza A Virus, H5N1 Subtype") OR (MH "Influenzavirus B") OR (MH "Influenza B Virus") OR (MH "Influenzavirus C") OR (MH "Enteroviruses") OR (MH "Paramyxoviruses") OR (MH "Respiratory Syncytial Viruses") OR (MH "Pharyngitis") OR (TX coronavirus* OR Coronavirid* OR parainfluenz* OR adenovirus* OR adenovirid* OR rsv) OR (TX Respiratory N2 Syncytial N2 Virus*) OR (TX corona N2 virus*)	22506	Respiratory Tract infections Terms
S3	S1 and S2	101	Base clinical Set
4	(MH "Double-Blind Studies") OR (MH "Intervention Trials") OR (MH "Preventive Trials") OR (MH "Randomized Controlled Trials") OR (MH "Single-Blind Studies") OR (MH "Triple-Blind Studies") OR (MH "Therapeutic Trials") OR (MH "Meta Analysis") OR (MH "Multicenter Studies")	56191	Study design Methodology terms

5	S3 and S4	4	FINAL Results
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**Appendix 2.** Details of author correspondence

<b>Study</b>	<b>Information requested</b>	<b>Response</b>
Aloia & Li-Ng, 2007	Amount of missing data for primary individuals with respiratory infection Was analyst blinded?	No response
Bergman et al., 2012	# of participants with $\geq 1$ event/trial arm Definition of acute respiratory infection	Concern regarding a potential conflict of interest; no data provided
Laaksi et al, 2010	# of participants with $\geq 1$ event/trial arm Methods for dealing with missing data Method of concealment Clarification re: whether or not outcome was known for those LTF Was analyst blinded?	No response
Li-Ng et al., 2009	Was analyst blinded?	No response
Murdoch et al., 2012	# of participants with $\geq 1$ lab confirmed infections/trial arm Confirmation of # of participants with $\geq 1$ clinical infections/trial arm Clarification re: whether or not outcome was known for those LTF	Author kindly responded to all questions and provided additional information on May 23, 2013.

## Appendix 3. Subgroup credibility assessment

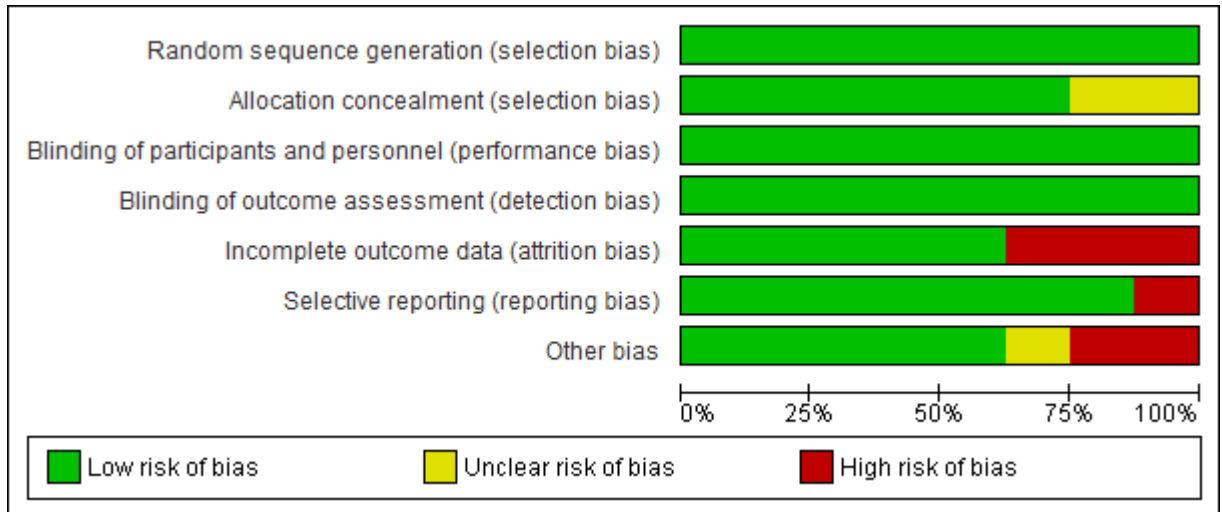
<b>Criteria</b>	<b>Age</b> (children vs. adults)	<b>Dosing</b> Frequency (daily vs. non-daily)	<b>Risk of bias in study</b> (High vs. low)
Variable measured at baseline	Y	Y	N
Comparison within study	N	N	N
<i>A priori</i> hypothesis	Y	Y	Y
Direction of effect specified <i>a priori</i>	Y	Y	Y
Small number of hypotheses tested	Y	Y	Y
Significant interaction test	N	N	N
Independent effect*	N (p=0.37)	N (p=0.23)	N (p=0.09)
Large subgroup effect size	N	N	N
Interaction consistent across studies	N	N/A‡	N/A‡
Interaction consistent across related outcomes	N/A†	N/A†	N/A†
Biological rationale	Y	Y	Y
Total Criteria Satisfied	5	5	4
Conclusion	Unlikely	Unlikely	Unlikely

\*low credibility when test for interaction yields  $p > 0.1$ , moderate credibility when  $p > 0.01 < 0.1$ , high credibility when  $p < 0.001$ [32]

‡ Subgroup analyses have not been conducted in other studies, no comparison is available

† Subgroup analyses could not be conducted with secondary outcomes due to the low number of studies

**Appendix 4.** Overall risk of bias of included trials



## Appendix 5. GRADE Evidence Profile

Quality assessment							No of patients		Effect		Quality	Importance
No of studies	Design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Vitamin D3 versus placebo or no treatment	Control	Relative (95% CI)	Absolute		
<b>Number of participants with 1 or more events</b>												
7	randomised trials	serious <sup>1</sup>	serious <sup>2</sup>	no serious indirectness	no serious imprecision <sup>3</sup>	none	361/989 (36.5%)	425/919 (46.2%)	RR 0.69 (0.45 to 1.05)	143 fewer per 1000 (from 254 fewer to 23 more)	⊕⊕○○ LOW	IMPORTANT
								41.4%		128 fewer per 1000 (from 228 fewer to 21 more)		
<b>Number of participants with 1 or more laboratory confirmed event</b>												
3	randomised trials	serious <sup>4</sup>	serious <sup>5</sup>	no serious indirectness	no serious imprecision	none	83/503 (16.5%)	125/480 (26%)	RR 0.67 (0.51 to 0.86)	86 fewer per 1000 (from 36 fewer to 128 fewer)	⊕⊕○○ LOW	IMPORTANT
								18.9%		62 fewer per 1000 (from 26 fewer to 93 fewer)		
<b>Symptom Severity (Better indicated by lower values)</b>												
2	randomised trials	no serious risk of	no serious inconsistency	no serious indirectness	serious <sup>6</sup>	none	81	82	-	SMD 0.04 higher (0.28 lower to 0.36)	⊕⊕⊕○ MODERATE	IMPORTANT

		bias								higher)		
<b>Duration (Better indicated by lower values)</b>												
2	randomised trials	no serious risk of bias	no serious inconsistency	no serious indirectness	serious <sup>6</sup>	none	81	88	-	MD 0.18 lower (0.71 lower to 0.35 higher)	⊕⊕⊕○ MODERATE	IMPORTANT
<b>Occurrence of any adverse events</b>												
4	randomised trials	no serious risk of bias <sup>7</sup>	no serious inconsistency	no serious indirectness	serious <sup>8</sup>	none	64/577 (11.1%)	55/545 (10.1%)	RR 0 (0.79 to 1.4)	101 fewer per 1000 (from 21 fewer to 40 more)	⊕⊕⊕○ MODERATE	IMPORTANT
								6.5%		65 fewer per 1000 (from 14 fewer to 26 more)		

<sup>1</sup> Two trials were at risk of recall and memory bias as a result of being post-hoc analyses of earlier trials. One trial was at risk of selective outcome reporting bias. Three trials were at risk of bias due to missing data.

<sup>2</sup> Considerable heterogeneity was detected (I<sup>2</sup>=94%).

<sup>3</sup> The random effects pooled estimate included 'no effect' and 'appreciable benefit' (RRR >25%).

<sup>4</sup> One trial was at risk of bias due to selective outcome reporting.

<sup>5</sup> Considerable heterogeneity was detected (I<sup>2</sup>=86%).

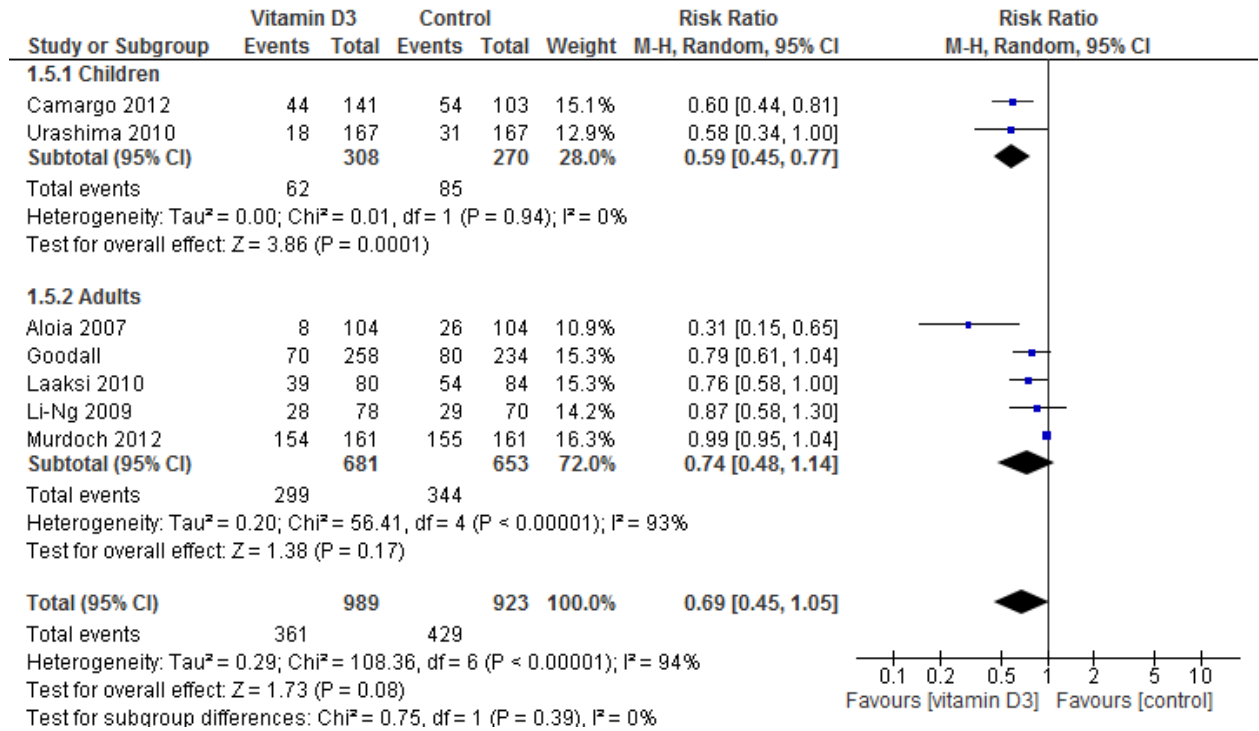
<sup>6</sup> The random effects pooled estimate included 'no effect' and 'appreciable benefit' (upper or lower arm of 95% CI crosses an effect size of 0.5 in either direction).

<sup>7</sup> One trial was at high risk of bias due to missing data.

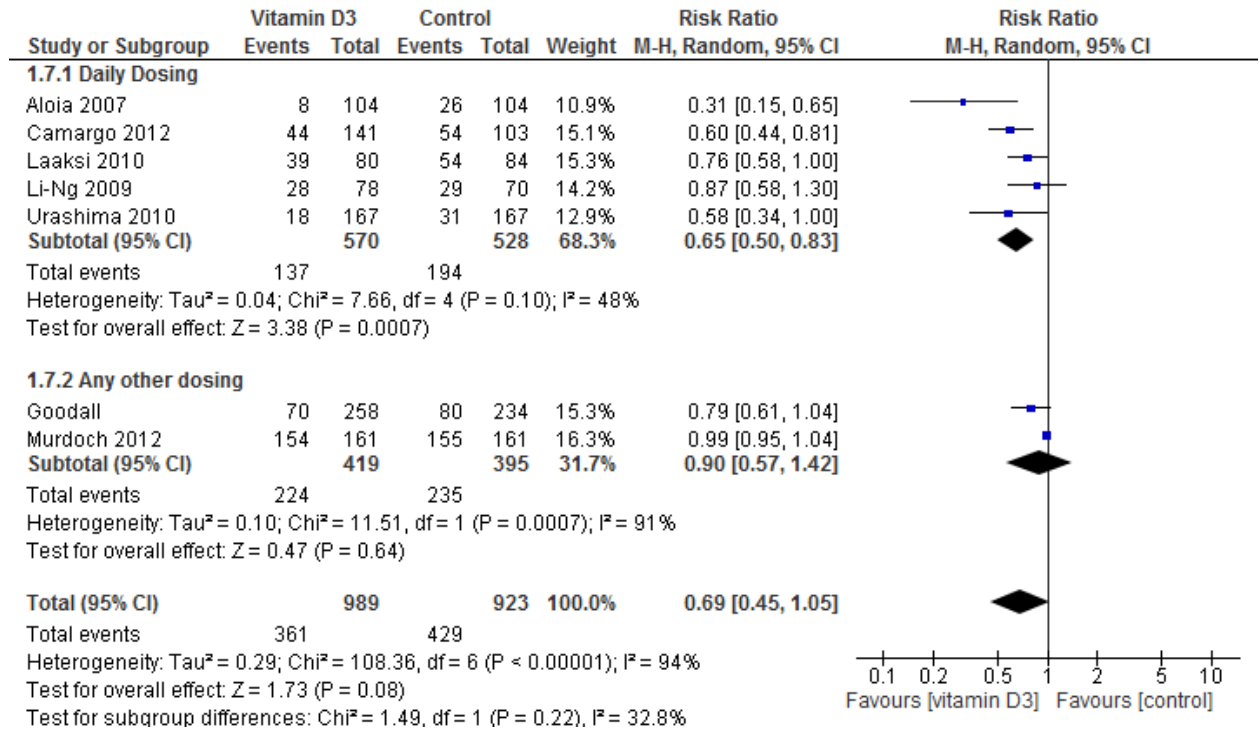
<sup>8</sup> The random effects model showed 'no benefit' and 'appreciable harm' (RRI>25%).



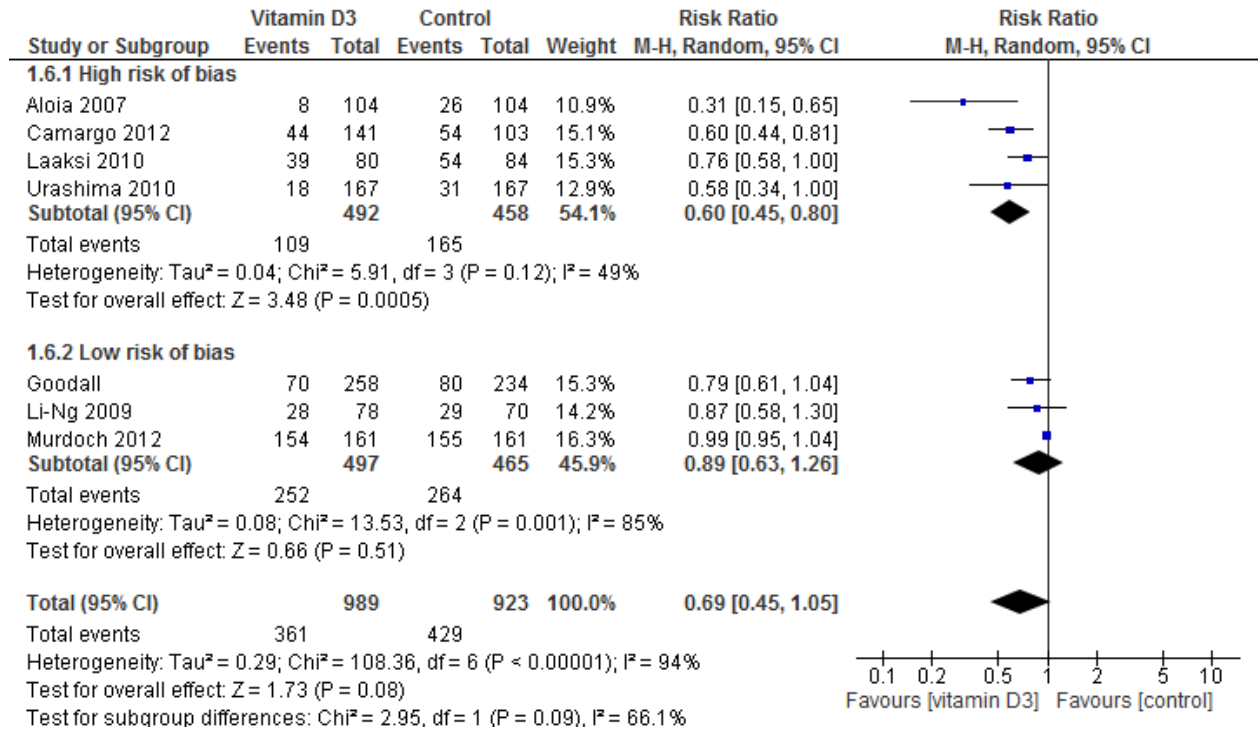
**Appendix 6.** Subgroup analyses assessing the number of participants with at least one ARI



**Appendix 6a.** Meta-analysis of the number of individuals with at least one event, by age, in randomized controlled trials of oral vitamin D3 supplementation for the prevention of acute respiratory infection. A pooled relative risk of less than one represents benefit from vitamin D3 supplementation. RR= relative risk, CI= confidence interval, df= degrees of freedom.



**Appendix 6b.** Meta-analysis of the number of individuals with at least one event, by frequency of dose, in randomized controlled trials of oral vitamin D3 supplementation for the prevention of acute respiratory infection. A pooled relative risk of less than one represents benefit from vitamin D3 supplementation. RR= relative risk, CI= confidence interval, df= degrees of freedom.



**Appendix 6c.** Meta-analysis of the number of individuals with at least one event, by trial risk-of-bias, in randomized controlled trials of oral vitamin D3 supplementation for the prevention of acute respiratory infection. A pooled relative risk of less than one represents benefit from vitamin D3 supplementation. RR= relative risk, CI= confidence interval, df= degrees of freedom.

**Appendix 7.** Description of assumptions applied to missing data in the intervention and control arms for sensitivity analyses.

<b>Assumptions applied to missing data</b>	<b>Intervention Arm</b>	<b>Control Arm</b>
<i>Most extreme benefit of intervention</i>	Lowest incidence among intervention arms of all included trials	Highest incidence among control arms of all included trials
<i>Neutral scenario</i>	Same incidence as the trial intervention arm	Same incidence as the trial control arm
<i>No benefit of intervention</i>	Assume same incidence as control arm	Assume same incidence as control arm