The Role of Daily High Dose Vitamin D in the Prevention of Post-Operative Vitamin D Deficiency in Children with Congenital Heart Disease

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Thesis submitted to the Faculty of Graduate and Postdoctoral Studies in partial fulfillment of the requirements for the Master's degree in School of Epidemiology

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List of Abbreviations

$1,25OH_2D$	1, 25 dihydroxyvitamin D
25OHD	25 hydroxyvitamin D
3-epi-25OHD	25-hydroxy-3-epi-vitamin D3
AI	Adequate Intake
CHD	Congenital Heart Disease
CPB	Cardiopulmonary Bypass
D2	ergocalciferol
D3	cholecalciferol
DEQAS	Vitamin D External Quality Assessment Scheme
ECMO	Extracorporeal Membrane Oxygenation
ICU	Intensive Care Unit
IM	Intramuscular
IOM	Institute of Medicine
IU	International Units
Kg	kilogram
LC-MS	Liquid Chromatography- Mass Spectrometry
nmol/l	nanomole per liter
MACCE	Major Adverse Cardiac and Cerebrovascular Event
PICU	Pediatric Intensive Care Unit
PRESS	Peer Review of Electronic Search Strategies
PRISMA	Preferred Reporting Items for Systematic Reviews and Met-analyses
PTH	Parathyroid Hormone
RACHS	Risk Adjusted classification for Congenital Heart Surgery
RCT	Randomized Controlled Trial
REDCap	Research Electronic Data Capture
RDA	Recommended Daily Allowance
SD	Standard deviation
UL	Tolerable Upper Intake Level
UV	Ultraviolet
VDD	Vitamin D deficiency
WHO	World Health Organization

Abstract

Background: Vitamin D deficiency (VDD) occurs in the majority of children following Congenital Heart Disease (CHD) surgery, and lower levels have been associated with post-operative cardiovascular dysfunction. Mechanistic studies have revealed that the high prevalence of VDD is due to poor pre-operative status and a 40% intraoperative drop in vitamin D. Available literature suggests that usual care with daily low dose vitamin D is ineffective and alternative regimens will be required to prevent post-operative VDD. **Objectives:** (1) To systematically review the pediatric clinical trial literature of high dose vitamin D, and (2) Determine whether pre-operative daily high dose vitamin D, based on the Institute of Medicine (IOM) Tolerable Upper Intake Level, can prevent post-operative VDD. **Methods:** (1) *Systematic review* - Medline, Embase and the Cochrane Central Register of Controlled trials were searched for clinical trials reporting 25OHD levels in children after high dose vitamin D (≥ 1000 IU). Descriptive and meta-analysis techniques were used to determine the study characteristics associated with 25OHD response and adverse events. (2) *Trial* – Design and initiate a dose evaluation trial. Recruit sixty two children with CHD into a double blind RCT and randomly assign to receive pre-operative cholecalciferol as usual (< 1 yr: 400 IU, > 1 yr 600 IU) or high doses (< 1 yr: 1600 IU, > 1 yr: 2400 IU). Primary outcome is immediate post-operative 25OHD concentration. Secondary outcomes include vitamin D related adverse events and measures of trial feasibility. Study data to be reviewed by the Data Safety Monitoring Board after the first 30 participants - this time point was also chosen for preparation of the thesis results (25OHD data not available). **Results:** (1) *Systematic review* **–** There were 88 eligible publications identified. Only two of six studies administering high dose daily supplementation (1000-4000 IU) to VDD children achieved group 25OHD levels above 75 nmol/L within 1 month; compared with nine of ten studies

using loading therapy (≥ 40000 IU). In meta-regression, baseline 25OHD, regimen type, dose, age, presence of illness, and time factors were associated with final 25OHD levels. Increased risk of hypercalcemia was calculated with doses ≥ 400 000 IU and group 25OHD levels above 200 nmol/L. There were no reported cases of nephrocalcinosis and no significant increase in hypercalciuria risk with high dose vitamin D. (2) *Trial* –During the 19 months of active recruitment, there were 68 eligible referrals, full study was discussed with 49, and 35 consented (accrual rate of 1.8 per month). Of the 35 participants, 4 were withdrawn and 1 was awaiting surgery. For the 30 participants who completed all study procedures, the median number of doses was 21 (IQR: 4, 40) with 45% and 16% receiving more than 30 and 60 doses, respectively. The study safety protocol successfully identified one patient with levels approaching the upper safety threshold (parents had started an alternative medicine with vitamin D). Intra-operative or post-operative hypercalciuria occurred in 12 (40%) study participants. No study participant had pre or post-operative hypercalcemia. Immediate postoperative blood was collected for 25OHD determination in 100% (n=30) of study participants (to be determined as part of future work). Protocol limitations identified were: inability to collect baseline 25OHD (n=12/35, 34.2%), poor drug compliance (n=9/30, 30%), and protocol deviations (n=6/30, 20%) and excessive coordinator time to achieve early recruitment. **Conclusion:** It is possible to recruit children with CHD into an RCT of high dose vitamin D. Due to both non-compliance and the short duration between enrollment and surgery the majority of CHD remain at risk for VDD following surgery despite pre-operative supplementation with doses approximating the IOM Tolerable Upper Intake Level. Based on our findings, an alternative dosing regimen utilizing loading therapy may be necessary to achieve therapeutic levels of vitamin D.

Acknowledgements

I would like to express sincere gratitude to my thesis advisory committee (Drs Menon, Fergusson and McIntyre) for the continuous high level support of my MSc project and related research. Their expression of interest, motivation, knowledge and patience were essential to the success of the work. I feel truly fortunate to have worked with such an experienced and wonderful group of individuals.

Besides my advisors, I would like to thank the many co-investigators without which the two projects would have either not happened or taken considerably longer. For the systematic review I would like to thank Margaret Sampson for her willingness to take on extra work and assist with search strategy development, and Katie O'Hearn, Klevis Iliriani and Dr. Supichaya Pojsupap for their insightful comments and dedication to screening and extraction – the number of citations and eligible trials was much more than we anticipated. Regarding the CHD trial there is an entire community of people to thank and acknowledge. First, thank you to Katie O'Hearn (PICU research coordinator) would joined our team part way through the study approval process; she brought an amazing personality, work ethic and valuable insight to the trial. Numerous other individuals went above and beyond to assist with this project including Drs. Margaret Lawson, Pavel Geier, Gyaandeo Maharajh, Stephanie Redpath, Jane Lougheed and the cardiovascular nurses (Tara Girolamo and Jocelyn Bill). Thank you seeing potential in this project and having the energy to persist with me. In addition to this group there are many staff at the CHEO Research Institute, pharmacy and laboratory would participated in the set-up and initiation of the trial – thank you. Finally, it is important to acknowledge and recognize the willingness of the patients and their caregivers to participate in this study and help us answer the question. I appreciate the trust they placed in both myself and the entire research team.

In addition to my formal thesis advisory committee I would like to acknowledge the support and mentorship received from the community of clinicians and researchers who constitute the Canadian Critical Care Trials Group. In particular I would like to thank Drs Sonny Dhanani and Jennifer Foster for reviewing the Heart and Stroke grant prior to submission and Dr. Patrician Fontela for reviewing the manuscript for the RCT protocol prior to submission to Trials.

Finally I would like to thank my wife Loren for all of her support through my graduate training. In addition to being a greater listener she often provided frequent advice and helped proofread the thesis. A better partner one could not ask for.

Contribution of authors

Author Contributions – Chapter 2: Background
J. Dayre McNally: Manuscript conceptualization and design, data collection (review of literature), drafted the manuscript and approved the final as submitted.
Kusum Menon**:** Made substantial contributions to manuscript design, critically reviewed and revised for important intellectual content, and approved the final version to be published.

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Author Contributions – Chapter 4: Protocol for Randomized Controlled Trial
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Kusum Menon, Dean Fergusson, Lauralyn McIntyre, Hope Weiler**:** All have significant clinical trial experience in PICU or on vitamin D. Each made significant contributions with study conceptualization and/or design. Critically revised the study protocol for submission to Heart and Stroke and/or Trials journal.
Katie O'Hearn: Contributed significantly to the study design in the areas of data collection, recruitment and consent, standard operating procedures for data monitoring, adverse event reporting and the Data safety monitoring board. Has lead a number of protocol modifications and critically reviewed the manuscript prior to submission.
Gyaandeo Maharajh, Stephanic Redpath, Margaret Lawson, Pavel Geier: Contributed to design of the study related to their specific areas of subspeciality expertise. All critically revised the grant and/or protocol manuscript for important intellectual content. GM and SR assisted with study design of procedures related to patient recruitment, data and research sample collection. ML and PG helped design the study safety procedures and adverse event analysis. ML has served as the study safety officer.

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CHAPTER ONE – THESIS OVERVIEW

1.1 SUMMARY OF POPULATON AND PROBLEM

Congenital Heart Disease (CHD) affects 1 in every 100 newborns with approximately 1500 Canadian children requiring heart surgery per year. Following surgery, these patients are often severely ill, requiring concentrated periods of expert medical care, consuming high-intensity resources, and spending weeks to months in hospital. New management approaches that reduce illness severity and speed recovery would benefit patients, families and the health care system. Vitamin D, well known for its role in bone health, is now recognized as a pleiotropic hormone important for the proper functioning of multiple organs, including the heart and lungs. A growing number of epidemiological studies in cardiovascular and adult critically ill populations have suggested vitamin D deficiency to be a modifiable risk factor for morbidity and mortality. Similarly, vitamin D deficiency has the potential to worsen illness following CHD repair through electrolyte disturbance, cardiac dysfunction, skeletal muscle weakness, altered immunity and impaired immumodulation. Until recently there were no studies reporting the prevalence of vitamin D deficiency in CHD patients admitted to the paediatric intensive care unit (PICU) following repair. Further, the potential relationship of vitamin D deficiency post CHD surgery with clinical outcomes has not been investigated. The CHEO PICU research team recently completed a multicentre study demonstrating that 70% of critically ill children, including a post-operative CHD group, are vitamin D deficient at PICU admission. We demonstrated that patients with lower levels of vitamin D on PICU admission were more likely to require cardiovascular support. Secondly, a prospective single-centre study at CHEO focusing specifically on CHD patients confirmed the high rates (85%) of post-operative vitamin D deficiency. Importantly, that study identified that most

children with CHD have borderline normal pre-operative vitamin D levels and that initiation of cardiopulmonary bypass (CPB) leads to an acute and sustained 40% decline in blood concentration. Similar to results from adult cardiovascular and ICU studies, lower post-operative vitamin D concentrations were associated with greater cardiovascular dysfunction. Our study findings, combined with the accumulating adult critical care literature, suggest that prevention of post-operative vitamin D deficiency could improve clinical outcomes following CHD surgery. As an inexpensive medication generally regarded as safe, vitamin D supplementation has the potential to be an ideal intervention for improving outcomes following CHD surgery.

1.2 HYPOTHESES

1. Vitamin D deficiency following surgery for congenital heart disease (CHD) contributes to critical illness pathophysiology and worsens clinical outcome.

2. For patients with CHD, a personalized vitamin D supplementation approach that maintains normal vitamin D levels throughout the pre and post-operative stages will decrease morbidity, mortality and health resource utilization associated with CHD surgery.

1.3 OBJECTIVES

1.3.1 Global Objective

The global objective is to identify supplementation strategies that will safely prevent post-operative vitamin D deficiency by significantly elevating levels pre-operatively.

1.3.2 Specific Objectives for the Thesis

A) Perform a systematic review of pediatric clinical trials reporting vitamin D supplementation meeting and exceeding the Institute of Medicine (IOM) Tolerable Upper Intake Level.

- Report on the vitamin D levels achieved with different high dose regimens
- Report on the occurrence of vitamin D toxicity with high dose regimens, including a comparison with study arms administering usual care.

B) Develop and initiate a pilot RCT evaluating whether a daily high dose vitamin D supplementation strategy based on the IOM recommendations can prevent post-cardiac surgery vitamin D deficiency, when compared with usual care supplementation.

- Develop a protocol acceptable for submission to Heart and Stroke Foundation
- Evaluate the feasibility and barriers to a larger outcome based RCT
- Compare the safety and toxicity of high dose and usual care dosing regimens

1.4 PRESENTATION OF THESIS WORK

The thesis is presented in manuscript style. Chapter 2 represents a manuscript published in the Translational Pediatrics (July 2013) entitled "Vitamin D deficiency in surgical congenital heart disease: prevalence and relevance" describing the population, problem, knowledge gaps and proposes future work. Chapter 3 represents a manuscript published in Pediatrics (January 2015) entitled "Rapid normalization of vitamin D levels: a meta-analysis" describing the results and interpretation of the systematic review and meta-

analysis. Chapter 4 contains a manuscript under review by Trials entitled “Prevention of vitamin D deficiency in children following cardiac surgery: a study protocol for a randomized dose evaluation trial”. The protocol manuscript provides an abridged version of the protocol that was submitted to the Heart and Stroke Foundation of Canada and awarded $138000 (Grant in aid, 2013). Permission has been obtained from the journals and have been reproduced with minor modifications to format but without modification of the content. Chapter 5 presents study findings after the first 30 participants completing all study procedures. To conclude the thesis, Chapter 6 provides an update of relevant literature, summarizes the results of the systematic review and RCT, and proposes the next steps for the field and research program.

2.0 BACKGROUND

2.1 POPULATION AND PROBLEM

Congenital heart disease (CHD) is a common condition with an estimated prevalence of 1 per 100 in the general population (1). A significant proportion of these children require one or more corrective surgeries over their lifetime, collectively leading to 15,000 procedures per year in North America (1). Post-operatively, these patients may suffer significant morbidities which include a pronounced systemic inflammatory response, coagulopathy, respiratory failure, electrolyte disturbances, arrhythmia, myocardial dysfunction, kidney failure, infection and endocrine imbalances (2-5). Interventions that target one or many of these pathophysiological states could prevent illness, speed recovery, and decrease chronic morbidity in this high risk pediatric population.

Emerging literature suggests vitamin D deficiency to be a highly prevalent problem in the immediate post-operative CHD population. This observation is immediately relevant to researchers and clinicians since vitamin D is well recognized to be a pleiotropic hormone important for the proper functioning of organs critical to post-operative illness and outcome. Considered inexpensive and safe, vitamin D supplementation could prove to be an ideal intervention for improving outcomes in children with significant CHD.

2.2 OVERVIEW OF VITAMIN D

2.2.1 Vitamin D axis

A schematic of the endocrine pathway is provided in Figure 2A (appendix 2.1). The vitamin D axis is primarily understood in the context of total body and serum calcium

homeostasis (6, 7). In response to low ionized calcium, the parathyroid glands increase parathyroid hormone (PTH) secretion. Increased serum PTH leads to activation of vitamin D through an inducible renal enzyme, converting 25 hydroxyvitamin D (25OHD) to 1,25 dihydroxyvitamin D (1,25OH_2D). The activated vitamin D, or calcitriol, works to restore serum calcium through bone breakdown, gastro-intestinal absorption, and increased renal reabsorption. Body stores of 25OHD are built and maintained through skin photosynthesis and dietary intake of pre-vitamin D that is immediately hydroxylated in the liver to 25OHD. Inadequate vitamin D intake through sun exposure and supplementation represents the most common reasons for diminished vitamin D axis functioning. Alternatively, or in addition, decreased renal or parathyroid function can impair 1,25OH_2D levels despite normal 25OHD levels.

2.2.2 Evaluation of vitamin D status

Circulating 25OHD is the accepted marker of vitamin D status, with three threshold ranges commonly cited in the literature. Generally, sufficiency is accepted as a value above 75-80 nmol/L, deficiency is defined as a value below 50 nmol/L, with severe deficiency occurring in the 25-30 nmol/L range (7-13). These thresholds are based not only on biochemical indicators of axis stress, but also represent the values below which bone health or calcemic symptoms develop. Briefly, when 25OHD concentrations fall into the 50 nmol/L range, maintenance of active hormone levels requires elevation of serum parathyroid hormone (PTH) and increased renal enzyme activity (14, 15). As 25OHD falls below the 25 to 30 nmol/L range, production of the active hormone (1,25OH_2D) falls and otherwise healthy individuals can develop electrolyte disturbances and clinically evident disease (rickets, seizures, myocardial disease) (15-17). Although overt clinical disease is often not

evident until values drop below 30 nmol/L, population based research has established improved bone health with values over 50 nmol/L (11). Further research confirming or refuting 50 nmol/L as the appropriate threshold for prevention of non-bone related disease is required.

The clinical and research assays available for 25OHD determination can be divided into two analytical approaches: Liquid Chromatography-Mass Spectrometry (LC-MS) and antibody based immunoassays (9, 18). Although considered equivalent by some, there is emerging evidence that LC-MS may be superior for infants and young children. Superiority of the LC-MS methods relates to the ability to resolve 25OHD from a number of vitamin D metabolites that occur at relevant concentrations in infants and young children, particularly 24,25 dihydroxyvitamin D and 25-hydroxy-3-epi-vitamin D3 (3-epi-25OHD) (19-22). Recent studies have shown that the antibodies used in most immunoassays cross react with one or more of these metabolites, and counting these toward the 25OHD total may be inappropriate as they appear to have reduced or absent clinical effects (23).

2.3 VITAMIN D DEFICIENCY IN CHD PATIENTS

Previously described roles for vitamin D in the maintenance of electrolyte homeostasis, cardiovascular health, inflammation and innate immunity have led multiple research groups to investigate and report on the prevalence of vitamin D deficiency among both critically ill children and post-operative CHD populations. Two studies on vitamin D in pediatric critical illness were published in 2012, one of which included a subgroup of 120 patients with CHD (24, 25). This study reported that approximately seven out of every ten critically ill children were vitamin D deficient, and subgroup analysis on the post-operative

CHD participants confirmed a 73% deficiency rate with a mean 25OHD level of 40 nmol/L (24). To identify potential centre specific effects, post-operative 25OHD levels in CHD patients were compared at the three Canadian cardiac surgery sites and did not identify any statistically significant differences (26). A third PICU cohort study was published later in 2012 that reported a 40% vitamin D deficiency rate in post-operative cardiac surgery patients (27). In 2013 two studies were published focusing specifically on the post-operative CHD population (26, 28). Graham *et al.* used blood remaining from a glucocorticoid study of 70 neonates with CHD to show that 84% had 25OHD levels below 50 nmol/L (28). The other study prospectively evaluated 58 CHD patients with a range of ages and heart defects found a mean post-operative value of 35 nmol/L and an 85% vitamin D deficiency rate (26) (findings tabulated and provided in appendix 2.2*)*.

Inspection of the 4 observational studies that included post-operative CHD patients demonstrates that all report statistically significant associations between lower vitamin D levels and need for cardiovascular support (vasopressor and/or inotropes). Findings from the sole PICU study that did not include post-operative cardiac patients also demonstrated a link between lower vitamin D levels and cardiovascular function (higher levels in patients with increasing CV-SOFA score, Cardiovascular Sequential Organ Failure Assessment, scores) (25). In addition, four studies showed an association between vitamin D levels and at least one other clinically important outcome measure including Pediatric Risk of Mortality III scores (25, 29), hypocalcemia (24) or calcium supplementation (27), fluid requirements (24), and PICU length of stay (24). Illness severity associations shown for the McNally et al. cohort study represent those for the entire group, while those for the study by Rippel and colleagues represent the CHD subgroup.

Additional evidence supporting vitamin D deficiency as a modifiable risk factor for poor outcome following CHD surgery can be found in a growing number of observational studies in adult ICU populations (30-38). A detailed evaluation of the adult critical care literature is beyond the scope of this article and has been reviewed elsewhere (39, 40). Briefly, the first adult publication on the topic in 2009 described 42 ICU patients, reported an average 25OHD level of 40 nmol/L, and demonstrated greater illness severity scores with lower hormone levels (30). Following a number of supportive small observational studies Braun *et al.* confirmed the association between lower admission vitamin D and mortality in a large observational study involving thousands of adult patients (41). Separately, this same research was also able to demonstrate pre-illness 25OHD concentration as a predictor of subsequent ICU related mortality (33). Although vitamin D deficiency has been associated with cardiovascular disease (36, 37, 42), the potential relevance of vitamin D deficiency to outcomes following adult cardiac surgery remains less well defined (43, 44). The best study to date comes from large prospective observational study published this year by Zitterman and colleagues, wherein they demonstrated that compared to normal 25OHD levels (75-100 nmol/L) having a value below 50 nmol/L was significantly associated with Major Adverse Cardiovascular and Cerebrovascular Events, or MACCE (43) .

2.4 FACTORS CONTRIBUTING TO POST-OPERATIVE VITAMIN D DEFICIENCY

There are multiple pre-operative, intraoperative and immediate post-operative factors that may contribute to post-operative vitamin D deficiency (Figure 2B, appendix 2.3).

2.4.1 Primary vitamin D deficiency

Children with CHD may be at increased risk for pre and post-operative vitamin D deficiency due to inadequate sun exposure and poor vitamin D intake that may be related to their underlying disease. Significant pre-surgical vitamin D deficiency rates were described in both pediatric studies that measured 25OHD levels pre-operatively (26, 28). This observation suggests either poor compliance with guidelines or that vitamin D requirements (metabolism) differ for CHD patients compared with healthy children. The question of compliance with current recommendations (9, 11, 12) for vitamin D intake and supplementation was addressed through a targeted vitamin D food frequency questionnaire in the prospective study performed by McNally *et al.* (26). This questionnaire demonstrated that up to 50% were not achieving a daily vitamin D intake of 400 IU. This non-compliance with Vitamin D recommendations occurred despite close supervision by a large group of health care providers and has also been reported in the general population (45, 46).

2.4.2 Secondary vitamin D deficiency

In addition to the mechanisms outlined above, blood vitamin D levels may be further reduced either intra-operatively or immediate post-operatively due to large circulating fluid shifts, blood loss, blood ultrafiltration, fluid administration and interstitial leak of vitamin D binding proteins due to inflammation (47-50). This occurrence, and timing, was explored by McNally *et al.* through the collection of serial blood samples through surgery and over the first two post-operative days (26). The major finding was a 40% intraoperative fall in serum 25OHD immediately following initiation of CPB. Similar blood levels before and after modified ultrafiltration (MUF) and insignificant 25OHD in the ultra-filtrate do not support a loss of Vitamin D through ultrafiltation. Other possibilities therefore include either a dilutional effect from the prime volume or absorption of 25OHD by components of the

bypass circuit (e.g., tubing, oxygenator membrane). No further change in group mean 25OHD levels occurred after PICU admission, evaluated over the critical first two post-operative days.

It is worth noting that in the retrospective study by Graham and colleagues an intra-operative decline in 25OHD concentration was not observed (28). The lack of even a small decline is not consistent with the study by McNally *et al.*, two recent adult CPB studies and a small neonatal case series describing calcitriol concentrations before and after initiation of Extracorporeal Membrane Oxygenation (26, 47, 51, 52). Zitterman *et al.* reported a significant drop in 25OHD level following adult heart transplantation, but the exact timing could not be determined as the first post-operative levels were measured on the sixth post-operative day (51). The study by Krishnan and colleagues, demonstrated up to a 40% drop in serum 25OHD in 19 adults following the CPB prime (47). A potential explanation for the contrast in findings between Graham *et al.* and the other four studies could be a result of differences in research blood collection, processing and storage. Blood collection approaches may be essential as 25OHD levels have been shown to be consistently 20% higher when measured from capillary blood compared to venous blood (53). Further, and as noted by Graham and colleagues, the pre-operative vitamin D level and choice of CPB prime fluids may also contribute to the differences in findings (28).

2.5 ROLE OF VITAMIN D DEFICIENCY IN CHD PATIENTS

The role of vitamin D as a modifiable risk factor for post-cardiac surgery outcome has biological plausibility due to the number of known organs and body pathways through which it could cause or worsen post-operative pathophysiology (Figure 2C, appendix 2.4).

2.5.1 Critical illness hypocalcemia

Hypocalcemia is a common problem following pediatric cardiac surgery (~30%), with a 2008 study demonstrating the need for calcium supplementation as a risk factor for morbidity and mortality (5). Calcium homeostasis is important for patient well-being as calcium initiates and propagates nerve conduction, muscle contraction, and contributes to intra-cellular signal transduction. In addition to the negative impacts of hypocalcemia on cardiovascular dysfunction, abnormal calcium homeostasis could impair gas exchange and influence ventilator requirements through nerve dysfunction and muscle weakness (54, 55). A number of pediatric studies have confirmed hypocalcemia to be risk factor for worse ICU outcomes and that critically ill children with hypocalcemia are more likely to have abnormalities of their vitamin D axis, including 25OHD deficiency (56-58). No studies have evaluated whether optimization of vitamin D status prevents or reduces the severity of critical illness hypocalcemia.

2.5.2 Cardiovascular dysfunction

Post-operative cardiovascular dysfunction is common following cardiac surgery with many children requiring the continuous infusion of one or more vasoactive medications to support blood pressure and maintain cardiac output (59). A role for vitamin D in pediatric cardiac health can be found in case reports and case series describing cardiomyopathy secondary to isolated severe vitamin D deficiency (60-65). A recent case series identified 16 children with treatment responsive cardiomyopathy secondary to isolated severe vitamin D deficiency (63). Vitamin D responsive subclinical cardiac dysfunction has also been described in children with rickets, with 50% of the cohort demonstrating ECG and

echocardiogram abnormalities at presentation (66). In addition to the indirect actions of vitamin D through calcium, it is well known that vitamin D influences myocyte structure and function via nuclear vitamin D receptors that alter gene and protein expression (67, 68). More recent research has also suggested that vitamin D may mediate structural and functional myocyte changes through non-nuclear VDRs (29). As an example, myocyte contractility has been observed to be favorably altered within minutes following $1,25OH_2D$ supplementation; an effect mediated through signal transduction pathways, enzymatic reactions and ion channels (69, 70). Further, vitamin D appears to play a role modulating peripheral vascular resistance directly through receptors on smooth muscle cells and indirectly through the renin-angiotensin-aldosterone system (32). Finally, numerous studies have recently suggested that vitamin D deficiency could serve as an effect modifier, augmenting the impact of other deficiency states (e.g., adrenal) and medications (corticosteroids) on cardiorespiratory function (26, 71, 72).

2.5.3 Immune dysfunction

Cardiac surgery uniformly leads to a post-operative systemic inflammatory response that can contribute significantly to low cardiac output and respiratory dysfunction (73, 74). There is good evidence that vitamin D metabolites play important immunomodulatory roles mediated through functional vitamin D receptors present on all major immune cell types. Specifically, vitamin D has been demonstrated to inhibit antigen-induced T-cell proliferation, antagonize the pro-inflammatory Th1 (T-helper) response, suppress macrophage release of pro-inflammatory cytokines, and alter gene expression of adhesion factors, decreasing adherence and chemotaxis of neutrophils (75-77). Vitamin D signaling is also known to play a role in innate immunity through the production of cathelicidins, which are important

endogenous antimicrobial peptides that, provide protection again multiple viral and bacterial pathogens (78-80).

2.6 VITAMIN D SUPPLEMENTATION REGIMENS

The cumulative body of basic science and clinical literature suggests that optimization of vitamin D status could improve clinical outcomes in CHD patients. Available evidence suggests that both primary deficiency and operative procedures potentially contribute to high post-operative deficiency rates. Although rapid restoration of vitamin D levels immediately following surgery would represent an attractive option for anesthesiology and intensivists, the lack an intravenous formulation of cholecalciferol or 25-hydroxyvitamin D prevents consideration of such an approach. Instead, the practioner caring for children with CHD will need to pre-operatively raise and maintain levels utilizing one of two approaches proven for other pediatric populations. Given the pharmacokinetics of vitamin D, prevention of post-operative deficiency patients may necessitate an understanding of both approaches, with application personalized based on patients factors such as compliance with daily intake and time prior to surgery.

Considering the primary literature, expert opinion and concerns about post-surgical inflammation, hypoparathyroidism and renal dysfunction, it would be ideal to target a post-operative 25OHD level above 75 nmol/L, with the goal of avoiding values below 50 nmol/L. Given the potential for a significant (40%) intra-operative decline, pre-operative levels in the 100-150 nmol/L range may be required to achieve these goals.

2.6.1 Daily low dose vitamin D supplementation

The most commonly used approach for building and maintaining levels of vitamin D relies on the daily consumption of an age specific low dose of ergocalciferol or cholecalciferol (ranging from 400 to 4,000 IU). To date there have been no pediatric trials of any vitamin D regimen in the CHD population, necessitating extrapolation of recommendations from the most recent guidelines or position statements for healthy children (9, 11, 12). Recently, at the request of agencies of the US and Canadian governments, the Institute of Medicine (IOM) assembled a committee to provide recommendation for intake of vitamin D based upon a rigorous and comprehensive review of literature. In the final report, the IOM provided two age specific doses: (i) Recommended Daily Allowance or Adequate Intake and (ii) Tolerable Upper Intake Level (11). As described in the IOM report, the Recommended Daily Allowance and Adequate Intake doses are intended to maintain blood 25OHD concentrations at or slightly above the 25OHD threshold (50 nmol/L) known to foster bone health. In calculating the age specific upper intake level, the IOM goal was to provide a daily dose that would significant elevate levels well above the cut-off for vitamin D deficiency while safely avoiding potential toxicity.

The efficacy and safety of the two age specific IOM dosing regimens have been supported by the publication of two well done trials since the IOM report (19, 81). First, a Canadian study confirmed previous work demonstrating that 2 or more months of daily dosing is required to achieve a new steady state 25OHD level (19). Second, both studies showed that with good compliance, 400 IU per day for 3 months will generate a mean pre-operative level of 80-90 nmol/L, with almost all participants elevated above 50 nmol/L. However, these studies also suggest that utilization of this dose in CHD patients could still leave 50% or more at risk for post-operative vitamin D deficiency. Both studies evaluated a

1,600 IU/day regimen, closely approximating the IOM 6 to 12 month old Tolerable Upper Intake Level. At this dose, the mean 25OHD concentrations achieved in the two studies were 157 and 180 nmol/L, with almost all generating 3 month levels above 90 nmol/L. Importantly, after consideration of unwanted metabolites, no child exceeded a 25OHD level above 250 nmol/L. Neither study demonstrated additional cases of hypercalcemia or hypercalciuria in the groups receiving doses above standard of care.

2.6.2 Intermittent high dose supplementation

Some circumstances may necessitate consideration of an alternative approach to the daily low dose vitamin D regimen. First, poor compliance with vitamin D supplementation occurs in a certain percentage of patients as previously demonstrated in the general population (45, 46). Second, in a small but consistent percentage of the CHD population, particularly neonates and young infants, the time between diagnosis and surgery will not allow for 2 to 3 months of low dose vitamin D intake.

The second approach to supplementation involves the delivery of a 2 to 3 month prescription of vitamin D either as a single dose or over a period of days (82-84). This regimen is commonly referred to as Stosstherapy (or megadose therapy) and generally involves the oral or intramuscular administration of 50,000 to 600,000 IU. Not surprisingly the route of administration contributes significantly to the rate and final 25OHD level achieved. When given orally, the loading dose is immediately absorbed into the circulation, undergoes rapid liver hydroxylation, and gives rise to a peak 25OHD within a few days (17, 85-87). In contrast, with intramuscular administration, the rise in 25OHD levels occurs over many weeks due to slow resorption from the muscle, and may be more variable (88).

There is significant experience with the high dose regimen in certain regions of the world and it has been recommended as part of the Australia and New Zealand position statement (89). However, it is important to point out that the available pediatric clinical trials have largely focused on healthy children (without or without vitamin D deficiency) and administered doses intended to prevent or treat vitamin D related bone disease. The safety of this dose regimen has received limited evaluation in children with cardiac dysfunction or acute illness particular at doses intended to achieve 25OHD levels in the 100 to 150 nmol/L range. As there is evidence to suggest that some populations at certain doses may develop hypercalcemia additional work in this area will be required to determine the high dose supplemention approach that safely maximizes 25OHD level in CHD patients (90).

2.7 VITAMIN D TOXICITY

2.7.1 Signs and symptoms

Vitamin D toxicity is a characterized by hypercalcemia and/or hypercalciuria, with the classic symptoms (lethargy, abdominal pain, anorexia, constipation, polyuria and nocturia) directly attributable to these abnormalities. In many instances symptomatology related to hypercalcemia and/or hypercalciuria is minor. However, as documented in case reports and case series the longstanding persistence of minor biochemical abnormalities or progression to severe electrolyte disturbances can give rise to more serious problems including dehydration, renal dysfunction and eventual nephrocalcinosis.

2.7.2 Toxic threshold levels

Presently no 25OHD level has been universally accepted as the threshold above which risk develops, with authors generally citing values between 250 and 750 nmol/L.

Although there is no evidence that children develop biochemical abnormalities or symptoms with 25OHD values at or slightly above 250 nmol/L, recent pediatric clinical trials of high dose vitamin D have focused on this threshold (19, 81). Application of this threshold for dosing studies is appropriate given that levels above this are supraphyiosological (cannot be achieved with excessive sun-exposure or healthy diets) and there is no evidence of benefit for 25OHD doses above 200 nmol/L (91, 92).

2.7.3 Risk factors for vitamin D related toxicity

Despite public and clinician concern regarding vitamin D toxicity, it is a rare event that generally occurs in the context of genetic susceptibility or inappropriate intake of high doses of vitamin D. Concern about the safety of daily vitamin D supplementation above 400 IU/day dates back to the 1950's when a rise in idiopathic infantile hypercalcemia (IIH) cases coincided with the population based implementation of increased daily vitamin D intakes to ~4,000 IU/day (93-96). This small epidemic led to a decrease in recommended daily intake to levels known to prevent rickets and hypocalcemic seizures (400 IU/day). It has recently been argued that many, perhaps all, cases of IIH were due to rare genetic conditions (<1:10,000) that increase susceptibility to vitamin D toxicity (97). Of these, patients with Williams syndrome can have heart defects as part of their constellation of symptoms and it would be prudent to avoid higher vitamin D intake in this subgroup (98).

There is a substantial body of low level evidence suggesting that high dose vitamin D giving rise to shorter term cumulative intake at or above 600,000 IU is excessive and can lead to hypercalcemia, hypercalciuria and eventual nephrocalcinosis. This anecdotal evidence is also supported by one prospective pediatric clinical trial that demonstrated significant

hypercalcemia rates among healthy infants who received intermittent, often repeated, high dose therapy with 600,000 IU (90). In most instances symptoms and biochemical abnormalities resolved following diagnosis and discontinuation of the vitamin D source allowing gradual decline of blood 25OHD levels below toxic levels. In some circumstances the nephrocalcinosis persisted despite discontinuation of vitamin D. A review of the literature on nephrocalcinosis shows that most cases associated with vitamin D have occurred in children with a rare genetic disorder called vitamin D resistant rickets and may be related to concurrent phosphate intake (99-103). Again, a review of case series and case reports demonstrate that otherwise healthy children only develop nephrocalcinosis following intentionally or unintentionally cumulative Vitamin D intake above 600,000 (99, 100, 104, 105). Our review of pediatric interventional trials on vitamin D supplementation identified 2 studies evaluating daily high dose vitamin D approximating below the IOM upper intake level and 4 with megadoses (100,000 to 150,000 IU range); none identified increased urinary calcium excretion or hypercalciuria (19, 81, 83, 106-108). Given these findings it would be prudent to avoid oral vitamin D dosing at or near 600,000 IU.

2.8 FUTURE DIRECTIONS

2.8.1 Absence of clinical trial evidence

As stated previously, there are no studies evaluating ergocalciferol (D2) or cholecalciferol (D3) dosing in CHD patients. Although sufficient evidence exists to support administration of vitamin D regimens 2 to 4 times above the current standard of care (400-600 IU/day), extrapolation of these safety findings from healthy infants to the CHD population may not be appropriate. CHD patients have unique metabolic demands, organ

dysfunctions, and known and unknown genetic abnormalities that may make them more or less susceptible to vitamin D toxicity (98, 109-111). Similar to the recent approach taken from other severely ill populations, including pediatric heart failure (112), pediatric acute lower respiratory tract infection (113, 114) and adult critical illness (86, 115) it would be prudent to test feasable dosing regimens as part of phase II clinical trials.

2.8.2 Supplementation with active vitamin D hormone

Although recognized as the best indicator of vitamin D status, post-operative 25OHD concentration may not accurately reflect vitamin D axis functioning and calcitriol levels in the immediate post-operative CHD patient. As shown in Figure, appendix 2.3, the post-operative CHD patient has many congenital, pre-operative and surgically acquired risk factors for low post-operative calcitriol for reasons beyond impaired 25OHD levels. Of these, we speculate that CPB may lead to a significant acute decline in blood calcitirol levels, as demonstrated in studies following adult cardiac transplant and initiation of neonatal ECMO (51, 52). Further, as described in other patient populations, pre-surgical or acquired dysfunction of the parathyroid and renal organs may limit or prevent conversion of 25OHD to calcitriol (7, 116). If future studies report a transient or persistent decline in calcitriol levels following CPB, administration of active vitamin D may also be required to achieve the goal of optimizing vitamin D status. Clinical evidence demonstrating cardiovascular benefit of calcitriol administration, in addition to 25OH, can be found in the end stage renal disease literature, where a vitamin D deficient state emerges due to reduced renal activation of vitamin D. In this population, the administration of an activated vitamin D analogue reduces the increase (50-10 fold) in cardiovascular disease (18, 117).

2.8.3 Nutrition and other vitamins

The impact of cardiac surgery and CPB on the other endocrine axis regulating cortisol, glucose, thyroid and vasopressin have been well described. However, the impact of cardiac surgery and cardiopulmonary bypass on other vitamins and blood metabolites remains less well defined. A better understanding of how these metabolites respond to CPB and post-surgical care would inform researchers regarding the need for a multivitamin supplement in patients undergoing CPB.

2.9 SUMMARY

Multiple observational studies have identified significant rates of post cardiac surgery vitamin D deficiency in a high-risk (CHD) pediatric population following CPB. Data from these same studies demonstrate that lower post-operative vitamin D are associated with a more protracted clinical course. Available data strongly suggest that the current approach to vitamin D supplementation will not reliably prevent post-operative vitamin D deficiency. The current lack of clinical trials evaluating the efficacy or safety of any alternative high dose vitamin D regimen prevents evidenced based recommendations. Therefore, it is important to conduct a systematic review of available literature prior to designing a clinical trial to explore alternative vitamin D supplementation strategies that will safely and effectively optimize post-operative vitamin D status in most CHD patients.

2.10 BRIDGING PARAGRAPH BETWEEN PUBLICATIONS

Prior to modifying current practice or embarking upon phase III clinical trials of high dose vitamin D, a fundamental step is a systematic review identifying all clinical trials of high dose vitamin D in children. In addition to identifying any clinical trials in the CHD populations such a review will prove crucial to understanding the short and longer term 25OHD response to different dosing regimens, the study and population characteristics that influence response, and what dosing regimens may be associated with adverse events. Understanding the different dosing regimens is particularly relevant to the CHD population where there is significant heterogeneity in lesion type, severity, comorbidity, age of presentation, and timing of surgery. Due to this heterogeneity, peri-operative optimization of vitamin D status is unlikely to be accomplished using currently approved doses and dosing regimens. Instead, individuals providing care for these children may need to understand and be comfortable with two or more dosing regimens so that care can be personalized and vitamin D deficiency is prevented for the vast majority of CHD patients.

In addition to uncertainty surrounding the appropriate dosing regimen for cholecalciferol, some research groups have also questioned whether other vitamin D metabolites, specifically calcitriol, might represent both a better marker of vitamin D status and more appropriate supplement given the availability of an IV formulation. Although limited, available research in cardiac surgery and critically ill populations have not demonstrated consistent advantage to the addition of $1,25(OH)_2D$ to 25OHD measurements for predicting clinical course (118-120). A recent pilot RCT of calcitriol supplementation in adult severe sepsis did not demonstrate differences between groups in plasma concentrations of cathelicidins or cytokine levels(121). Finally, there is convincing evidence that elevating

25OHD through cholecalciferol administration also increases 1,25$(OH)_2$D levels(122). Altogether the current wisdom supports trials of cholecalciferol prior to considering a more expensive metabolite with greater risk of toxicity(123).

Chapter 3 presents the result of a systematic review of clinical trials reporting vitamin D levels after receipt of high dose vitamin D. As the results of this review had applicability beyond the CHD population the publication was prepared with a more general pediatric audience in mind. Of additional note, the database we generated of all pediatric clinical trials of vitamin D has also been used as a resource for two other studies. First, we published a systematic review of clinical trials that evaluated how high dose vitamin D modifies asthma related outcomes; analysis of data from 5 studies demonstrated that high dose vitamin D decreased asthma exacerbations(124). Second, we have prepared a scoping review of high dose vitamin D clinical trials and created an online searchable database for clinicians and researchers (manuscript in preparation).

3.0 RAPID NORMALIZATION OF VITAMIN D: SYSTEMATIC REVIEW AND META-ANALYSIS

3.1 INTRODUCTION

The evidence for vitamin D in calcium homeostasis, cardiovascular and respiratory health, inflammation and innate immunity have lead to questions about whether deficiency might represent a modifiable risk factor in the prevention or recovery from acute and critical illness. A large body of observational literature from adult Intensive Care Unit (ICU) and cardiovascular populations have documented high Vitamin D deficiency (VDD) rates and association between blood 25-hydroxyvitamin D (25OHD) and organ dysfunction, health resource utilization and mortality. More recently pediatric observational studies have supported these findings in similar populations including asthma, ICU, and the post-surgical CHD (24-28).

Normalization of vitamin D status has the potential to speed recovery and improve outcomes in multiple acutely unwell pediatric populations. Most of the guidelines and clinical practice surrounding vitamin D dosing involves daily intake under 1000 IU (9, 11). As these standard dosing strategies target healthy children and require months to restore normal levels they are not applicable to the acute and critical care settings. Although other regimens that involve the administration of higher doses have been reported, there remains concern about both inadequate dosing and excessive doses leading to toxicity (82, 125). In the adult ICU setting, pilot trials of loading dose therapy have been performed, with results from a large trial evaluating clinical benefit completed but unpublished (86, 115, 122). No pediatric ICU studies have been completed at this time.

To inform clinical practice and future trials we have performed a systematic review with the goal of identifying all published pediatric trials reporting on the administration of high dose vitamin D (≥1000 IU). The objectives of this review were to: 1) assess the ability of different dosing regimens to normalize vitamin D status, 2) determine study characteristics that influence post-drug 25OHD levels, 3) determine what high dose regimens are associated with vitamin D related adverse events, and 4) use the findings to recommend a dosing regimen for clinical practice and future clinical trials in pediatric acute and critical care settings.

3.2 METHODS

Study objectives and protocol were determined *a priori* (PROSPERO protocol registration number: CRD42013006677) and reported according to PRISMA guidelines (Supplemental Information, Appendix S3.24) (126).

3.2.1 Eligibility Criteria

Studies were eligible for inclusion in the systematic review if they met all of the following criteria: i) an uncontrolled, controlled or randomized controlled trial (RCT) conducted in neonates, infants, children or adolescents; ii) the study administered at least one dose of cholecalciferol (D3) or ergocalciferol (D2) equal to or in excess of 1000 IU; iii) the study evaluated the effects of drug administration on 25OHD status. Studies were excluded if the population that was exclusively premature or low birth weight, had a genetic problem related to vitamin D metabolism, or were pregnant. Further, studies were excluded if they prescribed ultraviolet exposure, gave vitamin D as part of a food without precisely

controlling quantity, or administered vitamin D with another vitamin or drug (without a control arm).

3.2.2 Identification of Studies

Medline (1946 to 2014 Week 2), Embase (1974 to 2014 Week 3) and the Cochrane Central Register of Controlled Trials (December 2013) were searched using the Ovid interface. The MEDLINE search strategy was developed by a librarian experienced in systematic review searching (MS), and peer-reviewed by another librarian (LK), using the PRESS standard (127). The MEDLINE search was then adapted for the other databases. No date, language or study design limits were applied. We searched conference abstracts from 2010-2013 through Scopus. The search strategies are presented in Supplemental Information (Appendix S3.1-S3.6). The initial search was conducted on April 30, 2013 and updated on Jan 21, 2014. We also conducted a grey literature search by reviewing ongoing trials registered with Clinicaltrials.gov, the citations of all eligible articles, and 24 systematic reviews of vitamin D in children.

Unless otherwise noted, two of the study authors independently reviewed the citations sequentially through three sets of screening questions to determine eligibility (Supplemental Information, Appendix S3.7). Level 1 screening was performed using Mendeley (Mendeley Desktop, version 1.10.3) and those citations that could not be excluded were uploaded to DistillerSR^{TM} (Evidence Partners Incorporated, Ottawa, ON, Canada) for level 2 and 3 screening. Full texts of all potentially eligible citations were reviewed by two authors (KI/KO and DM). Conflicts between reviewers were resolved through discussion, with a third author available to resolve disagreement (MS). The eligibility of articles not in English, French or

Spanish was determined by a single author after written translation or with the assistance of a translator.

3.2.3 Data Collection and Risk of Bias

Data from eligible studies were extracted by one review author and verified by a second author by independent review of the article (DM, KI, KO, SP). Data were collected and managed using REDCap (Research Electronic Data Capture) hosted at the Children's Hospital of Eastern Ontario (128). Vitamin D or calcium data values that were only published graphically were extracted from figures using DigitizeIt software (http://www.digitizeit.de/, Germany). During the data collection process, 18 authors were contacted to clarify or request additional study 25OHD data, of which five responded. Study quality was described using the Cochrane risk of bias assessment tool (129).

3.2.4 Data Analysis and Reporting

Summary statistics and data from eligible studies and independent arms were described as text, through tables and figures. Clinical heterogeneity between studies was assessed using information on population (age, disease status, baseline vitamin D), dosing regimen (dose, frequency, form, route) and measurement features (time, assay type). Regimens were considered intermittent if they gave a vitamin D dose in excess of 40000 IU as a single administration (divided over two days) or was repeated with a frequency equal to or in excess of 1 month. Methodological heterogeneity was evaluated using information collected on study type (single arm, RCT, controlled non-RCT) and the Cochrane risk of bias tool. For specific dosing regimens, 25OHD response was presented using figures (Sigma

plot, version 12.3) and the success of each dosing regimen was defined as achieving a group 25OHD average > 75 nmol/L.

Given significant heterogeneity in post 25OHD levels, we performed random effects meta-regression to evaluate the contributions of specific study level population, dosing and methodological characteristics. This analysis included study arms reporting a group 25OHD level between 1 and 13 weeks of drug initiation and an accurate cumulative dose could be calculated. Assessment of heterogeneity and meta-analysis was performed using Comprehensive Meta-Analysis (version 2) with meta-regression performed using the PROC MIXED function in SAS (version 9.3). Analysis used group mean or median 25OHD levels, and within study variance using provided or calculated standard errors (130, 131). For age, if the median or mean was not provided we used the mid-point of the age range as an approximation (132). Initially, single variable random effects meta-regression was performed and potentially significant variables were then tested in a multivariable meta-regression analysis. A potential interaction was sought between cumulative dose and age, and an interaction term was included in the regression analysis to control for and evaluate how timing from single or divided loading doses to 25OHD measurement influences the level. No new variables were to be added to the multivariable model once the ratio of variables to eligible 25OHD measurements exceeded 10:1. The final multivariate model was used to calculate the predicted group 25OHD response to a loading dose (or stosstherapy) of drug among four age groups of vitamin D deficient (30 nmol/L) children.

3.3 RESULTS

3.3.1 Results of search

Figure 3A (appendix 3.1) shows the flow of studies through the identification and review process. A total of 2453 unique records were identified for screening. Of the 367 full text citations that remained after initial screening, 256 articles describing clinical trials were identified. Of these, 88 full text publications (17, 19, 81, 83, 84, 90, 106-108, 112, 133-211) and 10 conference abstracts (Supplemental Information, Appendix 3S.8-3S.9) met all population, dosing and 25OHD outcome-related eligibility criteria. Flow of eligible articles and study arms is presented in Supplemental Information, Appendix S3.10. The 88 full articles reported on 96 eligible study populations and included 199 different arms. Of these 199, three were ineligible due to UV exposure (n=2) or administration of active vitamin D (n=1). Of the remaining arms, 62 involved the administration of no vitamin D (e.g. placebo) or a dose under 1000 IU. Of the 134 high dose arms, 22% (29) and 78% (105) were from uncontrolled and controlled studies, respectively.

3.3.2 Patient populations

Tables 3A (appendix 3.2) and 3B (appendix 3.3) present the relevant clinical and methodological characteristics of the eligible high dose arms. Of the eligible high dose study arms, 73% involved administration of vitamin D to healthy children (49%), children with rickets (16%) or pediatric populations with subclinical VDD (8%). Populations of children with "other" disease states (e.g. HIV, arthritis, seizures) accounted for 19% of the study arms (Supplemental Information, appendix 3S.11). As shown in Table 3A, studies included children from all age ranges, with neonates being evaluated in 15% (n=20) of high dose study arms and adolescents in 50% (n=67). Vitamin D dosing regimens evaluating intermittent loading therapy accounted for 46% (n=62) of the eligible study arms, with daily regimens representing 38% (n=51). A minority of the eligible high dose arms (14%, n=19) described a

dosing regimen that varied dependent on factors including baseline 25OHD, weight or age. The number of participants in each arm ranged from 5 to 233, with a median size of 27 (IQR: 13, 40).

At least one measure of average post-drug group absolute 25OHD (or change) was available from all but one of the high dose study arms. Of the 134 high dose arms, 35% (n=48) and 76% (n=106) measured 25OHD within 1 and 3 months of study drug initiation. Tables in Supplemental Information (Appendix S3.12-S3.18) show relevant information on population, dosing regimen, and 25OHD response for each study arm reporting 25OHD within 3 months of study drug initiation (17, 19, 81, 84, 90, 106-108, 112, 133-141, 143-189, 191-194, 212, 213).

3.3.3 Evaluation of 25OHD response by dosing regimen

Six independent treatment arms were identified that evaluated response to daily vitamin D between 1000-4000 IU in a group of children who were vitamin D deficient and reported 25OHD levels within the first month (144, 151, 159, 180, 184, 188). As shown in Figure 3B (appendix 3.4) none of the arms achieved a group 25OHD above 75 nmol/L with the first measurement, and 2 (33%) achieved this target within the first month. A single weekly dosing regimen was identified that enrolled VDD children and performed blood work within 1 month; this study reported an increase in 25OHD from 22 to 143 nmol/L with 4 weekly doses of 60000 IU. Ten independent study arms were identified that evaluated oral loading doses with vitamin D deficient populations and measured 25OHD within a month (17, 151, 162, 165, 173, 176, 214, 215). As shown in Figure 3C (appendix 3.5), 9 (90%) achieved an average post-study drug group level above 75 nmol/l, with three arms exceeding

200 nmol/L(151, 162, 173). Five additional arms calculated 25OHD change following oral vitamin D loads and reported increases ranging from 45 to 73 nmol/L(163, 178, 182). All arms with more than one post study drug measurement demonstrated a decline between the first and subsequent measurements. Dosing regimens that reported multiple measurements during the first week after oral loading suggested that 25OHD peaks on day 3 and declines from day 3 to 7 by an average of 15% (Supplemental Information, appendix S3.19) (17, 176).

3.3.4 Evaluation of variable loading dose regimens

Seven independent arms were identified that evaluated 25OHD response in vitamin D deficient children using a variable intramuscular (IM) dosing strategy (10000 IU/kg) (84, 170, 171, 183, 216). The single 10000 IU/kg IM dosing regimen that reported 25OHD within one month of therapy achieved a mean group level above 75 nmol/L (216). No published studies or conference abstracts evaluating 25OHD response within a month of a variable oral load were identified. One of the conference abstracts, published by Frizzell *et al.*, evaluated response to an age based loading regimen (<3 yr:150000 IU, 3-12 yr: 300000 IU, >12 yr: 600,000 IU) among 40 children; approximately 6 weeks after treatment the group average increased from 27 nmol/L to 93 nmol/L, with at least one participant exceeding 300 nmol/L.

3.3.5 Factors associated with post study drug 25OHD levels

Significant heterogeneity in post study drug 25OHD was evident with group average levels ranging from 30 to 399 nmol/L, and a calculated I^2 value of 99. Single variable random effects meta-regression identified eight variables to be statistically significant, with two additional variables approaching significance (Table 3C, appendix 3.6). Multivariate random effects meta-regression performed using data available from 102 independent arms identified

7 variables independently statistically significant in either the main effects or through an interaction (Table 3D, appendix 3.7). The interaction term between age and cumulative dose determined that the 0.27 nmol/L increase in final group 25OHD per 1000 IU is reduced by 0.013 nmol/L for every one year rise in age. Similarly, the interaction term between dosing regimen and time demonstrated that the group mean 25OHD gradually decreases following a loading dose by 5.6 nmol/L per week (CI: 3.48, 7.7). Inclusion of the study type variable demonstrated that non-randomized controlled studies, but not uncontrolled studies, were associated with higher post drug 25OHD levels. After including the variable for study design, no other measure of study methodological quality from the Cochrane risk of bias tool was statistically significant. Exclusion of the obese or malabsorption studies did not significantly change any of the parameter estimates.

The final multivariate model was used to predict group 25OHD levels following 4 loading doses in 4 age groups of VDD diseased children (Table 3E, appendix 3.8). Regression analysis was also performed to model post study drug 25OHD standard deviation. Standard deviation was best predicted by the equation (SD = 0.42*final25OHD, $R^2 = 0.81$); no other variable significantly improved the model R^2 value.

3.3.6 Thresholds for potentially toxic vitamin D levels

Of the 88 eligible studies, 9 defined thresholds above which 25OHD was toxic or potentially toxic (range:125 to 374 nmol/L) (114, 139, 144, 152, 164, 169, 175, 206, 214). The most common definition was 250 (n=6) and another 2 used definitions of 374 and 375 nmol/L.

3.3.7 Adverse event analysis

There were 39 study arms reporting on high dose (≥1000 IU) vitamin D regimens that provided hypercalcemia data within 3 months of drug initiation. Information on relevant population, dosing and adverse events measurements are provided in Supplemental Information (appendix S3.20-S3.21). There were 23 study arms who received intermittent, weekly or daily high dose loading regimens. Significant heterogeneity in hypercalcemia rates was calculated (I^2=61%, see Figure 3D, appendix 3.9). Random effects meta-analysis identified a statistically significant difference in hypercalcemia rates between accepted daily dosing (2.6%, CI 1.1-5.9) and intermittent, weekly and daily high dose loading regimens (7.6%, CI: 4.1-13.7%, p=0.041). Further analysis identified higher hypercalcemia rates for the arms at or above 400000 IU (23.8%, CI: 16.3-33.3%) when compared with doses at or below 300000 IU (4.2%, CI: 2.0-8.8%, p=0.0001). Subgroup analysis using 25OHD data demonstrated that hypercalcemia was more likely among studies with average group levels above 200 nmol/L, compared to those below 200 nmol/L (3.9% vs 19.6%, p=0.006). Pooled hypercalcemia rates were similar for groups below 100 nmol/L and between 100 and 200 nmol/L. Further subgroup analysis by age was not possible due to the limited number of loading regimens administering doses above 300 000 IU.

For hypercalciuria (29 study arms; Supplemental information, appendix S3.22-S3.23) 13 distinct groups were identified that provided regimens corresponding to intermittent, weekly or daily loading doses. Of these, 10 reported no episodes of hypercalciuria and meta-analysis determined a pooled rate of 2.7% (CI: 0.8-8.9%). Exclusion of the study by Shajari and colleagues, reporting hypercalciuria in 28 of 30, reduced the pooled rate to 1.5% (CI: 0.5-4.5%). Of note, the Shajari study was an RCT and the daily dosing arms reported hypercalciuria in 23 (200 IU/day) and 25 (400 IU/day) of the 30 children (168). Finally, our

review did not identify any reported cases of nephrocalcinosis in the clinical trials administrating intermittent, weekly or daily loading dose regimens.

3.4 DISCUSSION

Evaluation of daily vitamin D administration demonstrated that a dosing strategy approximating the IOM Tolerable Upper Intake Level (1000-4000 IU) will not rapidly normalize vitamin D levels in deficient children. However, administration of a loading dose of > 40 000 IU can rapidly elevate 25OHD. Our analysis also identified baseline 25OHD, age, cumulative dose, regimen type, disease status, time from loading dose and study type as independent predictors of final 25OHDlevel. Adverse event analysis found no increased hypercalcemia or hypercalciuria risk with loading doses at or below 300 000 IU, while a significant increase in hypercalcemia risk was observed with doses at or above 400 000 IU.

This systematic review identified 88 full text publications reporting 25OHD levels following the prospective administration of high dose vitamin D to one or more groups of children. Daily administration and loading dose therapy each accounted for roughly 40% of the eligible study arms. The rarity of loading dose arms originating from North America may explain why vitamin D position statements from Canadian and American pediatric societies make no mention of this therapy (9, 113). Slightly more than 75% of the eligible study arms included healthy, VDD, or children with rickets or kidney disease. None of the studies were from an acute or critical care settings with the most relevant study being a pilot RCT suggesting long-term clinical benefit in stable outpatient congestive heart failure (112). Inspection of excluded studies did not identify any performed in the pediatric critical care

setting, with the most relevant evaluating high-dose intake in pneumonia and severe asthma (217-219).

Examination of post study drug 25OHD levels from high-dose study arms demonstrated a wide range of final group levels. To remove some heterogeneity related to clinical and methodological factors, we evaluated the short-term response to daily vitamin D approximating the IOM Upper Tolerable Intake Level (1000-4000 IU/day)(11). Overall, the results strongly advise that this approach will not normalize levels (> 75 nmol/L) in a time frame appropriate to potentially benefit acute and critically ill populations (144, 151, 159, 180, 184, 188). These findings are important as they will inform future studies, and help interpret the results of published RCTs. For example, these findings might call into question the validity of the pediatric RCT by Choudhary *et al.* evaluating the effect of 5 days of daily 1000 IU on recovery from pneumonia (217).

Conversely, there was convincing evidence that single or divided dose loading therapy is an effective means of rapidly raising 25OHD levels. We also observed that numerous studies generated levels well in excess of the 75 nmol/L target. Multiple study arms administering loading doses of vitamin D achieved potentially toxic levels (groups average ≥ 200 nmol/L) (151, 162, 165, 173). Three of these administered doses in excess of 200000 IU to neonates or infants, and the fourth evaluated 600000 IU in toddlers and preschool children (151, 162, 165, 173). In contrast, the administration of 50000 IU to a group of toddlers and preschool children did not achieve levels of 75 nmol/L in more than half (17). These results suggest that with appropriate dose selection, single or divided loading regimens have the ability to rapidly normalize vitamin D status and may explain the positive

benefits observed in clinical trials evaluating a loading dose in children with pneumonia and severe asthma (217-219).

This study also sought to further explain heterogeneity in post drug 25OHD levels due to population, dosing, and methodological characteristics. Single and multivariable random effects meta-regression identified that baseline vitamin D status, cumulative dose, age, regimen type, healthy vs. diseased status, and study type were significantly associated with post 25OHD level. Most importantly, we identified a statistically significant interaction between cumulative dose and population age, demonstrating that the 25OHD response per dose declines as age increases. Although this observation is most likely related to the high correlation between age and weight, differences in developmental pharmacokinetics may contribute (220). Further, our regression analysis identified lower post-study drug 25OHD levels in study arms originating from diseased populations, when compared to healthy children. There are multiple potential explanations including differential compliance, malabsorption, increased losses (e.g. capillary leak), and altered hepatic or end organ metabolism (48, 221-224).

Collectively, these findings suggest that rapid normalization of vitamin D status may require consideration of age (or weight), baseline 25OHD, and disease status. Prediction of 25OHD levels using the multivariate model suggested 50000 IU as appropriate in young infants, while doses in the 300 000 to 600 000 IU range may be required in adolescents. As weight-based dosing represents the standard of care in the pediatric medicine, these findings might be approximated to 10000 IU/kg. Review of published variable high dose regimens identified 7 independent pediatric populations having 25OHD measurements following the administration of 10 000 IU/kg IM vitamin D. These studies suggest that IM dosing might

rapidly normalize vitamin D status, although the lack of measurements within the first month and paucity of enteral studies prevents definitive conclusions. Regardless results from the IM studies are relevant as many acute and hospitalized patients suffer significant malabsorption and/or are not able to take food and medication enterally (88, 225). The need for pediatric studies evaluating 10000 IU/kg using the enteral route is reinforced by evidence from adult studies showing significant differences in short-term response between enteral and IM routes (91, 92).

This review also examined whether high dose loading regimens were associated with vitamin D related adverse events and toxicity. Vitamin D toxicity is a characterized by hypercalcemia and hypercalciuria with the classic symptoms (e.g. abdominal pain, anorexia, constipation, polyuria) directly attributable to these abnormalities. Presently, there is no accepted 25OHD threshold that identifies increased adverse event risk. The lack of certainty is emphasized by our finding that 90% of studies did not use or cite a specific threshold. For the few that did report, the most common value was 250 nmol/l (114, 139, 152, 164, 169, 175, 206, 214, 226). Review of these articles identified that the more recent trials did not select thresholds based on known toxicity, but the idea that supraphysiologic levels (not achievable with sun-exposure, healthy diets) are unlikely to be of benefit (81, 97, 98, 114, 141). Our analysis supports a 200 to 250 nmol/L threshold as dosing regimens with averages above 200 nmol/L, being associated with increased hypercalcemia risk.

To better inform selection of dosing regimens, we also sought to understand whether there was a cumulative loading dose associated with increased hypercalcemia and hypercalciuria. Our evaluation did not identify increased risk of hypercalcemia with loading doses at or below 300000 IU (4%) but did find a significantly higher risk for those at or

above 400 000 IU. In addition, our review identified only 3 cases of hypercalciuria among the 878 study participants who received intermittent, weekly or daily loading regimens (after exclusion of Shajari (168). Further, none of the eligible clinical trials reported a case of nephrocalcinosis with loading dose therapy. Taken together, these findings are consistent with the nephrocalcinosis literature, where most cases potentially associated with vitamin D have occurred in children with rare genetic disorders (99-103, 105) or following the intake of doses exceeding 600 000 IU in healthy children (85, 102, 227). Based on these findings we would suggest age or weight based loading doses, not exceeding 400000 IU or 25OHD levels 200 nmol/L. Of note, the increased hypercalcemia risk demonstrated with doses at or above 400000 IU is largely driven by multiple studies on young children and only one study administering 1.8 million IU to older children. Consequentially, our findings should not be interpreted to state that doses in the 400 000 to 600 000 IU range are toxic in adolescents. In fact, multiple adult studies, including pilot trials in the critical care setting, have not identified significant adverse events with loading dosing to adults in this range (86, 115, 122).

Although this systematic review summarizes a large body of literature and provides valuable information, a number of limitations must be acknowledged. First, accurate information on a number of potentially relevant characteristics was not available including race, ultraviolet exposure, diet, physical activity, compliance, and blood collection techniques (53, 226). Second, study size was often small, with the associated random error in the determination of group 25OHD levels potentially negatively influencing our ability to quantify associations. Third, there were relatively few studies compared to the number of potentially relevant characteristics and interactions. For example, due to the absence of

appropriate 25OHD measurements following intramuscular administration, no conclusions can be made about the rapidity at which this regimen achieves peak 25OHD levels. Further, to accommodate the discrepancy between potentially relevant factors and study number we were forced to combine patient groups into broad categories (e.g. diseased vs. healthy). As regression results generated using patient and study level variables are not always consistent, our results and recommendations will need to be affirmed through future clinical studies. Finally, our adverse event analysis was limited by lack of reporting in close to half of the studies for measures of hypercalcemia and hypercalciuria. Further, the lack of studies with loading doses at or above 400 000 IU to older children and adolescents prevents a more definitive statement of risk and benefit.

3.5 CONCLUSION

This systematic review provides valuable information on the ability of different dosing regimens to rapidly restore vitamin D levels. Our study findings indicate that age or weight-based loading therapy of 10000 IU/kg (maximum 400 000 IU) would be most appropriate. Given the absence of studies administering this dose enterally, and no studies on critically ill children, this dose along with vitamin D related adverse events including hypercalcemia and hypercalciuria should be evaluated in prospective RCTs prior to widespread use.

3.6 BRIDGING PARAGRAPH BETWEEN PUBLICATIONS

As described in Chapter 2, most children with CHD receive either no supplementation or doses based on the Recommended Daily Allowance or Adequate Intake suggested for healthy children. These doses are only intended to raise or maintain vitamin

D to levels that prevent bone disease and do not address extra-skeletal actions of vitamin D. Multiple research bodies and agencies have acknowledged that there may be situations and populations, like that identified for CHD, where higher daily dosing may be required or beneficial. Our systematic review evaluated all clinical trials administering daily high dose vitamin D in the 1000 to 4000 IU range – comparable to the IOM recommended Upper Tolerable Intake Level. This review suggested that given adequate time it should be possible to raise vitamin D levels high enough with IOM approved dosing to prevent post-operative vitamin D deficiency. As discussed in full detail in Chapter 4, 75 to 80% of CHD surgeries occur after 2 months of age, suggesting that it may be possible to prevent vitamin D deficiency with pre-operative administration of the IOM recommended Daily Upper Tolerable Intake Level. One specific population that may be at risk for inadequate response despite daily supplementation with the IOM high dose are neonates and young children who required immediate surgery. Further, there may be other factors that prevent adequate drug intake and increase vitamin D levels in children with surgical CHD. Evaluation of the entire surgical CHD population would allow us evaluate the feasibility of this approach in older children, and provide further support for our idea that this approach will not be effective in children. As such, we have designed a clinical trial intended to determine whether preoperative daily supplementation with the IOM Tolerable Upper Intake level can significant reduce the number of children with CHD who are vitamin D deficient post-operatively.

4.0 PREVENTION OF VITAMIN D DEFICIENCY IN CHILDREN FOLLOWING CARDIAC SURGERY: DEVELOPMENT OF A STUDY PROTOCOL FOR A DOSE EVALUATION RANDOMIZED CONTROLLED TRIAL

4.1 BACKGROUND

Congenital heart disease (CHD) is a common condition with an estimated prevalence of 1 per 100 in the general population. A significant proportion of these pediatric patients require one or more corrective surgeries over their lifetime, collectively leading to 15 000 procedures per year in North America (1). Post-operatively, these patients suffer significant morbidities, which may include a pronounced systemic inflammatory response, multiple organ failure, electrolyte disturbances, arrhythmia, infection and endocrine imbalances (2-5). Interventions that prevent or modulate post-operative pathophysiology may prevent illness, speed recovery, and decrease chronic morbidity in this high risk pediatric population.

Vitamin D status is well recognized as important to calcium homeostasis and musculoskeletal health. The importance of Vitamin D, and its physiological effects are well understood in the context of hypocalcemia(63, 228). Briefly, as serum calcium falls, the parathyroid increases parathyroid hormone (PTH) secretion leading to activation of vitamin-D through an inducible renal enzyme. The inducible renal enzyme works to convert serum 25-hydroxyvitamin D [25OHD] to 1,25 dihydroxyvitamin D [$1,25OH_2D$] and this actived metabolite circulates to the bone, gut and kidneys to restore homeostasis. It is now known that many cell types do not rely entirely on kidney production and have enzymes capable of converting 25OHD to its active form for both autocrine or paracrine

use. Circulating 25OHD is well accepted as the best marker for evaluating vitamin D status in the majority of health settings, including the general ICU population (8, 229). The generally accepted thresholds for defining vitamin D sufficiency is 75 nmol/L, with deficiency defined as below 50 nmol/L, and severe deficiency at 25 to 30 nmol/L.

Increasingly, vitamin D is accepted as a pleiotropic hormone important for the functioning of organ systems central to critical illness pathophysiology, including electrolyte homeostasis, cardiovascular health, inflammation and innate immunity (30, 39, 230). This has lead to the hypothesis that deficiency might represent a modifiable risk factor for critical illness. A growing number of observational studies in adult cardiovascular and intensive care populations have investigated this hypothesis and these studies have reported high vitamin D deficiency rates and associations between hormone level and organ dysfunction, health resource utilization and mortality (30-38). Further, a single moderately sized, interventional study on critically ill adults (VITdAL-ICU) suggests that rapid repletion of vitamin D may improve outcomes (122). This trial randomized 475 critically ill adults to an initial enteral 540 000 IU cholecalciferol loading dose (followed by monthly 90000 IU) or placebo doses. In this study there was a non-significant absolute risk reduction in hospital mortality in the vitamin D arm (7.0%, p=0.10). However, in the predefined subgroup of patients with vitamin D levels below 30 nmol/L at baseline this absolute difference became larger and statistically significant (-17.5%, p=0.01). Although large pediatric observational studies have also documented high rates of vitamin D deficiency and associations between hormone levels and clinical course within the PICU (24, 25), a high dose interventional trial in the PICU has yet to be undertaken (231).

Patients with CHD requiring surgery have been investigated as subgroups within large PICU studies and as distinct populations (24, 28). Analysis of the CHD patients enrolled in a large multicentre Canadian PICU reported a 70% deficiency rate and associations between vitamin D level and clinical course (24). Further, Graham *et al.* confirmed these observations with a secondary analysis of post-operative blood reporting not only that 84% of neonates with CHD were vitamin D deficient post-operatively, but that lower levels were associated with incrased need for inotropic agents (28). In addition, in a prospective longitudinal study we calculated an 85% vitamin D deficiency rate in the CHD population immediately following surgery, as well as an association between deficiency and post operative fluid and catecholamine requirements (26). Mechanistic studies determined the high rate of vitamin D deficiency to be secondary to borderline normal pre-operative 25 hydroxyvitamin D (25OHD) levels and an acute 40% intra-operative decline due to cardiopulmonary bypass (CPB), consistent with that described in an adult CPB study (47). In summary, the available data suggest that most CHD patients are vitamin D deficient following cardiac surgery and that the immediate post-operative levels are associated with subsequent clinical course.

A role for vitamin D in critical illness has biological plausibility, as there are multiple mechanisms through which deficiency could cause secondary pathophysiology. Hypocalcemia is a common problem following CHD repair (30%) and calcium replacement is associated with morbidity and mortality (5). Adult and pediatric ICU studies have shown that critically ill patients with hypocalcemia are more likely to have abnormalities of their vitamin D axis, including low 25OHD, hypoparathyroidism and/or renal dysfunction (56-58). A role for vitamin D in cardiac health can be found in case reports and case series

describing cardiomyopathy secondary to isolated severe vitamin D deficiency (60-63). A recent RCT of vitamin D supplementation in outpatient pediatric congestive heart failure showed improved cardiac function with a higher daily dose of vitamin D (112). Additionally, cardiac surgery with cardiopulmonary bypass uniformly leads to a post-operative systemic inflammatory response syndrome (73, 74). There is good evidence that vitamin D metabolites play important immunomodulatory roles mediated through functional vitamin D receptors present on all major immune cell types (75-77). Vitamin D signaling is also known to play a role in innate immunity, such as in the production of cathelicidins (78-80). Cathelicidins, important endogenous antimicrobial peptides, provide protection against multiple viral and bacterial pathogens. Prevention of vitamin D deficiency could decrease hospital acquired infections among CHD patients through improved innate immunity.

The current body of knowledge suggests that optimization of vitamin D status prior to and following CHD repair could improve clinical outcomes through reduced inflammation, fewer nosocomial infections, improved cardiac function, and faster post-operative rehabilitation and physical functioning (30-34, 38, 41, 51, 232). However, before these findings can be translated into clinical practice, a number of unknowns must be addressed. For instance, there have been no interventional studies establishing that prevention of post-operative vitamin D deficiency improves clinical outcomes in CHD patients. In addition, attempts to perform a large RCT would be premature as a dosing regimen that prevents post-operative vitamin D deficiency has not yet been identified. Moreover, there have been no vitamin D dosing studies or guidelines developed specific to the CHD population; presently children with CHD receive the same advice regarding

supplementation as healthy children (11). Although it is tempting to extrapolate recent safety data from high dose vitamin D studies on healthy children to the CHD population, this may be inappropriate. CHD patients have unique metabolic demands, organ dysfunctions, as well as known and unknown genetic abnormalities that potentially make them more or less susceptible to vitamin D toxicity (109-111, 233). To begin addressing these knowledge gaps, we have designed a pilot dose evaluation randomized controlled trial (RCT) with the goal of identifying a supplementation regimen that safely prevents post-operative vitamin D deficiency in children requiring cardiopulmonary bypass for CHD.

4.2 OBJECTIVES AND HYPOTHESES

4.2.1 Hypothesis

In pediatric patients requiring surgery for CHD, pre-operative supplementation with a daily high dose vitamin D regimen, modelled on the Institute of Medicine (IOM) Tolerable Upper Intake Level (UL), will significantly reduce post-operative vitamin D deficiency, when compared with usual care (11).

4.2.2 Study Objectives

1. The primary objective is to perform a double blind RCT to determine whether the pre-operative administration of daily high dose of vitamin D based on the UL from IOM, compared with usual care, results in a significant reduction in post-operative vitamin D deficiency in a pediatric population with CHD.

2. Secondary objectives –

a. Determine the barriers and feasibility of conducting a larger phase III RCT evaluating whether vitamin D supplementation improves clinical outcomes in children who require CHD surgery (blinding, recruitment, compliance).

b. Determine whether the pre-operative regimen of daily high dose vitamin D, compared with usual care, results in a greater number of vitamin D related adverse events (hypercalcemia, hypercalciuria).

c. Determine whether the pre-operative regimen of daily high dose vitamin D, compared with usual care, improves established markers of vitamin D axis functioning (active hormone levels, cardiac function).

4.3 METHODS

4.3.1 Sponsorship, approvals, principals

The Heart and Stroke Foundation of Canada is the Sponsor for the trial (Supplemental Information, appendix S4.1-S4.4). The study will be conducted in accordance with the ethical principles guided by Tri-Council Policy and the Declaration of Helsinki. The protocol is approved by Health Canada and the Children's Hospital of Eastern Ontario Research Ethics Board (REB reference: 13/03E). The trial will comply with the principles of Good Clinical Practice and will be carried out in accordance with applicable legislation and the Standard Operating Procedures of the CHEO Research Institute. The trial will be reported in line with the Consolidated Standards of Reporting Trials (CONSORT) 2010 guidelines (234) and the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) checklist (235). Protocol amendments

will be communicated as necessary to the study team, health care team and REB. A structured summary of the trial is provided in Table 4A (appendix 4.1).

4.3.2 Study design

The trial is a single centre, double blind, parallel, randomized, controlled dose evaluation trial comparing the efficacy and safety of two vitamin D dosing regimens in the prevention of post-operative vitamin D deficiency in children undergoing surgery for CHD.

4.3.3 Study population

Inclusion

1. Between 36 weeks gestational age and 18 years
2. CHD requiring surgery within the next 12 months
3. CHD requiring surgical correction with cardiopulmonary bypass (CBP)

Exclusion

4. Born less than 32 weeks gestational age
5. Disease preventing enteral feeds or drug administration prior to surgery
6. Confirmed or suspected Williams syndrome (a neurodevelopmental genetic disorder with symptoms that include cardiovascular problems and high blood calcium)
7. Proposed surgery to take place at another centre (outside of CHEO)

Justification of eligibility criteria

In our previous study, CHD patients who did not receive CPB had a minimal (<10%) intraoperative drop in 25OHD. Although prevention of vitamin D deficiency is important for these patients, they do not require pre-operative elevation into the upper normal physiological range. Very premature infants (<32 weeks) are at significantly increased risk for nephrocalcinosis (236, 237). CHD patients with Williams syndrome have a genetic susceptibility to hypercalcemia and current guidelines recommend against any vitamin D supplementation (97, 233).

4.3.4 Study Drug

Study drug distribution

Europharm (Quebec) will provide the study drug (vitamin D) in the required concentrations, prepared in indistinguishable vials for blinding purposes. The study drug will be analyzed as per Health Canada regulations. The CHEO Pharmacy will administer the study drug (based on randomization, participant age and whether the patient is breast or formula fed). Infants with CHD assigned to the usual care arm will be given either a placebo (0 IU/mL) solution if they are receiving vitamin D as part of formula, or they will be given a 400 IU/mL solution if they are breast-fed.

Proposed supplement doses (Interventions) to be tested and rationale

The doses for evaluation have been modelled on the two age specific intake levels recommended by the IOM (11) (Table 4B, appendix 4.2).

1. The *High dose group* is based on the age specific UL from the IOM. These doses were chosen to elevate 25OHD well above 50 nmol/L, while minimizing risk of vitamin D

toxicity (e.g. hypercalcemia, hypercalciuria). Patients under 1 year of age will receive 1600 IU/day, while those over 1 year of age will receive 2400 IU/day. Note – infants under 6 months of age in the high dose group will receive 600 IU/day more than the UL from IOM, while those between 6 and 12 months will receive 100 IU/day more than the UL.

2. The *Usual care group* will receive Adequate Intake (AI) for infants and Recommended Dietary Allowance (RDA) for children over 1 year (< 1 year, 400 IU;>1 year 600 IU/day). These doses were chosen by IOM to achieve blood 25OHD levels above 50 nmol/L in the vast majority of the healthy population.

4.3.5 Rationale for study design and interventions

Rationale for inclusion of the usual care arm –

Given that children with CHD receive the same vitamin D supplementation advice as healthy children (by default) it is tempting to conclude that usual care dosing will not be adequate to prevent vitamin D deficiency. Unfortunately, this conclusion may be wrong for the following reasons: (i) only 50% of previous study participants indicated daily vitamin D intake at or above 400 IU (24), (ii) compliance with vitamin D supplementation may be poor without motivation (research studies, vitamin D related disease), (iii) there is often uncertainty about vitamin D intake by caregivers, (iv) recommendations for usual care recently increased to 600 IU for children above 1 year (11). Given potential safety concerns regarding high doses of vitamin D in diseased population, it would be appropriate to properly evaluate the efficacy of usual care under ideal circumstances.

In addition, it is important to assess the baseline risk for vitamin D related adverse events in CHD patients receiving usual dosing. Although studies on healthy children have not identified adverse events (e.g. hypercalcemia, hypercalciuria) with doses at and slightly above the UL from IOM (19, 81), diseased populations including those with CHD may be predisposed at lower 25OHD levels.

Rationale for pre-operative daily enteral approach –

Our previous prospective study demonstrated that post CHD surgery vitamin D deficiency occurs due to borderline normal pre-operative values and a consistent cardiopulmonary bypass induced intraoperative decline (26). As there is no intravenous form of either cholecalciferol or 25OHD maintenance of appropriate post-operative levels will require elevation of pre-operative levels into the high normal range using enteral supplementation. Two basic approaches for enteral restoration and maintenance of vitamin D stores have been described. First, representing usual care, is the daily consumption of a relatively low dose of cholecalciferol (400 to 4000 IU/day). The second option is a single or divided megadose of vitamin D (100,000 to 600,000 IU) given intermittently throughout the year (83, 238). As safety concerns regarding the megadose approach in children have not been adequately addressed we have chosen to first evaluate regimens based on daily consumption (90).

4.3.6 Anticipated duration of study drug and peri-operative 25OHD levels

Duration of study drug – Vitamin D supplementation is generally initiated within a week of birth, so the duration of preoperative therapy would be from birth (or diagnosis of CHD) to the time of surgery. Timing of surgery is very dependent on the type of CHD

lesion. Consistent with the literature, our recent observational study of perioperative vitamin D status demonstrated 8 months as the median age at surgery, with 2.4 months being the 25th percentile. Based on these findings, we anticipate that it should be possible for 75 to 80% of patients to receive study drug for more than 2 months (the time required to achieve a new 25OHD steady state with high dose daily supplementation). Whether this duration of study drug can actually be achieved pre-operatively, and the 25OHD levels achieved, will be reported as part of the pilot study.

Peri-operative 25OHD levels - Given the 40% intra-operative decline, pre-operative levels above 90 nmol/L will be required to maintain post-operative levels above 50 nmol/L (the value at which sufficient substrate to synthesize the active metabolite is available). The ability of certain vitamin D intake levels to achieve this pre-operative value can be inferred from recently completed dosing studies on healthy children level (19, 81). These studies have shown that usual care dosing for 2 to 3 months will achieve pre-operative levels of 90 nmol/L in only 40-50%. In contrast, studies evaluating doses approximating our higher daily intake level (1600 IU/day) achieved mean 25OHD levels of 130 to 150 nmol/L; suggesting that 80% or more of CHD patients could achieve pre-operative levels of 90 nmol/L or above.

4.3.7 Subject recruitment

Potentially eligible study participants will be identified in the ambulatory clinics (cardiology, cardiovascular) or inpatient wards (including pediatric intensive care and neonatal intensive care unit) by a member of the health care team or study staff. A research coordinator will provide study information and obtain informed consent as well as applicable

assent from each participant (Supplemental Information, appendix 4S.5-4S.19). Study staff will provide support to participants and encourage adherence to intervention protocols.

4.3.8 Randomization, blinding and stratification procedures

We will use a computer-generated randomization sequence. Only the CHEO pharmacy will have access to the randomization sequence and will be responsible for participant randomization and allocation. Only the pharmacist will know the identity of the study drug administered to a specific patient. Given the expected recruitment (2 to 3 per month) and potential impact of season on 25OHD, randomization will be performed in permuted blocks (4 within each stratum). We will blind patients, families, investigators, hospital staff, and research personnel to treatment arm. Blinding was considered necessary to avoid: (i) families altering the outpatient dose, and (ii) a number of secondary outcome measures are potentially subjective (echocardiography, timing of extubation, need for fluids or catecholamine infusion). Within each age group the two interventions will be indistinguishable (vial, volume, colour, taste, consistency and smell). Participants will be stratified into whether or not they are expected to receive at least 8 weeks of study drug prior to surgery. This stratification should guarantee that an equal number of CHD patients who will not receive 8 weeks of oral dosing end up in both the high and low dose arms. We will further stratify by age (under or over 1 year of age).

4.3.9 Co-interventions

We will not protocolize post-operative co-interventions as the study is single centre and CHEO has standardized approaches to the common post-operative complications and adverse events (e.g. hypocalcemia, junctional ectopic tachycardia, necrotizing enterocolitis

constipation, sedation, catecholamine administration, etc). As the study is blinded, protocolization of co-interventions is less relevant and differences should relate to random chance or drug effects.

4.3.10 Diagnostic and clinical outcome measures

Blood 25OHD –

The primary objective will be evaluated using immediate post-operative blood 25OHD concentrations (collected on the day of ICU admission) with a level lower than 50 nmol/L used to define deficiency (7, 13). This is a well-established cut-off based on: (i) knowledge that the parathyroid and renal organs need to compensate for 25OHD levels below 50 nmol/L, and (ii) clinical studies showing increased risk of bone, cardiovascular, immune and other disease entities once concentrations fall below this level. 25OHD will be determined using a LC-MS assay from a laboratory participating in the Vitamin D External Quality Assessment Scheme (DEQAS) (24, 239).

Vitamin D related adverse events –

We will report, by intervention, on the occurrence of clinically significant adverse events. However, a measurable difference in clinically significant adverse events between the high dose and usual care arms of the study is unlikely. Therefore to enhance our ability to evaluate for potential toxicity we will use two well accepted surrogate outcome measures:

- *Hypercalcemia:* Will be defined as an ionized calcium level above 1.40 mmol/L (or above 1.45 mmol/L for children under 8 weeks) (Table 4C, appendix 4.3). We will assess calcium in blood collected immediately before surgery and throughout the

post-operative course (measurements are standard of care and any single episode of hypercalcemia not related to parenteral administration of calcium will be considered an adverse event) (190).

- *Hypercalcuria* – We will identify hypercalcuria using calcium:creatinine ratios defined using age specific norms and thresholds (Table 4C, appendix 4.3) (190, 240, 241). Measurements will be performed on urine collected in the operating room immediately prior to surgery and on the first post-operative day.

Vitamin D axis function –

We will evaluate vitamin D axis function through changes in blood $1,25OH_2D$ (24) levels from blood collected at specified times following surgery. Based on our previous work we anticipate a 40% intra-operative decline in $1,25OH_2D$ levels (229) and some children will experience a transient decline in $1,25OH_2D$ levels into the deficient range (<50 pmol/L). Given adequate 25OHD levels and an otherwise properly functioning vitamin D axis we would anticipate restoration (or maintenance) of active hormone levels into the normal range within 12 hours of surgery. Impaired vitamin D axis function will be defined as an inability to maintain active hormone levels in the normal range at any point from blood work collected after the first post-operative day.

Organ function and ICU outcome measures –

Post-operative cardiovascular and immune function will be measured and compared between the two groups. Cathelicidin levels, an endogenous antimicrobial peptide, will be used as a surrogate measure of innate immune function (78, 79, 242). Clinically relevant measures of cardiac organ function will include echocardiograms (e.g. ejection fraction),

inotrope requirements (e.g. vasopressor need, maximum inotrope score) and fluid resuscitation (positive fluid balance in first 48 hours). Further we will also evaluate standard PICU clinical outcome measures including time to extubation, PICU and hospital length of stay.

Phase III Study Feasibility –

We will determine the feasibility of a subsequent multi-centre large interventional study through an evaluation of study consent and accrual rate, protocol deviations and violations, proportion of adequate allocation concealment and blinding, proportion of study drug compliance, and proportion of study drop out/withdrawals.

4.3.11 Study Procedures

A summary of study procedures, biological sample collection and metabolite measurements has been provided as a flow diagram (Figure 4A, appendix 4.4) and the biochemical measurements on research specimens are summarized in Table 4D (appendix 4.5).

1. Prior to initiation of study drug –

Urine – After consent is obtained and the study participant is waiting for the pharmacy to prepare the study drug we will gather a urine sample for determination of calcium:creatinine ratios. Where developmentally appropriate the participants will be asked to provide urine into a container. Urine bags will be placed on younger children. If the child is unable to provide a urine sample during the time it takes to fill the study drug prescription, the participant will be provided with a container or urine bag and asked to provide an outpatient urine sample.

Blood - Neonates and other study participants requiring surgery within 2 months of diagnosis and enrollment will have 0.5 - 1 mL of blood collected prior to (or within 2 days) of starting study supplement for determination of 25OHD. Where possible, unused or discard blood will be obtained from the laboratory (243). If unused blood is not available, research blood will be collected at the time of clinically indicated blood work, or not at all. These patients will not have blood collected for research purposes again until they are taken to the operating room.

* Note -We will request initiation samples but children will still be enrolled if these samples cannot be collected or the families do not want these procedures.

2. During period of study drug administration -

All outpatient participants will have blood collected at the time of standard pre-surgical blood work (two to three weeks prior to surgery) for both 25OHD and Ionized calcium. These samples are collected to ensure that patients do not go for surgery with potentially toxic levels of vitamin D. The safety officer (pediatric endocrinologist) will review the ionized calcium level on the patients chart and follow up if required as per the safety measures outlined below (Figure 4B, appendix 4.6). For those participants who will receive study drug for more than 6 months we will also perform additional blood work to ensure that potentially toxic levels are not maintained for long periods prior to surgery. The timing of this blood work will not be specified and will occur as part of clinically indicated blood-work during regularly scheduled clinic appointments

3. Intraoperative biological samples and measurements–

Blood - All study participants will have 2 mL of blood collected in the operating room following anesthesia and intubation, but prior to skin incision and initiation of cardiopulmonary bypass. Pre-operative ionized calcium will be determined. Remaining sample will be processed to plasma, aliquoted and stored at -80^{o}C for determination of 25OHD at the end of the study.

Urine – All study participants will have urine collected after insertion of the urinary catheter and the calcium:creatinine ratio will be determined. Study results will not appear on the patient hospital chart, but will be labeled with the study ID number and forwarded to the study investigator and safety officer for review.

4. Post-operative biological samples and other study measurements

Blood - All study participants will have 2 mL of blood collected following separation from cardiopulmonary bypass (at admission to PICU). Table 2 shows the biomarkers to be measured. Further study participants will have 2 mL of blood collected on post-operative days 1, 3, 5 and 10 in the PICU. Samples will be collected from arterial or central venous catheters at the time of clinically indicated blood work. If these catheters have been removed, blood will be collected at the time of clinically indicated venipuncture. If patients are discharged to the ward before the day 10 research sample is collected, a discharge sample will be collected at the time of discharge and no further research blood will be gathered. To limit the volume of research blood collected, neonates will only have 1 mL of blood collected post operatively on days 3, 5, and 10.

Urine – All study participants will have urine collected from the urinary catheter on the first post-operative day. Calcium and creatinine concentrations will be determined.

Echocardiography – A comprehensive exam will be performed immediately post-operatively (standard of care) and on the first post-operative day by a trained technician or pediatric cardiologist. Between group comparison will evaluate for differences in left-ventricular in (LV) end-diastolic diameter, LV end-systolic diameter, LV ejection fraction (112).

4.3.12 Case report form

All trial information will be stored in a secure electronic database to maintain confidentiality. Data entry methods are in place to promote data quality. In addition, protocol deviations, including discontinuation of study participation will be recorded. The case report form will be developed using REDCap (128). Research Electronic Data Capture is a secure web application for building and managing online surveys and databases. The following data will be entered electronically:

(a) Questionnaire - On the day of surgery the research coordinator will collect the participant diaries and unused study supplement. The patient diaries will contain information on which days the patient was or was not given the study drug, why not, and whether there were difficulties. Information will also be collected on prescribed medications, nutrition, additional supplement use, and symptoms associated with vitamin D intoxication (e.g. constipation, abdominal discomfort).

(b) Operative details - The research assistant will extract detailed operative information, including: cardiac lesion type, surgery performed, RACHS score (244), total fluid intake and output, blood product and fluid administration and loss, hypothermia, need for deep hypothermic circulatory arrest (duration), aortic cross clamp times, CPB circuit volumes, CPB circuit constituents, CPB time, occurrence of intraoperative hyper or hypocalcemia,

administration of parenteral calcium, need for catecholamines following separation from CPB, occurrence of intra-operative arrhythmias.

(c) *PICU course* - Clinically relevant information on clinical course and organ dysfunction will be collected, including: death, ECMO, PRISM illness severity (245), cardiovascular dysfunction (fluid bolus requirements, inotrope/catecholamine use, arrhythmia), renal dysfunction (urine output, creatinine measurements, need for dialysis), hypocalcemia and calcium administration, duration of mechanical ventilation and duration of PICU stay.

4.4 STATISTICAL ANALYSIS

4.4.1 Sample size justification

Based on our observational studies and findings from recent dose evaluation studies on healthy children, we estimate that no more than 40% of the usual care arm will have post-operative 25OHD levels above 50 nmol/L. Based on the 25OHD levels achieved with 1600 IU/day in recent studies on healthy infants we anticipate that 80% of the high dose arm will have post-operative levels above 50 nmol/L. Therefore group sample sizes of 28 in both treatment arms will be required to achieve 80% power to detect an absolute difference between the group proportions of 0.40. The test statistic used is the two-sided Fisher's exact test and the significance level of the test was targeted at 0.05. Assuming a 10% drop out rate, approximately 62 patients (total) will need to be recruited.

4.4.2 Comments on power for evaluating vitamin D related adverse outcomes:

1. Hypercalcemia – Our previous observational study (n=58) identified no cases of pre-operative or immediate post-operative hypercalcemia (26). With a baseline rate in the usual

care arm between 0 and 10% our sample size would be sufficient to show a statistically significant absolute difference between groups if the rate in the high dose arm exceeded 30%.

2. Hypercalciuria – Information on baseline rates of hypercalciuria prior to or following cardiac surgery with usual care vitamin D intake is not available. The proposed sample size would be sufficient to demonstrate a 35% absolute difference in proportions with baseline pre or post-operative rates up to 20%.

4.4.3 Statistical procedures

The analyses will be conducted using SAS software (Copyright SAS Institute Inc., Cary, NC, USA) and a p-value less than 0.05 will be considered statistically significant.

Descriptive statistics - Treatment groups will be described and compared using: (i) means with standard deviations or medians with inter-quartile range values for continuous variables or (ii) frequencies with percentages for categorical variables. Statistically significant differences will be determined using Chi-square and Fisher's exact tests for categorical variables, and t-tests or nonparametric tests (e.g. Wilcoxon) for continuous variables, as appropriate.

Primary outcome – The primary analytical approach will be to evaluate all randomized patients in an intention to treat analysis. Differences in the primary outcome measure, proportion with 25OHD < 50 nmol/L, between the treatment groups will be evaluated using the Fisher's exact test. Logistic regression analysis will be used if important variables are unevenly distributed between groups. We anticipate minimal missing data, as over 95% of participants from the recently completed observational study had an immediate post-operative sample(26).

Secondary outcomes - Secondary analyses will be evaluated between groups based on data type. Outcome measures that are continuous will be evaluated using the t-test, Wilcoxon sign rank test (where appropriate) or through linear regression analysis if important variables are not evenly distributed between groups. Binary secondary outcome measures (e.g. hypercalcemia, hypercalciuria) will be compared between the two treatment groups using Fisher's exact or Chi-square. For the analysis of outcomes measures that represent time to event (e.g. restoration of $1,25OH_2D$ levels to normal range, time to extubation, PICU length of stay/discharge) we will apply the log rank test. If randomization does not lead to equal distribution of important variables (e.g. weight) the analysis will be expanded to multiple regression modeling (e.g. logistic, linear, Cox proportional hazard).

Subgroup analysis - The well-known pharmacology of enteral vitamin D dosing shows that up to 2 months of regular daily intake is required to build body stores and achieve steady state blood levels of vitamin D. Consequently, neonates or other infants enrolled into the study who receive surgery within two months of birth or CHD diagnosis will be analyzed separately. Within this subgroup analysis, the primary objective remains reporting of proportions (in the usual care and high dose groups) that are vitamin D deficient post-operatively. However, given that these participants will receive study drug for a very short period we anticipate that the proportion with 25OHD levels above 50 nmol/L will remain low in the high dose group. Our program goal at this stage is to identify a dosing regimen that prevents post-operative vitamin D deficiency in 75% of CHD patients. Given this goal, and an estimated prevalence of 25% we would need 12 neonates (or children who receive < 2 months) to generate a confidence interval that excludes 75%. *Feasibility* – Most neonates with CHD who require cardiac surgery within the first few weeks of life have serious cardiac

lesions that can limit enteral nutrition and medication delivery. Anticipating that most of these patients will not significantly elevate 25OHD levels with daily enteral intake at IOM high dose this study will provide important information on the willingness of health care providers to provide enteral study drug. This information will allow us to consider alternative dosing regimens for future studies based on single or divided doses representing a months or more worth of daily dosing (e.g. 5000-10,000 IU/kg). Only those children completing the entire study protocol will be included in analyses.

4.5 DATA AND SAFETY MONITORING

4.5.1 Data Safety Monitoring Board

In order to assess possible changes in risk/benefit ratio to study subjects and to obtain independent oversight of study conduct, an external Data and Safety Monitoring Board (DSMB) will be established to oversee the progress of the study. The DSMB will be composed of representatives from statistics, nephrology and endocrinology. External DSMB study reviews will be conducted after half of the participants (n=30) have completed all study procedures. The DSMB will review and monitor the study procedures and potential risks with a focus primarily on safety. Serious Adverse Events (SAEs) will be reviewed by the DSMB members in order to determine whether additional safety measures should be initiated. There are no predefined criteria for stopping the study, although the DSMB may recommend changing study drug concentration or stopping the study based on SAE or 25OHD data. If there are significant deviations from major study assumptions the DSMB or study investigators may choose to evaluate 25OHD levels and stop the study early.

The principal investigator and his co-investigators will be responsible for maintaining and assessing subject safety in the study, monitoring the presence and severity of adverse events, and monitoring compliance with study drug use. Information on specific vitamin D related adverse events will be obtained by laboratory findings, including 25OHD, ionized calcium and urine calcium:creatinine levels.

4.5.2 Safety measures and clinically relevant research findings

A flow diagram depicts the safety measures in place and response to clinically relevant research findings for individual study participants (Figure 4B, appendix 4.6). A standard operating procedure has been developed for adherence to the following safety measures. To avoid vitamin D overdose and related toxicities we have selected a supplement level recently proven to be safe in healthy children and will target the period of high dose supplementation to 6 months, and no more than 12 months. Study participants with elevated blood calcium and/or vitamin D levels will be identified and contacted by the safety officer. For 25OHD, although 500 nmol/L is generally considered the definitive acute toxicity threshold, we have chosen to intervene with 25OHD levels above 200 nmol/L as this value is supraphysiological and exceeds our study goal. The following details the actions that will be taken with abnormal values:

a) For 25OHD above 200 nmol/L with evidence of hypercalcemia (vitamin D toxicity): discontinue study drug immediately, repeat the values (fasting), and refer to endocrinology.

b) For 25OHD above 200 nmol/L without hypercalcemia: study drug will be reduced by 50%

c) For 25OHD above 250 nmol/L, without hypercalcemia: study drug will be discontinued

d) For hypercalcemia with 25OHD under 200 nmol/L: repeat the bloodwork (fasting) and refer to endocrinology

Both post-operative hypo and hypercalcemia will be managed by the clinical team as required. Study participants with persistently elevated blood calcium levels (for more than two days, not explained by intravenous calcium administration) will be referred to endocrinology. As prolonged exposure to hypercalciuria (> 3 months) could theoretically cause nephrocalcinosis, we will have ultrasounds performed prior to hospital discharge on all patients with elevated immediate pre-operative urine calcium to creatinine ratios. Any study participant with nephrocalcinosis will be referred to the nephrology service for further assessment. Participants that have study drug discontinued or decreased will be retained in the study, have peri-operative biological samples collected as outlined, and will be included in the analysis using intention to treat methodology.

4.6 DISCUSSION

Research by our group and others has documented not only that 4 out of every 5 CHD patients have inadequate blood levels of vitamin D following surgery, but an association between immediate post-operative hormone levels and clinical course (24, 26, 28). Altogether, these findings and similar results in adult critical care and CHD surgery populations, suggest that optimization of vitamin D status following CHD repair could lessen inflammation, reduce nosocomial infection and improve cardiac function (30-34, 38, 41, 51, 232). As an inexpensive medication (~$15/month) that is generally regarded as safe,

vitamin D has the potential to be an ideal intervention for improving outcomes following CHD repair. As protocols and guidelines should be evidenced based, well-designed clinical trials are essential to adjust clinical practice. This trial aims to determine whether a pre-operative proposed dosing strategy will be sufficient to elevate pre-operative 25OHD and prevent post-operative vitamin D deficiency. Further clinical study will be required to investigate how normalization of vitamin D levels impacts the clinical course of patients with CHD requiring cardiac surgery.

Trial Status

At the time of writing 62% of the target sample size had been enrolled and the anticipated study completion date is January 2016.

CHAPTER 5 – PROGRESS TO DATE WITH CONDUCT OF THE DOSE EVALUATION RCT

5.1 RESULTS

The primary objective of this study is to determine whether pre-operative supplementation with daily high dose vitamin D approximating the Tolerable Upper Intake Level can prevent post-operative vitamin D deficiency in children with CHD. As 25OHD are not yet available for the patients (and group assignments are unknown) we will instead describe our experiences with study drug intake, compliance and protocol violations. In addition we will described the other, equally important, feasibility objectives (i.e. recruitment, adverse events, collection of research samples).

5.1.1 Study initiation and time period for thesis

The thesis proposal was submitted in 01-Aug-2012. The Graduate Studies Committee requested minor modifications (15-Oct-2012) which were resubmitted on 23-Nov-2012. Official approval for the thesis was received from the Graduate Studies Committee on 11-Jan-2013. CHEO Research Ethics Board and Health Canada applications were submitted on 12-Dec- 2012 and 18-Dec- 2012, respectively. The 'No Objection letter' was received from Health Canada on 24-Jan-2013 (Supplemental Information, appendix S5.1). Notice was received of CHEO REB approval on 25-Mar-2013 and annual renewal has been requested and granted twice, most recently on 04-Mar-2015 (Supplemental Information, appendix S5.2). Following these approvals, study drug was requested from Europharm and was received by the CHEO pharmacy on 09-May-2013. The start-up DSMB meeting was held on 17-May-2013 where the terms of reference (Supplemental Information, appendix S5.3-S5.6) and reporting documents (Supplemental Information, appendix S5.7-8) were reviewed and

approved. The only significant adjustment to the document was the addition of post-operative 25OHD levels included to the interim analysis. With all study procedures and approvals in place the Cardiology and Cardiovascular service were approached about initiating screening and recruitment. Due to significant recent turn over in administrative and nursing staff they requested that active screening for study participants begin in September 2013 after clinical training was complete.

The date of 30-Apr-2015 was selected as the end time point to describe study results for the thesis as this was the date when the first 30 participants had completed all study related procedures and the DSMB report was to be prepared. The chair's summary of the DSMB meeting on 16-Jun-2015 can be found in Supplemental Information (appendix 5S.9).

5.1.2 Study recruitment

Participant flow

Although official active study recruitment was planned for Sept-2013 the first study participant was recruited early on 22-July-2013. This family approached the cardiovascular team about the trial after reading a newspaper article describing the findings of our initial study (Supplemental Information, appendix S5.10-S5.11). From 01-Sep-2013 to 30-Apr-2015 there were 19 months of active recruitment (excluding 2 weeks over each of the 2013 and 2014 Christmas seasons where research coordinators were unavailable).

A participant flow diagram is shown in Figure 5A (appendix 5.1). During the 19 months of active recruitment research staff were contacted about 86 children of whom 67 were determined to meet study eligibility criteria. Of the 67 eligible patients there were 18 instances where research staff did not discuss the study with the family and/or patient. For

the 49 children where the study was presented in full by a member of the research staff, consent and/or assent was given by 35 participants. The consent rate was calculated as 52% (35/67) for all referred eligible patients, and 71% (35/49) for those approached by study staff.

Explanation of excluded patients, declined participation and withdrawals

1. The two most common reasons referred patients were not eligible was either that the patient had CHD but did not require surgery (n=15) or had a lesion type that necessitated more complex surgery at another centre (n=3).
2. For the instances where study participation was not discussed with eligible children and families, refusal by the most responsible physician to allow study participation (n=5) or family unwillingness to discuss research due to stress (n=3), lack of interest (n=2) or inability to make decision (n=1) accounted for half of the cases. In 6 cases, the study was not discussed with the family as the research staff was unavailable (n=2), there was insufficient time after referral for consent and initiation of study procedures (n=2) or study staff were unable to make contact with family (n=2). In one circumstance, the family agreed to discuss the study with research staff but became distressed at the beginning of the conversation stating that they were unaware of the need for surgery. Study procedures were reviewed and it was confirmed that the most responsible physician (cardiology) had informed study staff that the family was aware of the need for surgery.
3. The reason for refusal after presentation of full study protocol was available for 9 of 15 cases (Table 5A, Appendix 5.3). In three of the cases the family vocalized that the need for daily supplementation and compliance contributed to their decision not to participate. In one case the mother decided not to participate as she did not think the

father would reliably give the medication and did not want to negatively impact the study. In two other instances the caregivers did not want the added responsibility of another medication to give or track.

4. One family requested almost immediate withdrawal after enrolment over concerns that study drug might be worsening gastroesophageal reflux. For the remaining three cases withdrawal from the study occurred when the surgical plan changed and the patients were referred to the Hospital Sick Children in Toronto for more complex procedures.

Concurrent with randomization the 35 patients were stratified into four groups by age and expected duration of study drug. At time of randomization, 24 (69%) were under one year of age and 7 (20%) were expected to receive study drug for more than two months at time of enrollment. Of the randomized patients, 4 were ultimately withdrawn and 1 has not yet had surgery.

Recruitment of 35 patients over a 19 month study period represents an average accrual rate of 1.84 patients per month. Figure 5B (appendix 5.2) compares actual to target recruitment rate. Actual and target recruitment rates were well matched over the first 6 months, with accrual declining to 1.6 patients per month over the final 12 months.

5.1.3 Study participant characteristics

Baseline patient information is presented in Table 5B (appendix 5.4). Our enrollment survey found that the vast majority (78%, n = 27) of families identified themselves as being Caucasian and that 40% (n=14) reported taking a vitamin D supplement. Addition of the patients who were formula fed increased the percentage receiving vitamin D supplementation

to 71% (n=25). At time of enrollment, the median age of the population was 3.0 months, with 74% (n=26) being under 1 year of age. Three patients (9%) had confirmed genetic syndromes. Age was also determined using the day of surgery (median 5.5 months, IQR: 1.5, 37.4) with 23% (n=7) and 71% (n=26) having surgery before 1 and 12 months of age, respectively. The majority of the study participants were in RACHS category 2 (n=13) or 3 (n=11). The primary cardiac lesion is listed for the study participants in Table 5B (appendix 5.5)

5.1.4 Duration of study drug intake, compliance and protocol deviations

Duration of study and compliance

The median time between enrollment and surgery for the 30 study participants completing all study procedures was 27 days (IQR: 6, 56). The number of study participants who could have received study drug for more than 30 and 60 days between enrollment and surgery was 50% (n=15) and 26% (n=8), respectively. The study participant withdrawn by family did so on day 3, while the withdrawals due to change in surgical plan occurred on days 12, 78 and 208. When available, study drug intake was recalculated using information from pharmacy for inpatient participants (n=10) and study diary for outpatients (n=16). The recalculated median number of doses received was 21 days (IQR: 4, 40) with receipt of more than 30 and 60 doses occurring in 45% (n=14) and 16% (n=5), respectively. The median per dose compliance rate was 97% (IQR 72%, 100%). Further, 11 participants reported having missed more than 10% of the doses, with 9 of 30 missing more than 20%. Excluding the 10 inpatients where drug was not dispensed to family, study vials were returned by 13 of the

remaining 20 participants who underwent surgery. There were no cases where the volume of study drug returned exceeded that reported in the diary by more than 2 doses.

Protocol deviations

Protocol deviations with regard to study drug administration occurred in 6 patients. (Table 5A, appendix 5.3). On three occasions pre-operatively the caregivers decided to modify or change the regimen in a manner that would have reduced the potential rise in vitamin D. Pre-operatively one other family decided to start an immune supplement with additional vitamin D (described in more detail in section 5.1.6). During the post-operative period two separate participants received a short course of study drug resulting in 6 extra doses (both cases). In one instance the family did not return the study drug as requested and continued to give it despite instructions, and in the second case a physician wrote an order to re-initiate daily study drug and it was provided by pharmacy without approval from study staff or their knowledge.

5.1.5 Research related testing and biological sample collection

Research blood was properly collected, processed and stored for 97% (n=29) of patients at time of PICU admission and 93% (n=28) on the first post-operative day. One of either the PICU admission or first post-operative day blood samples was available for 100% of the participants. The number of additional research related biological samples collected at the initiation, intra-operative and post-operative time points has been summarized in Figure 5C (appendix 5.6). With the exception of the optional collection of enrollment urine and blood samples, more than 90% of the required research samples were collected.

5.1.6 Safety procedures and adverse events

Due to the possibility of vitamin D related adverse events, blood and urine samples were collected at specific time points. Results are shown in Figure 5D (appendix 5.7).

Mid-treatment – Pre-operatively, three participants were identified as receiving study drug in excess of 6 months. For one of the three, the 25OHD concentration was reported at 199 nmol/L. An interview with the family determined that since enrollment they had started an over the counter immune supplement and had not declared it with any of the research related telephone calls or appointments. The label indicated it contained vitamin D and, if accurate, the patient was receiving an additional 400 IU per day. As the family wanted to both continue the immune supplement and stay in the study, the decision was made to unblind to increase confidence the appropriate study drug solution was being provided. This confirmed the participant was in the high dose arm and receiving the 1600 IU/mL formulation; the family was asked to stop taking vitamin D study drug (~2 weeks) to allow the 25OHD level to decline slightly and then reinitiate using the provided 1200 IU/mL solution.

Pre-surgical samples – As per protocol, ionized calcium and 25OHD concentrations were determined on 20 participants (100% of required cases). None of these patients had elevated 25OHD concentrations or met the study definition of hypercalcemia.

Intra-operative and Post-operative samples –

Intraoperative blood and urine samples were collected on all 30 study participants. No participant was documented to have hypercalcemia, while 7 had elevated urine calcium to creatinine ratios. As per protocol, 6 of the 7 participants had a kidney ultrasound and no cases of nephrocalcinosis were documented. The one abnormal urine value that did not have a kidney ultrasound was not received by the safety officer until after hospital discharge. The

patient had no clinical evidence of kidney dysfunction or urine abnormalities with their post-operative blood or urine samples; upon review with nephrology it was considered unnecessary to arrange for an outpatient ultrasound.

A post-operative urine sample was collected on 93% (n=28) of participants. Both of the participants with missing samples had surgery late in the week and due to an unremarkable clinical course were discharged from hospital the beginning of the following week; as they do not live in the Ottawa area we have been unable to arrange follow-up to obtain a post-operative urine sample. Five patients met criteria for post-operative hypercalciuria and had their findings (blood work and intra-operative urine measurements) discussed with a nephrologist who requested repeat urine measurements in all cases. Four of the patients had a repeat urine collection, while the fifth patient refused to provide a repeat urine sample prior to hospital discharge (will be attempted at a future clinic appointment). Three of the four repeat samples showed resolution of hypercalcuuria and the wrong analysis was performed by the laboratory on the fourth (urine albumin to creatinine ratio); attempts will be made to collect urine at future clinic appointments.

5.1.7 Study modifications

Table 5D (appendix 5.8) describes the protocol changes or initiatives and intended purpose(s). A number of specific changes were intended to improve accrual rate, lengthen time from enrollment to surgery (to increase cumulative study drug intake), or increase patient safety. Two major efforts were made to increase recruitment rate. First, it was recognized that approximately 10-15% of CHD surgeries at CHEO are from patients who reside in Kingston. As these patients are followed by cardiology in Kingston and are

presented by videoconference they are generally only seen once by the CHEO team approximately 2 weeks prior to surgery. Our research team liaised with the pediatric cardiologist in Kingston and obtained REB approval to recruit patients; unfortunately the Kingston clinic did not recruit any patient from their clinic over a 12 month period. Second, we attempted to initiate recruitment at a second site. One of the investigators (Dr. Dermot Doherty) on the observational study of vitamin D in CHD (26) moved to Ireland (Dublin) and works in an ICU that provides post-operative care to children following CHD surgery. Dr. Doherty was a co-investigator on the Heart and Stroke Grant and was interested in having Ireland as a second site for the study. Despite evidence of high vitamin D deficiency rates in their PICU (246) and agreement from the PICU and endocrinology services we were unable to convince the cardiovascular surgery team to enroll patients under 1 year of age. As the majority of CHD patients are under 1 year of age we decided not to proceed. In addition there were multiple protocol modifications made at CHEO intended to allow for either earlier access to families or easier follow-up. In addition to increasing accrual rate these efforts were designed to increase the duration of time on study drug prior to surgery. Analysis of whether there had been an increase in duration of time from enrollment to surgery suggested minimal to no change for the more recent study participants (Figure 5E, appendix 5.9). For example, the average mean period of time between enrollment and surgery increased from 37 (SD 29) to 48 (SD 64) days, while the percentage of study participants who received study drug for more than 30 days decreased from 53% (8/15) to 47% (7/15).

5.2. DISCUSSION

This section discusses our experience with the set-up and initiation of a phase II dose evaluation double blind randomized controlled trial comparing pre-operative daily high dose

vitamin D supplementation to usual care in children with surgical CHD requiring cardiopulmonary bypass. The primary objective is to determine whether the high dose regimen can significantly reduce the prevalence of post-operative vitamin D deficiency. As 25OHD concentrations have not yet been determined from stored biological samples it is not possible to comment on the extent to which post-operative vitamin D status differs between groups. Instead we will focus the discussion on feasibility issues(study set-up, patient recruitment, study drug intake, safety procedures) and vitamin D related adverse events.

5.2.1 Study set-up and recruitment

The thesis objectives of designing, obtaining approvals for, and initiating a Health Canada regulated clinical trial was successfully achieved. Once regulatory approvals were in place the pilot study also demonstrated that the families of children with surgical CHD were willing to participate in a RCT of high dose vitamin D. Although below what we have reported for observational studies of vitamin D in ill children (24, 26, 247), the 70% consent rate is very similar to what has been reported for other studies evaluating hormones in surgical CHD(248, 249). For example, in recent placebo controlled trial of insulin for the management of post-operative hyperglycemia Agus and colleagues reported a 69% consent rate(248). Similarly, in their RCT investigating the benefits of post-operative thyroid supplementation, Portman and colleagues reported that 68% of those screened and eligible agreed to participate(249). Although the consent rate for approached patients was encouraging and consistent with recent literature, the study accrual rate was below that anticipated at the beginning of the study (1.8 vs. 2.5 per month per site). While below our target, the per month accrual rate is similar to what has been reported in the aforementioned multicenter studies of thyroid and insulin(248, 249). Our expectation of a higher consent and

accrual rate was based on widespread public interest in vitamin D and the fact that most children would already be receiving some form of supplementation. Evaluation of the participant flow diagram identified two main reasons the accrual rate was below target. First, and most importantly, instead of the estimated 100 eligible patient referrals for the 19 month period the study team received only 67. Discussion with our cardiovascular surgery team determined that there has been a decline in the number of children having surgery for CHD at CHEO over the past few years. Second, was our observation that the research team was unable to discuss study participation in 26.9% (n=18/67) of eligible cases. Patient and/or parent refusal to discuss research is well known, but occurred in only 9% (n=6). Instead, physician refusal and inappropriately timed referrals (i.e. late) represented the most common reasons an eligible patients was not approached about study participation. Some of these barriers may be modifiable, and as our institution gains further training and experience with CHD research on high dose vitamin D, the proportion of eligible patients who do not have study participation discussed may decrease.

Finally, we documented that 4 of the 35 patients enrolled were ultimately withdrawn from the study. For three of the cases, the withdrawal occurred because the surgical plan changed and the child was to receive their operation in Toronto. Although a problem for our single center study, this change in the care plan may not negatively impact a large Canadian multicentre study if data and biological samples were collected at all sites.

5.2.2 Sample collection and performance of safety procedures

Primary objective -

In addition to recruitment, the one study procedure most important to the success of the study is the ability to collect biological samples for determination of post-operative vitamin D status. As anticipated based on the CHD observational study(26), immediate post-operative blood was collected and properly processed for 96.6% of study participants. For the single patient without a PICU admission sample, blood was intentionally not collected as the patient had a prolonged period of resuscitation immediately following return to the PICU. For this patient we will use their post-operative day 1 sample as the preceding observational study did not indicate a significant difference between 25OHD levels at PICU admission and post-operative day 1(26). Finally, although it will not be possible to comment on post-operative vitamin D status for all withdrawn patients we did collect a mid-treatment sample from one participant prior to withdrawal; using this sample we will be able to comment on steady state 25OHD levels after approximately 6 months of study drug intake.

Safety procedures and adverse events -

As part of this trial we were able to design, set-up, and implement a "real time" safety protocol intended to evaluate for, document and minimize adverse events related to elevated vitamin D. It is well accepted that vitamin D toxicity is related to elevated 25OHD levels and that months of daily intake at or above the daily Tolerable Upper Intake Level would be required before levels rose sufficiently to increase risk of toxicity (11, 91, 250, 251). Given expected variability in CHD lesion type and duration of study participation prior to surgery we designed a safety protocol personalized to patient duration of study drug intake.

Regardless of patient type our safety protocol and adverse event analysis included intra-operative (pre-surgery) and immediate post-operative evaluation for hypercalcemia and

hypercalciuria. Intra-operative and post-operative blood calcium levels were available for 100% of study participants, and no cases of hypercalcemia occurred. The absence of peri-operative hypercalcemia matches what was reported in our observational study of CHD and is consistent with the 2.6% rate calculated for daily dosing regimens in our systematic review (26, 231). Similarly, evaluation for peri-operative hypercalciuria was equally successful with 100% and 93% of participants having intraoperative and post-operative urine samples sent for calcium:creatinine ratio, respectively. Altogether 40% of study participants had hypercalciuria on either their intra-operative or post-operative sample. This hypercalciuria rate contrasts significantly with the pooled 2.5% rate observed in the systematic review(231). Although different from what is observed in other pediatric populations, this high rate of peri-operative hypercalciuria was not unexpected and was one of the primary reasons the dose evaluation study was performed as a controlled trial. In deciding upon the study design we speculated that due to the abnormal physiology, diuretic administration, and kidney dysfunction an unknown, but potentially significant, proportion could have hypercalciuria. Having a control arm is essential to demonstrating whether hypercalciuria was related to CHD surgery or vitamin D. The closest data for comparison comes from the recently completed RCT by Amrein and colleagues, where they compared hypercalciuria rates in critically ill adults (n=475) who received placebo or a 540000 IU cholecalciferol load(122). This well powered study demonstrated no difference between the two groups, with hypercalciuria occurring in 25% of all patients at each time points(122). Once enrolment is complete and all participants have completed study procedures it will be important to evaluate for potential differences in the occurrence of hypercalciuria between study arms (and vitamin D level). In June 2015, the DSMB did compare hypercalciuria and serious

adverse event rates by study arm and reported back no safety concerns or reasons to discontinue the study. Regardless of whether there are differences in peri-operative hypercalcuria rates it is important to emphasize that no participant with elevated intraoperative urine calcium levels had nephrocalcinosis and that all isolated episodes of post-operative hypercalcuira appeared insignificant and/or transient in nature. The lack of nephrocalcinosis with high dose vitamin D is consistent with the absence of any reported episodes in the clinical trials identified as part of the systematic review(231). Further, our review of nephrocalcinosis case series and cohort literature suggested that those cases associated with high dose vitamin D involved either massive doses (>600000 IU) or children with genetic abnormalities of their vitamin D axis (99-103).

5.2.3 Study drug intake and projected 25OHD levels

Despite the lack of analysis and presentation of 25OHD results, important information is available from data collected on time from enrolment to surgery and cumulative study drug intake. Our study goal was to raise vitamin D levels pre-operatively to above 90 nmol/L, such that they would remain above 50 nmol/L after the 40% intraoperative decline. This idea was supported from the results of two recent well done clinical trials demonstrated that daily dosing in the 1200 to 1600 IU range achieved group means between 100 and 150 nmol/L in healthy infants(19, 81). Further supporting the dose selection was a more recent publication by Lewis and colleagues on a group of healthy school age children(252). Given a baseline 25OHD concentration of 70 nmol/L the group of children receiving 2000 and 4000 nmol/L increased their 25OHD levels by approximately 40 and 80 nmol/L, respectively. Importantly, these studies clearly demonstrate that change is time dependent with at least 2 months required to achieve new steady state (19, 81, 252).

Based on previous observations that the majority of CHD surgeries occur at or after 3 months of age we projected that ~75% of the study participants could receive 2 or more months of daily supplementation prior to surgery(26). Instead, the average (median) number of doses prior to surgery was only 27 days, with just 25% achieving two months of intake. The negative impact this will have on pre-operative vitamin D status can be estimated from the three RCTs and our systematic review of all high dose pediatric trials(19, 81, 231, 252). Based on the evidence from the three aforementioned clinical trials, dosing for 20 to 30 days may only be sufficient to raise levels by 20 to 30 nmol/L. However, this may be an overestimate of 25OHD response given our systematic review observation that disease children have a blunted response (per dose) when compared to healthy children (231). Inserting the study data into the equation generated from our systematic review and meta-analysis (baseline 25OHD of 60 nmol/L; cumulative dose of 40000 IU) returns a group average pre-operative 25OHD concentration of 70 nmol/L and standard deviation of 35 nmol/L.

Based on the short duration of study drug intake we expect the 25OHD levels to show that the majority of study participants do not raise pre-operative levels enough to prevent post-operative vitamin D deficiency. Although we did foresee that pre-operative daily high dose supplementation would not be effective in certain surgical CHD subgroups, the results of our pilot study suggest that the subgroup may represent the majority instead of the minority.

5.2.4 Protocol limitations, deviations and modifications

In addition to being unable to obtained adequate cumulative drug intake prior to surgery our study demonstrated that attempts to utilize daily supplementation for a trial on CHD patients presents other scientific, economic and potential patient safety issues. First, in contrast to the success with collection of immediate post-operative biological sample, baseline blood and urine were collected for only 43% of study participants in whom it was desired. During study design, the risk and benefits of requiring a baseline 25OHD and urine calcium were debated by the investigators, REB and DSMB committee. Although all agreed that this information would be beneficial it was ultimately decided to make these investigations optional instead of mandatory. The single most important factor that played into this decision was consistent feedback during our previous studies that parents would decline participation if additional venipuncture for blood work was required (26, 247). These concerns are validated in a recent study by Menon and Ward where additional blood work was one of the most common reasons given for declining participation (253). Although it would have been an option to make baseline 25OHD mandatory and wait until the next set of clinically indicated blood work for initiation of study drug this would have further reduced cumulative dosing. Ultimately, the lack of baseline 25OHD data may end up as a significant study limitation.

Second, although we were able to recruit at an accrual rate similar to other surgical CHD trials involving hormones there were significant challenges not immediately evident through inspection of the flow diagram(248, 249). Unlike most other trials of surgical CHD where the intervention is given intra-operatively or during PICU admission, our intervention was given pre-operatively and requires early initiation to achieve the greatest chance of success. Shortly following the initiation of recruitment it became apparent that most patients

were not being referred early enough and that most patients would not consent at the time of the first research encounter. Armed with the knowledge that the duration of time between enrolment and surgery matters, significant efforts were made to lengthen the time from recruitment to surgery. These efforts included REB approval for study staff to speak with potentially eligible patients prior to appointments, follow-up up with patients by telephone or home visit after appointments, and delivery of study drug by mail. Interestingly, although the study coordinators were of the impression that these changes were of value, no improvement in accrual rate or average duration of drug intake was shown. Importantly, the significant coordinator effort required to identify and repeatedly meet with families has resulted in coordinator time expenditure well in excess of the 2 hours budgeted for the study.

Third, we observed issues surrounding the administration of study drug to participants(254, 255). Included among the issues were anticipated problems with study drug compliance. Although the majority of caregivers gave the medication as instructed, approximately 30% gave less than 80% of the ordered doses.. Understanding and neutralizing poor compliance is important as it can significantly impair trial power, regardless of whether the outcome is biochemical or clinical (256, 257). In addition to problems with reduced study drug intake we also documented problems with protocol deviations by both families and physicians that resulted in increased vitamin D intake. Our observations also support the consideration of an alternative dosing regimen that is less susceptible to deviation and modification by families and health care staff.

CHAPTER 6: CONCLUSIONS

6.1 Summary of literature and update

Recent studies have demonstrated that many children with CHD have post-operative vitamin D deficiency and that these lower levels may place them at risk for greater post-operative morbidity (24, 26-28). The limited work in this area is supported by a recent systematic review, wherein the authors report a statistically significant association between vitamin D status and at least one post-operative outcome in 26 of 31 surgical studies (258). Although undesirable, the high prevalence of vitamin D deficiency also presents an opportunity as optimization could represent a simple and inexpensive means to improve short and/or long term outcomes. The idea that vitamin D deficiency could be relevant to critically ill patients received a significant boost recently with the publication of the VITdAL-ICU study suggesting a clinically meaningful but statistically insignificant absolute mortality reduction of 7% (p=0.10) with rapid normalization of vitamin D status in critical ill adults(122). As literature in other populations emerges, physicians and health care workers providing care for critically ill children, including those with CHD, are beginning to worry about vitamin D deficiency and ask whether and how they can optimize status(259). As a novel area of research, work has been limited and our systematic review confirmed that absence of clinical trials in the CHD or general PICU populations(231). The body of work completed for this thesis represents the next steps to address the knowledge gaps surrounding how to prevent or treat vitamin D deficiency in surgical CHD patients.

6.2 Summary of thesis work and findings

The primary project for the thesis was the design, set-up and initiation of a pilot dose evaluation RCT to determine whether pre-operative supplementation with daily high dose

vitamin D, when compared with usual care, significantly reduces the rate of post-operative deficiency. Although there were other dosing regimens to consider, it was decided to begin clinical trial work in this area with a daily dosing regimen as it had the potential to be effective in approximately 75% of the population, is the only method of vitamin D supplementation recommended by the Canadian Pediatric Society, and is the only dosing regimen approved by Health Canada. The systematic review confirmed our suspicion that daily supplementation is the favoured regimen, as only one trial evaluating intermittent loading dose therapy has been performed and published (twice) in North America(135, 136). The thesis reports on our experience after the first 30 study participants completed all surgical procedures. Although vitamin D levels were not available for discussion there were important and relevant results available on study drug intake, safety and feasibility outcomes.

The majority of our initial observations related to the design, set-up, initiation and recruitment into the RCT were positive. First, the high consent rate suggests that patients and families are concerned about post-operative vitamin D deficiency and see value in a study of high dose vitamin D. Second, comparison with the CHD literature demonstrates that we could recruit study participants at a rate similar to other pediatric trials of hormone therapy (248, 249). Third, we confirmed our ability to collect biological samples for measurement of post-operative 25OHD and vitamin D related adverse events. Fourth, the safety procedures and adverse event analysis proved valuable. Especially meaningful was the finding that 40% of study participants had at least one episode of peri-operative hypercalciuria analysis. This rate is considerably higher than the 2.5% rate calculated as part of the systematic review and meta-analysis for healthy and less sick pediatric populations (231), and affirms our original decision to include a control arm in the dose evaluation study. Without the usual care arm it

would not be possible to appraise whether the high hypercalciuria rate was related to critical illness or high dose vitamin D.

Although there were many positives observed with set-up, initiation, recruitment and sample collection many of other findings raise considerable concern that an intervention based on the pre-operative daily administration of the IOM Tolerable Upper Intake Level may not be the best choice for an eventual phase III trial. The primary concern was that the majority of participants could not be enrolled with adequate time to administer the desired cumulative dose of vitamin D. Only 25% of participants (instead of 75%) achieved the desired 2 month cumulative intake of study drug. Additional concerns about the pre-operative daily high dosing regimen included the significant coordinator time required for early identification and recruitment, the inability to routinely measure baseline 25OHD status, and multiple instances of family or health care provider deviation from study protocol. These problems emphasize the need to explore alternative dosing strategies as the current strategy not only impacts the scientific integrity of the work but may place the patients at risk.

Prior to the initiation the dose evaluation RCT we recognized a subgroup of CHD patients (neonates) who were unlikely to adequately elevate 25OHD levels with the daily high dose regimen. Originally we believed that this group would be the minority (<25%) and would largely be made of those neonates and young infants presenting with lesions that require surgery within a few weeks of birth (or presentation). Our systematic review confirmed our concerns and demonstrated that high dose daily supplementation may not be adequate for the CHD patient presenting within a month of surgery(231). Fortunately, the systematic review did convincingly establish that it is possible to raise 25OHD levels within

48 hours with the administration of a single large enteral dose of cholecalciferol. Importantly, the adverse event analysis performed as part of the systematic review did show a greater risk for hypercalcemia with high dose regimens exceeding the Tolerable Upper Intake Level. Importantly, a detailed analysis revealed that this increased risk only occurred in the setting young children who received doses equal to or exceeding 400000 IU and achieved group mean 25OHD levels exceeding 200 nmol/L.

6.3 Proposed next steps

In conclusion, the preliminary findings suggest that it is possible to set-up and recruit into a study of high dose vitamin D in CHD but that this regimen may not achieve our goal of mitigating post-operative vitamin D deficiency. Therefore, prior to proceeding with a phase III RCT it will be important to consider an alternative dosing regimen. Based on available data we anticipate the need for a second dose evaluation RCT investigating loading dose cholecalciferol immediately prior to surgery. For this study, inpatients would receive their dose sometime in the week leading up to surgery, while outpatients would receive their dose at the pre-surgical appointment. Timing the dose with the pre-surgical visit would allow for collection of baseline research blood. Further using the knowledge generated from our systematic review it would be possible to personalize each participants dose to account for weight/age and baseline 25OHD level. The recent development and marketing of point-of-care devices (e.g. Qualigen, Nanospeed) that can assess 25OHD levels within 10-15 minutes would further make it possible to measure and respond to vitamin D status within the same patient encounter. This approach should reduce research coordinator time associated with aggressive patient recruitment and follow-up, improve study drug compliance, reduce the potential for patient and caregiver protocol deviations, reduce the variability observed with

the implementation of the intervention, allow for the routine collection of baseline blood, and lead to a more consistent 25OHD response with better separation of the study arms.

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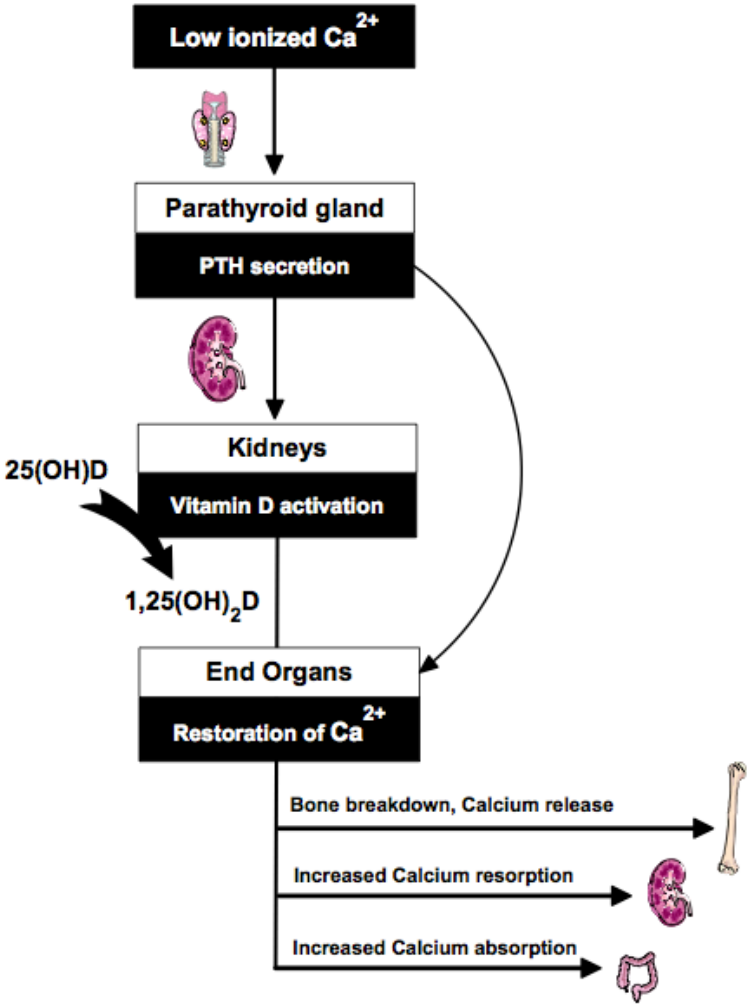

Figure 2A. Overview of vitamin D parathyroid renal axis—Functioning of the axis is demonstrated in the context of calcium homeostasis. In response to low calcium, the parathyroid glands increase parathyroid hormone (PTH) secretion. Increased PTH leads to activation of vitamin-D through an inducible renal enzyme, converting 25 hydroxyvitamin D (25OHD) to the active hormone or 1,25 dihydroxyvitamin D ($1,25OH_2D$).

Table 2A. Findings reported within the 4 published PICU observational studies that included CHD patients.

	McNally (24)	Rippel (27)	Graham (24)	McNally (26)
Study population				
PICU (n)	326	316		
CHD subgroup (n)	122	210	70	58
25OHD <50 nmol/L				
PICU deficiency rate, %	69%	34.5%		
CHD deficiency rate, %	73%	40.5%	84%	86%
Illness severity†				
Catecholamine use	+			+
Inotrope score		+	+	+
Hypocalcemia	+	–		–
Calcium supplementation		+		–
Fluid requirements	+			+
Mechanical ventilation, %	–	–		
Mechanical ventilation, time	–	–		+
PRISM/PIM‡	+	–		
Lactate		–	–	
PICU LOS	+	–	–	+/–
Hospital LOS		–	–	–
Mortality	–	–		

Legend. If the relationship between a marker of illness severity was tested in more than one study it was included. A significant association or p <0.05 was reported as (+), a trend or P value between 0.05-0.10 as (+/-) and no association as (-). An empty cell indicates that this information was not provided or was not applicable. † The illness severity associations indicated for the McNally, 2013 study represent those for the entire PICU cohort, and those for the study by Rippel and colleagues represent the CHD subgroup. ‡ PRISM and PIM represent illness severity scores. Abbreviations: 25OHD, 25-hydroxyvitamin D; CHD, Congenital heart disease; LOS, Length of Stay; PICU, Pediatric intensive care unit; PIM, Pediatric Index of Mortality; PRISM, Pediatric Risk of Mortality.

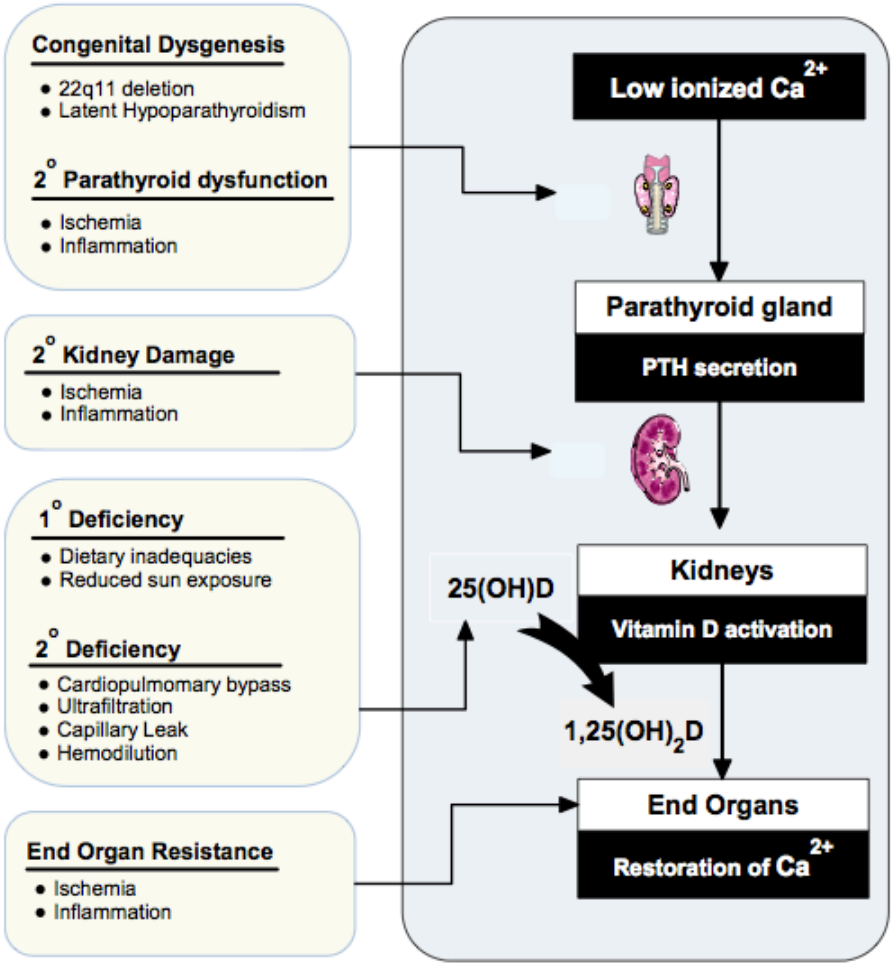

Figure 2B. Factors contributing to Vitamin D deficiency. Demonstrates some of the congenital and acquired risk factors that could impair vitamin D axis functioning following cardiac surgery for congenital heart disease

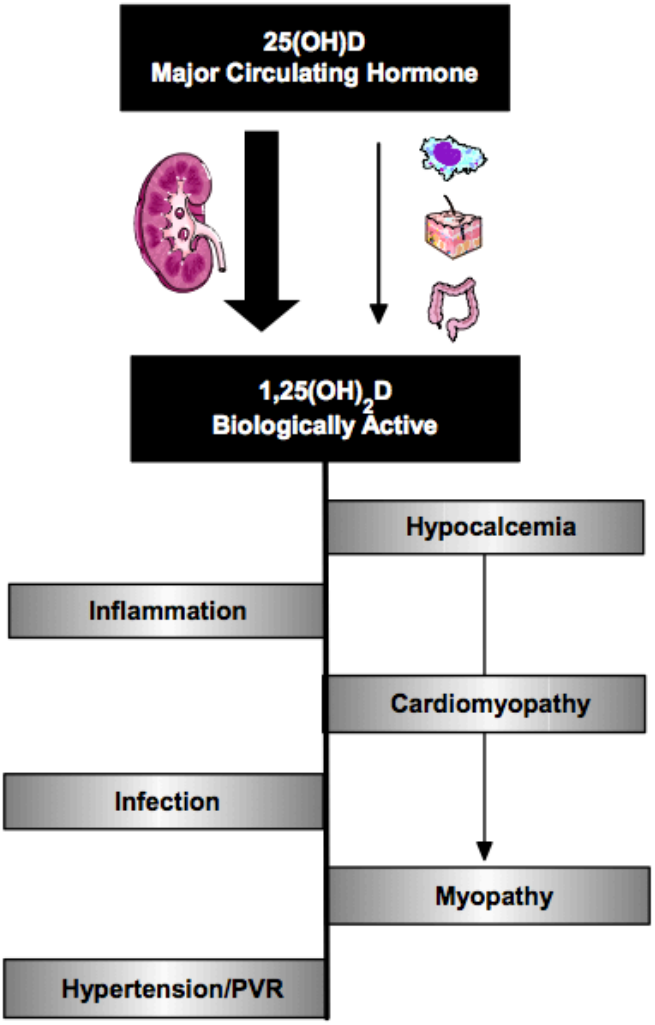

Figure 2C. Vitamin D deficiency mediated pathophysiology relevant to post-operative Congenital Heart Disease patients.

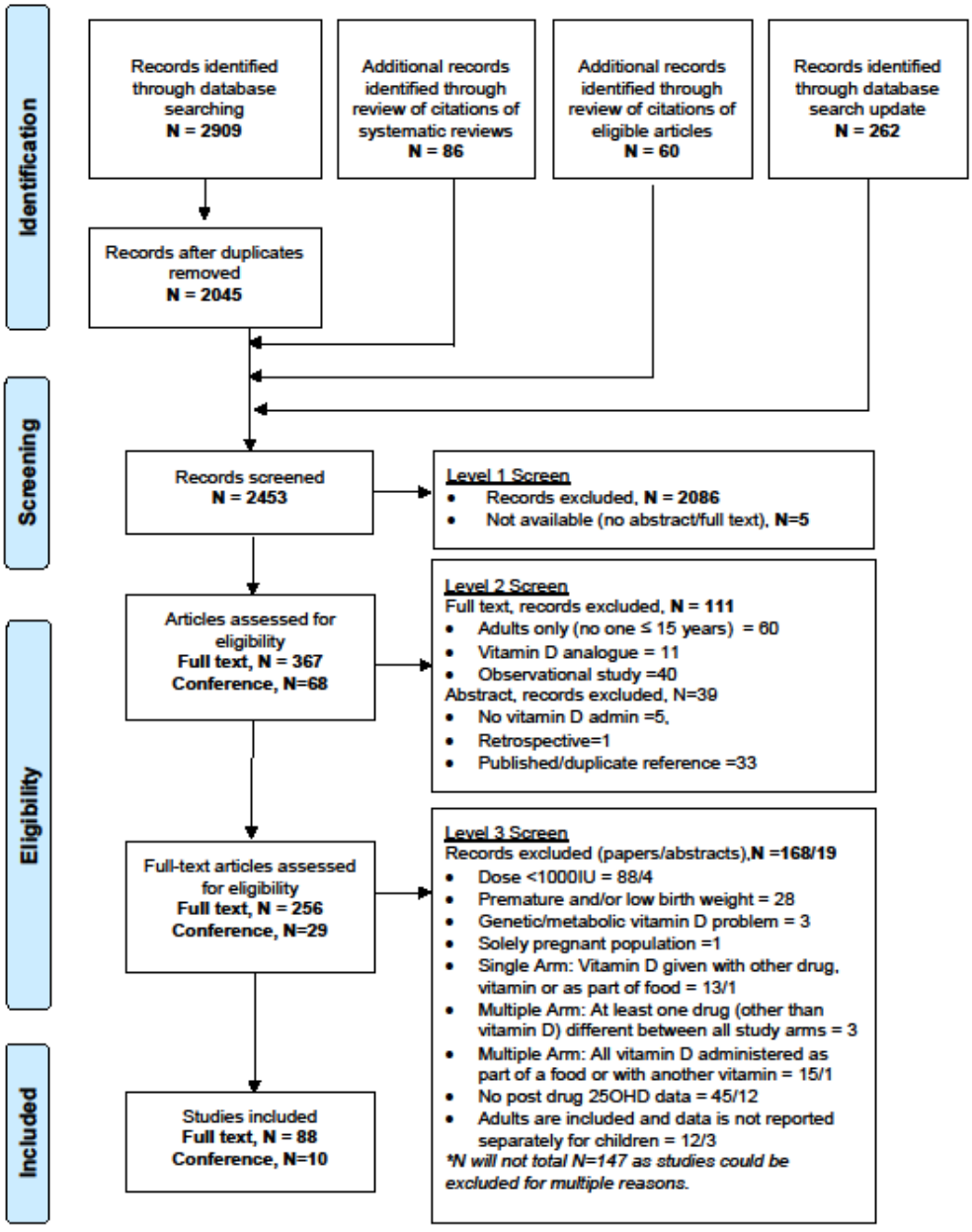

Figure 2A: Flowchart of study selection based on inclusion and exclusion criteria. The stages of a systematic selection scheme include identification, screening, eligibility, and final included studies. Numbers will not total 168 because studies could be excluded for multiple reasons.

Study Characteristic	Vitamin D Dosing Regimen, *n*		
	Daily (*n* = 51)	Weekly (*n* = 19)	Intermittent (*n* = 64)
Age group[a]			
Neonates	12	1	7
Infants	7	2	14
Toddlers	17	4	28
School age	33	18	37
Adolescents	23	17	33
Diagnostic category			
Healthy	25	12	29
VDD (no rickets)	4	1	6
Rickets	3	0	19
Malabsorption	3	0	2
Renal disease	2	1	2
Other disease[b]	14	5	6
Dosing regimen			
Variable	4	2	13
Constant	47	17	51
1000–4000 IU	41	4	0
4000–10 000 IU	1	1	0
10 000–40 000 IU	2	6	0
40 000–1 000 000 IU	3	6	51
Enteral	51	19	49
Intramuscular	0	0	15
Vitamin D_2 (ergocalciferol)[c]	12	3	9
Vitamin D_3 (cholecalciferol)	35	16	49
Geography			
North America	23	5	2
Central/South America	0	0	2
Europe	15	1	12
East Asia	2	0	0
Rest of Asia	4	4	9
Africa	0	0	11
Australia/New Zealand	0	0	2
Middle East	7	9	26

Table 3A. Patient, dosing and study characteristics of high dose study arms.

[a] Counts will exceed 134 as population could include multiple age groups. [b] Supplementary Table 8 lists other diseases. [c] In 18 cases vitamin D form was unclear.

Study Characteristic	Vitamin D Dosing Regimen, *n*		
	Daily (*n* = 51)	Weekly (*n* = 19)	Intermittent (*n* = 64)
Year			
1970–1979	1	0	1
1980–1989	9	0	6
1990–1999	2	0	8
2000–2009	11	10	16
2010–2013	28	9	33
Study design			
Single arm	8	1	20
RCT/quasi-RCT[a]	36	15	37
Controlled, other	7	3	7
25(OH)D assay			
Immunoassay	37	18	47
LC-MS/MS	8	1	9
Unclear	6	0	4
25(OH)D measurement			
Within 3 months	41	10	51
Within 1 month	17	3	28
Randomized trial quality			
Low risk	15	1	8
Medium risk/unclear	20	14	28
High risk	1	0	1
Cochrane risk of bias[b]			
Generation adequate	26 (13)	8 (7)	26 (15)
Concealment adequate	17 (22)	7 (8)	13 (27)
Blinding adequate	19 (9)	8 (2)	12 (7)
Outcome report complete	33 (6)	15 (1)	45 (5)
Outcome not selective	26 (5)	7 (2)	24 (7)

Table 3B: Assessment of Study Design and Methodological Quality. LC-MS/MS, liquid chromatography – tandem mass spectrometry. [a] Only 1 study arm originated from a quasiRCT. [b] Values represent the number of arms, while values inside parenthesis indicate unable to determine.

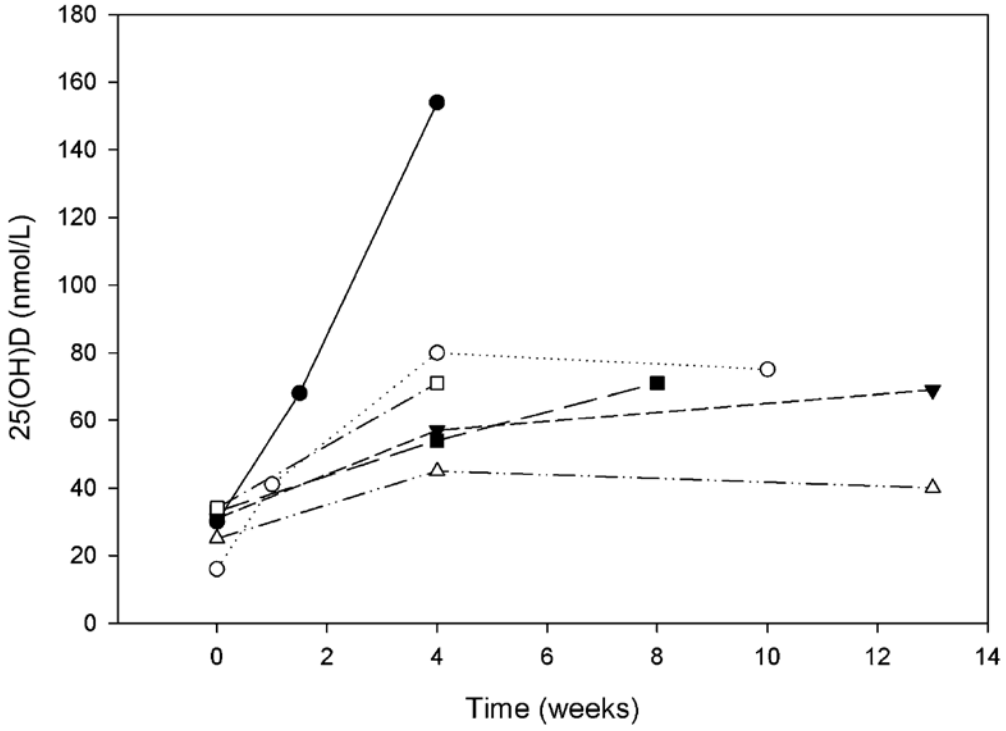

Figure 3B: Short term 25(OH)D response to high dose daily vitamin D intake. Six study arms evaluated 25OHD response in VDD children within 1 month of initiating dosing that approximated the Institute of Medicine's daily Tolerable Upper Intake Level (1000-4000 IU). (●) Holst-Gemeiner 1987; (o) Markestad 1987; (Δ) Leger 1989; (▼) Vervel. 1997; (■) Dong. 2010; (□) Park 2010.

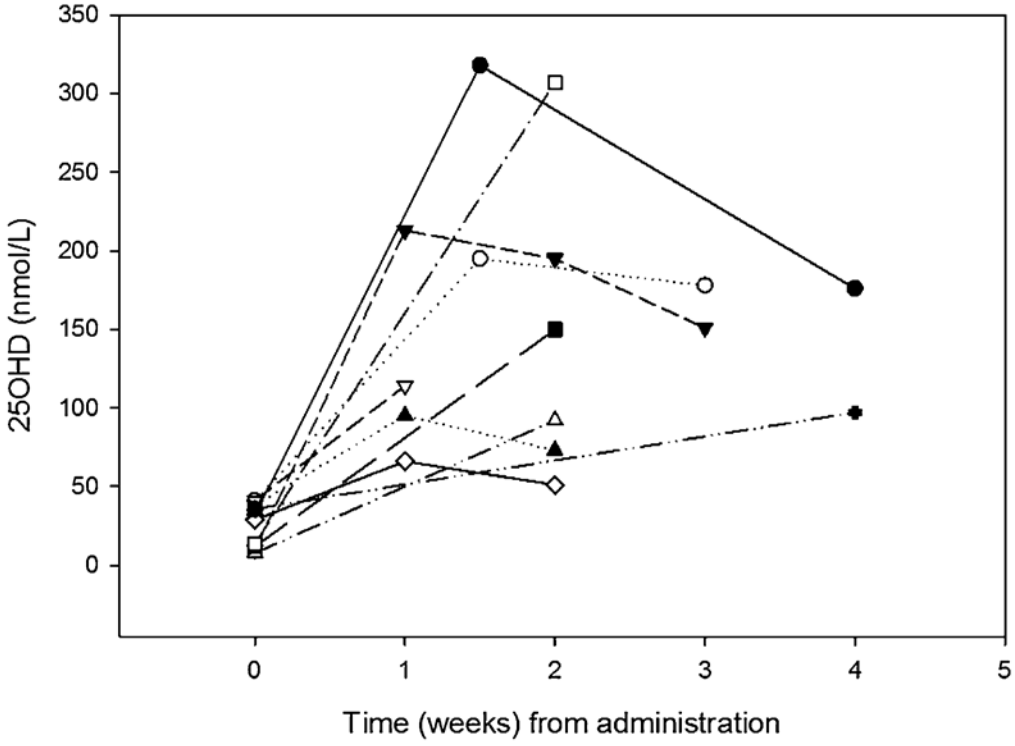

Figure 3C: Short term 25(OH)D response to vitamin D loading therapy. Ten study arms were identified that evaluated 25(OH)D response in VDD children within 1 month of administering a loading dose of vitamin D. (●) Holst-Gemeiner, 1987; (□) Zeghoud, 1994; (▼) Stogmann, 1985 (o) Raghuramulu, 1982; (■) Zeghoud, 1994; (v) Manaseki-Holland, 2012; (▲) Thacher, 2010; (Δ) Zeghoud, 1994; (◊) Thacher, 2006; (Cross) Kari, 2013.

	β	SE	95% CI
Population			
Pre-25(OH)D (per nmol/L)	0.58	0.22	0.15 to 1.01
Age (per year)	−2.66	0.79	−4.21 to −1.11
Diseased (versus healthy)	−14.5	9.4	−32.9 to 3.92
Regimen			
Cumulative dose (per 1000 IU)	0.12	0.019	0.083 to 0.16
Intramuscular versus enteral	−7.52	19.68	−46.03 to 30.99
Vitamin D_3 versus vitamin D_2	−9.68	13.86	−36.85 to 17.49
Single load versus placebo	61.08	11.26	39.01 to 83.15
Regular/repeat versus placebo	38.68	9.9	19.28 to 58.08
Loading dose versus other	36.88	10.01	17.26 to 56.5
25(OH)D measurement			
Time from initiation (in weeks)	−4.02	1.07	−6.12 to −1.92
Immunoassay versus LC/MS	4.98	12.15	−18.83 to 28.79
Study type			
Uncontrolled versus RCT	44.68	12.25	20.67 to 68.69
Non–randomized-controlled versus RCT	52.82	13.50	26.38 to 79.26
Study quality			
Overall assessment			
High versus low risk	21.84	9.97	2.30 to 41.38
Medium/unclear versus low	23.80	11.20	1.85 to 45.75
Components			
Allocation generation (adequate versus not)	−34.89	8.98	−52.49 to −17.29
Allocation concealment (adequate versus not)	−16.89	9.59	−35.69 to 1.91
Blinding (adequate versus not)	−15.88	9.69	−34.87 to 3.11
Outcome reporting (adequate versus not)	9.47	11.76	−13.58 to 32.50
Selective reporting (adequate versus not)	−1.16	9.31	−19.41 to 17.09

Table 3C: Single-Variable Meta-regression of Post-Study Drug 25(OH)D. The β estimate provides the change (per 1 nmol/L) in post study drug 25OHD for each variable. Study quality was determined used the Cochrane Risk of Bias tool. LC/MS – Liquid chromatography/Mass Spectrometry.

	Model 1		Model 2	
	β	(95% CI)	β	(95% CI)
Intercept	41.3	(24.0 to 58.6)	28.34	(12.2 to 44.4)
Baseline 25(OH)D	0.79	(0.54 to 1.05)	0.84	(0.62 to 1.06)
Age (per year)	−0.68	(−1.80 to 0.44)	−0.54	(−1.5 to 0.42)
Diseased (versus healthy)	−18.6	(−29.0 to −8.15)	−19.5	(−28.6 to −10.4)
Dose (per 1000 IU)	0.29	(0.22 to 0.36)	0.27	(0.21 to 0.34)
Loading dose (versus other)	32.6	(10.1 to 55.1)	43.8	(22.6 to 65.0)
Time from initiation (weeks)	−0.54	(−2.10 to 1.01)	0.02	(−1.37 to 1.41)
Cumulative dose × age	−0.014	(−0.020 to −0.008)	−0.013	(−0.019 to −0.007)
Loading dose × time (weeks)	−5.27	(−7.98 to −2.57)	−5.6	(−7.7 to −3.48)
Study type				
Uncontrolled (versus RCT)	—	—	−3.53	(−19.13 to 12.07)
Non–randomized-controlled (versus RCT)	—	—	34.95	(21.19 to 48.71)

Table 3D: Multivariate Meta-Regression Predicting Post-Study drug 25(OH)D.

Model 1 considered relevant population, dosing and 25(OH)D measurement variables. Model 2 also considered study design and quality features. The β estimate represents the change (per 1 nmol/L) in post-study drug 25(OH)D levels.

Age Group	50 000 IU	150 000 IU	300 000 IU	600 000 IU
Infant (3 mo), nmol/L	86 (35)	112 (45)	152 (60)	232 (91)
Preschool age (2 y), nmol/L	83 (34)	108 (43)	144 (57)	217 (85)
School age (9 y), nmol/L	76 (29)	93 (34)	118 (43)	168 (61)
Adolescents (15 y), nmol/L	66 (24)	73 (27)	82 (31)	101 (40)

Table 3E: Predicted final group 25OHD following vitamin D loading therapy (IU).

The predicted group 25(OH)D one week after 4 different loading doses of vitamin D are shown. The population was considered to be unhealthy and to have an average baseline 25(OH)D level of 30 nmol/L. Predicted SDs are shown in parentheses.

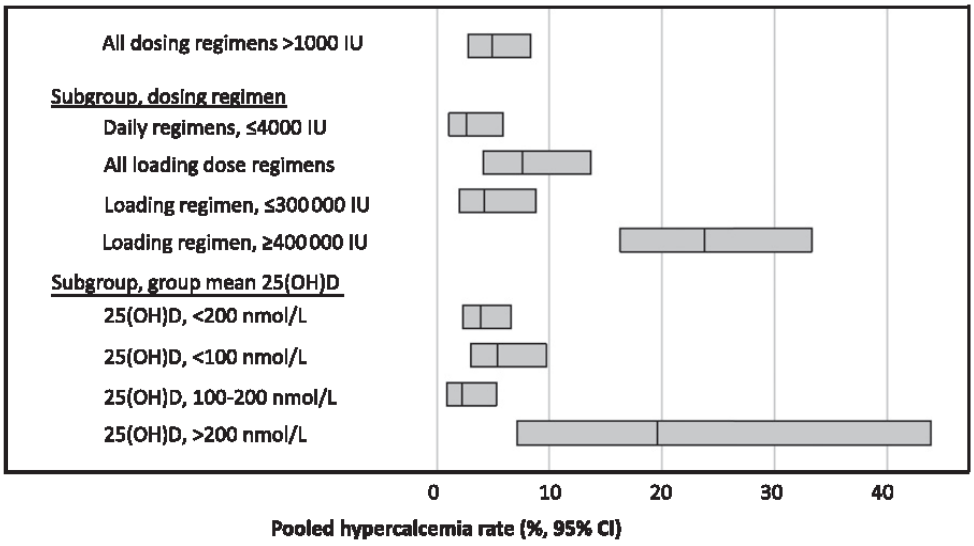

Figure 3D: Forest plot of hypercalcemia rates by dosing regimen. Random-effects meta-analysis was used to calculate pooled hypercalcemia rates and 95% CIs for the all high dose vitamin D regimens (≥1000 IU) and regimen subgroups. Point estimates are shown as the vertical lines in the boxes and 95% CIs are represented by the edge of the boxes. The y-axis describes the various subgroup analysis.

Data Category	Information
Primary registry, trial identifying #	Clinicaltrials.gov Identifier - NCT01838447
Date of registration in primary registry	April 11, 2013
Secondary indentifying numbers	HICCUPS, VitaminDinCHD-01, 13/03E
Sources of monetary support	Heart and Stroke Foundation of Canada Operating Grant, Children's Hospital of Eastern Ontario Research Institute
Primary sponsor	Heart and Stroke Foundation of Canada
Secondary sponsor	Children's Hospital of Eastern Ontario Research Institute
Contact for public queries	JDM, Pediatric Critical Care, Children's Hospital of Eastern Ontario, Ottawa, Canada
Contact for scientific queries	JDM, Pediatric Critical Care, Children's Hospital of Eastern Ontario, Ottawa, Canada
Public title	Prevention of vitamin D deficiency after congenital heart disease surgery: a dose evaluation trial
Scientific title	Prevention of vitamin D deficiency in children following cardiac surgery: a study protocol for a randomized dose evaluation trial
Country of recruitment	Canada, single academic center
Health problem under investigation	Prevention of vitamin D deficiency after congenital heart disease surgery
Key inclusion and exclusion criteria	Ages eligible for study: Corrected gestational age > 36 weeks and <18 years. Inclusion criteria: has CHD requiring surgical correction with cardiopulmonary bypass within 12 months Exclusion criteria: born at <32 weeks gestational age; cardiac or gastrointestinal disease preventing enteral feeds or drug administration prior to surgery; has confirmed or suspected Williams syndrome; proposed surgery to take place at another centre
Study type	Single centre, double blind, parallel, randomized, controlled dose evaluation trial
Date of first enrolment	July 2013
Target sample size	62
Recruitment status	Recruiting as of June, 2015
Primary outcome	Using immediate post-operative vitamin D status, (i.e. blood 25OHD) we will determine whether the pre-operative administration vitamin D compared with usual care results in a significant reduction in post-operative vitamin D deficiency in a pediatric population with CHD.

Table 4A: WHO Trial Registration Data Set – Structured Summary.

A: Breastfed infant or over 12 months of age

Age Group	Volume	Standard Dose Group		High Dose Group	
	mL	IU per day	Concentration	IU per day	Concentration
0-1 year	1	400 IU	400 IU/mL	1600 IU	1600 IU/mL
1-17 years	1	600 IU	600 IU/mL	2400 IU	2400 IU/mL

B: Formula fed and under 12 months of age

Age Group	Volume	Standard Dose Group		High Dose Group	
	mL	IU per day	Concentration	IU per day	Concentration
0-1 year	1	None*	Placebo, 0 IU/mL	1200 IU*	1200 IU/mL

Table 4B: Vitamin D supplementation strategy. *Does not include the vitamin D intake from formula (400 IU/day). ** We will not increase the vitamin D dose in those children who turn 1 year after initiating study drug. Further details on study drug: (1) Isoform - cholecalciferol (D3), (2) Route – Enteral, (3) Form – Solution (4) Frequency – Daily, (5) Duration – from time of CHD diagnosis to day of operation (started no more than 6 months prior to surgery date). Euro-pharm has agreed to provide the study drug in 50 mL vials with the following concentrations to achieve blinding. Concentrations will include: placebo, 400 IU/mL, 600 IU/mL, 1200 IU/mL, 1600 IU/mL, 2400 IU/mL.

Age specific thresholds for pH corrected ionized calcium levels	
Age (months)	**95th % (mmol/L)**
< 2	1.45
> 2	1.4
Age specific threshold for corrected total calcium level	
Age (months)	**mmol/L**
<3	2.8
>3	2.7
Age specific thresholds for elevated calcium-creatinine ratio	
Age (year)	**95th % Ca:Cr ratio (mmol/mmol)**
< 1	2.2
1-2	1.5
2-3	1.4
3-5	1.1
5-7	0.8
7-17	0.7

Table 4C: Safety thresholds for ionized calcium, total corrected calcium and elevated calcium-creatinine ratio.

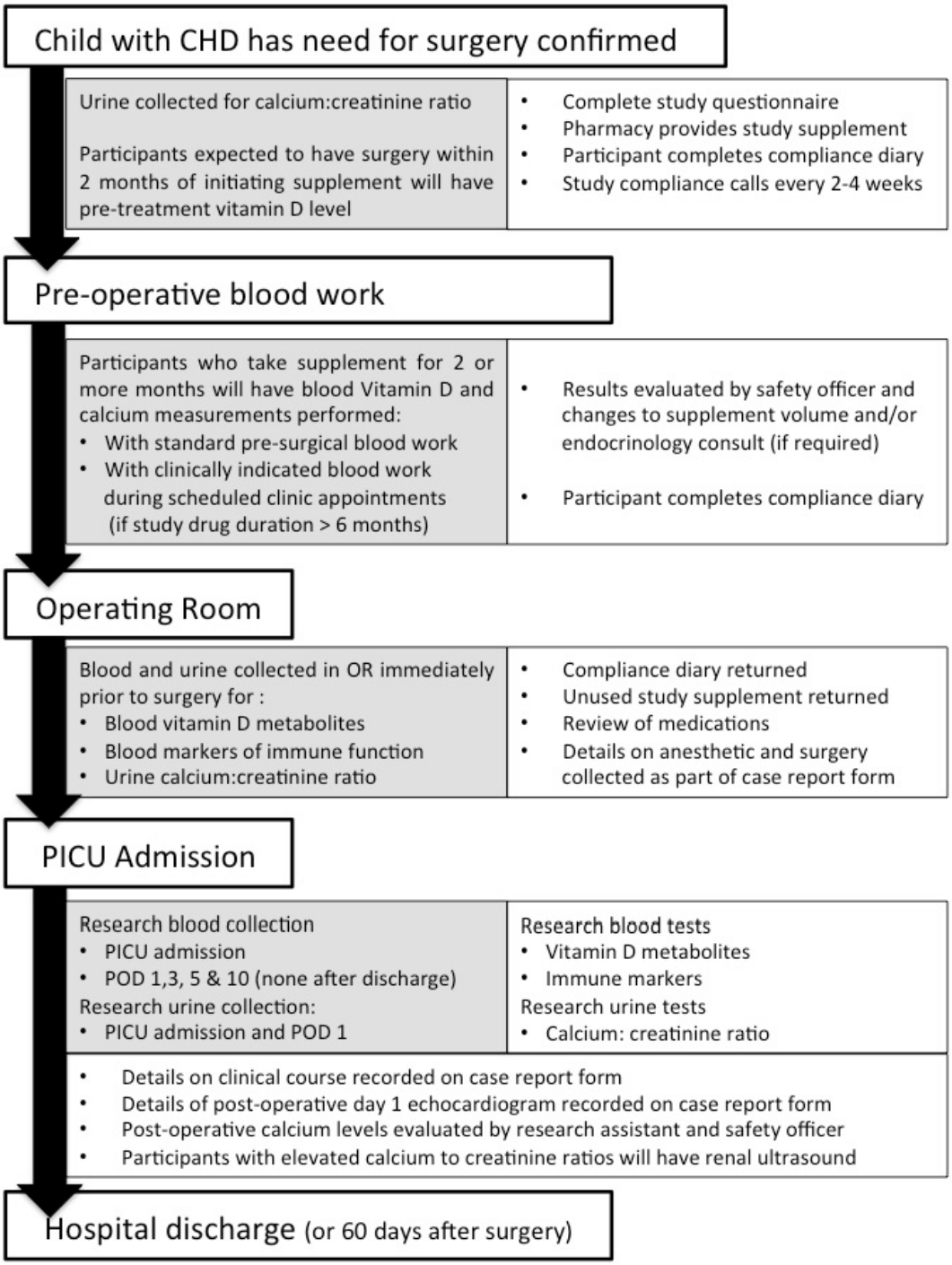

Figure 4A. Study related procedures and measurements

Sample	Timepoint				
	Pre-treatment[1]	During study drug administration[2]	Pre-operative[3]	Operating room prior to surgery	Post-operative
Blood	25OHD*	25OHD ***** **Ionized calcium**	25OHD * Ionized calcium	25OHD * Ionized calcium VDBP PTH Immune function (e.g cathelicidin) Immune activity (e.g. cytokines)	25OHD * Ionized calcium VDBP PTH Immune function (e.g. cathelicidin) Immune activity (e.g. cytokines)
Urine	Calcium: Creatinine	-	-	Calcium: Creatinine ratio	Calcium: Creatinine

Table 4D: Biochemical measurements on research specimens. [1] neonates or anticipated < 2 months of supplement (optional). [2] any patient who receives > 6 months of supplement. [3] all outpatients who present for a pre-operative appointment. *25OHD will be measured to indicate vitamin D status. The 25OHD level determined at the time of standard pre-operative blood work, for safety purposes, will be ordered through the laboratory.

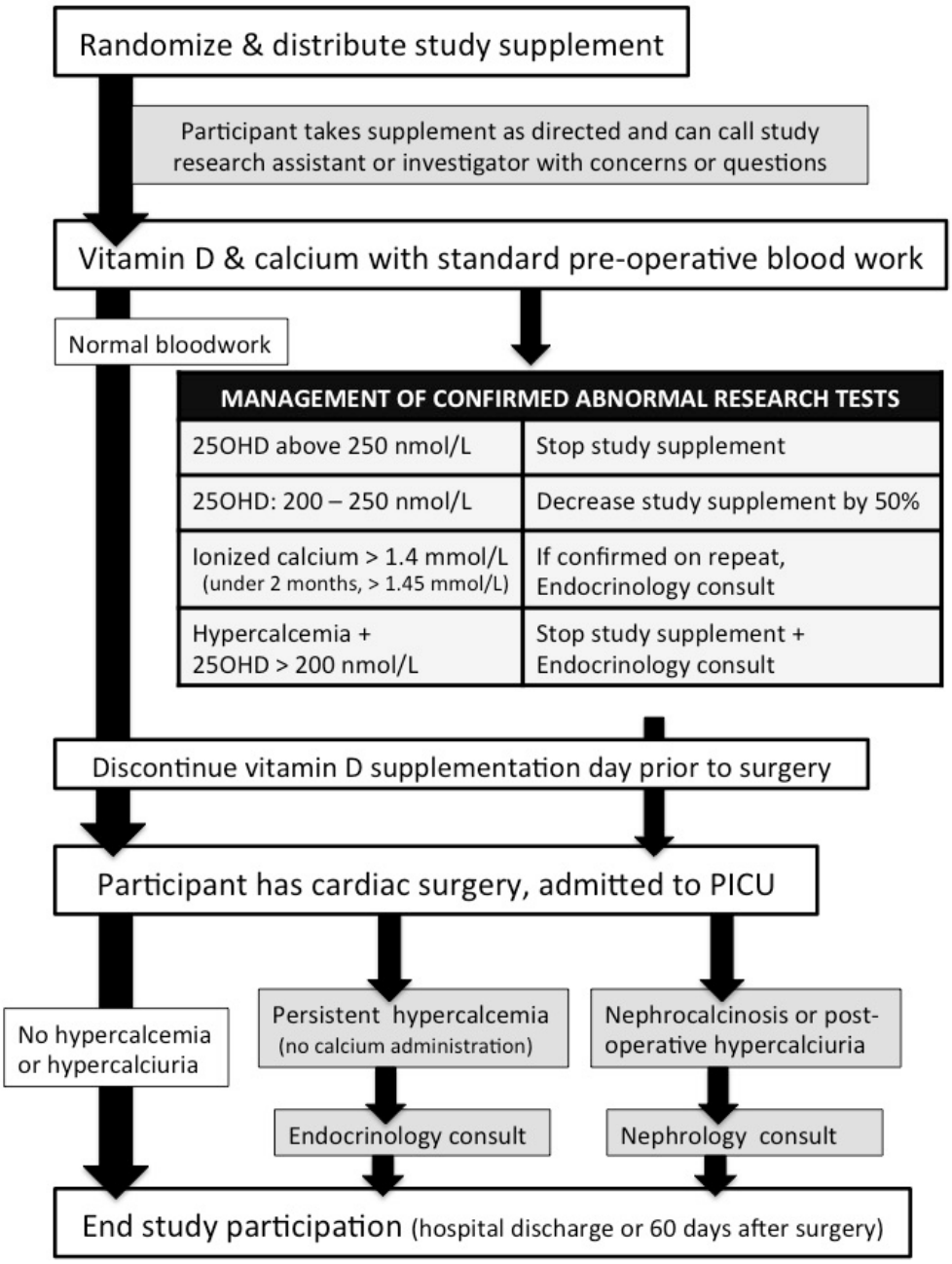

Figure 4B. Flow diagram of study safety measures

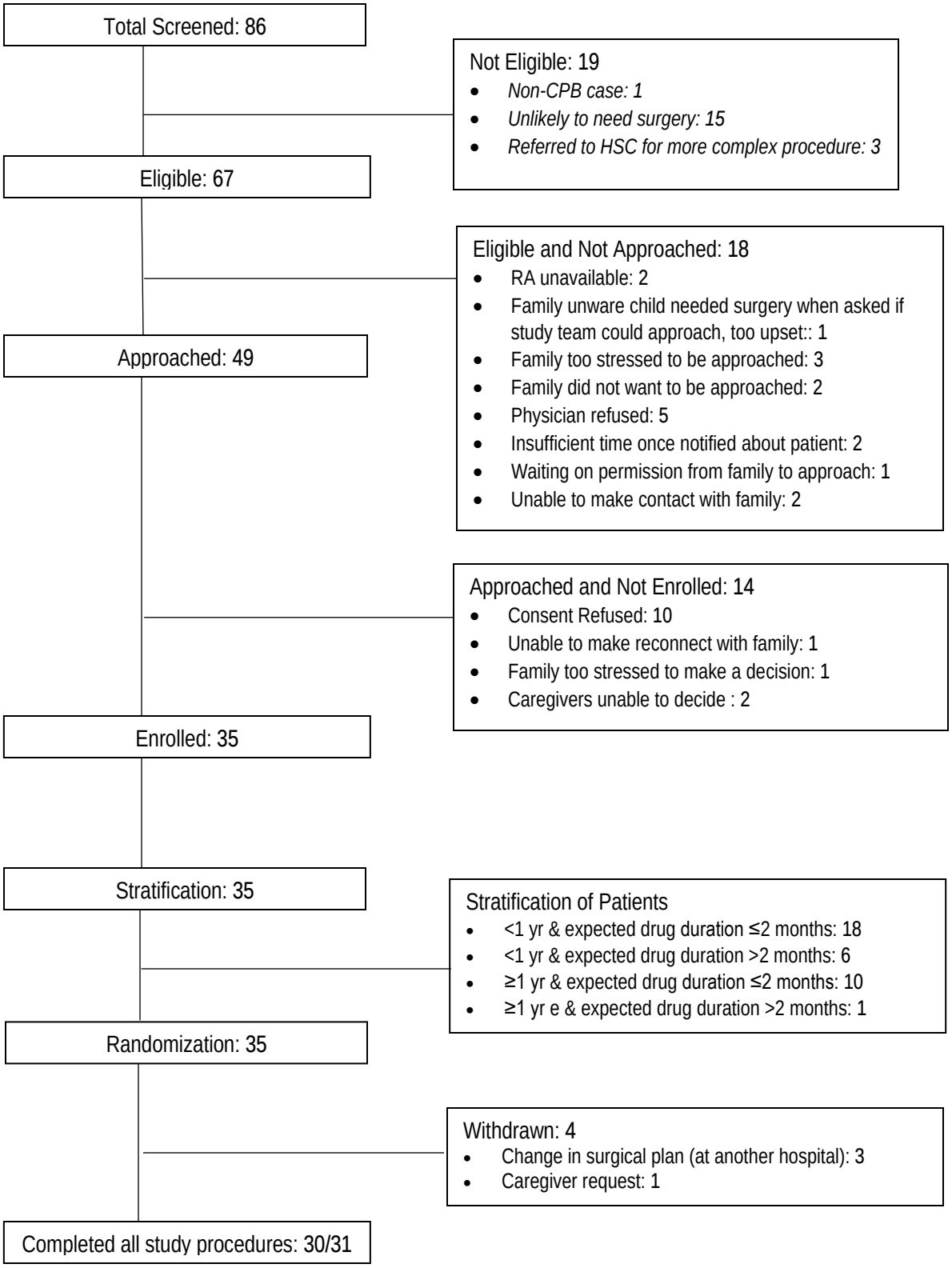

Figure 5A. Participant Flow Diagram (CONSORT)

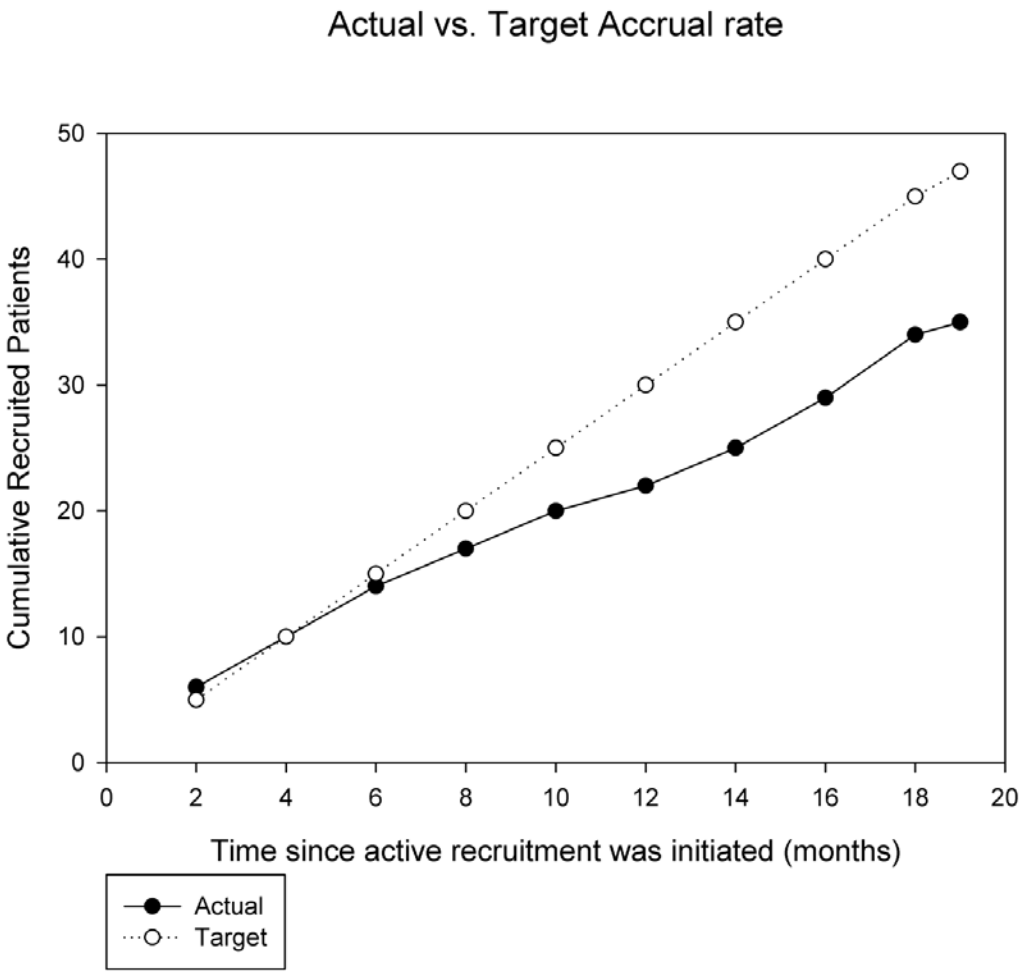

Figure 5B. Actual vs target recruitment rate

Issues during Recruitment
Parents only wanted to participate if guaranteed to be in the high-dose group
Parents only wanted to participate if guaranteed to be in the low-dose group
Mom declined participation due to patient's multiple medical issues. Mom not want to have to track anything else (i.e. track drug on calendar) or worry about giving a daily dose.
Mom declined participation as she felt she already had too many things to worry about with feeding patient and did not want to also worry about giving daily vitamin D.
Mother said that she didn't want to make a decision about the study until she spoke with the father. The patient goes back and forth between two homes, and mother wanted there to be consistency in giving the Vitamin D. Mom concerned dad would not give the study drug. Unable to make further contact with mom before surgery.
Unable to make contact with family after introducing study
Family too stressed to make a decision
Caregivers unable to decide whether to participate (n=2)

Study drug related protocol deviation after enrollment
Surgical plan changed, withdrawn since patient no longer eligible
Surgical plan changed, withdrawn since patient no longer eligible
Surgical plan changed, withdrawn since patient no longer eligible
Family did not return study drug on OR day. Cardiovascular surgery nurse asked family to return drug and calendar at post-operative appointment. The family brought the drug and calendar at this time and study team realized that the parents continued to give study drug to patient after surgery (6 doses)
Family did not return study drug on OR day. The research assistant called the mother and left a voicemail for her to return the drug bottle to post-operative appointment. At the post-op appointment, the mother told the CVS RN that she had brought the bottle back and given it to the PICU staff. Neither the PICU pharmacy nor the main pharmacy had any record of receiving the unused drug.
Mom thought the study drug was making patient constipated. For the last 10 days of enrolment, mom gave baby pharmacy vitamin D instead of the study drug.
CVS RN took the study drug bottle from the patient’s bedside and gave it to the study coordinator. The study coordinator returned the bottle to pharmacy; however, pharmacy had no record of this but did acknowledge that staff may have just thrown the unused drug away without documenting it. Post-operatively, a PICU resident mistakenly wrote an order in the chart that said to continue vitamin D as per study. As a result, the patient received an additional 6 doses of study drug while in the PICU post-op.
Mom gave patient an Immune Tonic (recommended by a naturopath) that contained vitamin D while patient was on study drug.
Mom thought study drug was making patient vomit, so began to alternate several days of study drug, and then several days of a vitamin D drop
Patient was enrolled in NICU. RN did not see study order in chart so patient received regular vitamin D initially instead of study drug (for one day only).
Patient and family are from up North and did not have a telephone. Study staff was unable to do compliance calls. At pre-operative appointment on 02-Mar-2015, father stated that he had stopped giving the study drug sometime in January but that he still wanted to be in the study.

Table 5A: Recruitment issues, withdrawals and protocol deviations

Patient Characteristic	Result (n = 35)
Age (months)	3.0 (0.4, 32.5)
Under 1 year	74.3 % (26)
Under 1 month	31.4 % (11)
Weight (kg)	4.5 (3.7, 12.9)
Male sex	60 % (21)
Formula feed	43 % (15)
Breastfed/other	57 % (20)
Vitamin D supplement	40 % (14)
Breastfed only, supplement	71% (8)
Season	
Summer	20 % (7)
Fall	31.4 % (11)
Winter	31.4 % (11
Spring	17.1 % (6)
Ethnicity *	
Caucasian	77.7 % (27)
North American Indian	5.7 % (2)
Oriental	8.6 % (3)
Other †	8.6 % (3)
Genetic Syndrome	
Trisomy 21	5.7 % (2)
VACTERL	2.8 % (1)

Table 5B: Study participant characteristics at baseline. Values are percentages with counts or medians with interquartile ranges.

Heart Defect /Surgery	No
RACHS category 1 (n=5)	
Atrial septal defect	4
Atrial septal defect and PAPVR	1
RACHS category 2 (n=13)	
VSD	6
Tetralogy of fallot	6
Double inlet left ventricle	1
RACHS category 3 (n=11)	
Complete or transitional AVCD	4
Tricuspid atresia	3
Pulmonary insufficiency, severe	1
Pulmonary stenosis	1
TGA (with VI) and severe PS	1
Double outlet right ventricle	1
RACHS category 4 (n=6)	
Hypoplastic or interrupted aortic arch	4
Transposition Great Arteries and VSD	2
* 8 more defects to insert	

Table 5C: List of participant cardiac lesions by RACHS category. AVCD = Atrioventricular canal defect; PAPVR = Partial Anomalous Venous Return; PS= Pulmonary stenosis; RACHS = Risk Adjusted classification for Congenital Heart Surgery; TGA = Transposition of Great Arteries; VI= Ventricular Inversion; VSD = Ventricular septal defect.

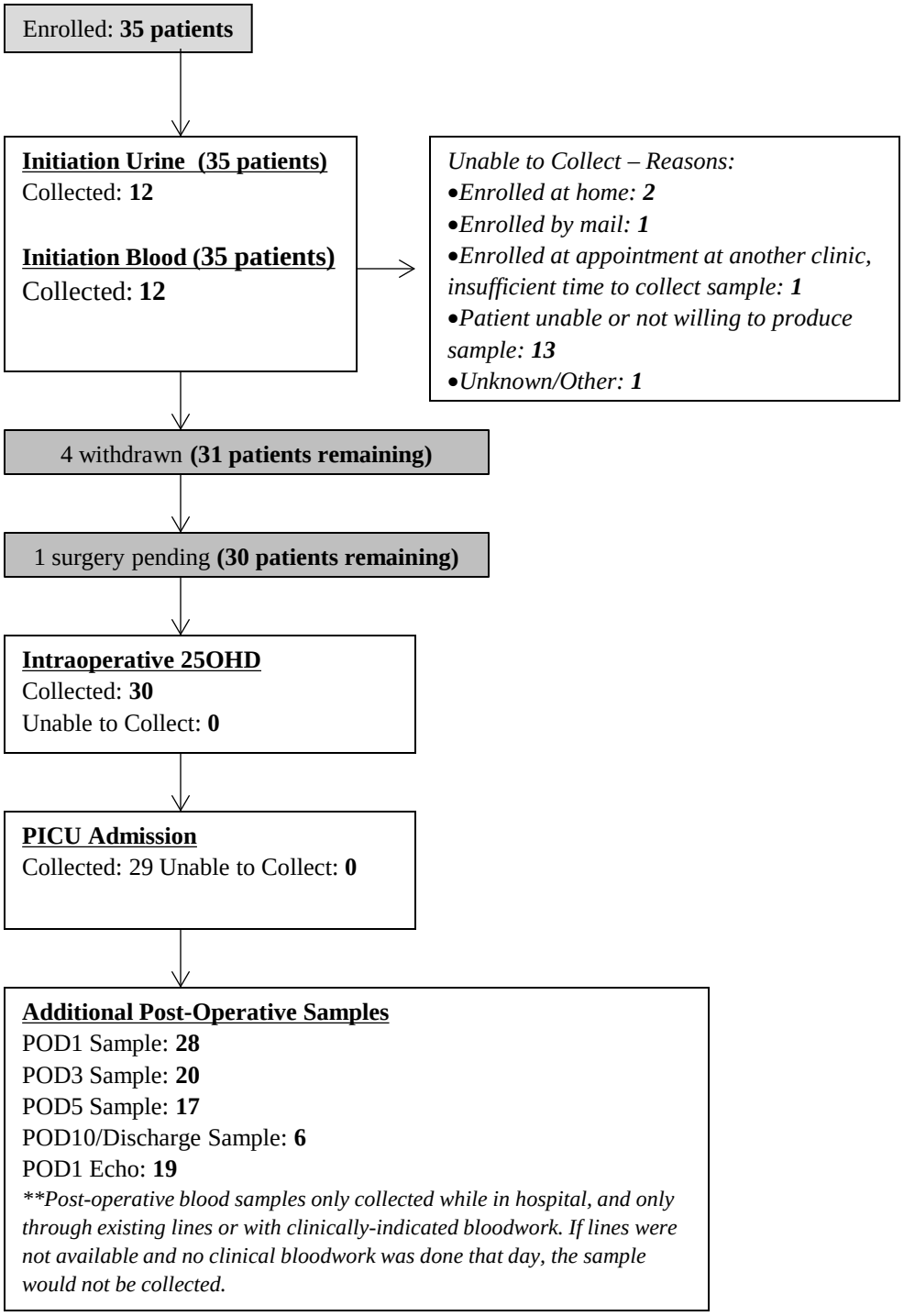

Figure 5C: Flow diagram of collected research samples

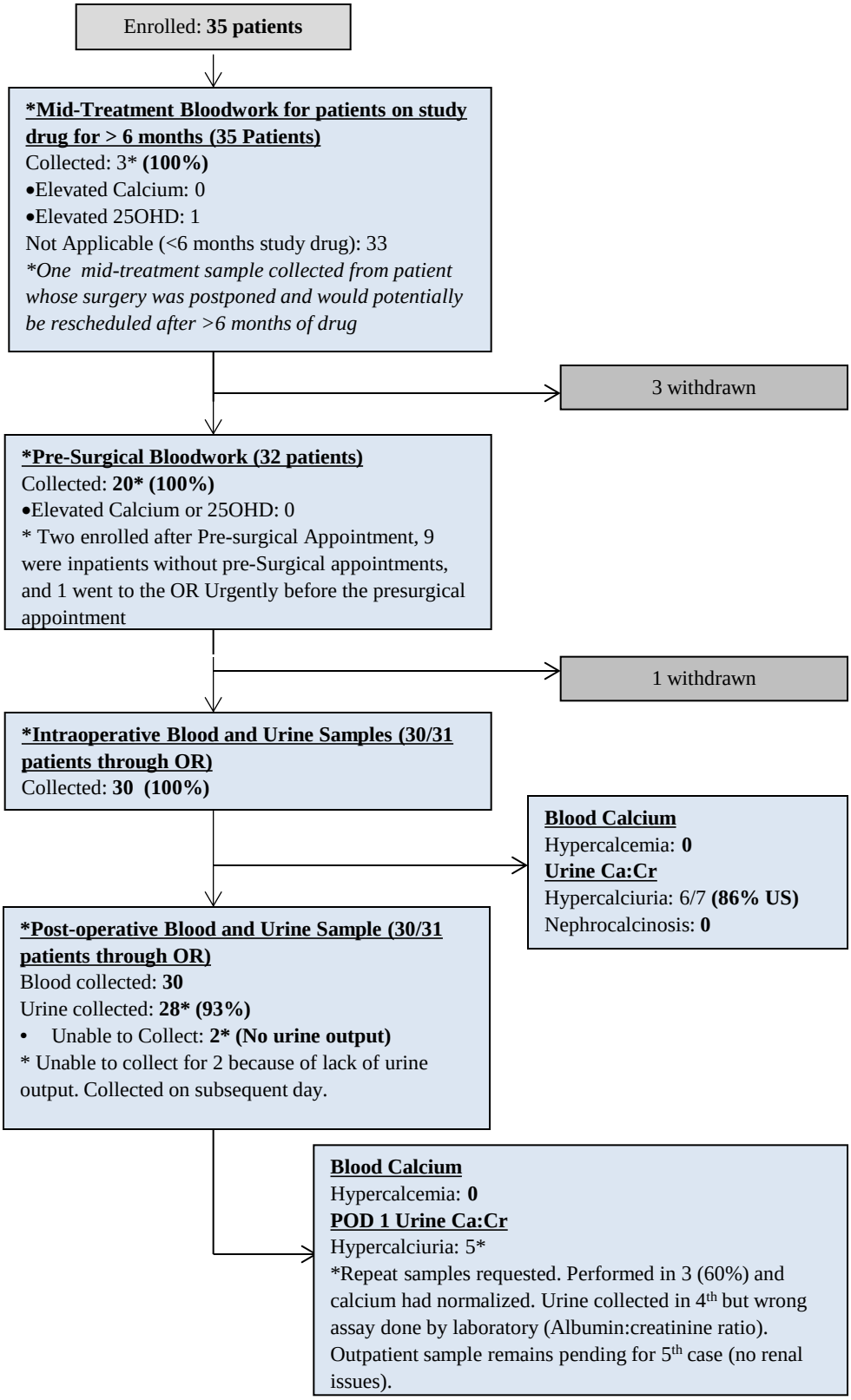

Figure 5D: Flow diagram of safety procedures and adverse events

Change	Goal/Reason
Changed from paper-based CRF to electronic based CRF in REDCap	REDCap is a secure, web-based application designed to support data collection for research studies. Pre-defined ranges for all data values will be set up in this application to allow data entry personnel to validate data as soon as it is entered and send data queries immediately. Missing data will be similarly managed
Developed an SOP for ensuring adherence to study safety measures, and for facilitating participant compliance with study protocol	
Procedure put in place to allow for collection of initiation urine sample at home in cases where patient is unable to provide a sample during enrolment at CHEO	To increase the number of initiation samples collected while minimizing the amount of time families need to spend at CHEO for the enrolment process
Study team became aware that CVS collects an ionized calcium sample at the pre-surgical appointment, thus a research sample was not required. Study safety officer now to review clinical pre-surgical calcium result.	Minimize the amount of blood drawn.
Obtained REB approval to visit families at home to introduce study and obtain informed consent. Also obtained permission from REB to contact families by telephone to explain the study and distribute the consent form and study documents to contacted families by mail.	To improve recruitment rate while minimizing the amount of time families need to spend at CHEO to enroll To maximize the amount of time enrolled patients will be on study drug by initiating contact with eligible families sooner
Additional study site initiated (Kingston General Hospital). REB approval from Kingston obtained, modification submitted to CHEO REB and approved.	To increase the length of time that patients are on study drug. By enrolling patients into the study while they are being followed at KGH, patients >2 months out from surgery can be enrolled and and extend the duration of vitamin D intake
Pre-surgical Vitamin D samples sent to TOH instead of HSC	Reduce time from sample collection to reporting of result. This change helped to improve patient safety by ensuring that 25OHD result from pre-surgical appointment is available for review by the safety officer prior to surgery.
Attempt made by principal investigator to add additional study site in Dublin, Ireland	To increase recruitment rate

Table 5D: List of study protocol modifications and initiatives

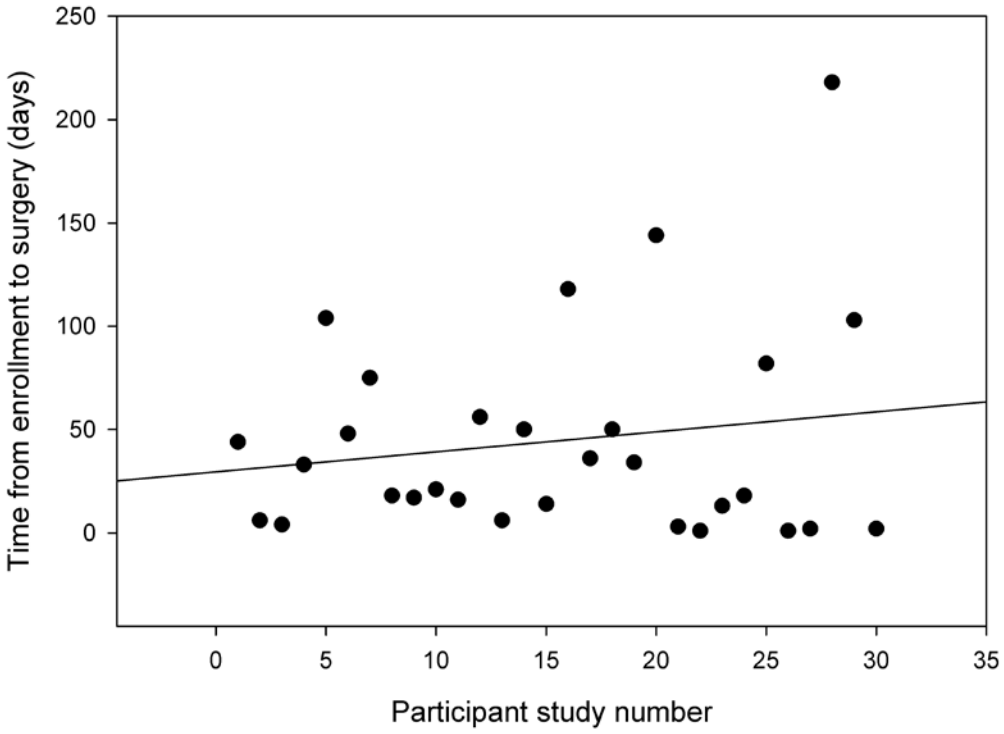

Figure 5E. Change in duration of enrollment over recruitment period

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52. (Single arm or pilot or cross-over or n-of-1).tw.
53. Double-blind Method/ or Single-blind Method/
54. (clin$ adj25 trial$).ti,ab.
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56. Placebos/
57. 50 and (or/51-56)
58. limit 50 to clinical trial, all
59. or/57-58
60. ((single adj2 dose) or bolus or stoss* or single day or mega*).tw.
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64. 59 or 63
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35. Hypercalcemi$.tw.
36. (Burnett$ adj2 syndrome$).tw.
37. Hypercalciuri$.tw.
38. exp Vitamin d/ae
39. (Side effect* or adverse effect$).tw.
40. or/23-39
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44. 42 or 43
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22. Calcium/bl, ur
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27. Vitamin D/to
28. No-Observed-Adverse-Effect Level/
29. upper limit$.tw.
30. UL.tw.
31. (excess$ or toxic$).tw.
32. (noael or noel).tw.
33. (no observed adj2 effect$).tw.
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42. Hypercalcemi$.tw.
43. (Burnett$ adj2 syndrome$).tw.
44. Hypercalciuri$.tw.
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48. Dose-Response Relationship, Drug/
49. exp Vitamin D/
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52. ergocalciferol$.tw.
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69. (avitaminosis and (d or d2 or d3)).tw.
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71. upper limit$.tw.
72. UL.tw.
73. (excess$ or toxic$).tw.
74. (noael or noel).tw.
75. (no observed adj2 effect$).tw.
76. exp Calcification/
77. Hypercalcemia/
78. exp Stone Formation/
79. Calcinosis/
80. Hypercalcemi$.tw.
81. (Burnett$ adj2 syndrome$).tw.
82. Hypercalciuri$.tw.
83. exp Vitamin d/ae
84. (Side effect* or adverse effect$).tw.
85. ((single adj2 dose) or bolus or stoss* or single day or mega*).tw.
86. Dose-Response Relationship, Drug/
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SUPPLEMENTAL TABLE 6 Screening Criteria

Level	Screening Criteria
1	1. The citation is not a review article (or case report) 2. The study is in humans, and children are included 3. The study administers at least 1 dose of vitamin D (cholecalciferol, ergocalciferol) to the patient 4. The citation does not represent a conference abstract
2	1. At least 1 study arm (group) includes children 2. At least 1 dose of vitamin D (ergocalciferol and/or cholecalciferol) was administered 3. Vitamin D was administered at ≥1 doses determined by the investigators
3	1. One or more study arms provide vitamin D supplementation at a dose that meets or exceeds the lowest age-specific pediatric Institute of Medicine's daily Tolerable Upper Intake Level (≥1000 IU) 2. Study population is not solely premature and/or low-birth-weight infants 3. Study population is not solely patients with genetic or metabolic problems related to vitamin D metabolism 4. Study population is not solely pregnant women/adolescents 5. Single-arm study exclusions: a. Vitamin D was not administered as part of a drug regimen that included medications or vitamins other than calcium b. Vitamin D was not given as part of an intervention that included a fixed exposure to UV or sun c. Vitamin D was not administered as part of a food or multivitamin 6. Multiple-arm study exclusions: a. At least 1 drug (other than ergocalciferol or cholecalciferol) was not different between all study arms b. All study arms administered ergocalciferol or cholecalciferol as part of an intervention that did not include a fixed exposure to UV or sun c. All study arms did not administer ergocalciferol or cholecalciferol as part of a food or multivitamin 7. Study provides information on blood/serum 25(OH)D levels

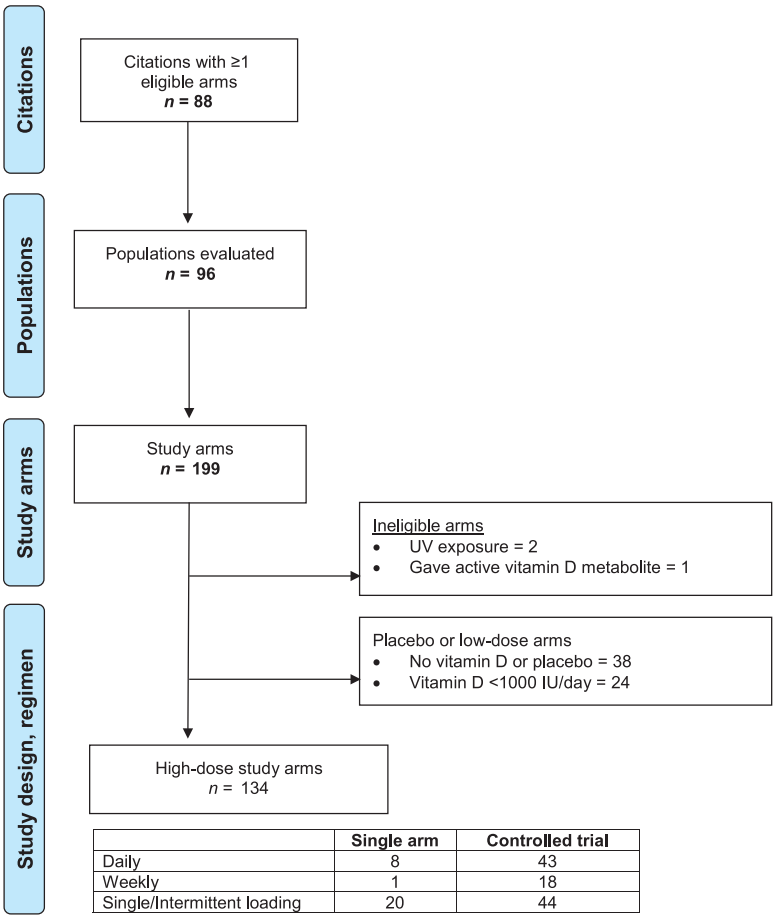
SUPPLEMENTAL TABLE 7 Novel Conference Abstracts Describing ≥1 High-Dose Regimens

Study and Population	Dosing Regimen	Relevant Results
Frizzell et al[a]		
	Age-based	• At 5.6 wk (range 3–39) after treatment the median 25(OH)D increased from 26.5 nmol/L (range 0–49) to 93 nmol/L (range 24–319)
Healthy, VDD 0.1–7.5 y olds	Loading dose:	• No patient developed hypercalcemia (highest corrected calcium 2.71 mmol/L)
	<3 y: 150 000 IU 3–12 y: 300 000 IU >12 y: 600 000 IU	• Two asymptomatic infants aged <3 mo had supraphysiologic 25(OH)D levels (269 and 319 nmol/L) after treatment, without sequelae
Gottschlich et al[b]		
Diseased 0.7–18.4 y olds with burns to >25% total body surface area	Daily: placebo + multivitamin 400 IU/d vs daily: 100 IU/kg per day vitamin D_2 + multivitamin 400 IU/d vs daily: 100 IU/kg per day vitamin D_3 + multivitamin 400 IU/d	• Over 10% of study subjects had low serum 25(OH)D levels at discharge and % deficiency worsened at 1 y follow-up for placebo (75%), vitamin D_2 (56%) and vitamin D_3 (25%)
Homola et al[c]		
Diseased with malabsorption (cystic fibrosis)	Daily: 5000 IU vitamin D_2 3×/wk for 12 wk vs daily: 2000 IU vitamin D_3/d for 12 wk	• Comparison of vitamins D_2 and D_3 in achieving a 25(OH)D level of 75 nmol/L proved significantly higher effectiveness of vitamin D_3 ($P < .001$). Vitamin D_3 reached 86 ± 23 nmol/L, vitamin D_2 reached only 48 ± 11 nmol/L
Lai et al[d]		
Healthy 2 d olds	Loading dose: 40 000 IU	• 25(OH)D (median [range]) levels increased from 62 nmol/L (17–176) on day 2 to 119 nmol/L (27–366) at 6 wk; 7 newborns had levels >250 nmol/L at 6 wk
Mondal et al[e]		
Diseased, with nutritional rickets aged 6 months to 6 years	Weekly: 60 000 IU/wk for 10 wk vs loading dose: 600 000 IU	• At baseline, 96.67% in weekly group and 93.55% in single-dose group had serum 25(OH)D <20 ng/mL; at the end of treatment 70% from weekly group and 71% from single-dose group achieved serum 25(OH)D levels >20 ng/mL • After completion of treatment 2 children from weekly group and 1 child from single-dose group had excess serum 25(OH)D levels (>100 ng/mL) without any symptoms or hypercalcemia; 2 children from each group developed asymptomatic hypercalcemia • Nine children with baseline elevated urinary calcium:creatinine ratios continued to have elevated urinary calcium:creatinine ratios throughout the study period; no child other than these 9 children developed hypercalciuria during the study period
Potter et al[f]		
Healthy adolescents	Daily: 400 for 16 wk vs daily: 2000 IU/d for 16 wk	• Mean (±SD) plasma 25(OH)D values at baseline and 16 wk were 28.7 ± 8.6 and 55.2 ± 21.5 nmol/L for the 400-IU group and 32.6 ± 8.7 and 85.5 ± 33.5 nmol/L for the 2000-IU group
Saffari et al[g]		
Obese versus nonobese, children versus adolescents	Loading dose: 300 000 IU intramuscularly	• Reported pretreatment 25(OH)D levels for both obese (13.5 + 7.2) and nonobese (14.5 + 7.2); units of measurement not reported; posttreatment 25(OH)D increased to 29.6 + 8.6 and 33 + 8.5 in obese and nonobese groups
Shakiba et al[h]		
Healthy 12–15 y olds	Loading dose: 50 000 IU, 3 × mo over 7 mo	• Prevalence of vitamin D sufficiency before and after intervention was 40% and 97%, respectively • There was no major clinical side effect that needed medical intervention, but headache, abdominal pain, and nausea were among prevalent complaints

SUPPLEMENTAL TABLE 7 Continued

Study and Population	Dosing Regimen	Relevant Results
Siafarikas et al[i]		
Healthy, VDD	Daily: 200–5000 IU/d for 15–90 d	• 25(OH)D was unchanged in 67.7%, improved in 19.6%, and worsened in 12.8%; 59.8% of patients remained vitamin D insufficient (25[OH]D 27.5–78)
Wingate et al[j]		
8–18 y olds with Crohn disease	Daily: 400 IU vs daily: 2000 IU	• 2000-IU group was more likely than 400-IU group to achieve levels >75 nmol/L (75% vs 34%); mean 25(OH)D levels were 90 vs 67 nmol/L

[a] Frizzell C, Verge C, Woodhead H, Walker J, Neville K. Stoss therapy (single, high-dose cholecalciferol) in childhood vitamin D deficiency. *J Paediatr Child Health*. 2010. 46 (Supp 2): 4.
[b] Gottschlich MM, Mayes T, Khoury J, Kagan R. Differential effects of three vitamin D supplementation practices on clinical outcome postburn. *J Burn Care Res*. 2011; 32 (Supp 2):S73.
[c] Homola L, Holcikova, Mikolasek P, Pavelka J. Vitamin D supplementation in children with cystic fibrosis: Sunshine, cholecalciferol and ergocalciferol. *J Cyst Fibr*. 2011. 10 (Supp 2): S76.
[d] Lai YY, Sebastien R, Thomas SD, Andersen C. Low dose stoss therapy in newborns of pregnant women with vitamin d deficiency. *J Paediatr Child Health*. 2013. 49(Supp 2): S95.
[e] Mondal K, Seth A, Dhanwal N et al. A randomized controlled trial on safety and efficacy of single intramuscular V/S staggered oral dose of 600,000 IU vitamin D in treatment of nutritional rickets. *Osteoporos Int*. 2011. 25(4 Supp):S524.
[f] Potter LH, Pollock N, Guo D et al. Effect of 400 IU and 2000 IU daily vitamin D supplementation on total IgE and fire ant venom specific IgE in African American adolescents over 16-weeks. *Ann Allergy, Asthma Immunol*. 2010. 105(5 Supp):A113.
[g] Saffari F, Shahroodi P, Oveisi S, Esmailzadehha N. Response to vitamin D therapy in obese vs. Non-obese children and adolescents. *Horm. Res. Paediatr*. 2013 80(Supp 1): 215–216.
[h] Shakiba M, Nafei Z, Tefagh S, Goldansaz N, Mirzaee M. Result of mass vitamin d therapy in growing girl in sunny city of yazd during academic year. *Acta Paediatr Int J Paediatr*. 2011. 100 (Supp s463): 30.
[i] Siafarikas A, Pascoe E, Banfiel S, et al. An evidence based approach to vitamin D deficiency in the child and adolescent refugee population of Western Australia (WA). *Bone*. 2009. 44(Supp 1): S89–S90.
[j] Wingate KE, Jacobson K, Issenman R, et al. The effect of two doses of vitamin D3 (400 IU vs. 2000 IU/d) on serum 25-hydroxyvitamin D in children with Crohn's disease. *FASEB J*. 2013. 27(Supp 1): 347.2.



	Single arm	Controlled trial
Daily	8	43
Weekly	1	18
Single/Intermittent loading	20	44

SUPPLEMENTAL FIGURE 5
Flow of study arms.

SUPPLEMENTAL TABLE 8 Other Populations by Regimen Type

Number of Arms	First Author, Year	Population
Daily		
1	Cataneda, 2012	Obesity
1	Cayir, 2013	Recurrent AOM
2	Hillman, 2008	Juvenile arthritis
1	Kilpinen, 2007	Cerebral palsy/neuromuscular disorder
2	Leger, 1989	Congenital hypothyroidism
1	Lewis, 2012	Chronic persistent asthma
1	Mikati, 2006	Epilepsy
1	Raghuramulu, 1982	Protein-energy malnutrition
1	Shedeed, 2012	Heart failure
1	Tau, 1986	Congenital hypothyroidism
1	Marchisio, 2013	Recurrent AOM
1	Poomthavorn, 2013	Obesity
1	Principi, 2013	Recurrent AOM
Weekly		
2	Kakalia, 2011	HIV
1	Majak, 2009	IgE-dependent asthma
1	Osunkwo, 2012	Sickle cell
1	Kelishadi, 2013	Obese with metabolic syndrome
Stoss therapy		
1	Arpadi, 2012	HIV
1	Arpadi, 2009	HIV
1	Kazemi, 2009	Seizure disorder
1	Raghuramulu, 1982	Protein-energy malnutrition
1	Soliman, 2008	β-Thalassemia
1	Zeghoud, 1995	Various: infection, allergy, psych, etc

The populations evaluated with the identified eligible high-dose vitamin D regimens are presented. Duplicate or overlapping populations identified are as follows: (1) Leger 1989 and Tau 1986, (2) Arpadi (2009 and 2012), (3) Marchisio 2013 and Principi 2013. AOM, acute otitis media; IgE, immunoglobulin E; psychiatric, .

SUPPLEMENTAL TABLE 9 Study Arms Administering No Drug or Placebo With 25(OH)D Measurements Between 1 and 13 Weeks of Study Initiation

First Author, Year	Location	Study Design	Population, Age[a]	*n*, *N*[b]	Dose, IU	Cumulative Dose	Time, wk	25(OH)D		
								Before	Absolute	Change
Ala-Houhala, 1985	Europe	RCT	Healthy, neonates	32, 92	0	0	8	33 (18)	24 (15)	—
Tsybysheva, 1988	East Asia	CT	Healthy, neonates	15, 51	0	0	4	49 (11)	50 (8)	—
Tau, 1986	Europe	RCT	Hypothyroid, newborns	13, 25	0	0	4	22 (8)	17 (7)	—
Leger, 1989	Europe	RCT	Hypothyroid, newborns	13, 24	0	0	4	25 (10)	15 (10)	—
Zeghoud, 1995	Europe	RCT	Acute illness, 10–17 y	6, 15	0	0	2	30 (9)	29 (9)	—
Guillemant, 1998	Europe	RCT	Healthy, 13–16 y	32, 56	0	0	9	42 (10)	44 (8)	—
Thacher, 1999	Africa	RCT	Healthy, 1–14 y	41, 123	0	0	12	40 (43)	45 (33)	—
Duhamel, 2000	Europe	RCT	Healthy, 10–15 y	35, 68	0	0	13	49 (26)	42 (19)	—
Dahifar, 2006	Middle East	RCT	VDD, 11–15 y	8, 15	0	0	3	19 (5)	35 (10)	—
Dahifar, 2007	Middle East	RCT	VDD, 11–15 y	8, 15	0	0	3	19 (5)	35 (11)	—
Kilpinen-Loisa, 2007	Europe	qRCT	CP/NMD, 9–18 y	23, 45	0	0	10	43 (26–81)	37 (24–74)	—
Maalouf, 2008	Middle East	RCT	Healthy, 10–17 y	9, 25	0	0	2	89 (78, 125)	92 (77, 127)	—
Arpadi, 2009	North America	RCT	HIV, 6–16 y	29, 59	0	0	4	61 (SEM 5)	55 (SEM 5)	—
Majak, 2009	Europe	RCT	IgE asthma, 6–12 y	18, 54	0	0	12	79 (7)	78 (7)	—
Majak, 2009	Europe	RCT	IgE asthma, 6–12 y	18, 54	0	0	12	78 (9)	76 (7)	—
Ghazi, 2010	Middle East	RCT	Healthy, adolescents	70, 210	0	0	8	29 (18)	24 (14)	—
Rich-Edwards, 2011	East Asia	RCT	Healthy, 9–11 y	101, 744	0	0	7	20 (10)	20 (10)	—
Arpadi, 2012	North America	RCT	HIV, school age/ adolescents	29, 59	0	0	4	59 (SEM 5)	58 (SEM 6)	—
Carnes, 2012	Australia	RCT	Healthy/VDD, 15–17 y	8, 22	0	0	13	43 (6)	56 (38–75)	—
Hill, 2012	North America	RCT	Healthy, 9–14 y	66, 323	0	0	12	72 (SEM 2.2)	66 (SEM 3)	—
Manaseki-Holland, 2012	Asia, other	RCT	Healthy, 1–11 mo	70	0	0	1		41 (29–54)	—
Shedeed, 2012	Middle East	RCT	Heart failure, all ages	38, 40	0	0	6	35 (6)	37 (4)	—
Shroff, 2012	Europe	RCT	Renal disease, 0–17 y	23, 49	0	0	13	52 (17)	68 (25–137)	—
Abrams, 2013	North America	RCT	Healthy, 4–9 y	32, 64	0	0	8	69 (18)	75 (31)	—
Khadgawat, 2013	Asia, other	RCT	Healthy, 10–14 y	255, 796	0	0	12	29 (13)	27(13)	—
Rianthavorn, 2013	Asia, other	RCT	Renal disease, 0–18 y	10, 20	0	0	1	35 (14)	25 (23)	—
Belenchia, 2013	North America	RCT	Healthy, 9–19 y	23, 49	0	0	12	49 (20)	56 (6)	—
Kelishadi, 2013	Middle East	RCT	Obese-metabolic, 10–16 y	25, 50	0	0	12	45 (6)	48 (5)	—
Lewis, 2013	North America	RCT	Healthy, 9–13 y	66, 323	0	0	12	72 (19)	—	−8 (−14, 0)
Aggarwal, 2013	Asia, other	RCT	Rickets, 12–18 y	22, 67	0	0	12	36 (14)	54 (54)	—

Only the first group 25(OH)D measurements after week 1 are shown. 25(OH)D levels are provided in nmol/L. Measures of dispersion are provided as SDs (XX), interquartile ranges (XX, XX), or low–high ranges (XX–XX). Duplicate or overlapping study populations: (1) Arpadi et al (2009 and 2012), (2) Tau et al 1986 and Leger et al 1989. CT, controlled trial; CP, cerebral palsy; NMD, neuromuscular disease; qRCT, quasi randomized controlled trial; RCT, randomized controlled trial; UC, uncontrolled.

[a] Age is at time of enrollment.

[b] *n* represents the number of participants in the study arm who had 25(OH)D testing; *N* represents the number of participants enrolled in study.

SUPPLEMENTAL TABLE 10 Study Arms Administering Doses <1000 IU With 25(OH)D Measurements Between 1 and 13 Weeks of Study Initiation

First Author, Year	Location	Type	Population, Age[a]	*n*, *N*[b]	Dose, IU	Cumulative Dose	Drug (Vitamin) Form	Route	Time, wk	25(OH)D		
										Before	Absolute	Change
Kunz, 1982	Europe	CT	Healthy, neonates	13, 29	500	21 000	—	Oral	6	33 (13)	93 (20)	—
Ala-Houhala, 1985	Europe	RCT	Healthy, neonates	31, 92	400	22 400	—	Oral	8	26 (20)	47 (22)	—
Vervel, 1997	Europe	RCT	Healthy, neonates	40, 80	500	15 000	D_2	Oral	4	30 (15)	56 (11)	—
Shajari, 2009	Middle East	RCT	Healthy, neonates	30, 90	200	14 000	D_3	Oral	10	NR	NR	NR
Shajari, 2009	Middle East	RCT	Healthy, neonates	30, 90	400	28 000	D_3	Oral	10	NR	NR	NR
Dong, 2010	North America	RCT	Healthy, 14–18 y	24, 49	400	11 200	D_3	Oral	4	33 (10)	45 (5)	—
Rich-Edwards, 2011	East Asia	RCT	Healthy, 9–11 y	140, 744	300	13 700	D_3	Oral	7	20 (10)	50 (15)	—
Rich-Edwards, 2011	East Asia	RCT	Healthy, 9–11 y	137, 744	300	13 700	D_3	Oral	7	25 (10)	73 (25)	—
Rich-Edwards, 2011	East Asia	RCT	Healthy, 9–11 y	109, 744	300	13 700	D_3	Oral	7	28 (10)	53 (15)	—
Holmlund-Suila, 2012	Europe	RCT	Healthy, neonates	38, 113	400	28 000	D_3	Oral	10	52 (14)	88 (18)	—
Hill, 2012	North America	RCT	Healthy, neonates	64, 323	400	33 600	D_3	Oral	12	72 (SE 2)	79 (SE 3)	—
Putman, 2013	North America	RCT	Healthy, 11–19 y	25, 56	200	15 400	D_3	Oral	11	70 (16)	72 (29)	—
Gallo, 2013	North America	RCT	Healthy, neonates	39, 132	400	12 000	D_3	Oral	4	56	70 (16)	—
Khadgawat, 2013	Asia, other	RCT	Healthy, neonates	263, 796	600	50 400	D_3	Oral	12	29 (13)	57 (16)	—
Gallo, 2013	North America	RCT	Healthy, neonates	39, 132	800	24 000	D_3	Oral	4	56	85 (28)	—
Lewis, 2013	North America	RCT	Healthy, neonates	64, 323	400	33 600	D_3	Oral	12	71 (20)	—	5 (−2, 12)

Only the first group 25(OH)D measurements after week 1 are shown. 25(OH)D levels are provided in nmol/L. Measures of dispersion are provided as SDs (XX) and SEs (SE XX). Duplicate or overlapping study populations: Shajari et al 2009 only provided group VDD rates. CT, controlled trial; NR, not reported; qRCT, quasi randomized controlled trial; RCT, randomized controlled trial; UC, uncontrolled.

[a] Age is at time of enrollment.

[b] *n* represents the number of participants in the study arm who had 25(OH)D testing; *N* represents the number of participants enrolled in the full study.

SUPPLEMENTAL TABLE 11 Study Arms Administering Daily Doses Between 1000 and 4000 IU With 25(OH)D Measurements Within 1 Month of Study Initiation

Author, year	Location	Study Design	Population, Age[a]	*n*, *N*[b]	Dose, IU	Cumulative Dose	Drug (Vitamin) Form	Route	Time, wk	25(OH)D	
										Before	After
Holst-Gemeiner, 1978	Europe	CT	Healthy, 0–8 d	10, 21	1200	12 000	D_3	Oral	1.5	30 (7)	68 (33)
Raghuramulu, 1982	Asia, other	CT	Healthy, 1–5 y	5, 10	2000	20 000	D_3	Oral	1.5	65	90 (60)
Raghuramulu, 1982	Asia, other	CT	Malnourished, 1–5 y	13, 27	2000	20 000	D_3	Oral	1.5	60	112 (115)
Markestad, 1984	Europe	UC	Rickets, 4–24 mo	17, 17	1700–4000	11 900–28 000	D_2	Oral	1	16 (10)	41 (13)
Tau, 1986	Europe	RCT	Congenital hypothyroid, 0–30 d	12, 25	1200	33 600	D_2	Oral	4	22 (9)	57 (12)
Leger, 1989	Europe	RCT	Congenital hypothyroid, 0–30 d	11, 24	1200	36 000	D_2	Oral	4	25(8)	45 (25)
Vervel, 1997	Europe	RCT	Healthy, 3–6 d	40, 80	1000	30 000	D_2	Oral	4	31 (13)	57 (15)
Zeghoud, 1997	Europe	RCT	Healthy, 0–6 d	40, 80	1000	30 000	D_2	Oral	4	30 (14)	59 (14)
Dong, 2010	North America	RCT	Healthy, 14–18 y	25, 49	2000	56 000	D_3	Oral	4	47 (14)	54 (9)
Park, 2010	North America	UC	Healthy, 12–14 y	13, 13	1000	56 000	D_3	Oral	4	34 (10)	71 (16)
Gallo, 2013	North America	RCT	Healthy, 0–30 d	38, 132	1200	36 000	D_3	Oral	4	65 (56–73)	116 (41)
Gallo, 2013	North America	RCT	Healthy, 0–30 d	16, 132	1600	48 000	D_3	Oral	4	64 (53–77)	122 (61)

Only the first group 25(OH)D measurements after week 1 are shown. 25(OH)D levels are provided in nmol/L. Measures of dispersion are provided as SDs (XX) and 95% CIs (XX–XX). Duplicate or overlapping study populations: (1) Vervel et al 1997 and Zeghoud et al 1997, (2) Tau et al 1986 and Leger et al 1989. CT, controlled trial; RCT, randomized controlled trial; UC, uncontrolled.

[a] Age is at time of enrollment.

[b] *n* represents the number of participants in the study arm who had 25(OH)D testing; *N* represents the number of participants enrolled in the full study.

SUPPLEMENTAL TABLE 12 Study Arms Administering Daily Doses of 1000 to 4000 IU With 25(OH)D Measurements Between 1 and 3 Months of Study Initiation

First Author, Year	Location	Study Design	Population, Age[a]	*n*, *N*[b]	Dose, IU	Cumulative Dose	Drug (Vitamin) Form	Route	Time, wk	25(OH)D		
										Before	After	Change
Kunz, 1982	Europe	CT	Healthy, newborns	16, 29	1000	42 000	—	Oral	6	38 (18)	135 (45)	—
Ala-Houhala, 1985	Europe	RCT	Healthy, newborns	29, 92	1000	56 000	—	Oral	9	27 (18)	70 (23)	—
Kilpinen-Loisa, 2007	Europe	CT	CP/NMD, 9–18 y	22, 45	1000	50 000	D_3	Oral	10	46 (26–82)	56 (39–88)	—
Gordon, 2008	North America	RCT	Healthy-VDD, 8–24 mo	12, 40	2000	84 000	D_2	Oral	6	39 (18–50)	110	—
Gordon, 2008	North America	RCT	Healthy-VDD, 8–24 mo	14, 40	2000	84 000	D_3	Oral	6	34 (18–50)	103	—
Rich-Edwards, 2011	East Asia	RCT	Healthy, 9–11 y	92, 744	1950[c]	13 700	D_3	Oral	7	20 (10)	30 (10)	—
Castaneda, 2012	North America	UC	Healthy, 12–18 y	22, 22	2000	168 000	D_3	Oral	12	72 (21)	—	25 (18)
Castaneda, 2012	North America	UC	Obesity, 12–18 y	27, 27	2000	168 000	D_3	Oral	12	63 (12)	—	15 (16)
Emel, 2013	Middle East	CT	Rickets, infants/toddler	21, 42	2000	84 000	D_3	Oral	6	34 (12)	64 (25)	—
Sheedeed, 2012	Middle East	RCT	Heart failure, infants/toddlers	42, 80	1000	42 000	D_3	Oral	6	34(6)	56(6)	—
Shroff, 2012	Europe	RCT	Renal disease, 0–17 y	24, 49	600–8000	—	D_2	Oral	13	50 (19–73)	97 (16–153)	—
Holmlund-Suila, 2012	Europe	RCT	Healthy, newborns	38, 113	1200	84 000	D_3	Oral	10	54 (15)	124 (30)	—
Holmlund-Suila, 2012	Europe	RCT	Healthy, newborns	37, 113	1600	112 000	D_3	Oral	10	53 (15)	153 (40)	—
Hill, 2012	North America	RCT	Healthy, 9–14 y	65, 323	1000	84 000	D_3	Oral	12	70 (SE 3)	92 (SE 3)	—
Hill, 2012	North America	RCT	Healthy, 9–14 y	64, 323	2000	168 000	D_3	Oral	12	66 (SE 2)	100 (SE 4)	—
Hill, 2012	North America	RCT	Healthy, 9–14 y	64, 323	4000	336 000	D_3	Oral	12	70 (SE 2)	141 (SE 7)	—
Belenchia, 2013	North America	RCT	Healthy, 9–19 y	21, 49	4000	360 000	D_3	Oral	12	48(16)	86 (SE 6)	—
Abrams, 2013	North America	RCT	Healthy, 4–9 y	32, 64	1000	56 000	D_2	Oral	8	69 (19)	90 (26)	—
Putman, 2013	North America	RCT	Healthy, 11–19 y	29, 56	1000	77 000	D_3	Oral	11	73 (30)	75 (17)	—
Khadgawat, 2013	Asia, other	RCT	Healthy, 10–14 y	258, 796	1000	84 000	D_3	Oral	12	30 (14)	69 (22)	—
Lewis, 2013	North America	RCT	Healthy, 9–13 y	65, 323	1000	84 000	D_3	Oral	12	71 (20)	—	22 (13, 33)
Lewis, 2013	North America	RCT	Healthy, 9–13 y	64, 323	2000	168 000	D_3	Oral	12	66 (7)	—	32 (16, 48)
Lewis, 2013	North America	RCT	Healthy, 9–13 y	64, 323	4000	336 000	D_3	Oral	12	70 (18)	—	80 (39, 108)

Only the first group 25(OH)D measurements after week 1 are shown. 25(OH)D levels are provided in nmol/L. Measures of dispersion are provided as SDs (XX), SEs (SE XX), interquartile ranges (XX, XX), and low–high ranges (XX–XX). Duplicate or overlapping study populations: CT, controlled trial; CP, cerebral palsy; NMD, neuromuscular disease; RCT, randomized controlled trial; UC, uncontrolled; —, not reported/not measured.

[a] Age is at time of enrollment.

[b] *n* represents the number of participants in the study arm who had 25(OH)D testing; *N* represents the number of participants enrolled in the full study.

[c] Rich-Edwards gave 1950 IU vitamin D_3 for 7 d and then measured at 7 wk.

SUPPLEMENTAL TABLE 13 Study Arms Administering a Constant Loading Dose(s) of Vitamin D With 25(OH)D Measurement Within 3 Months of Study Initiation

First Author, Year	Location	Study Design	Population, Age[a]	*n*, *N*[b]	Dose, IU	Cumulative Dose	Drug Form	Route	Time, wk	25(OH)D		
										Before	Absolute	Change
Holst-Gemeiner, 1978	Europe	CT	Healthy, newborns	11, 21	200 000	200 000	D_3	0	1.5	32	318 (205)	—
Raghuramulu, 1982	Asia, other	RCT	Healthy, 1–5 y	5, 10	600 000	600 000	D_3	0	1.5	96	268 (SE 35)	—
Raghuramulu, 1982	Asia, other	RCT	Malnutrition, 1–5 y	14, 27	600 000	600 000	D_3	0	1.5	41	195 (SE 37)	—
Stogmann, 1985	Europe	CT	Rickets, 4–21 mo	5, 10	200 000	400 000	D_2	0	1	10	213	—
Markestad, 1987	Europe	UC	Healthy, 1–21 mo	43, 43	600 000	600 000	D_2	0	2	52 (28)	399 (237)	—
Zeghoud, 1994	Africa	RCT	Healthy, newborns	15, 30	100 000	100 000	D_3	0	2	8	92 (42)	—
Zeghoud, 1994	Africa	RCT	Healthy, newborns	15, 30	200 000	200 000	D_3	0	2	12	150 (55)	—
Zeghoud, 1994	Africa	UC	Healthy, newborns	30, 30	600 000	600 000	D_3	0	2	14	307 (77–692)	—
Zeghoud, 1995	Europe	RCT	Acute illnesses, 10–17 y	9, 15	100 000	100 000	D_3	0	2	30 (16)	—	47 (6)
Oliveri, 1996	South America	UC	Healthy, 5–11 y	30, 79	150 000	150 000	D_2	0	6	47 (27)	62 (35)	—
Guillemant, 1998	Europe	RCT	Healthy, 13–16 y	24, 56	100 000	100 000	D_3	0	9	42 (10)	44 (8)	—
Thacher, 1999	Africa	RCT	Rickets, 1–14 y	41, 123	600 000	600 000	NR	0	12	35 (15)	65 (30)	—
Thacher, 1999	Africa	RCT	Rickets, 1–14 y	41, 123	600 000	600 000	NR	0	12	33 (13)	90 (33)	—
Duhamel, 2000	Europe	RCT	Healthy, 10–15 y	33, 68	100 000	100 000	D_3	0	13	45 (23)	58 (28)	—
Ozkan, 2000	Middle East	RCT	Rickets, 4–19 mo	12, 40	300 000	300 000	D_3	1	4	21 (5)	—	51 (16)
Ozkan, 2000	Middle East	RCT	Rickets, 4–19 mo	13, 40	300 000	300 000	D_3	0	4	21 (5)	—	45 (13)
Ozkan, 2000	Middle East	RCT	Rickets, 4–19 mo	15, 40	600 000	600 000	D_3	0	4	21 (5)	—	62 (26)
Thacher, 2006	Africa	UC	Rickets, 15–48 mo	16, 16	50 000	50 000	D_2	0	1	29 (6)	66 (21)	—
Tau, 2007	South America	UC	Healthy, 1–16 y	18, 18	100 000	100 000	D_2	0	4	73 (15)	88 (11)	—
Shajari, 2009	Middle East	RCT	Healthy, neonates	30, 90	50 000	100 000	D_3	0	10		NR	NR
Arpadi, 2009	North America	RCT	HIV, 6–16 y	29, 59	100 000	100 000	D_3	0	4	62 (SE 6)	87 (SE 4)	—
Bereket, 2010	Middle East	UC	Rickets, infants/toddlers	22, 22	300 000	300 000	NR	0	13	19 (5)	45 (10)	—
Ghazi, 2010	Middle East	RCT	Healthy, adolescents	70, 210	50 000	100 000	D_3	0	8	32 (22)	60 (27)	—
Ghazi, 2010	Middle East	RCT	Healthy, adolescents	70, 210	50 000	50 000	D_3	0	8	28 (15)	31 (15)	—
Thacher, 2010	Africa	UC	Rickets, 2–12 y	12, 13	50 000	50 000	D_3	0	1	38 (28–60)	95 (SE 8)	—
Thacher, 2010	Africa	RCT	Healthy, 2–6 y	12, 23	50 000	50 000	D_3	0	1	65 (53–85)	131 (SE 9)	—
Mallet, 2010	Europe	UC	Healthy, 11–18 y	20, 64	200 000	200 000	D_2	0	2	64 (20)	96 (16)	—
Thacher, 2010	Africa	RCT	Healthy, 19–59 mo	11, 23	50 000	50 000	D_3	0	1	70 (37–83)	135 (SE 11)	—
Hari, 2010	Asia, other	UC	Renal disease, 1–16 y	42, 42	200 000	600 000	D_3	0	6	45 (CI: 32–53)	121(CI: 110– 140)	—
Carnes, 2012	Australia	RCT	Healthy/VDD, 15–17 y	7, 22	150 000	150 000	D_3	0	13	39 (10)	65 (40, 96)	—
Carnes, 2012	Australia	RCT	Healthy/VDD, 15–17 y	7, 22	300 000	300 000	D_3	0	13	38 (9)	67 (58, 87)	—
Dogan, 2012	Middle East	UC	Rickets, 0.25–15 y	30, 30	300 000	300 000	D_3	1	4	16 (6)	94 (30)	—
Emel, 2012	Middle East	CT	Rickets, infants/toddlers	21, 42	150 000	150 000	D_3	0	6	36 (13)	126 (49)	—
Arpadi, 2012	North America	RCT	HIV, 6–16 y	30, 59	100 000	100 000	D_3	0	4	61 (SEM 4)	87 (SE 5)	—
Manaseki-Holland, 2012	Asia, other	RCT	Healthy, 1–11 mo	69, 1524	100 000	100 000	D_3	0	1	41 (29–54)	114 (86–155)	—
Kari, 2013	Middle East	UC	Renal disease, 2–16 y	19, 19	300 000	300 000	D_3	0	4	36 (19)	97 (33)	—
Aggarwal, 2013	Asia, other	RCT	Rickets, 12–18 y	23, 67	600 000	600 000	D_3	0	12	35 (26)	82 (60)	—
Aggarwal, 2013	Asia, other	RCT	Rickets, 12–18 y	22, 67	600 000	600 000	D_3	0	12	49 (45)	78 (77)	—

Only the first group 25(OH)D measurements after week 1 are shown. 25(OH)D levels are provided in nmol/L. Measures of dispersion are provided as SDs (XX), SEs (SE XX), interquartile ranges (XX, XX), 95% CIs (CI: XX–XX), and low–high ranges (XX–XX). Stogmann et al 1985 gave two 200 000 IU doses separated by 48 h; Manaseki-Holland et al's 2012 baseline 25(OH)D measurements were estimated from the placebo group at 1 week; Mallet et al's 2010 pre-25(OH)D administration levels were estimated from the arm that only had predrug levels determined. CT, controlled trial; NR, not reported; RCT, randomized controlled trial; UC, uncontrolled; —, not reported/not measured.

[a] Age is at time of enrollment.

[b] *n* represents the number of participants in the study arm who had 25(OH)D testing; *N* represents the number of participants enrolled in the full study.

SUPPLEMENTAL TABLE 14 Study Arms Administering Daily Doses >4000 IU and 25(OH)D Measurements Within 3 Months of Study Initiation

First Author, Year	Location	Study Design	Population, Age[a]	*n*, *N*[b]	Dose, IU	Cumulative Dose	Drug (Vitamin) Form	Route	Time, wk	25(OH)D		
										Before	Absolute	Change
Tsybysheva, 1988	East Asia	CT	Renal disease, 8–16 y	12, 51	20 000	600 000	D_2	0	4	43 (8)	168 (23)	—
Stogmann, 1985	Europe	CT	Rickets, 4–21 mo	5, 10	9600	57 600	D_2	0	1	6	97	—
Dahifar, 2006	Middle East	RCT	Healthy-VDD, 11–15 y	7, 15	50 000	1 000 000	D_3	0	3	19 (5)	57 (11)	—
Dahifar, 2007	Middle East	RCT	Healthy-VDD, 11–15 y	7, 15	50 000	1 000 000	D_3	0	3	19 (5)	57 (11)	—
Boas, 2009	North America	UC	Malabsorption, 6–17 y	6, 6	50 000	700 000	D_3	0	2	54 (13)	139 (54)	—
Poomthavorn, 2013	Asia, other	UC	Obese, 6–18 y	72, 72	20 000	560 000	D_2	0	6	52 (12)	142 (41)	—

Only the first group 25(OH)D measurements after week 1 are shown. 25(OH)D levels are provided in nmol/L. Measures of dispersion are provided as SDs (XX). Duplicate or overlapping study populations: Dahifar et al 2006 and Dahifar et al 2007. CT, controlled trial; RCT, randomized controlled trial; UC, uncontrolled; —, not reported/not measured.

[a] Age is at time of enrollment.

[b] *n* represents the number of participants in the study arm who had 25(OH)D testing; *N* represents the number of participants enrolled in the full study.

SUPPLEMENTAL TABLE 15 Study Arms Administering Constant Weekly Dose of Vitamin D and 25(OH)D Measurements Within 3 Months of Study Initiation

First Author, Year	Location	Study Design	Population, Age[a]	*n*, *N*[b]	Dose, IU	Cumulative Dose	Drug (Vitamin) Form	Route	Time, wk	25(OH)D	
										Before	After
Gordon, 2008	North America	RCT	Healthy/VDD, 8–24 mo	14, 40	50 000	300 000	D_2	0	6	35 (18–50)	110
Maalouf, 2008	Middle East	RCT	Healthy, 10–17 y	8, 25	14 000	28 000	D_3	0	2	83 (80, 106)	97 (92, 104)
Maalouf, 2008	Middle East	RCT	Healthy, 10–17 y	9, 25	14 000	28 000	D_3	0	2	123 (100, 150)	120 (109, 143)
Majak, 2009	Europe	RCT	Asthma, 6–12 y	18, 54	1000	12 000	D_3	0	12	80 (8)	82 (6)
Ashraf, 2011	North America	UC	Healthy, adolescents	14, 14	50 000	400 000	D_2	0	8	27 (11)	64 (30)
Garg, 2013	Asia, other	CT	Healthy, 10–15 y	238, 511	60 000	240 000	D_3	0	4	22 (11)	153 (36)
Garg, 2013	Asia, other	CT	Healthy, 10–15 y	139, 511	60 000	360 000	D_3	0	6	18 (8)	120 (28)
Garg, 2013	Asia, other	CT	Healthy, 10–15 y	134, 511	60 000	480 000	D_3	0	8	33 (14)	146 (36)
Kelishadi, 2013	Middle East	RCT	Obese-metabolic, 10–16 y	25, 50	50 000	300 000	D_3	0	12	46 (5)	80 (5)

Only the first group 25(OH)D measurements after week 1 are shown. 25(OH)D levels are provided in nmol/L. Measures of dispersion are provided as SDs (XX), interquartiles ranges (XX, XX), or low–high ranges (XX–XX). Maalouf et al 2008 compared the same vitamin D dose in different solutions (Vigantol oil versus ethanol solutions). CT, controlled trial; RCT, randomized controlled trial; UC, uncontrolled.

[a] Age is at time of enrollment.

[b] *n* represents the number of participants in the study arm who had 25(OH)D testing; *N* represents the number of participants enrolled in the full study.

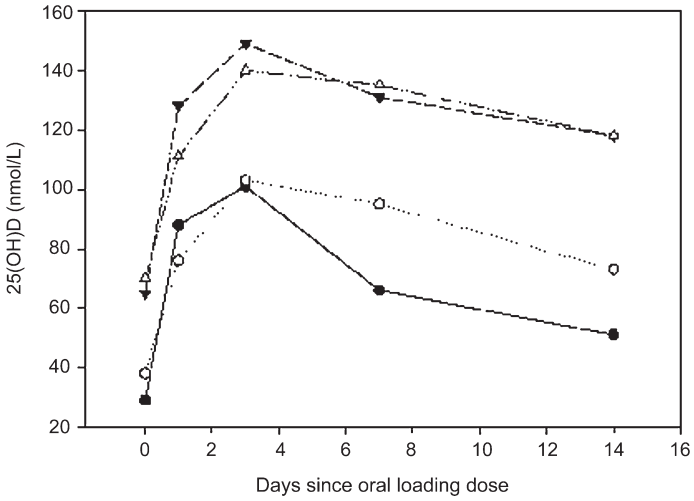
SUPPLEMENTAL TABLE 16 Study Arms Administering Variable Weekly or Loading Dose Regimens of Vitamin D and 25(OH)D Measurements Within 3 Months of Study Initiation

First Author, Year	Location	Study	Population, Age[a]	Dose, IU	Route/Form	Time, wk	25(OH)D		
							Before	Absolute	Change
Goncerzewicz, 1985	Europe	CT	Malabsorption, all ages	• 12 000/kg	PO/D_3	1	140 (58)	—	350 (245)
Kazemi, 2009[b]	Middle East	RCT	Seizure, 3–18 y	• Group 1: 50 000 IU • Group 2: 100 000 IU • Group 3: 150 000 IU	PO/D_3	8	62(24)	87 (28)	—
Soliman, 2011	Middle East	UC	VDD, 12–18 y	• 10 000 IU/kg (max, 600 000 IU)	IM/D_3	12	23 (12)	69 (23)	—
Soliman, 2008	Middle East	UC	Thalassemia, 12–19 y	• 10 000 IU/kg (max, 600 000 IU)	IM/D_3	13	20 (11)	53 (24)	—
Soliman, 2008	Middle East	UC	VDD, 12–19 y	• 10 000 IU/kg (max, 600 000 IU)	IM/D_3	13	17 (11)	67 (32)	—
Soliman, 2012	Middle East	UC	Rickets, toddlers	• 10 000 IU/kg (max, 600 000 IU)	IM/D_3	13	17 (7)	64 (18)	—
Soliman, 2012	Middle East	UC	Rickets, 12–19 y	• 10 000 IU/kg (max, 600 000 IU)	IM/D_3	13	20 (6)	70 (13)	—
Soliman, 2010	Middle East	UC	Rickets, toddlers	• 10 000 IU/kg (max, 150 000 IU)	IM/D_3	13	16 (12)	70 (22)	—
Soliman, 2012	Middle East	UC	VDD, school-age	• 10 000 IU/kg	IM/D_3	4	28 (12)	77 (34)	—

Only the first group 25(OH)D measurements after week 1 are shown. 25(OH)D levels are provided in nmol/L. Measures of dispersion are provided as SDs (XX). CT, controlled trial; D_2, ergocalciferol; D_3, cholecalciferol; IM, intramuscular; max, maximum; PO, oral; RCT, randomized controlled trial; UC, uncontrolled; —, not reported/not measured.

[a] Age is at time of enrollment.

[b] Authors combined 25OHD measurement at 8 weeks for all three arms.



SUPPLEMENTAL FIGURE 6

Immediate 25(OH)D response to an oral load of vitamin D. Four study arms were identified that evaluated 25(OH)D response within 1 week of an oral loading dose of vitamin D. (●) Thacher et al, 2006[81]; (○) Thacher et al, 2010[73]; (▼) Thacher et al, 2010[73]; (Δ) Thacher et al, 2010.[73]

SUPPLEMENTAL TABLE 17 Study Arms Administering Loading, Weekly, or Excessive Daily Dose Vitamin D With Hypercalcemia Data Reported Within 3 Months of Drug Initiation

First Author, Year	Location	Study Design	Population, Age[a]	Dose, IU	Form/Route	Time, wk	25(OH)D		Hypercalcemia	
							Before	Absolute	Definition	% (n/N[b])
Single or divided loading dose regimens										
Stogmann, 1985	Europe	CT	Rickets, 4–21 mo	400 000[c]	D_2/NR	1	10	213	10.5 mg/dL	1/5
Markestad, 1987	Europe	UC	Healthy, 1–21 mo	600 000	D_2/oral	2	52 (28)	399 (237)	11.2 mg/dL	14/43
Zeghoud, 1994	Africa	RCT	Healthy, newborns	100 000	D_3/oral	2	8	92 (42)	11.2 mg/dL	0/13
Zeghoud, 1994	Africa	RCT	Healthy, newborns	100 000	D_3/oral	13	8	44 (25)	11.2 mg/dL	0/13
Zeghoud, 1994	Africa	RCT	Healthy, newborns	200 000	D_3/oral	2	12	150 (55)	11.2 mg/dL	0/15
Zeghoud, 1994	Africa	UC	Healthy, newborns	600 000	D_3/oral	2	14	307 (77–692)	11.2 mg/dL	0/30
Oliveri, 1996	South America	UC	Healthy, 5–11 y	150 000	D_2/oral	6	47 (27)	62 (35)	NR	0/79
Ozkan, 2000	Middle East	RCT	Rickets, 4–19 mo	300 000	D_3/IM	4	21 (5)	Δ 51 (16)	NR	0/12
Ozkan, 2000	Middle East	RCT	Rickets, 4–19 mo	300 000	D_3/oral	4	21 (5)	Δ 45 (13)	NR	0/13
Ozkan, 2000	Middle East	RCT	Rickets, 4-19 mo	600 000	D_3/oral	4	21 (5)	Δ 62 (26)	NR	3/10
Tau, 2007	South. America	UC	Healthy, 1–16 y	100 000	D_2/oral	4	73 (15)	88 (11)	10.6 mg/dL	0/11
Arpadi, 2009	North America	RCT	HIV, 6–16 y	100 000	D_3/oral	4	62 (SE 6)	87 (SE 4)	NR	0/29
Mallet, 2010[d]	Europe	UC	Healthy, 11–18 y	200 000	D2/oral	2	64 (20)	96 (16)	10.1 mg/dL	0/17
Mallet, 2010[d]	Europe	UC	Healthy, 11–18 y	200 000	D_2/oral	12	64 (20)	58 (13)	10.1 mg/dL	0/17
Carnes, 2012	Australia	RCT	Healthy/VDD, 15-17 y	150 000	D_3/oral	13	39 (10)	65 (40, 96)	NR	0/7
Carnes, 2012	Australia	RCT	Healthy/VDD, 15–17 y	300 000	D_3/oral	13	38 (9)	67 (58, 87)	NR	0/8
Kari, 2013	Middle East	UC	Renal disease, 2–16 y	300 000	D_3/oral	4	36 (19)	97 (33)	10 mg/dL	0/19
Aggarwal, 2013[e]	Asia, other	RCT	Rickets, 12–18 y	600 000	D_3/oral	4	35 (26)	82 (60)	5.4 mg/dL ionized	0/19
Aggarwal, 2013[e]	Asia, other	RCT	Rickets, 12–18 y	600 000+ calcium	D_3/oral	4	49 (45)	78 (77)	5.4 mg/dL ionized	2/20
Weekly loading dose regimens										
Maalouf, 2008	Middle East	RCT	Healthy, 10–17 y	14 000/wk	D_3/oral	8	83 (80, 106)	116 (99, 170)	10.7 mg/dL	1/9
Maalouf, 2008	Middle East	RCT	Healthy, 10–17 y	14 000/wk	D_3/oral	8	123 (100, 150)	126 (101, 151)	10.7 mg/dL	0/8
Very high dose daily loading dose regimens										
Stogmann, 1985	Europe	CT	Rickets, 4–21 mo	9600/d	D_2/oral	1	6	97	10.5 mg/dL	1/5
Tsybysheva, 1988	East Asia	CT	Renal disease, 8–16 y	20 000/d	D_2/oral	13	43 (8)	300 (30)	NR	3/13

Only the first group 25(OH)D and hypercalcemia data between 1 and 13 weeks after initiation are shown. 25(OH)D levels are provided in nmol/L. Measures of dispersion are provided as SDs (XX), SEs (SE XX), interquartile ranges (XX, XX), and low–high ranges (XX–XX). CT, controlled trial; D_2, ergocalciferol; D_3, cholecalciferol; IM, intramuscular; NR, not reported; RCT, randomized controlled trial; UC, uncontrolled trial.

[a] Age is at time of enrollment.

[b] n represents the number of participants who had hypercalcemia; N represents the number of participants reported on or enrolled in the arm.

[c] Stogmann et al 1985 gave two 200 000 IU doses separated by 48 h.

[d] Mallet et al 2010 had 2 study arms because the study gave identical doses to different groups and performed analysis at separate times.

[e] Aggarwal et al 2013 reported on 2 patients with hypercalcemia and hypercalciuria in the discussion (data reported as ionized).

SUPPLEMENTAL TABLE 18 Study Arms Administering Vitamin D Doses <4000 IU With 25(OH)D Measurements Between 1 and 13 Weeks of Study Initiation

First Author, Year	Location	Study Design	Population, Age[a]	Daily Dose, IU	Form/Route	Time, wk	25(OH)D		Hypercalcemia	
							Before	After	Definition	% (n/N^b)
Markestad, 1984	Europe	UC	Rickets, 4–24 mo	1700–4000	D_2/oral	1	16 (10)	41 (13)	11.2 mg/dL	0/9
Tau, 1986	Europe	RCT	Congenital hypothyroid, 0–30 d	1200	D_2/oral	4	22 (9)	57 (12)	10.6 mg/dL	3/6
Leger, 1989	Europe	RCT	Congenital hypothyroid, 0–30 d	1200	D_2/oral	4	25(8)	45 (25)	10.6 mg/dL	4/10
Kilpinen-Loisa, 2007	Europe	CT	CP/NMD, 9–18 y	1000	D_3/oral	10	46 (26–82)	56 (39–88)	10.8 mg/dL	0/21
Shroff, 2012	Europe	RCT	Renal disease, 0–17 y	600–8000	D_2	13	50 (19–73)	97 (16–153)	NR	0/20
Holmlund-Suila, 2012	Europe	RCT	Healthy, neonates	400	D_3/oral	10	52 (14)	88 (18)	11.3 mg/dL	0/35
Holmlund-Suila, 2012	Europe	RCT	Healthy, newborns	1200	D_3/oral	10	54 (15)	124 (30)	11.3 mg/dL	0/35
Holmlund-Suila, 2012	Europe	RCT	Healthy, newborns	1600	D_3/oral	10	53 (15)	153 (40)	11.3 mg/dL	0/37
Putman, 2013	North America	RCT	Healthy, 11–19 y	1000	D_3/oral	11	73 (30)	75 (17)	10.5 mg/dL	0/29
Lewis, 2013	North America	RCT	Healthy, 9–13 y	<2000	D_3/oral	12	66 (7)	Δ 32 (16, 48)	10.6 mg/dL	3/265
Lewis, 2013	North America	RCT	Healthy, 9–13 y	4000	D_3/oral	12	70 (18)	Δ 80 (39, 108)	10.6 mg/dL	0/58
Putman, 2013	North America	RCT	Healthy, 11–19 y	200	D_3/oral	11	70 (16)	72 (29)	10.5 mg/dL	0/25
Gallo, 2013	North America	RCT	Healthy, 0–30 d	1200	D_3/oral	4	65 (56–73)	116 (41)	6 mg/dL ionized	0/32
Gallo, 2013	North America	RCT	Healthy, 0–30 d	1600	D_3/oral	4	64 (53–77)	122 (61)	6 mg/dL ionized	0/14
Gallo, 2013	North America	RCT	Healthy, neonates	400	D_3/oral	4	56	70 (16)	6 mg/dL ionized	0/32
Gallo, 2013	North America	RCT	Healthy, neonates	800	D_3/oral	4	56	85 (28)	6 mg/dL ionized	0/32

25(OH)D levels are provided in nmol/L. Measures of dispersion are provided as SDs (XX) and 95% CIs (XX–XX). Overlapping study populations were identified for the reports by Tau et al (1986) and Leger et al (1989), and only 1 set was included in the meta-analysis. Data from Shroff et al 2012 were not used in the meta-analysis because the reported dosing range overlaps multiple groups. Lewis et al provided hypercalcemia data separately for the highest dose group and combined for the 4 other dosing groups <2000 IU. CT, controlled trial; CP, cerebral palsy; NMD, neuromuscular disease; NR, not reported; RCT, randomized controlled trial; UC, uncontrolled.

[a] Age is at time of enrollment.

[b] *n* represents the number of participants who had hypercalcemia; *N* represents the number of participants reported on or enrolled in the arm.

SUPPLEMENTAL TABLE 19 Study Arms Administering Intermittent, Weekly, or Daily Loading Dose Vitamin D With Hypercalciuria Data Reported Within 3 Months of Study Initiation

First Author, Year	Location	Study Design	Population, Age[a]	Dose, IU	Form/Route	Time, wk	25(OH)D		Urine		
							Before	Absolute	Measurement	Units, threshold	Hypercalciuria, n/N[b]
Single or divided loading dose regimens											
Oliveri, 1996	South America	UC	Healthy, 5–11 y	150 000	D_2/oral	6	47 (27)	62 (35)	Interval Ca/Cr	mg/mg, NR	0/79
Arpadi, 2009	North America	RCT	HIV, 6–16 y	100 000	D_3/oral	4	62 (SE 6)	87 (SE 4)	Interval Ca	mg/kg/d, NR	0/29
Mallet, 2010	Europe	UC	Healthy, 11–18 y	200 000	D_2/oral	2	64 (20)	96 (16)	Interval Ca/Cr	mmol/mmol, 0.7	0/20
Mallet, 2010	Europe	UC	Healthy, 11–18 y	200 000	D_2/oral	12	64 (20)	58 (13)	Interval Ca/Cr	mmol/mmol, 0.7	1/17
Soliman, 2011	Middle East	UC	VDD, 12–18 y	10 000/kg	D_3/IM	12	23 (12)	69 (23)	Interval Ca/Cr	NR, NR	0/40
Soliman, 2012	Middle East	UC	Rickets, toddlers	10 000 IU/kg	D_3/IM	13	17 (7)	64 (18)	Interval Ca/Cr	NR, NR	0/45
Soliman, 2012	Middle East	UC	Rickets, 12–19 y	10 000 IU/kg	D_3/IM	13	20 (6)	70 (13)	Interval Ca/Cr	NR, NR	0/36
Aggarwal, 2013	Asia, other	RCT	Rickets, 12–18 y	600 000	D_3/oral	4	35 (26)	82 (60)	Ca/Cr	NR, NR	0/19
Aggarwal, 2013	Asia, other	RCT	Rickets, 12–18 y	600 000 + calcium	D_3/oral	4	49 (45)	78 (77)	Ca/Cr	NR, NR	2/20
Weekly loading dose regimens											
Garg, 2013	Asia, other	CT	Healthy, 10–15 y	60 000	D_3/oral	4	22 (11)	153 (36)	Spot Ca/Cr	mg/mg, 0.35	0/214
Garg, 2013	Asia, other	CT	Healthy, 10–15 y	60 000	D_3/oral	6	18 (8)	120 (28)	Spot Ca/Cr	mg/mg, 0.35	0/136
Garg, 2013	Asia, other	CT	Healthy, 10–15 y	60 000	D_3/oral	8	33 (14)	146 (36)	Spot Ca/Cr	mg/mg, 0.35	0/132
Only reported proportion VDD within first 3 mo											
Shajari, 2009	Middle East	RCT	Healthy, neonates	100 000*	D_3/oral	10	NR	NR	Spot Ca/Cr	mg/mg, 0.21	28/30

Only the first group 25(OH)D and hypercalciuria data between 1 and 13 wk after initiation are shown. 25(OH)D levels are provided in nmol/L. Measures of dispersion are provided as SDs (XX) and SEs (SE XX). Aggarwal et al 2013 reported on two patients with hypercalcemia and hypercalciuria in the discussion. Garg 2013 had 3 separate groups that received weekly dosing for variable durations. Mallet et al 2010 has 2 study arms given identical doses to 2 different groups and performed analysis at separate times. Shajari et al 2009 measured 25(OH)D at 10 wk but only reported as % VDD. Ca/Cr, calcium/creatinine ratio; CT, controlled trial; NR, not reported; RCT, randomized controlled trial; UC, uncontrolled.

[a] Age is at time of enrollment.

[b] n represents the number of participants who had hypercalciuria; N represents the number of participants reported on or enrolled in the arm.

SUPPLEMENTAL TABLE 20 Study Arms Administering Daily Vitamin D Doses <4000 IU With Hypercalciuria Data Reported Within 3 Months of Study Initiation

First Author, Year	Location	Study Design	Population, Age[a]	Daily Dose, IU	Form/Route	Time, wk	25(OH)D		Urine		Hypercalciuria, *n*/*N*[b]
							Before	After	Measured	Units, threshold	
Reported group average 25(OH)D levels within first 3 mo											
Holmlund-Suila, 2012	Europe	RCT	Healthy, neonates	400	D_3/oral	10	52 (14)	88 (18)	Spot Ca/Cr	mmol/mmol, 2.2	13/35
Holmlund-Suila, 2012	Europe	RCT	Healthy, newborns	1200	D_3/oral	10	54 (15)	124 (30)	Spot Ca/Cr	mmol/mmol, 2.2	12/35
Holmlund-Suila, 2012	Europe	RCT	Healthy, newborns	1600	D_3/oral	10	53 (15)	153 (40)	Spot Ca/Cr	mmol/mmol, 2.2	14/37
Khadgawat, 2013	Asia, other	RCT	Healthy, 10–14 y	1000	D_3/oral	12	30 (14)	69 (22)	Spot Ca/Cr	mg/mg, 0.21	0/243
Khadgawat, 2013	Asia, other	RCT	Healthy, 10–14 y	600	D_3/oral	12	29 (13)	57 (16)	Spot Ca/Cr	mg/mg, 0.21	0/233
Gallo, 2013	North America	RCT	Healthy, neonates	400	D_3/oral	4	56	70 (16)	Spot Ca/Cr	mmol/mmol, 3.3	2/27
Gallo, 2013	North America	RCT	Healthy, neonates	800	D_3/oral	4	56	85 (28)	Spot Ca/Cr	mmol/mmol, 3.3	5/29
Gallo, 2013	North America	RCT	Healthy, 0–30 d	1200	D_3/oral	4	65 (56–73)	116 (41)	Spot Ca/Cr	mmol/mmol, 3.3	4/27
Gallo, 2013	North America	RCT	Healthy, 0–30 d	1600	D_3/oral	4	64 (53–77)	122 (61)	Spot Ca/Cr	mmol/mmol, 3.3	2/27
Lewis, 2013	North America	RCT	Healthy, neonates	0	D_3/oral	12	72 (19)	$\Delta -10$	Spot Ca/Cr	mg/mg, 0.22	3/265
Lewis, 2013	North America	RCT	Healthy, neonates	400	D_3/oral	12	71 (20)	$\Delta 5$	Spot Ca/Cr	mg/mg, 0.22	
Lewis, 2013	North America	RCT	Healthy, 9–13 y	1000	D_3/oral	12	71 (20)	$\Delta 22$	Spot Ca/Cr	mg/mg, 0.22	
Lewis, 2013	North America	RCT	Healthy, 9–13 y	2000	D_3/oral	12	66 (7)	$\Delta 32$	Spot Ca/Cr	mg/mg, 0.22	
Lewis, 2013	North America	RCT	Healthy, 9–13 y	4000	D_3/oral	12	70 (18)	$\Delta 80$ (39, 108)	Spot Ca/Cr	mg/mg, 0.22	0/58
Only reported proportion VDD within first 3 mo											
Shajari, 2009	Middle East	RCT	Healthy, neonates	200	D_3/oral	10	NR	NR	Spot Ca/Cr	mg/mg, 0.21	25/30
Shajari, 2009	Middle East	RCT	Healthy, neonates	400	D_3/oral	10	NR	NR	Spot Ca/Cr	mg/mg, 0.21	23/30

25(OH)D levels are provided in nmol/L. Measures of dispersion are provided as SDs (XX). Lewis et al 2013 provided hypercalciuria data separately for the highest dose group (4000 IU) and combined for the 4 other dosing groups <2000 IU. Ca/Cr, calcium/creatinine ratio; NR, not reported; RCT, randomized controlled trial.

[a] Age is at time of enrollment.

[b] *n* represents the number of participants who had 25(OH)D testing; *N* represents the number of participants enrolled in the full study.

SUPPLEMENTAL TABLE 21 PRISMA Checklist

Checklist Number	Topic	Reported on Page
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PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses.

GRANT-IN-AID

AWARD AGREEMENT (the "Agreement")

2013/2014

Principal Investigator: James Dayre McNally

Project Title: Prevention of post-operative vitamin D deficiency in children with CHD: A dose evaluation trial

Funding period: 2013-2015

Project No.: G-13-0002519

Grantee Institution: Children's Hospital of Eastern Ontario Research Institute

1. Representations and Warranties of the Principal Investigator

In accepting a grant-in-aid from the Heart and Stroke Foundation of Canada ("**HSFC**" or the "**Foundation**"), the Principal Investigator, being the person in charge of the research being granted this award (the "**Award**"), makes the following representations and warranties:

(a) that they have read and will comply with the terms of the Foundation's Grant-in-Aid Submission Guidelines (the "**GIA Submission Guidelines**") and Grant-in-Aid Award Management Guidelines (the "**GIA Award Management Guidelines**" and, together with the GIA Submission Guidelines, the "**Guidelines**");

(b) that they have read and understand the following sections of the Guidelines:
   (i) Section 8 – which outlines the right of the Foundation to participate in licensing and royalty profits from any discoveries made as a result of its support;
   (ii) Section 9 – confirming that the Award does not cover indirect costs of research;
   (iii) Section 10, that refers to the Foundation's Open Access to Research Outputs Policy; and
   (iv) Section 14, governing personal financial gain to the applicant from the outcome of the research.

(c) It has obtained the express written authorization of the grantee institution to conduct the research being funded under this Award in the form attached as Schedule A to this Agreement;

(d) with respect to human and animal experimentation, as per Section 7 of the GIA Submission Guidelines:
   (i) all investigations involving human subjects have been endorsed by the Principal Investigator's institution's ethics review board, or other clearly designated body, as ethical;

(ii) all investigations involving human subjects conform to the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans and/or Human Pluripotent Stem Cell Research: Guidelines for CIHR-Funded Research;
(iii) the research protocol and the care of animals for laboratory experimentation has been approved by the grantee institution's animal care committee and conforms to the Guiding Principles for Animal Experimentation as enunciated by the Canadian Council on Animal Care; and
(iv) for all investigations involving human or animal subjects, the research has been reviewed and conforms with the guidelines outlined in Health Canada's Laboratory Biosafety Guidelines and that the research will not be undertaken until it has been accepted as meeting the requirements regarding biological and chemical hazards by such a review.

Under no circumstances will the Foundation fund research that involves human embryos that are created solely for research purposes or the derivation of stem cells from cloned human embryos that are created solely for research purposes.

(e) all sources of funding, both active and applied for, and a complete list of all commercial research and development activities in which the Principal Investigator is engaged have been inputted into CIRCUlink. This includes all private, public, and commercial research even if not related to the research pursuant to which this Award relates;
(f) The Principal Investigator shall not, without prior written approval by the Foundation:
  (i) use the name of the Foundation or any trademarks owned or licensed by the Foundation, including, without limitation, use in any press releases, advertisements, or publicity;
  (ii) use the name of the Foundation in association with the raising of funds for a private or public company, partnership, or any other business arrangement; or
  (iii) indicate in any manner that the Foundation is in "association" with or "partner" of "joint venturing" with any other person or agency.
(g) funds received under this Agreement will only be spent on matters which are directly related to the research project pursuant to which this Award has been granted;
(h) neither the Principal Investigator, nor any of its affiliates, have received funding from other sources which would constitute a duplication of the funding being awarded under this Agreement;
(i) any matching funding for this project was correctly reported in the grant-in-aid application; and
(j) If matching funding becomes available after the signing of this Agreement, that funding will be reported to the Foundation immediately using the appropriate section of CIRCUlink.

For greater clarity, the Guidelines are incorporated by reference and form part of this Agreement.

2. Notice Requirements

The Principal Investigator must notify the Foundation immediately, in writing, if any of the following occur:

(a) There is any significant deviation from the awarded budget;
(b) If the Principal Investigator ceases to be an academic staff member at the original grantee institution during the term of this Award;
(c) If the site where the research is being conducted changes; and
(d) If the Principal Investigator cannot carry out the research or fulfill the purpose for which the Award was granted.

Notice must be given to the following address:

By email to: PeerReview@hsf.ca

OR

By Post to: Heart and Stroke Foundation of Canada
1402-222 Queen Street
Ottawa, Ontario K1P 5V9
Attention: Manager, Peer Review

3. Publication of Results

Results of the research must be made freely available to the public through appropriate scientific channels, and all publications will bear the statement: "This work was supported by a Grant-in-Aid from the Heart and Stroke Foundation of Canada." Notwithstanding the foregoing, discoveries that are patentable or protected as trade secrets need not be disclosed to the public if disclosure would result in loss of protection.

4. Reporting Requirements

The following are the reporting requirements of the Principal Investigator during and following termination of this Award:

a) Progress Report – A satisfactory progress report must be filed with the Foundation on an annual basis by August 1st of the funding year. The report must be completed in the prescribed form and must be filed via CIRCUlink. Failure to submit a satisfactory progress report in the prescribed form may result in termination of funding.

b) Final Report - Following termination of the Award, a satisfactory final report must be filed with the Foundation on or before August 1st of the termination year. The report must be completed in the prescribed form and must be filed via CIRCUlink.
c) Close-Out Report – A satisfactory close-out-report must be filed with the Foundation before the first anniversary of the project completion date. The report must be completed in the prescribed form and must be filed via CIRCUlink.

5. Limitations on Obligations

The Foundation reserves the right, in its sole discretion, to decrease or eliminate the funds awarded under this Agreement at any time. The granting of the Award is subject to the Foundation receiving a fully executed copy of this Agreement and the Grantee Institution Authorization Form attached as Schedule A.

6. Termination

The Foundation may terminate this Agreement at any time if:

(a) in its sole discretion, there are insufficient funds available to support the Award; or
(b) the Principal Investigator is in breach of any of its representations and warranties under this Agreement and such breach is not cured to the satisfaction of the Foundation within 30 days of the Principal Investigator being notified of the breach.

In the event the Agreement is terminated in accordance with this section, the Principal Investigator will return to HSFC the balance of any money awarded but not yet spent under this Agreement.

7. No Joint Venture or Partnership

Nothing in this Agreement shall be construed or interpreted to make HSFC and the Principal Investigator partners or joint venturers, or to make one an agent or representative of the other, or to afford any rights to any third party other than as expressly provided herein. None of HSFC or the Principal Investigator is authorized to bind the other to any other contract, agreement or understanding. HSFC is not responsible and specifically disclaims any liability for any claim, judgment, award for damages, settlement, negligence, or malpractice arising from any research or investigation related to the Award.

8. Assignment

This Agreement may not be assigned or transferred by the Principal Investigator without the prior written consent of HSFC, and any assignment without consent shall be null and void. This Agreement shall enure to the benefit of and be binding upon the respective permitted successors and assignees of the Principal Investigator.

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Patient Information and Consent Form

Prevention of Post-Cardiac Surgery Vitamin D Deficiency in Children with Congenital Heart Disease: A Pilot Dose Evaluation Randomized Controlled Trial.

Study Doctor: Dr. Dayre McNally

Other Doctor's Involved: Dr. Kusum Menon, Dr. Gyaandeo Maharajh, Dr. Margaret Lawson, Dr. Stephanie Redpath, Dr. Pavel Geier, Dr. Jane Lougheed, Dr/ John Smythe

Address: Pediatric Intensive Care Unit, CHEO,
401 Smyth Road, Ottawa, ON K1H 8L1

Phone Number: 613 - 737-7600, ext 3553

Our research group would like to invite you to take part in a research study on vitamin D. You are being invited to participate because your child is less than 18 years of age and is scheduled to have surgery for congenital heart disease (CHD) at the Children's Hospital of Eastern Ontario (CHEO).

Your decision to participate or not in this study will not affect the care your child receives at CHEO. Before agreeing to participate in this study, it is important that you read and understand the following explanation of the proposed study procedures. The following information describes the purpose, procedures, benefits, discomforts, risks and precautions associated with the study. It also describes your right to decline to participate or withdraw from the study at any time. In order to decide whether you wish to participate in this research study, you should understand enough about its risks and benefits to be able to make an informed decision. This is known as the informed consent process. Please ask the study doctor or study staff to explain any words you don't understand before signing this consent form. Make sure all your questions have been answered to your satisfaction before signing this document. If you agree to have your child participate, you will receive a copy of this form to keep for your records.

Background and purpose of study

Vitamin D is a nutrient and hormone best known for its role in maintaining body calcium levels and bone strength. Recently, vitamin D has been reported to also be important for maintaining the health of the heart, lungs and immune systems. Recent research, including work at CHEO, has shown that 4 out of every 5 children with Congenital Heart Disease have low levels of vitamin D following

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cardiac surgery. Further, this research demonstrated that children have low levels due to borderline normal levels before surgery and close to a 50% drop due to the life saving cardiac surgical procedures. We are concerned that low levels of vitamin D may contribute to some of the common problems that develop after CHD surgery, including low calcium levels, poor heart function, and inflammation. Our goal is to identify an approach to vitamin D supplementation that safely prevents post-operative vitamin D deficiency.

What is the current standard of treatment for vitamin D?

A minimal daily vitamin D intake has been recommended by the Institute of Medicine and endorsed by Health Canada (400 IU for infants, 600 IU over 1 year). At present, most children with Congenital Heart Disease receive this minimal vitamin D supplement dose prior to surgery. Our research suggests that this approach may not maintain adequate vitamin D following cardiac surgery.

What is the proposed alternative Vitamin D treatment?

The goal of this research study is to evaluate whether a higher daily dose of vitamin D can **safely** prevent vitamin D deficiency following cardiac surgery (1600 IU for infants, 2400 IU over 1 year). **The higher daily dose of vitamin D will be compared to the current standard minimum dose recommended by Health Canada.** The higher dose of vitamin D **we have chosen** is based on results from studies evaluating vitamin D supplementation in healthy children **and recommendations from Health Canada**. These studies showed that children receiving the higher dose were more likely to achieve adequate vitamin D levels and were no more likely to have unwanted side effects (elevated blood and urine levels of calcium). **For children with Congenital Heart Disease** it is unclear whether the higher daily dose of vitamin D will similarly elevate vitamin D levels and avoid unwanted side effects in.

If you agree to participate in this study, your children would be assigned to one of two study groups: one group would receive the low dose of vitamin D (usual care) and the second group would receive the higher dose of vitamin D. This will allow us to compare those subjects who receive the high dose and those who receive usual care. Health Canada and the CHEO Research Ethics Board has given approval to test the safety and effectiveness of the higher dose of vitamin D.

Objectives of study

a. To determine whether the daily intake of a higher dose of vitamin D can decrease the number of children with CHD who have inadequate vitamin D levels following surgery, **as compared to minimum standard vitamin D supplementation recommended by Health Canada.**
b. To determine whether the daily intake of a higher dose of vitamin D supplement significantly increases blood and urine calcium levels.

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c. To determine whether the daily intake of a higher dose of vitamin D supplement improves the functioning of the vitamin D axis.

How many people will take part in this study?

The study doctors will be inviting 62 children with CHD having cardiac surgery at CHEO.

Eligibility to Participate

The doctor in charge of this study, or a member of the study staff, has discussed with you the requirements for your child's participation in the study. Some of the requirements are:

- Your child must be less than 18 years of age
- Your child must have Congenital Heart Disease and require cardiopulmonary bypass and cardiac surgery within 12 months
- Your child will be admitted to the Pediatric Intensive Care Unit (PICU) after surgery.

Your child cannot participate in this study if:

- Your child is known or suspected to have William's syndrome (a rare genetic disorder that can lead to elevated blood calcium levels).
- Your child has a health problem that prevents ingestion of the vitamin D supplementation
- Your child has previously participated in this study.

Study Procedures

Following your decision to participate we will ask you to take either the usual standard daily dose or a high daily dose of vitamin D prior to surgery; when possible the supplement would be introduced a minimum of two months prior to surgery. You will be 'randomized' into one of the study groups described below. Randomization means that you are put into a group by chance. Neither you nor your doctor can choose the group you will be in. You will have an equal (one in two) chance of being placed in any group. The purpose of randomization is to ensure that those receiving the high and low dose of vitamin D supplement are identical in every other respect. That way, we can know for certain that any differences that we observe between the two groups are due to the study medication and nothing else. If your child develops a problem during the course of the study that might be related to vitamin D, the treating physician can request to know the dose of vitamin D your child was assigned.

In this study all participants will receive at least the **Health Canada** recommended minimum daily dose of vitamin D (usual care). **As infant formula contains the minimum daily dose of vitamin D,** some study participants **assigned to the usual care group will** receive a vial that contains a solution without any vitamin D (also called placebo). The placebo is required so that it is not obvious these children are receiving the low dose supplement.

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Complete a short questionnaire. You will be asked to complete a questionnaire booklet regarding age, current medications, diet and supplements, family background, sun exposure habits and ethnicity. This will take about 5-10 minutes. For outpatient participants this can be completed while you are waiting for the CHEO pharmacy to provide study supplement.

Record supplement intake and permit study staff to contact the caregivers every 2 to 4 weeks. You will be provided with a calendar. We ask that you mark on the calendar every day that your child received the supplement. If the supplement was held, we will ask that you record the reason and duration. You will also be asked to contact study staff if more than 2 days of supplement is held. For those children who are at home prior to surgery we also request permission to have the study staff contact you every 2 to 4 weeks. The purpose of this phone call will be to answer questions related to the study and encourage the daily intake of the supplement.

Permit a small amount of blood to be taken prior to surgery.

1. For neonates, or other children who require surgery within two months of starting the study, we will collect 1 mL or a bit less than a quarter of a teaspoon of blood at the initiation of supplement. To avoid unnecessary discomfort blood will either be collected from arterial or central lines or at the time of venipuncture for clinically indicated blood work.
2. **For children, who have received study supplement for more than 6 months we will measure calcium and vitamin D during scheduled cardiology or cardiovascular clinic appointments if your physician is ordering bloodwork for another purpose.**
3. As part of this study, we will collect blood for measurement of calcium and vitamin D levels at the time of standard pre-surgical blood work **(approximately 3 weeks prior to surgery).** These measurements will be used to make sure children do not have unnecessarily high levels of vitamin D or calcium and allow for adjustment of the supplement dose.

Permit small quantities of blood to be taken in operating room and after surgery. In the operating room, immediately prior to surgery, all patients have lines placed for monitoring and blood collection. Prior to surgery, 2 mL (less than half a teaspoon of blood) will be collected from these lines. Further, after the operation is complete we will take blood at the time of admission to PICU, and on the 1st, 3rd, 5th and 10th post-operative days. If your child is discharged from PICU to the pediatrics ward before day 10, a sample will be collected on the day of discharge and no additional blood will be collected for research purposes. Neonates will only have 1 mL or a bit less than a quarter of a teaspoon of blood collected on the 5th and 10th post-operative days.

Permit small quantities of urine to be taken in operating room and after surgery. In the operating room, immediately prior to surgery, all patients have a tube placed in the bladder for measurement of urine output. This tube remains in place following admission to the Pediatric Intensive Care Unit (PICU). We request permission to collect 5 mL of urine from this tube immediately prior to surgery and once on the day following surgery. This urine will be used to determine whether children who receive the higher dose of vitamin D expel higher amounts of calcium.

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Permit the study doctors to use the collected research blood to determine vitamin D status and measures of vitamin D function. The collected blood will only be used for the express purpose of the research question(s). No other investigations will be allowed without your consent.

Echocardiography (an ultrasound that provides an image of the heart). We request permission to have cardiology evaluate heart function on the day following cardiac surgery through echocardiography. **This often occurs as part of routine care.** This information will be recorded in the research chart.

Permit collection of information from your child' medical chart. Your child's medical chart will be reviewed by the study staff and some information will be recorded (e.g. type of heart disease, duration of surgery, calcium levels, calcium administration, and measures of organ dysfunction).

Additional appointments or changes to care. There are no additional appointments planned as part of the study. With the exception of the study supplement, phone calls and additional blood tests, there will be no other changes to the care provided by the CHEO cardiovascular program. Certain additional (research) tests will be performed to ensure patient safety. If abnormalities are identified with these tests, study participants may be recommended to have additional blood work or to see physician specialists that work in the area of vitamin D (**endocrinologists, nephrologists**).

Study procedures summarized in the attached flow diagram.

Potential Risks

The purpose of this study is to compare the usual daily dose of vitamin D to a higher daily dose. Based on our current knowledge we do not know whether the higher dose of vitamin D will be significantly better than the usual dose in terms of preventing post-operative vitamin D deficiency and side effects. The study would be stopped if we learned that this was not in fact true.

Toxicity with vitamin D supplementation has been described and occurs due to high levels of blood calcium. Vitamin D overdose and hypercalcemia (a high level of calcium in the blood) leads to symptoms such as poor appetite, nausea, vomiting, increased urination, weakness, and nervousness. With very high blood levels, calcium can accumulate in the kidneys leading to impaired kidney function. Vitamin D toxicity has been described in two distinct settings. The first setting involves individuals with rare genetic conditions with abnormal handling of body calcium (1 in 10,000 in the general population). For example, some children with Congenital Heart Disease have a syndrome that makes them more prone to high blood levels of calcium. Patients known or expected to have William's syndrome will be excluded from the study. The second setting involves the intentional or unintentional intake of unnecessarily high vitamin D doses. The table below shows the dose and intake intervals linked to toxicity.

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Dose	Interval of intake	Link to toxicity
>600,000 IU	Single or repeat dosing	Strong
10,000-40,000 IU/day	One to four months	Strong
3,000-10,000 IU/day	Months to years	Weak/unclear

Recent studies on vitamin D supplementation have demonstrated that doses of 1600-2000 IU/day (infants) and 2500-3000 IU/day (above 1 year of age) do not increase risk of high blood calcium concentrations or increase urine calcium levels. However, we do not know whether these doses are safe in children with Congenital Heart Disease who require cardiac surgery.

To compare the effectiveness and safety of the two vitamin D doses we need to collect blood from your child at regular intervals. Blood drawing can cause pain, discomfort, bleeding, bruising or infection at the site of the needle. To avoid these complications research blood will be taken from the venous or arterial lines placed in all of these patients for monitoring. If your child's lines are removed or are not available, we will collect the research blood at a time when they have regular blood work. No patient will receive needle poke solely to collect research blood. Analgesic (numbing or pain blocking) cream can be used to decrease the pain and discomfort of blood tests.

Alternatives to Participating in the Study

Your child does not have to take part in this study to have cardiac surgery. In the absence of other medical conditions, if you choose not to participate your physician will encourage you to take the **Health Canada recommended minimum** daily dose of vitamin D.

Possible Benefits

Your child may or may not benefit from participating in this study. However, our hope is to change clinical practice or to take better care of our children with Congenital Heart Disease in the future. Your participation in this study may eventually provide the study doctors with valuable information about how to safely prevent vitamin D deficiency in children undergoing cardiac surgery for Congenital Heart Disease.

Voluntary Participation

Taking part in this study is entirely voluntary. Your decision to participate or not in this study, will not affect the care you receive at CHEO. We will inform you of any new information that might influence your decision to participate in the research project. You may be asked to sign a revised consent form if this occurs.

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Withdrawal from the Study

In some cases, your study participation could be discontinued by the study doctor without your consent, at any time for any of the following reasons.

- The research blood work shows elevated levels of calcium and vitamin D
- The study doctor feels it is in your best interest
- You need additional medication that would interfere with the study
- You do not follow the study staff's instructions

You are free to withdraw your child from the study at any time and there will be no penalty to you or your child. You may also choose to have all of the blood and urine specimens destroyed and information collected as part of the research chart withdrawn. Withdrawing from the study will not affect the care you receive at CHEO.

Confidentiality

Your child's personal information will be kept strictly confidential, except as required or permitted by law. **Studies like this one are regulated by Health Canada and therefore** representatives of Health Canada and the CHEO Research Ethics board will have access to your child's personal information. Your child's medical records may be reviewed by the investigators or delegates, the Research Ethics Board, and regulatory authorities for the purpose of verifying clinical trial procedures and/or data. **CHEO internal monitoring research staff may review your research chart and medical records for quality improvement purposes.** Further, there may be instances where risk to the participant is identified. In this case, information will be shared with appropriate medical personnel in order to initiate care.

You or your child will not be identified in any data publication or presentation of this study. Any personal information about you that leaves the hospital will be coded so that it cannot be identified by name. There is a small risk of unwanted release of information from your research records. Health and research records have been used against patients and their families. For example, in Canada, insurance companies may deny insurance to patient's with a certain illness or those that have genetic risk of disease. Your hospital medical records cannot, however, be released unless required or permitted by law or if you sign a release of information. The researchers of this study will protect your research records so that your name, address and phone number will be kept private. Your child's medical information may be held and processed on a computer in a locked office at CHEO or the CHEO Research Institute.

By signing this consent form, you authorize the record review, information storage and data transfer described above.

If the research uncovers information that might be helpful to your child's current or future health (e.g. high blood calcium or vitamin D levels) the information will be disclosed to you by study

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doctor. This physician will contact you to discuss what these results might mean. This physician may request repeat blood work to confirm the findings. Based on the initial or repeat blood work, this physician may recommend you decrease or discontinue the study supplement and refer you to a physician expert in vitamin D (endocrinologist). The cardiovascular surgery team will be made aware of the need for this additional blood work, change in study protocol, or referral to endocrinology. It is possible that the study doctor may decide that no additional action is necessary.

Study Costs

You will not be charged for any test or research procedure required for this study. You will not be paid to participate in this research study. The vitamin D supplement will be provided to you free of charge as long as you participate in this study. If additional blood work or hospital visits are required you may request compensation and will be provided with a parking voucher.

The study doctor(s) will not receive any financial benefit from your participation in this study.

Compensation

In the event that your child suffers injury as a direct result of participating in this study, normal legal rules on compensation will apply. By signing this consent form, you are in no way waiving your legal rights or releasing the investigators from their legal and professional responsibilities.

Study Results

With the exception of potentially important findings, results from the various study related tests will not be made immediately available to you as they will have no clinical value. At your request, you can receive a copy of the study results at the end of the study.

Questions

If you would like more information, please contact the investigator: Dr. Dayre McNally, Telephone: 737-7600, ext. 3553.

The CHEO Research Ethics Board (REB) has reviewed and approved this research project. The REB is a committee of the hospital that includes individuals from different professional backgrounds. The board reviews all research that takes place at the hospital. Its goal is to ensure the protection of the rights and welfare of people participating in research. The Board's work is not intended to replace a parent or child's judgment about what decisions and choices are best for them. You may contact the Chair of the Research Ethics Board, for information regarding patient's rights in research studies at (613) 737-7600 (3272), although this person cannot provide any health-related information about the study.

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Option

The blood samples obtained for this study will be used for the express purpose of the research question(s). We would like your permission to use remaining blood to answer related questions on the importance of nutrition, hormones to heart dysfunction and critical illness.

I agree to have any remaining blood used to answer related research questions ☐ Yes ☐ No

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Patient Information and Consent Form Signature Page

To become a part of this study, you or your legal representative must sign this page. If capable, patients over 14 years of age will be asked to sign this form.

By signing this page, you are confirming the following:

- You have read all of the information in this Patient Information and Consent Form, and you have been offered time to think about it.
- All of your questions have been answered to your satisfaction.
- You voluntarily agree to have your child be a part of this study, to follow the study procedures, and to provide necessary information to the doctor, nurses or other staff members as requested.
- You can freely choose to stop your child from being a part of this study at any time.
- You have received a copy of this Patient Information and Consent Form to keep for yourself.
- If you so wish, you may have a copy of the final study results

_____
Name of Subject (printed)

_____ _____
Signature of Subject (≥ 14 yrs) **Date**

_____
Printed Parents Name

_____ _____
Parental Signature for children <14 years **Date**

_____
Printed Name of person conducting consent

_____ _____
Signature of person conducting consent **Date**

If the patient is ≤ 14 years old, he/she should be informed about this study at a level he/she can understand. If the patient is able, he/she should give assent to participate in the study and personally sign and date the assent form. This is in addition to the signature of the patient's legal/authorized representative.

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Study related procedures and measurements

Child with CHD has need for surgery confirmed

Urine collected for calcium:creatinine ratio Participants expected to have surgery within 2 months of initiating supplement will have pre-treatment vitamin D level	• Complete study questionnaire • Pharmacy provides study supplement • Participant completes compliance diary • Study compliance calls every 2-4 weeks

Pre-operative blood work

Participants who take supplement for 2 or more months will have blood Vitamin D and calcium measurements performed: • With standard pre-surgical blood work • With clinically indicated blood work during scheduled clinic appointments (if study drug duration > 6 months)	• Results evaluated by safety officer and changes to supplement volume and/or endocrinology consult (if required) • Participant completes compliance diary

Operating Room

Blood and urine collected in OR immediately prior to surgery for : • Blood vitamin D metabolites • Blood markers of immune function • Urine calcium:creatinine ratio	• Compliance diary returned • Unused study supplement returned • Review of medications • Details on anesthetic and surgery collected as part of case report form

PICU Admission

Research blood collection • PICU admission • POD 1,3, 5 & 10 (none after discharge) Research urine collection: • PICU admission and POD 1	Research blood tests • Vitamin D metabolites • Immune markers Research urine tests • Calcium: creatinine ratio
• Details on clinical course recorded on case report form • Details of post-operative day 1 echocardiogram recorded on case report form • Post-operative calcium levels evaluated by research assistant and safety officer • Participants with elevated calcium to creatinine ratios will have renal ultrasound	

Hospital discharge (or 60 days after surgery)

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Making a difference in the lives of children and youth

Faire une différence dans la vie des enfants et des adolescents

Children's Hospital of Eastern Ontario
Centre hospitalier pour enfants de l'est de l'Ontario

Assent Form

Prevention of Post-Cardiac Surgery Vitamin D Deficiency in Children With Congenital Heart Disease: A Pilot Dose Evaluation Randomized Controlled Trial.

Study Doctor: Dr. Dayre McNally

Other Doctor's Involved: Dr. Kusum Menon, Dr. Gyaandeo Maharajh, Dr. Margaret Lawson, Dr. Stephanie Redpath, Dr. Pavel Geier, Dr. Jane Lougheed.

Address: Pediatric Intensive Care Unit, CHEO,
401 Smyth Road, Ottawa, ON K1H 8L1

Phone Number: 613 - 737-7600, ext 3553

Your doctor or nurse will have explained all about this trial to you in a lot more detail than is here, but this information sheet is for you to keep and to help you remember what you have been told. If there are words that you do not understand, please ask your doctor or nurse. You will also be given a separate sheet where some of these words are explained in more detail. You or an adult may contact the research doctor or nurse at 613-737-7600 ext 3553 if you want to discuss any of this further when you get home.

Invitation

You are being invited to be part of a research study. It is up to you if you want to be in this study. No one will make you be part of the study. Even if you agree now to be part of the study, you can change your mind later. No one will be mad at you if you choose not to be part of this study. If you decide not to take part in this study it will not affect the care you receive.

Why Are We Doing This Study?

You are being invited to be a part of this study because you were born with a heart problem that requires surgery within 12 months. We recently learned that the machines used to perform surgery on the heart cause almost all children to have low blood levels of a substance called vitamin D. We believe that having low blood levels of vitamin D following surgery increases risk of infection and places extra stress on the heart and lungs. Our recent study suggests that the usual daily intake of vitamin D recommended for children without heart disease may not protect children who require heart surgery from low blood levels of vitamin D. The goal of this study is to determine whether a higher daily intake of vitamin D can safely prevent low blood levels of vitamin D following surgery.

What Will Happen in This Study?

If you agree to participate, you and your parents will be asked questions about your health, food intake and medications. You and your parents will be given a bottle of vitamin D with instructions to take a certain amount every day prior to surgery. Some children will take a lower amount of vitamin D, while other children will take a higher amount. You and your doctors will not know which group you are in. We will ask you to mark down on a calendar every day you take the study supplement and we will call you every 2 to 4 weeks to answer questions and encourage you to keep taking the study supplement. To compare the children taking low and high amounts of vitamin D we need to do tests on your urine and blood. First, we will ask you to give us some urine prior to starting the study supplement. Second, **we need to make sure your blood levels of vitamin D and calcium are safe. To do this we will do some tests 2 to 3 weeks before surgery (at the same time your surgeon wants blood work to prepare for surgery). For children who take study drug for more than 6 months we will also check the vitamin D and calcium levels during a visit, but only if the heart doctor is doing blood work for other purposes.** On the day of your surgery we will measure your vitamin D levels before and after the surgery. After surgery we will also look for signs that the blood levels of vitamin D were too high or too low. To do this we will need to collect some blood, urine, and take some pictures of your heart (echo). We will also collect information from your medical chart.

Will it Hurt?

- No, we have designed our study so that there will be no additional needles. We will collect the blood required for the tests at the same time as the bloodwork your doctor has ordered.
- We will ask that you give us a sample of urine prior to starting the vitamin D supplement. This will not hurt and we will only ask you to pee into a container.
- As part of the study we will take some images of your heart on the day following surgery. This is called an echo or ultrasound. It will not hurt, but may feel uncomfortable.

How will it Help Me?

We do not know if you will benefit from being in the study. We hope it will help us to find out why some people get this disease and why others don't.

Who Is Doing This Study?

Dr. Dayre McNally and other doctors from CHEO, including your heart doctors, will be doing this study. They will answer any questions you have about the study. You can also call CHEO at (613) 737-7600 Ext 3553 if you are having any problems with your tummy. If you get sick, the study doctors will make sure that you are taken care of.

Who Will Know I Am in the Study?

Only your doctor and people who are involved in the study will know who is in the study. When the study is finished, the doctors will write a report about what was learned. This report will not say your name or that you were in the study. We will make sure that all your medical information is kept private. Your parents and you do not have to tell anyone that you are in the study if you don't want to.

Do I Have to Be in the Study?

You do not have to be in this study if you do not want to. You can have as much time as you want to decide to be part of the study. You can discuss the study with your parents. Your participation in this study is voluntary. No one will be upset with you if you say no. Even if you say yes, and you want to change your mind, it is okay.

Can I stop the Study?

Even if you agree now to be part of the study, you can change your mind later. No one will be upset with you if you choose not to be part of this study.

Will My Information Be Kept Private?

Your identity will remain a secret, unless the law says we have to reveal it, for a very good reason for example, if you are at risk. We will make sure that all your medical information is kept private. Your name will not be used, only your initials. Any personal information that leaves the hospital will be coded so that you are not named.

Who can answer Questions?

The CHEO Research Ethics Board is a group of people who reviews all research that takes place at CHEO. The Board's job is to protect people taking part in research. You may contact the Chair of the Research Ethics Board, if you have any questions at (613) 737-7600 (3272), but this person cannot give you any information about the study.

If you would like more information, please call Dr.Dayre McNally at (613) 737-7600 Ext. 3553.

☐ Check box if verbal assent obtained

	Yes	No
I have been told about the study in detail	☐	☐
I have had the chance to talk to my Doctor about the study.	☐	☐
I understand that all of the information collected will be kept private.	☐	☐
I understand I will be given a signed copy of this consent form	☐	☐
I understand that I am able to stop being in the study at any time.	☐	☐
I agree to have my private information kept in a computer database which is password protected	☐	☐
I agree to have Dr. McNally or his study staff contact me when further information is needed.	☐	☐

Signature

If I put my name at the end of this form, it means that I agree to be in the study.

_____	_____	_____
Child's Name printed	**Child's Signature**	**Date**

_____	_____	_____
Parental Name printed	**Parental Signature, children < 14 yr**	**Date**

_____
Printed Name of Person Conducting Consent

_____	_____
Signature of Person Conducting Consent	**Date**

Health Canada / Santé Canada

Therapeutic Products Directorate
5th Floor, Holland Cross, Tower B
Address Locator# 3105A
OTTAWA, Ontario
K1A 0K9

Your file / Votre référence

24 January 2013

Our file / Notre référence 9427-C2270-44C

Dayre McNally, MD
Children's Hospital of Eastern Ontario
401 Smyth Road, Room 3445
OTTAWA, Ontario
K1H 8L1
(613) 737-7600 Ext. 3553

No Objection Letter RE: Protocol # VITAMINDINCHD-01

Dear Dr. McNally:

I am pleased to inform you that the information and material to support your Clinical Trial Application for **VITAMIN D**, control number **161404**, received on January 2, 2013, have been reviewed and we have no objection to your proposed study. I would remind you of the necessity of complying with the *Food and Drug Regulations,* Division 5, in the sale of this product for clinical testing. In addition, the regulations impose record keeping responsibilities on those conducting clinical trials. You are also reminded that all clinical trials should be conducted in compliance with the Therapeutic Products Directorate's *Guideline for Good Clinical Practice.*

Please note that Health Canada has implemented electronic reporting of adverse drug reactions and is currently in pilots with some sponsors. Those sponsors who have established electronic connection with Canada Vigilance Production stream should submit their reports only to the appropriate Directorates: TPD, BGTD or MHPD (i.e. a report no longer needs to be sent in duplicate to multiple directorates). For the sponsors who have not yet established this connection, they should continue submitting their reports by fax or by courier. The following website will provide you further clarification on Health Canada's adverse drug reactions reporting requirements:
http://www.hc-sc.gc.ca/dhp-mps/alt_formats/pdf/prodpharma/applic-demande/guide-ld/ich/efficac/e2a_pre_notice_avis-eng.pdf

Consistent with Health Canada's Notice - *Registration and Disclosure of Clinical Trial Information* of November 30, 2007, sponsors are encouraged to register their clinical trials within 21 days of the trial's onset, using a publicly available registry that conforms with international standards for registries such as: Clinicaltrials.gov (www.clinicaltrials.gov); Current Controlled Trials (www.controlled-trials.com).

Should you have any questions concerning this letter, please contact the Office of Clinical Trials (613) 941-2132.

Yours sincerely,

CHEO Children's Hospital of Eastern Ontario
Centre hospitalier pour enfants de l'est de l'Ontario

2012 FINAL APPROVAL LETTER

CHEO Site Investigator:	Department/PSU	Date of Final Approval	Valid until
Dr. Dayre McNally	Critical Care	March 25, 2013	March 15, 2014

Protocol Title:	REB #:	Other Study #:	Date of REB Meeting (Initial Review)	Deadline for Annual Renewal Submission
Prevention of Post-Cardiac Surgery Vitamin D Deficiency in Children with Congenital Heart Disease: A Pilot Dose Evaluation Randomized Controlled Trial	13/03E		January 09, 2013	February 18, 2014

c.c.:	Consent Form Version and Date *Additional items listed below
,CHEO RI Administration	March 22, 2013, Version #3

Protocol Date	Protocol Version	Assent Form Version and Date *Additional items listed below
March 22, 2013	Amendment #3	March 22, 2013, Version #3

Date of Health Canada Non-Objection Letter, and Control #	Recruitment Poster Version and Date
January 31, 2013, Control #161404	Not applicable

Investigators Brochure/Drug Monograph Version and Date	Recruitment Pamphlet Version and Date
Product Monograph, January 12, 2012	Not applicable

SITE Specific Restrictions	Other Study Documentation Received by REB
None.	Case Report Form - Version 1 Example of Compliance Calendar/Diary - Version 1 Patient Questionnaire DSMB Terms of Reference - December 17, 2012, Version 1 Patient information Sheet

This protocol was approved at a meeting of the CHEO Research Ethics Board in which the quorum rules were met and only those REB members who were independent of the investigator(s) conducting the study voted on the final decision.

In fulfilling its mandate, the CHEO REB is guided by: Tri-Council Policy Statement; ICH Good Clinical Practice Practices: Consolidated Guideline; Applicable laws and regulations of Ontario and Canada (e.g., Health Canada Division 5 of the Food and Drug Regulations & the Food and Drugs Act - Medical Devices Regulations).

Final approval is granted with the understanding that the investigator agrees to comply with the following requirements:

- The investigator must conduct the study in compliance with the protocol and any additional conditions set out by the Board.
- The investigator must not implement any deviation from, or changes to, the protocol without the approval of the REB except where necessary to eliminate a immediate hazard to the research subject, or when the change involves only logistical or administrative aspects of the study (e.g., change of telephone number or research staff). As soon as possible, however, the protocol deviation form and, if appropriate, the proposed protocol amendment(s) should be submitted to the Board for review.
- The investigator must, prior to use, submit to the Board changes to the study documentation, e.g., changes to the informed consent letters, recruitment materials. Should major revisions to the consent form be made, the investigator agrees to re-consent those subjects who have originally consented to the study and who wish to continue on the study.
- For clinical drug or device trials, investigators must promptly report to the REB all adverse events that are both serious and unexpected (SAEs). For SAE reports on CHEO patients, the investigator must also comply with the hospital-wide Policy regarding, Procedures For Considering Medical Error In The Differential Diagnosis of Severe Adverse Events (SAE) Associated with the Drugs Administered in a Clinical Trial (see http: //cheonet/data/1/rec_docs/3792_Medical%20Error%20Policy%20revised%20january%2020061.doc).
- For all other research studies, investigators must promptly report to the REB all unexpected and untoward occurrences (including the loss or theft of study data and other such privacy breaches).
- Investigators must promptly report to the REB any new information regarding the safety of research subjects (e.g., changes to the product monograph or investigator's brochure for drug trials). Where available, any reports produced by Data Safety Monitoring Board should be submitted to the REB.
- Investigators must notify the REB of any study closures (temporary, premature or permanent), in writing along with an explanation of the rationale for such action.
- Investigators must submit an annual renewal report to the REB 30 days prior to the expiration date stated above.
- Investigators must submit a final report at the conclusion of the study.
- Investigators must provide the Board with French versions of the consent form, unless a waiver has been granted.

For complete procedures relating to the CHEO REB Procedures and Application Forms, please refer to the REB website at http://www.cheori.org/en/researchethicsboard or contact Sharon Haig, Ethics Coordinator at shaig@cheo.on.ca or 613-737-7600 ext. 2128.

Regards,

Prevention of post-cardiac surgery vitamin D deficiency in children with Congenital Heart Disease: A pilot dose evaluation Randomized Controlled Trial

Data and Safety Monitoring Board (DSMB)

Terms of Reference

Membership:

Dr. Tim Ramsay, Chair
Scientist, Ottawa Hospital Research Institute
Email: tramsay@ohri.ca

Dr. Leanne Ward, MD
Endocrinologist, Scientist, CHEO Research Institute
Email: Lward@cheo.on.ca

Dr. Janusz Feber, MD
Pediatric Nephrologist, CHEO
Email: jfeber@cheo.on.ca

Conflict of interest:

1. DSMB members will have no major financial or intellectual conflict of interest that could prevent them from objectively reviewing the interim data and giving advice to the Steering Committee.
2. DSMB members will disclose to the Chair any other conflicts that they consider relevant.

Rationale for DSMB:

Not all randomized controlled trials require a DSMB as the installation adds a level of complexity and imparts a burden that may compromise trial efficiency. Although vitamin D supplementation is considered beneficial at standard doses, the impact of high dose supplementation on children with Congenital Heart Disease requiring surgery is unknown. As this population of children is high risk, frequently suffering severe morbidity and mortality in the pre and post-operative periods, a DSMB would be appropriate. Review of comparative data by the DSMB would help ensure that the intervention is not leading to unexpected harm.

Roles and Responsibilities:

The Data and Safety Monitoring Board (DSMB) is an independent group of experts who will function independently and at arms length from the Study investigators and the Steering Committee. The primary responsibilities of the DSMB are to:

1. Periodically review and evaluate the accumulated study data for participant safety.
2. Make recommendations to the Steering committee based on these reviews regarding the continuation, modification or termination of the trial.
3. Comment on the relevance of new external published data from other trials that may impact on patient safety or efficacy of the study treatments
4. DSMB members must maintain strict confidentiality concerning all privileged trial results, and during all phases of DSMB review and deliberations. With respect to the Vitamin D study the DSMB may communicate only with the Principal Investigator, or another member of the Steering committee.
5. No member of the DSMB should have direct involvement with the conduct of the study. No member should have financial, proprietary, professional or other interests that may affect impartial, independent decision-making by the DSMB.

Frequency of meetings:

1. Meetings will be scheduled after the completion of study procedures on 32 study participants. Alternatively, reviews will be conducted at least yearly and focused primarily on safety issues.
2. Between meetings the DSMB chair will receive information about post-randomization serious adverse events that are potentially related to study medication.
3. The DSMB Chair may request a full meeting of the committee at any time.
4. Face-to-face meetings are preferable but conference calls or electronic communication are acceptable alternatives with the agreement of the DSMB members. If the DSMB have further questions that might require clarification from additional data, this will be communicated to the PI & the SC for approval. A quorum, i.e. all three DSMB members, must be present at the meetings.
5. The study coordinator will facilitate and arrange cost reimbursement for teleconferences and couriers.

Structure of meetings:

1. First, an open session with the Principal Investigator (PI), study coordinator and the study statisticians to review accrual, data timeliness and quality, completeness of follow-up and adjudication, and any proposals for changes in the study protocol or study duration. In addition, the PI will be responsible for reporting any new external evidence (especially results from other relevant ongoing trials) that bear on the conduct of the Example trial. No unblinded information will be revealed during this session.
2. Second, a closed session will be held with only DSMB members present review data on safety, and the status of statistical monitoring boundaries.

3. Third, an open session with the blind PI and staff from the project office to deliver and discuss the DSMB comments and recommendations and to decide on the timing of the next meeting.
4. Finally, no unblinded information will be revealed at meetings of the DSMB or provided to any third party. The DSMB can request unblinding based on concern.

Minutes:

1. Minutes will be taken at all meetings and maintained. This will be considered confidential until the end of this trial.
2. After each meeting, the DSMB Chair will provide the PI with a letter stating the general outcome of this meeting and suggested changes to the study design or conduct. For example, this letter may simply contain the statement that the trial should continue as planned and rationale for recommendations will be included. This report will not include confidential information. The DSMB Chair or designee is responsible for drafting and forwarding the summary report to the PI and Steering committee members within two weeks of the meeting.
3. Copies of a DSMB letters will be forwarded by the PI to the CHEO REB

Items to be reviewed by the DSMB:

SAE Reporting

1. As described in the study protocol for SAE documentation and reporting, all potentially related unexpected SAE will be reported to Health Canada and the CHEO REB. Further, and as per CHEO REB policy, we will report all potentially vitamin D related SAE, with a review of study drug administration and patient use. Copies of these reports will be made available to the chair of the DSMB.
2. Given the high frequency of baseline SAE in this population, and lack of reported SAE with daily vitamin D intake at the proposed doses, we will not provide individual SAE reports to the DSMB.
3. For the interim and final DSMB meeting our study statistician we will provide a summary of SAE for each of the study arms (blinded). We have prepared a preliminary reporting form that will be modified at the request of the DSMB after the pre-study meeting.

Study Reports

1. Interim reports for review by the DSMB will include data on recruitment, baseline demographics, study safety outcomes, specific clinical outcomes, and related serious adverse effects. An agreed upon review package (see DSMB form) which contains the appropriate data summary by treatment will be provided by the study statistician two weeks prior to any scheduled review. Again, DSMB members will review data only by masked study group (such as X vs Y rather than experimental vs control).

Decisions about stopping the trial for safety:

1. The committee shall recommend stopping the study based on a comparison of the study reports. The DSMB may request unblinding of the groups. The members of

the DSMB may recommend stopping the trial only if they find that one strategy is clearly more harmful, however harm must be considered in light of potential benefit. *This decision will be based on clinical judgment, and not statistical analysis (no stopping rules will be provided).*

2. If the DSMB decides that a definitive conclusion to stop can be made based on safety issues, they will immediately notify the PI and the members of the Steering Committee to discuss the results.

Study – Prevention of post-cardiac surgery vitamin D deficiency in children with congenital heart disease: a pilot dose evaluation randomized controlled trial – **Dr. Dayre McNally (Principal Investigator)**

DSMB-Regular Report Form

Start date: June 2013

	Drug A	**Drug B**	**Overall**
Total Participants Screened	**N**		
Total Participants Screened Failures - N	**N**		
Reasons:	xx - Will not require cardiopulmonary bypass xx - Corrected gestational age < 36 xx - Born at GA < 32 xx - Surgery planned outside of CHEO xx - Unable to receive enteral nutrition or drugs xx – William's syndrome (or highly suspected) xx – Surgery to occur more than 12 months in future xx - other		
Patients enrolled	**N**		
Patients eligible for rescreening	**N**		
Patients refused or unable to be consented	**N**		
Participants no longer on protocol after randomization (excluding those who reached a primary endpoint) - N (%)	**N (%)**		
	Reasons:	xx – patient withdrew consent xx – MD withdrew x – toxicity identified (pre-surgical workup) x – other	
Demographics & Baseline Clinical Characteristics (Case Report Form completed)	**N=xx**	**N=xx**	
Age – N (%)			
Under 1 year			
Over 1 year			
Expected supplement time – N (%)			
More than 2 months			
Less than 2 months			
Pre-Treatment Urine Sample Collected – N(%)			
Pre-treatment hypercalciuria – N(%)			
Treatment/Compliance			
Months of study drug treatment (median, IQR))			
Duration of study drug – N (%)			
Under 2 months			
Above 2 months			
Percentage days drug consumed – N(%)			
Above 90%			
80 to 90%			
Under 80%			

Study – Prevention of post-cardiac surgery vitamin D deficiency in children with congenital heart disease: a pilot dose evaluation randomized controlled trial – **Dr. Dayre McNally (Principal Investigator)**

DSMB-Regular Report Form

	Drug A	Drug B	Overall
Mid Treatment Safety outcomes			
Mid-treatment levels			
Blood 25OHD (median, range)			
200 to 250 nmol/L – N (%)			
Above 250 nmol/L – N (%)			
Mid-treatment, pre-surgery ionized Ca^{2+}			
Ionized calcium (median, range)			
Hypercalcemia for age – N (%)			
Vitamin D toxicity			
Elevated calcium and 25OHD – N (%)			
Mid Treatment, pre-surgical safety outcomes			
Mid-treatment, pre-surgery 25OHD			
Blood 25OHD (median, range)			
200 to 250 nmol/L – N (%)			
Above 250 nmol/L – N (%)			
Mid-treatment, pre-surgery ionized Ca^{2+}			
Ionized calcium (median, range)			
Hypercalcemia for age – N (%)			
Vitamin D toxicity			
Elevated calcium and 25OHD – N (%)			
Post-treatment pre-operative safety outcomes			
Pre-operative ionized calcium (median, range)			
Pre-operative, hypercalcemia – N(%)			
Pre-operative, calcium/creatinine (median, range)			
Pre-operative hypercalciuria – N(%)			
Post-treatment post-operative safety outcomes			
Post-treatment, post-operative hypocalcemia – N(%)			
Post-treatment, post-operative hypercalcemia – N(%)			
Post-operative, calcium/creatinine (median, range)			
Post-operative hypercalciuria – N(%)			
Post-treatment outcome measures and SAE			
PICU admission catecholamine requirement– N (%)			
Length of mechanical ventilation (median, IQR)			
Confirmed post-operative infection – N (%)			
Arryhthmia, requiring medication/electricity – N (%)			
Required dialysis or renal replacement – N (%)			
Received study related ultrasound – N (%)			
Evidence of nephrocalcinosis – N (%)			
Related, unexpected SAE – N (%)			
Related SAE – N (%)			

Ottawa Hospital
Research Institute
Institut de recherche
de l'Hôpital d'Ottawa

July 7, 2015

Timothy O. Ramsay, B.Sc., PhD

Scientific Director,
The Ottawa Methods Centre

Scientist,
Clinical Epidemiology Program (CEP),
Ottawa Hospital Research Institute (OHRI)

Assistant Professor,
University of Ottawa, Faculty of Medicine

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501 Smyth Road
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Ottawa, ON, Canada
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☎ 613-737-8899 x79830
613-739-6938
tramsay@ohri.ca

Dear Dr. McNally,

This letter is to inform you that the DSMB for your vitamin D deficiency after congenital heart disease surgery met on June 16, 2015. The DSMB consisted of myself (Dr. Tim Ramsay) as well as Drs. Leanne Ward and Janusz Feber. The committee considered the interim report you provided, which summarized safety and other outcomes for the first 30 patients. While the committee unanimously agreed that there are no safety issues of concern at this time, it would like to request a change for the next interim report and to make a suggestion for your study protocol.

The change requested is that future reports provide baseline data for the ionized calcium and calcium/creatinine outcomes similar to the mid-treatment and post-treatment values reported in the current report. If possible, it would also be good to provide pre-treatment vitamin D levels.

The suggestion for the protocol is that it would be good to standardize follow-up for those study participants identified as having hypercalciuria. While the current strategy of follow-up by a nephrologist seems perfectly adequate to guarantee patient safety, the committee feels that when it comes time to publish it will look better if you can say that follow-up urine samples were taken at prescribed regular intervals and the follow-up ultrasound was done at a consistent time. The committee suggests an interval of three months for the follow-up ultrasound.

Sincerely,

Tim Ramsay, Chair
Scientist, Clinical Epidemiology Program, Ottawa Hospital Research Institute
Scientific Director, Ottawa Methods Centre

THE GLOBE AND MAIL

Study links vitamin D to post-surgical recovery times

AMBER DAUGHERTY
The Globe and Mail
Published Monday, Jul. 01, 2013 5:30PM EDT
Last updated Monday, Jul. 01, 2013 5:30PM EDT

Logan Irvine is a happy four-and-a-half-year-old boy. He loves watching Disney shows, cheering on his favourite team (the Ottawa Senators) and kicking balls around the yard.

But he gets tired faster than other kids his age. He needs to take breaks during physical activities, something his father, Graham Irvine, encourages. “We take every day where he’s healthy and happy, we take it all as absolute gold,” Irvine said.

Logan was born eight weeks premature with Tetralogy of Fallot, a congenital heart disease that is present when the baby is still in the womb. It’s a combination of four defects in the heart that affect its structure and cause a lack of oxygen in the blood.

When Logan was born, his condition required immediate surgery. His first two months of life were spent in the hospital.

When he was just six months old, Logan underwent his second heart surgery – a cardiac corrective surgery during which doctors installed the pulmonary artery and valve he was missing.

Two years ago, Logan had his third surgery; doctors replaced what thay had already put in. In a few years, those components will need replacing yet again.

Each time Logan has had surgery, he has spent anywhere from a few days to a couple months in recovery. That’s tough on kids going through surgeries and on their families.

But a new study from the Children’s Hospital of Eastern Ontario Research Institute in Ottawa suggests there may be a way to decrease that amount of recovery time, so children who require congenital heart disease surgery can get better, and get home, sooner.

The study, led by Dr. Dayre McNally, showed that patients who went into the procedure with normal or slightly below normal levels of vitamin D had 40 per cent less vitamin D in their system after surgery.

For the study, McNally took blood samples from 58 children before they went into cardiac surgery, at different points during surgery, and once they were recovering. He found the immediate drop in vitamin D levels happened when the children were hooked up to what is called the heart-lung machine, or the

cardiopulmonary bypass machine, which allows surgeons to work on the heart.

Because the children's vitamin D levels decreased, McNally says, they needed more recovery time post-surgery.

"We've known for 100 years that vitamin D is important for bone health," McNally said, "but more recently, there's rising or compelling evidence that vitamin D is important for other organs – the heart, the lungs, the immune system. All of these organ systems can become quite unwell after surgery."

"The relevance of this finding is that it presents an opportunity to help these organs stay healthier after surgery if we can make sure the kids aren't vitamin D-deficient post-operatively," he said.

McNally said they only have theories at this point as to why the cardiopulmonary bypass machine causes a decrease in vitamin D levels.

"In some ways, it's a concerning finding," McNally said. "In other ways it presents an opportunity. We have vitamin D, which is safe and simple and cheap, so we have this modifiable problem, fixable problem," he said.

The researchers have received funding from Heart and Stroke Foundation of Canada and are embarking on further studies this summer. They hope to optimize vitamin D levels in children before they go into surgery, to see if that helps maintain vitamin D levels after surgery and shorten the kids' recovery time.

"The idea here is if we can optimize the vitamin D levels prior to going to surgery, and maintain levels in the normal range, that the heart will be healthier and the lungs will be healthier [post surgery]," McNally said.

Graham Irvine is excited by the news of the study.

"A parent doesn't want to see their child suffer for any more time than they have to. We took this as a positive," he said. "If this could in fact help promote his recovery that much faster, we'd be all for it."