

Effect of High-Dose Vitamin D Supplementation on Bone Density in Youth with
Osteogenesis Imperfecta: A Randomized Controlled Trial

Laura Plante
School of Dietetics and Human Nutrition
McGill University, Montreal
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ABSTRACT

Background: Osteogenesis imperfecta (OI) is a genetic disease characterized by fragile bones and short stature. A recent study showed serum 25-hydroxyvitamin D (25OHD) concentrations to be positively associated with lumbar spine areal bone mineral density (LS-aBMD) in patients with OI.

Objectives: To assess whether high-dose vitamin D supplementation will result in significantly higher LS-aBMD z-scores after one-year; and to evaluate the effect of vitamin D supplementation on lower limb muscle power assessed through jumping mechanography.

Methods: Patients were randomized in equal number to receive either 400 or 2000 international units (IU) of vitamin D supplements and stratified according to baseline bisphosphonate treatment status and pubertal stage.

Design: A one-year double blind randomized controlled trial conducted at the Shriners Hospital for Children in Montreal.

Participants: Sixty children and adolescents with OI (female, n=35; male, n=25; age 5.9 to 18.9 years; mean 11.7 years, SD 3.2) participated.

Results: At baseline, average serum 25OHD concentration was 65.6 nmol/L (SD 20.4) with no difference seen between treatment groups ($p=0.77$). Inadequate serum 25OHD concentrations (<50 nmol/L) were measured in only 21% of patients at baseline. Supplementation resulted in higher serum 25OHD concentrations in almost all participants (90%) with significantly higher increases seen with 2000 IU (mean [95% C.I.] = 30.5 nmol/L [21.3; 39.6] vs 15.2 nmol/L [6.4; 24.1], $p= 0.02$). No significant changes were detected in aBMD measurements or in lower limb muscle power between treatment groups from baseline to final visit.

Conclusions: Supplementation with either 400 or 2000 IU of vitamin D translates into significant increases in serum 25OHD concentrations in children with OI. However, increases in baseline serum 25OHD concentrations already within a healthy range (≥ 50 nmol/L) do not translate into increases in aBMD z-scores in children with OI.

Keywords: Osteogenesis imperfecta, vitamin D, bone mineral density

RÉSUMÉ

Mise en contexte : L'ostéogénèse imparfaite (OI) est une maladie génétique caractérisée par une fragilité des os et une petite taille. Une étude récente montra une association positive entre la concentration sanguine de 25-hydroxyvitamine D (25OHD) et la densité minérale osseuse (DMO) surfacique du rachis lombaire chez les patients atteints par l'OI.

Objectifs: 1) Évaluer l'impact d'une forte dose quotidienne de supplément de vitamine D sur la DMO surfacique du rachis lombaire après un an; 2) Évaluer l'effet d'un supplément de vitamine D sur la puissance musculaire des membres inférieurs par l'entremise d'une plateforme de sauts. Devis de recherche: Un essai contrôlé, randomisé, à double insu d'un an complété à l'hôpital Shriners pour enfants à Montréal. Participants: Soixante enfants et adolescents diagnostiqués avec l'OI (femelle, n=35; male, n=25; âgé de 5.9 à 18.9 ans; moyenne 11.7 ans, SD 3.2) ont participé.

Méthodologie: Les patients furent randomisés à part égales afin de recevoir 400 ou 2000 unités internationales (UI) de vitamine D et stratifié selon leur statut de traitement par bisphosphonates ainsi que leur stade pubertaire.

Résultats: Au départ, la concentration sanguine moyenne de 25OHD était de 65.6 nmol/L (SD 20.4) sans différence entre les deux groupes ($p=0.77$). Au commencement de l'étude, 21% des patients présentèrent un résultat sanguin inadéquat en 25OHD (<50 nmol/L). Les suppléments de vitamine D ont entraînés une augmentation des concentrations sanguines de 25OHD chez presque tous les participants (90%) dont une hausse significativement plus élevée avec 2000 UI (moyenne [95% I.C.] =30.5 [21.3; 39.6] vs 15.2 [6.4; 24.1], $p=0.02$). Aucun changement significatif fut détecté entre le début et la fin de l'étude pour la DMO ni pour la puissance musculaire.

Conclusion: Un supplément de vitamine D de 400 ou de 2000 UI se traduit par une hausse significative de la concentration sanguine de 25OHD chez les jeunes atteints par l'OI. Cependant, augmenter la concentration sanguine de 25OHD déjà à un niveau sain (≥ 50 nmol/L) n'affecte pas la DMO chez les jeunes avec l'OI.

Mots-clés: Ostéogénèse imparfaite, vitamine D, densité minérale osseuse

CONTRIBUTION OF AUTHORS

Laura Plante was the primary author of this Thesis under the supervision of Dr. Frank Rauch and Dr. Hope Weiler.

F. Rauch, Dr. Francis Glorieux and H. Weiler were responsible for the conception of this project.

L. Plante and Dr. Louis Nicolas Veilleux were responsible for recruiting study participants as well as for conducting jumping mechanography tests.

L.Plante collected all data relevant to the study and followed participants for the one-year trial.

L. Plante completed all statistical analyses of the data with the help, supervision and direction of F. Rauch and H. Weiler.

L. Plante contributed to the interpretation of the data and reviewed relevant literature. Both Thesis supervisors (F. Rauch and H. Weiler) contributed to reviewing and editing the present Thesis draft.

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LIST OF ABBREVIATIONS

25OHD:	25-hydroxyvitamin D
BMD:	Bone mineral density
DRI:	Dietary Reference Intake
DXA:	Dual-energy x-ray absorptiometry
FFQ:	Food frequency questionnaire
IFITM5:	Interferon-induced transmembrane protein 5
IU:	International Units
LS-aBMD:	Lumbar spine areal bone mineral density
nmol:	Nanomole
OI:	Osteogenesis imperfecta
PEDF:	Pigment epithelium-derived factor
PICP:	Procollagen type I C-terminal propeptide
PINP:	Procollagen type I N-terminal propeptide
pQCT:	Peripheral quantitative computed tomography
PTH:	Parathyroid hormone
SERPINF1:	Serpin peptidase inhibitor, clade F, member 1
sCTX:	Serum type I C-telopeptide
uNTX:	Urinary N-telopeptide of type I collagen
VDR:	Vitamin D receptor

CONVERSION FACTORS

1 ng/mL of 25OHD = 2.5 nmol/L

1 µg of vitamin D = 40 IU

LITERATURE REVIEW

1.1 Introduction

Osteogenesis imperfecta (OI), also known as brittle bone disease, is a genetic disease characterized by low bone mineral density (BMD) and increased risk of fractures [1]. The worldwide prevalence of OI is estimated to be approximately 1 per 10,000 births [2-4]. To this day, twelve distinct types of OI have been distinguished based on genetic mutations and phenotype [5-8]. Clinical manifestations of the disease vary in severity depending on the type of OI diagnosed. Type I OI, the mildest genetic mutation, has been shown to hold a 95-fold increase in long-bone fracture risk [9]. Clinical treatment for all types of OI aims at minimizing fractures and improving physical function. Bisphosphonates are widely used to increase BMD in children with OI [10-13], leading to increments in lumbar spine areal BMD (LS-aBMD) [10, 14, 15]. Adequate intake of calcium and vitamin D is likely to optimize response to bisphosphonate treatments by preventing symptomatic hypocalcemia [13, 16] and by optimizing substrate availability for bone mineral accretion [17-19].

The best clinical indicator of vitamin D status is serum 25-hydroxyvitamin D (25OHD) concentration which reflects both endogenous sources and exogenous vitamin D synthesis [20]. General recommendations for healthy serum 25OHD concentrations in individuals the general population range from 50 to 75 nmol/L [21-23]. Lower concentrations of 25OHD have been documented to affect anywhere from 27% to 52% of young OI patients in North America [24-26]. Modifiable risk factors for vitamin D deficiency, defined as 25OHD below 30 nmol/L, in children with OI have encouraged nutritional support especially in regard to nutrient intake [25, 27]. However, the most suitable intakes and status targets for serum 25OHD concentrations are yet to be established for this population.

One of the functional outcomes of healthy vitamin D status is BMD. A recent meta-analysis evaluating the impact of vitamin D supplementation on BMD in healthy

children showed a significant increase on LS-aBMD when baseline 25OHD concentrations were below 35 nmol/L [28]. Thus, vitamin D supplementation may be clinically warranted in children affected by bone disorders and presenting with deficient serum 25OHD concentrations [28-30]. Since higher BMD helps reduce the incidence of fractures in OI, vitamin D treatment may prove to be a beneficial long term adjuvant in the disease management [10, 31].

Previous retrospective studies conducted at the Shriners hospital for Children in Montreal evaluated whether serum 25OHD concentrations in their OI population had any relationship to BMD or histomorphometric measurements of bone [24, 32]. No relationship was found between serum 25OHD concentrations and histomorphometric measures of bone mass, metabolism and mineralization in 71 patients diagnosed with OI types I, III or IV [24]. However, assessment of 315 children with OI found serum 25OHD concentrations were positively correlated with LS-aBMD z-scores [32]. Retrospective data collection showed that for every 1 nmol/L increase in serum 25OHD concentration, LS-aBMD z-score increased by 0.008 in children with mild to severe OI types (I, III and IV) [32]. Carefully conducted randomized controlled trials are necessary to evaluate the potential for improvements in LS-aBMD through increases in serum 25OHD concentrations in this population's youth.

In addition to its impact on bone, vitamin D also has a positive influence on muscle mass [33]. This relationship has been shown in pre and post menarcheal healthy girls however it has yet to be studied in the OI population [34, 35]. Through its effect on muscle size and function, vitamin D plays an important role in acquisition of muscle strength [33]. Potentially, muscular improvements could help decrease the risk of fractures and lead to a better understanding of muscle and bone interactions in this population.

The aim of this study was to contribute to the limited data on the impact of high-dose vitamin D supplementation on BMD measurements in children with OI. To do so, a

randomized, double-blind, controlled trial compared the impact of 2000 IU to 400 IU of vitamin D supplementation over one-year. The secondary objective was to evaluate the effect of a yearly vitamin D supplementation on lower limb muscle power as assessed through jumping mechanography. This study also serves as a starting point for future nutritional studies in this population.

1.2 Osteogenesis imperfecta

1.2.1 Pathogenesis

The extracellular matrix of bone is made up of 90% type I collagen fibers and constitutes the basis for bone deposition [36]. Therefore, most extracellular matrix defects are due to mutations in collagen type I fibers which is the main genetic autosomal dominant cause for OI [5].

The collagen type I molecule is made up of three polypeptide chains including two $\alpha 1$ and one $\alpha 2$ chain [1]. Each polypeptide chain contains exactly 1014 amino acids consisting of a repeating sequence of three amino acids one of which is glycine [37]. For collagen type I to form properly, the three polypeptide chains must tightly intertwine and create a triple helical structure (Figure 1; p. 39) [1]. Glycine being the smallest of all amino acids, it is the only one able to fit within the triple helix and allow for adequate collagen formation [37].

Autosomal dominant forms of OI (type I-V) are attributable to mutations affecting either the quantity or the structure of type I collagen [38]. The genes coding for collagen type I's $\alpha 1$ and $\alpha 2$ chains are *COL1A1* and *COL1A2* respectively. A haploinsufficiency of the *COL1A1* gene leads to the mildest OI phenotype, type I [37]. Glycine substitutions on the other hand, impact gene structure and lead to various OI phenotypes ranging from mild to lethal depending on the α chain affected, the type of amino acid substituted and the position of the mutation within the α chain [8, 37]. Moreover, gene defects

affecting posttranslational collagen processing have also been found to cause different OI types with distinct histology findings (type V and VI) [38-40].

1.2.2 Genotypes and phenotypes

1.2.2.1 Autosomal dominant OI

Every healthy gene is made up of two alleles inherited from each parent [41]. Haploinsufficiency occurs when only one of the two required allele's for a gene is available [41]. Nonsense or frameshift mutations in *COL1A1* lead to premature termination codons in which case only half the required amount of collagen type I protein is produced [8, 9]. This mutation leads to the mildest OI phenotype known as type I [8, 9, 37]. Extraskelatal manifestations of type I OI include blue sclera, found in 92% of cases assessed [9], and hearing impairment initiated around 30 years of age found in 35% of cases assessed [5]. A recent retrospective study of OI type I patients showed the following skeletal characteristics: vertebral compression fractures found in 71% of cases, a 10-fold increased risk of scoliosis and a 95-fold increased risk of long bone fracture compared to a healthy population [9]. Mean measured LS-aBMD z-scores were of -3.0 in this population without bisphosphonate treatment [9]. Nonetheless, individuals with type I OI tend to be of normal stature and do not present with severe bone deformations [8].

OI types II to IV are caused by structural mutations in type I collagen [8]. Glycine substitutions cause over 80% of these mutations, whereas splice-site mutations are accountable for the other 20% [38]. Splice site mutations give rise to premature termination codons and result in mild phenotypes. Structural mutations in type I collagen lead to OI phenotypes that range from mild to lethal.

Glycine substitutions in collagen type I's $\alpha 1$ chain are more likely to result in lethal perinatal type II OI [38]. Charged or branched side chain amino acid substitutions occurring beyond the first 200 helical residues also result in lethal OI type II [38].

Phenotype of OI type II includes clinical findings of “beaded” ribs, dark blue sclera, macrocephaly combined with under mineralized skull as well as bowing of the long bones [38, 42]. Perinatal lethality in type II OI is caused by respiratory insufficiency [42].

Collagen mutations in the form of a glycine substitution within the helical domain of either the $\alpha 1$ or the $\alpha 2$ chain can cause OI types I to IV [37]. OI type III patients present the most severe skeletal defects whereas OI type IV patients present with mild to moderate skeletal deformities and varying stature [37]. OI type I patients diagnosed with haploinsufficiency mutation presented with statistically higher LS-aBMD, thicker cortical widths and greater heights when compared to patients with helical mutations [37]. Haploinsufficient OI patients have an average z-score height of -1.1 compared to -4.2 for type I, III and IV OI patient with glycine substitutions [37, 43].

OI type V patients are known to present with moderate to severe bone fragility [1]. Histological examinations in OI type V show mesh-like lamellation which differs from the thin and slightly irregular lamellae seen in type I and in types III & IV respectively [6]. Moreover, hyperplastic callus tend to develop after fractures or surgical procedures in patients with OI type V [6]. This OI type has been diagnosed in about 5% of all OI cases seen in hospitals [1]. In 2012, whole-exome sequencing of an OI type V patient and her parents allowed for the discovery of a de novo mutation within the gene encoding interferon-induced transmembrane protein 5 (*IFITM5*) [40]. The *IFITM5* gene, also known as Brill (bone-restricted interferon-induced transmembrane protein-like protein), is likely implicated in early mineralization but its exact mechanism in relation to collagen has yet to be elucidated [8, 40]. However, *IFITM5* knockout mice exhibit bent bones, 15-25% shorter long bones and less calcified mandibles [40].

1.2.2.2 Autosomal recessive OI

Originally, all OI cases were due to collagen type I mutations and were classified based on clinical differences. With time, new cases of OI emerged presenting with

distinct histological characteristics and lacking any *COL1A1* or *COL1A2* mutations [1, 44]. Therefore, new types of OI were coined with the identification of a second mechanism behind OI pathogenesis present in posttranslational collagen processing [39].

OI type VI presents as a moderate to severe form of the disease [1]. This type of OI is distinguishable through histological evaluation of bone lamellae since it showcases an abnormal fish-scale lamellation pattern as well as an increased amount of osteoid when compared to normal individuals [7]. At the Shriners Hospital for children in Montreal, OI type VI has been diagnosed in 8 of the 195 patients (4%) with OI who underwent a bone biopsy [1]. Recently, genetic analysis has identified a causative stop mutation in *SERPINF1* (serpin peptidase inhibitor, clade F, member 1) [39, 45]. *SERPINF1* encodes pigment epithelium-derived factor (PEDF) of which undetectable serum levels can be used to diagnose OI type VI [8]. *SERPINF1* mutations have been shown to cause premature termination codons and subsequent loss of PEDF expression [46]. How this leads to under mineralized bone in extracellular matrix has yet to be explained. PEDF has however been shown to up regulate osteoprotegerin, an osteoclast inhibitor, therefore possibly increasing the number of osteoclast cells in OI type VI patients [39].

1.2.3 Treatment

There is no known cure for the genetic bone disorder that is OI. Treatment aims at providing long term autonomy by alleviating the burden of frequent fractures [47]. Since clinical manifestations of the disease can be quite heterogeneous, an individualized multidisciplinary approach is best to corroborate optimal treatment [47].

Nonsurgical management aims at preventing and treating fractures while improving physical ability and locomotion [47]. This type of management can include physical therapy, rehabilitation and orthotic devices such as braces and splints [47].

Braces and splints are used to improve function, to slow progression of deformities and to prevent fractures [48]. Physical activity is encouraged to reduce immobility induced bone loss and has been shown to significantly improve aerobic capacity and muscle force in this population [49, 50]. However, in order for physiological benefits to be maintained, exercise regimens must be continuous [49].

Orthopedic surgeries are conducted on patients with severe bone deformities and high fracture risks [51, 52]. To correct for bowing of the long bones, intramedullary rods are inserted in the bone marrow canal to enhance stability and bone alignment [51, 52]. Risk of scoliosis in children with the mildest form of OI is 10-fold greater than in the general population [9] and more common even in OI types III and IV [53]. Progression of scoliosis can lead to pulmonary insufficiency [53, 54]. For best results, corrective surgery through spinal fusion should be sought when curvature is below 60° [53]. Surgical procedures however do not improve the patient's low BMD which is the root cause of fractures.

1.2.3.1 Bisphosphonates

Bisphosphonates are the most widely used pharmacological treatment for low bone density disorders including OI [50, 55]. The exact method by which bisphosphonates transfer from circulation to bone has yet to be confirmed however bisphosphonates are known to bind to hydroxyapatite on the surface of bone and inhibit its dissolution [55, 56]. Bisphosphonate molecules resist to both chemical and enzymatic hydrolysis within the body and by interfering with protein prenylation they inhibit osteoclast function [50, 55]. Consequently, bone resorption is reduced which leads to increased BMD [55, 56]. Bisphosphonates attach to bone mineral however they do not absorb evenly between bones nor are they absorbed evenly within bone structures (Figure 2; p. 40) [50, 55]. Studies show that bisphosphonates have a higher affinity for trabecular bone compared to cortical bone and their absorption is highest

within the spine [55]. The impact of bisphosphonates on BMD z-scores is most significant within the first 2 years of treatment [11, 16].

Resulting from bisphosphonates' positive impact on BMD, fracture incidence is significantly reduced with initiation of this pharmacological treatment in both human and mouse models [10, 13, 31]. Administration of bisphosphonates in the treatment of severe cases of OI was first initiated at the Shriners Hospital for children in Montreal which still follows the largest known population of children with the disorder [10]. Mean yearly incidence of fractures in young OI patients is shown to decrease anywhere from 38% to 74% pre and post initial bisphosphonate treatments [10, 13]. The use of bisphosphonates increased BMD in OI patients by an average of 41.9% per annum, mainly through increases in cortical bone thickness [10]. Subsequent research has also shown that bisphosphonates decrease chronic bone pain and increase vertebral BMD in children with OI [50].

There are several different types of bisphosphonates in use for the treatment of low BMD. Nitrogen containing bisphosphonates (risedronate, zoledronate, pamidronate, alendronate) have a greater absorption affinity to bone than do non nitrogen containing bisphosphonates (etidronate, clodronate) [55, 56]. A one-year study comparing pamidronate to zoledronate intravenous treatments in young OI patients showed a significantly greater increase in LS-aBMD with the later treatment (42.7 % versus 34.7 %, $p=0.013$) [57]. Nowadays, zoledronate is the preferred treatment for OI since it has the highest binding affinity to hydroxyapatite [56] and requires less frequent administrations [13, 57]. Treatment protocol for zoledronic acid infusions is 0.05 mg/kg over 45 minutes every 6 months [13]. In order to avoid hypocalcemia, daily infusions should not exceed 4.0 mg and pre infusion serum calcium and 25OHD concentrations should be assessed [13].

In addition to BMD, muscle force also improves secondary to bisphosphonate treatments. Young patients with OI reported increased stamina and strength post initial

bisphosphonate treatment [58]. Four months following initial treatment, results showed that maximal isometric grip force of the patient's non dominant hand had increased by a median value of 18% (range 8% - 48%) compared to expected increase of 5% in healthy children [58].

1.3 Vitamin D

1.3.1 Sources, metabolism and function

Vitamin D is a fat soluble vitamin available in two isoforms, vitamin D₂ and vitamin D₃. This vitamin is the only one which can be obtained through endogenous synthesis. Exposure to ultraviolet-B sun rays triggers the endogenous conversion of 7-dehydrocholesterol, a substance present within the lipid layer of epidermal keratinocytes, to previtamin D₃ also known as cholecalciferol (Figure 3; p.41) [59]. Cholecalciferol then binds to vitamin D binding protein and undergoes hydroxylation firstly in the liver and secondly in the kidney to reach its biologically active form under the endocrine system control [59]. In humans, it is estimated that up to 90 % of vitamin D needs are derived from endogenous production [60]. However, numerous factors affect skin derived vitamin D synthesis including seasonal variations, geographical latitude, sun protection and skin color [61]. Ultraviolet radiation (UVR) skin exposure is measured by the minimal amount of exposure required to cause slight erythema known as the minimum erythema dose [59]. Exposure of 40% of the body to a quarter of each person's minimum erythema dose provides 1000 International Units (IU) of vitamin D which is sufficient to cover the need of individuals at all stages of life [21, 59, 60].

Exogenous sources of vitamin D include plant and animal derived foods. Plant derived foods provide vitamin D₂ in the form of ergocalciferol whereas animal food sources provide vitamin D₃ in the form of cholecalciferol [61]. Sources of vitamin D₂ include mushrooms but they provide negligible amounts of this nutrient with ~41 international units (IU) and ~12 IU per cup of shiitake and white mushrooms respectively [62]. On the other hand, sources of vitamin D₃, can help meet dietary requirements

mainly through consumption of fatty fish (~458 IU/75 g) and eggs (natural: ~43 IU/whole egg; bioenriched: 200 IU/whole egg) [62-64].

Since few foods naturally contain vitamin D, fortification programs started being implemented in both Canada and the United States of America in the 1930's in an attempt to prevent rickets [22]. In the United States of America, milk is voluntarily fortified at 385 IU/L, whereas Canada mandates milk fortification at 350-450 IU/L [22]. Moreover, in Canada all plant-based milk substitutes must be fortified accordingly in order to provide a nutritionally equivalent alternative to cow's milk [65, 66] Fortified milk can also be used in the production of yogurts and cheeses and all margarines in Canada are fortified with vitamin D at about 530 IU per 100 g [65]. In 2008, it was reported that 75% of all breakfast cereals sold in the U.S.A. were fortified with vitamin D although the form of vitamin D is unspecified [22]. In February 2011, Canada's Food and Drug Regulations authority permitted the addition of vitamin D₂ containing yeast to baked products including breads, bagels and dough [67]. Fortifications are expected to evolve in Canadian baked products and can reach up to 90 IU of vitamin D₂ per 100 g of product [67]. It is important to note that neither form of vitamin D is bioactive. Therefore, hydroxylation is required to obtain a metabolically active vitamin in the form of calcitriol (1,25OH₂D) (Figure 3; p.41) [61].

Two hydroxylation steps are required for both endogenously and exogenously derived vitamin D sources to be activated (Figure 3; p.41). The first hydroxylation occurs in the liver by mitochondrial and microsomal vitamin D 25-hydroxylase enzymes [68]. The liver converts vitamin D into 25-hydroxyvitamin D (25OHD) using the CYP2R1 enzyme metabolizing both endogenous and exogenous sources with comparable efficiency [22]. Following suit, renal hydroxylation converts 25OHD into 1,25-dihydroxyvitamin D (1,25(OH)₂D) by means of the CYP27B1 enzyme inducing a 1 α -hydroxylation [22, 69]. This enzyme's gene is also expressed in a number of vitamin D target tissues including bone, however their contribution to activation of 25OHD is unclear [66]. A separate hydroxylation can occur converting 25OHD to 24,25-

dihydroxyvitamin D ($24,25(\text{OH})_2\text{D}$) through the 24-hydroxylase (CYP24A1) enzyme [22, 69]. Vitamin D is biologically active in the form of $1,25(\text{OH})_2\text{D}$ whereas research has found $24,25(\text{OH})_2\text{D}$ to have little if any physiological role [22]. In serum, 25OHD is the predominant circulating metabolite and measuring its concentration allows for the most accurate assessment of both endogenous and exogenous stores of vitamin D [68]. Moreover, the serum half-life of 25OHD is 2 to 3 weeks long which allows for assessment of vitamin D concentrations over longer periods whereas $1,25(\text{OH})_2\text{D}$ has a half-life of merely 4 hours and varies greatly based on physiological need since it is tightly regulated by parathyroid hormone (PTH) levels [20, 60, 70].

The main role of endocrine derived $1,25(\text{OH})_2\text{D}$ is to maintain calcium and phosphate homeostasis thereby ensuring proper bone mineralization [66]. Acting upon the ileum and kidneys, $1,25(\text{OH})_2\text{D}$ increases active absorption of calcium to 30-40% as well as phosphorus to 80% [71]. If vitamin D concentrations are suboptimal (<50 nmol/L), absorption of calcium has been shown to decrease by as much as 65% [72]. When calcium levels are low, vitamin D collaborates with parathyroid hormone (PTH) to mobilise calcium from bone through activation of osteoclasts [22, 73]. Moreover, $1,25(\text{OH})_2\text{D}$ along with PTH can also stimulate calcium reabsorption within the renal distal tubules to maintain serum calcium levels within normal range [22].

1.3.2 Vitamin D recommendations for children

The Dietary Reference Intake (DRI) for adequacy of vitamin D has been extensively reviewed by the Institute of Medicine in 2011. These dietary recommendations assume minimal sun exposure [22]. The establishment of the DRI value for adequacy of vitamin D was determined through literature review of numerous factors. For instance, 25OHD serum levels below 30 nmol/L were found to correlate with increased risk of rickets, decreased BMC in children and impaired fractional calcium absorption [22]. In addition, serum 25OHD levels at or above 50 nmol/L presented

optimal fractional calcium absorption yet were found to show little added health benefits [22].

For children and adolescent life stages, ensuring normal bone accretion was of main concern in establishing the DRI's [22]. However, the DRI's established are the same from age 1 up to age 70 for both men and women. The studies examined by the Institute of Medicine to establish governmental vitamin D recommendations utilized a combination of small studies totaling a little over 3000 subjects. Reviewed research showed the consumption of 400 IU of vitamin D to maintain serum blood 25OHD concentrations at or above 50 nmol/L and without cases of vitamin D deficiency at that intake level [22]. Hence, the intake level of 400 IU was set as the Estimated Average Requirement with the requirements curve following a normal distribution. Consequently, governmental recommendations for vitamin D's Recommended Dietary Allowance for all children between the ages of 1 to 18 years old should be of 600 IU daily [22].

Debate surrounding adequate vitamin D requirements for children and adults is ongoing. Vieth (2011) explored data assessing over 13 000 adults which is four times more subjects than was used by the Institute of Medicine in conducting the DRIs. This population research showed that a positive correlation between serum 25OHD concentrations and BMD presented a steep positive slope reaching a plateau between 90 and 100 nmol/L which is double the governmental aim for the established Recommended Dietary Allowance for vitamin D intake [74]. Although concentrations above 50 nmol/L are known to prevent diseases such as rickets, further health benefits are believed to exist above 50 nmol/L serum 25OHD [28]. Recent research has looked into vitamin D's role in cancer prevention through its ability to subdue cell proliferation and prevent cells from becoming autonomous [59]. Also, the biologically active form of vitamin D (1,25(OH)₂D) has been shown to act upon keratinocytes by inhibiting growth and promoting cell differentiation therefore proving to be a potent treatment for psoriasis [75]. Thus, the endocrine society recommends serum 25OHD concentrations of children

be maintained at or above 75 nmol/L in order to provide aforementioned non-skeletal benefits [76].

1.3.3 Vitamin D status in children with OI

Vitamin D metabolites play a crucial role in regulating calcium and phosphate homeostasis [77]. Notably, this vitamin is required for normal absorption of calcium by the enterocytes in the proximal intestine and plays a key role in bone mineralization through both growth and maintenance [22, 75]. Children with OI may be particularly sensitive to vitamin D deficiencies considering their bone disorder may make it more difficult to compensate for nutritional inadequacies.

Although sun exposure allows for cutaneous vitamin D synthesis, a recent retrospective study conducted at the Shriners Hospital for children in Montreal showed that 52% of OI patients were deficient in vitamin D as defined by a serum 25OHD concentration below the recommended 50 nmol/L [24, 26]. Dietary assessments in children with OI have shown mean intakes of vitamin D at 1024 IU per day compared to healthy Canadian children, found to consume a daily mean of 216 IU and 292 IU for females and males respectively [27, 78]. National dietary assessment of healthy American children shows mean intakes of vitamin D at 216 IU per day [79]. According to the collected data, children with OI are consuming over 3 times more vitamin D than healthy children. This discrepancy is likely attributable to this population's increased awareness of the importance of vitamin D in their diet. However, the increased dietary intake of vitamin D does not translate into higher serum 25OHD concentrations. Previous studies have shown widespread deficiencies in serum 25OHD concentrations in the OI population ranging anywhere from 80% to 28% [24, 27, 30, 32]. Contrastingly, Canadian Health Measures Surveys have found healthy children had normal mean serum 25OHD concentrations between 65.2 and 73.4 nmol/L [27, 80]. A possible explanation for the lack of higher serum 25OHD concentration in the OI population is that endogenous synthesis of vitamin D is not contributing as much as it is in healthy

children. Previous studies have suggested this be due to reduced sun exposure secondary to the restricted mobility of children with OI [32].

Previous data collected at the Shriners Hospital for children in Montreal showed that serum 25OHD concentrations were independently associated with LS-aBMD in patients with OI [32]. The study showed that for every increase in 25OHD concentrations of 1 nmol/liter, LS-aBMD z-score increased by 0.008 [32]. In spite of these results, optimal vitamin D concentration in patients with OI has yet to be determined in regard to maximizing BMD. Currently, no studies have shown that higher than normal recommendations for vitamin D intake would benefit patients with OI. Given the scarcity of data relating vitamin D needs in children with OI, and the limited evidence relating vitamin D intake with bone health outcomes, the standard approach in clinical care is to aim for the Recommended Dietary Allowance for vitamin D. The Recommended Dietary Allowance for children over 4 years of age is 600 IU per day with the Tolerable Upper Intake Level set between 3000 to 4000 IU per day [21].

1.4 Bone

1.4.1 Development

Bones serve many functions in the body. A person's height and movements will be highly determined by the length and shape of their bones. For locomotion, muscles attach to long bones and account for the mechanical properties of everyday actions [81]. Bones also serve as organ protectors and enclose vital bone marrow. Moreover, bones can be readily resorbed in order to adjust for ion disruptions within the blood system [81].

1.4.2 Bone cells

Cells involved in ossification stem from both mesenchymal stem cells and hematopoietic cells [36]. The three main bone cells are known as osteoblasts, osteocytes and osteoclasts. Osteoblast cells originate from mesenchymal stem cells

and are responsible for bone matrix synthesis as well as subsequent bone mineralization [36, 82]. As ossification occurs and layers of bone are formed, osteoblast cells are incorporated into the newly formed bone matrix where they become known as osteocytes [83]. Osteocytes create a communication network through canals called canaliculi and control bone mineral content by acting as regulators in the exchange of minerals [36]. Osteoclasts, on the other hand, are derived from hematopoietic cells. [82]. They play a major role in bone resorption through secretion of bone-resorbing enzymes that allow for the release of minerals into the blood system [83]. Osteoclast and osteoblast cells work in concert to allow for bone remodeling [84, 85]. Osteoblast cells utilize the cavity formed by osteoclast cells during previous bone resorption to initiate osteoid formation and mineralization [85].

1.4.3 Modeling and remodeling

The articular-epiphyseal growth plates develop during fetal skeletal development and allow bone elongation to occur. Bones, as it is well known, are continuously being remodeled even throughout adult life when growth in length has ceased [86]. This process is known as bone remodeling and about 20% of all cancellous bone surface is undergoing remodeling at any point in time [85]. The cells involved in bone remodeling are part of the basic multicellular unit which is made up of both osteoclast and osteoblast cells [85]. The skeleton is continually undergoing resorption by osteoclasts followed by new bone formation taken up by osteoblasts (Figure 4; p.42) [87].

Initiation of bone remodeling can be attributed to numerous stimuli including bone microdamage as well as hormonal or cytokine regulation [88, 89]. The initiation phase of bone remodeling attracts osteoclast cells to the bone surface where they differentiate into multinucleated cells and remain active for ~12 days [85, 89]. The transition phase follows and osteoblast cells are activated while osteoclast cells undergo apoptosis [89]. The final phase of bone remodeling consists of the termination phase during which

osteoblasts form unmineralized bone called osteoid [85, 89]. Mineralization of the newly formed osteoid begins ~15 days post its formation by osteoblast cells [85].

Bone remodeling is defined as the resorption and formation of bone through the coupling action of both osteoclasts and osteoblasts on the same bone surface (Figure 4; p.42) [86]. Bone modeling is referred to when bone formation is continuous such as seen in growing children [90]. Thus, bone accrual is more efficient during bone modeling where osteoblasts enable new bone formation without the coupling action of the osteoclasts [86, 90].

Although the intricacies of the coupling process is unknown, bone growth in length must be closely related to cross-sectional bone growth in order to maintain bone strength [90]. Bone growth in width is acquired through bone modeling on the periosteum which increases bone size as well as bone mineral content [90]. During bone formation, osteoblast cells lay down osteoid, made up mainly of collagen type I, which in turn serves as a template to deposit calcium and phosphorus crystals [91-93].

1.4.4 Cortical and trabecular bone

Cortical or compact bone forms the outer shell of bone which surrounds trabecular bone and makes up about 80% of skeletal mass (Figure 5; p.43) [94, 95]. Hence, cortical bone comprises the diaphysis of long bones providing strength through its high resistance to bending and torsion [95]. Cortical bone has a slower turnover rate than trabecular bone thus houses bones' long term mineral reserve [95].

Trabecular or spongy bone is found in the epiphysis and metaphysis of long bones and throughout the interior of short bones (Figure 5; p.43) [95]. Trabecular bone is made up of a structural mesh of short and parallel strands of bone fused together [95]. Trabecular bone helps withstand compressive forces, namely in the spine, by being less dense and more elastic than cortical bone [95]. This bone type constitutes

most of the axial skeleton and is predominantly found in the ribs, spine and skull [95, 96]. Trabecular bone accounts for the remaining 20% of skeletal mass, yet 80% of bone surface [95]. In the human skeleton, trabeculae are typically 100–150 μm thick, whereas cortical bone thickness varies between 1 and 5 mm [96].

1.4.4 Histomorphometry in children with OI

As discussed previously, varying OI genotypes present with different histomorphometric parameters [37]. However, structural parameters of bone including cortical width and trabecular bone volume are lower in all OI types when compared to healthy controls [37]. Bone biopsy specimens have also revealed distinct histology in type V and type VI OI patients respectively presenting a mesh-like and fish-scale bone pattern under polarized light [6, 7].

1.4.5 Serum and urinary bone turnover markers

There are two main categories of biochemical markers of bone turnover; formation and resorption. Bone formation markers include serum bone specific osteocalcin, serum bone specific alkaline phosphatase, serum procollagen type I N-terminal propeptide (PINP) and serum procollagen type I C-terminal propeptide (PICP) (Figure 1; p.39) [97-99]. Bone specific alkaline phosphatase has been previously utilized to assess bone formation in metabolic bone diseases where abnormal remodeling occurs [100]. A product of osteoblasts, it has a relatively long half-life in serum (~2 days) and is less affected by diurnal variations when compared to other bone markers including PINP and PICP [100, 101].

Collagen type I is the most abundant collagen found in organic bone matrix [36]. Type I collagen biosynthesis takes place in osteoblasts during bone matrix production [102]. The cleavage of both carboxy and amino terminal propeptides, allowing type I collagen to attach to fibrils, is thus related to the rate of bone formation. Following their

cleavage, both PINP and PICP are released into circulation where they can be detected through assay [102]. PINP's half-life is estimated to be around one hour according to *in vivo* studies whereas PICP has a serum half-life of only 6 to 8 minutes [103, 104]. Pediatric serum PINP concentrations show median reference ranges varying from 76 to 748 µg/L with peaks measured during pubertal growth spurt [105].

On the other hand, bone resorption markers include serum collagen type I C-telopeptide (sCTX), urinary N-telopeptide of type I collagen (uNTX) and urinary-free deoxypyridoline [10, 97]. Although all bone resorption markers can be found in both serum and urine, serum CTX and urine NTX are most commonly used since they tend to be more stable in those forms [106]. Both sCTX and uNTX are type I collagen degradation fragments rendered by bone resorption processes and sCTX has a serum half-life of 5 to 7 days [97]. High levels of either degradation product signals high bone turnover which can denote loss of BMD when not coupled with high bone formation markers [97]. In a recent retrospective study, 315 participants diagnosed with OI were analyzed for 25OHD serum concentrations prior to receiving any bisphosphonate treatment [32]. This study showed a negative correlation between serum 25OHD concentrations and uNTX/Creatinine ratios, thus illustrating the potential detrimental effects of low vitamin D levels on bone resorption, even with bisphosphonate treatment [32].

Biochemical bone turnover markers have been used as complimentary tools in assessing bone formation in combination with BMD measurements in numerous studies [10, 97, 107, 108]. A case-control study conducted with adult OI patients showed significant increases in all bone turnover markers studied with the exception of sCTX [98]. Bone resorption as well as bone formation markers were shown to be 50 to 200 percent higher in the OI patients therefore corroborating higher bone turnover rates in this population [98].

The use of biochemical markers of bone turnover enable earlier assessment of statistically significant change during intervention studies than do BMD measurements

[108]. For example, a randomized study of 214 healthy postmenopausal women showed significant changes in bone turnover markers as early as 1 month post treatment initiation whereas significant BMD changes were only detected after 1 year of treatment [108].

Research also suggests that biochemical markers of bone resorption have shown potential regarding fracture prediction in patients with osteoporosis and are positively correlated with multiple fractures in children [109, 110]. Changes affecting bone mass result from changes seen in bone formation as well as in bone resorption. For example, a study assessing the impact of bisphosphonate treatments in children with OI showed a decrease in urinary excretion of serum PINP as well as a decrease in serum concentrations of bone specific alkaline phosphatase [10]. The former, a measure of bone resorption, showed a greater decrease than the latter and significant increases in bone mass were measured corroborating the changes measured by bone formation markers [10].

Bone turnover markers can be affected by numerous factors both controllable and uncontrollable. For example, resorption markers, such as sCTX and uNTX, show diurnal variations in the form of peaks between 2 and 8 am [97, 111, 112]. In order to control for such variations, blood samples should be collected within a predetermined time frame and respected for all participants at every follow-up. Moreover, during periods of growth bone turnover is higher than in adulthood therefore, bone turnover markers will be higher [97, 113].

The skeleton is responsive to mechanical loading as well, hence physical activity can cause increases in bone turnover markers for up to 72 hours post exercise [97, 114]. Bone turnover markers also undergo seasonal variations mainly seen throughout the winter months when vitamin D synthesis is lessened [115]. Recent fractures allow us to attribute causality for increased bone turnover rates seen through increased formation and resorption of bone markers [97, 98]. It is important to assess covariates,

both controllable and uncontrollable, when measuring changes in bone turnover markers especially in our OI population in whom fractures are more common.

1.4.6 Bone mineral density measurements

Density is a quantitative measure of the physical property of an element as defined by its mass per unit volume. In the case of bone, it represents the amount of bone mineral per volume of bone (g/cm^3). A BMD test measures how much calcium, phosphorus, magnesium and other types of minerals are in an area of your bone. BMD measurements are mostly conducted in order to diagnose osteoporosis and hence help prevent fractures [116]. Bone strength, consequently, is closely related to both bone density and bone quality [116].

1.4.7 Dual energy x-ray absorptiometry

Dual-energy x-ray absorptiometry (DXA) is the most widely used technique to help assess areal BMD (g/cm^2) in the population [117]. This x-ray machine allows for quick and reproducible measurements all while causing minimal radiation to the subject and minimal scatter to the practitioner [116]. DXA is recognized as the gold-standard technique for measuring BMD and also allows for whole body composition assessment [116].

The DXA technique relies on the transmission of two different x-ray photon energies passing from the beneath the bed where the patient lies and captured by a detector arm above the bed [118]. This machine measures the attenuation of x-rays as measured after passing through the body. Hence, it is assumed that the more the x-rays are attenuated, the denser the tissue through which it passed [118]. The DXA distinguishes two types of tissue; bone (higher attenuation) and soft tissue (lower attenuation) [118]. From this method, DXA measures bone area (cm^2), bone mineral content (g), BMD (g/cm^2) as well as body composition (lean and fat mass). Bone density

measured through DXA does not reflect true volumetric density but rather a calculated area density based on bone mineral content and area [118]. According to the International Society for Clinical Densitometry quality control should include weekly DXA phantom scanning in order to assess appropriate system calibration [119].

1.4.7.1 Limitations

DXA measurement limitations include the challenges of positioning [116]. Guidelines must be followed for positioning in order to control for measurement errors from one measurement to the other. For example, slight rotation of the spine will result in larger vertebral area assessed by the x-rays [116]. This variation will not affect bone mineral content but will decrease BMD values since DXA evaluates density based on a two dimensional equation (g/cm^2) [116]. Lumbar spine areal BMD for patients with scoliosis is thus affected since straight positioning cannot be achieved [116]. This must be considered in patient with OI since scoliosis is frequent due to vertebral compression fractures [1, 9].

Analysis of the DXA images can also cause measurement discrepancies and radiology technicians should undergo routine evaluations and perform precision analysis trainings if needed [119]. Moreover, the reference group assessed to create z-score curves excluded children whose weight, height and/or body mass index fell outside the 3rd to 97th percentile range [117]. Therefore, erroneous values can arise for individuals who do not meet the reference populations' anthropometric criteria [116]. This may be an issue for OI children for whom height, weight and consequently body mass index is often compromised by smaller bones and vertebral fractures. One study showed 90% (27/30) of OI patients assessed had heights below the 3rd percentile according to their sex and age [10].

1.4.8 Peripheral quantitative computed tomography

Peripheral quantitative computed tomography (pQCT) is a bone densitometry measurement that allows for true volumetric BMD (g/cm^3 or mg/cm^3) to be assessed in both the radius and the tibia. This test, unlike the DXA, provides three dimensional images of the bone through 360 degrees x-rays scans of the peripheral limb assessed. The pQCT scan takes a number of x-ray projections around either the forearm or the lower leg and reconstructs a slice of the limb as an image [94]. Subsequently, pQCT quantifies the attenuation of its single energy x-ray in order to determine bone mineral content as well as BMD [94, 120]. At the radius, the 4% and 66% sites of the bone length can be measured according to the reference line known either as the distal portion of the still open growth plate or as the middle of the ulnar border of the articular cartilage (Figure 6; p. 44) [121].

Although restricted to the forearm and lower leg, the pQCT test allows for bone compartments to be distinguished and measured separately. Hence, a three dimensional evaluation of cortical and trabecular bone can be conducted. Distinguishing between cortical and trabecular bone can allow for more precise information as to whether the treatment is affecting bone turnover or bone modeling. A compartmental distinction within bone allows for distinct assessments to be made proving especially advantageous when no overall BMD changes can be detected by DXA. Moreover, pQCT imaging provides further physiological information pertaining to muscle and fat tissues. Bone size and shape can also be evaluated through the use of this technique [121]. Detailed reference database have been established allowing for calculation of age- and sex- specific z-scores of volumetric BMD measurements conducted by pQCT [121].

1.4.9 Z-score

The DXA and the pQCT machines measure bone parameters such as BMD and compare it to measures of a large reference group rendering a score called the z-score [122]. The z-score is a measure that compares BMD and other bone parameters to those of a reference group of individuals whose age, sex and ethnicity are matched [117, 122]. Z-scores allow for comparisons of bone measurements to a healthy population thus indicative of the degree of severity with which a person differs from the healthy norm. In 2007, pediatric reference data for DXA bone measurements were obtained from a sample of 1335 American children thus providing the first large scale ethnic-specific pediatric reference values [117]. Z-score is measured as such,

$$\mathbf{Z\text{-score}} = \frac{x - \bar{x}}{s}$$

where \bar{x} is the population mean and s is the populations' standard deviation

$$\mathbf{Z\text{-score}} = \frac{\text{BMD value obtained} - \text{mean BMD value for sex, age and ethnicity matched population}}{\text{standard deviation of the distribution of same population}}$$

Children below the 3rd percentile or above the 97th percentile for height, weight or body mass index are excluded from the reference group [117]. A BMD z-score of 0 means that the person's BMD is at the median of that population's BMD. The z-score curves include values from +2 to -2 which respectively represent the 97th percentile and the 3rd percentile of measurements within that population [117]. Low bone density for chronologic age according to sex and ethnic group are diagnosed with z-score values below -2 [119, 120]. In a recent retrospective study conducted with 71 OI patients (ages 1.4 to 17.5 years), mean LS-aBMD z-scores were found to be -4.6 [24].

1.5 Musculoskeletal system

Bone, muscles, tendons and ligaments work together to create an entity referred to as the musculoskeletal system enabling movement and posture [123]. Tendons attach muscles to bone whereas ligaments attach bones to bones [123]. Three different types of muscle are distinguished including skeletal, smooth and cardiac muscle [123]. The three muscle groups are distinguished based on structure and functional capabilities [124]. Skeletal muscles are involved in the musculoskeletal system [124]. They are voluntary muscles as opposed to smooth muscles which are involuntary and controlled by the autonomic nervous system [124, 125]. The latter include all gastrointestinal tract muscles as well as muscles involved in the circulatory system [123, 124]. The cardiac muscle, as the name describes, include all muscles in the heart.

1.5.1 Muscle and bone interaction

The functional relationship between muscle and bone has provided researchers with an array of study avenues throughout life cycles including growth, development, and aging [126]. The mechanical stimuli that muscles impart upon bone tissue are physiologically undeniable, yet the nature of the stimuli that induces bone formation has yet to be determined with certainty [126].

Diminished muscle function has been shown to cause bone catabolism [126-128]. Disorders affecting muscle function correlate with low bone densities. Namely, Duchenne muscular dystrophy, a neuromuscular disorder causing skeletal muscle wasting and weakness, is known to induce low bone mass [128, 129]. The BMD of 36 affected boys was assessed over 7 years and showed a strong correlation to diminished muscle function [128]. Femur BMD mean z-scores were of -1.6 and progressively worsened to a mean of -3.9 as mobility decreased over time [128].

Increased immobilization due to fractures occurs in conditions such as OI and causes loss of muscle as well as bone mass [130]. Substantial bone loss during

immobilization is caused by increased bone resorption and decreased bone formation [131]. This period is referred to as bone remodeling transient and persists for 3 to 6 months in healthy children but has yet to be studied and established in children with OI [131]. However, secondary to their impaired bone mineralization, it can be assumed that this population would exhibit increased bone remodeling transient time. This issue is important since the decrease of BMD occurs at the fracture site but also at sites adjacent to the fracture [131]. Since bone growth depends on weight bearing and muscle pull, muscle mass will inevitably be impacted by immobilization [132]. A longitudinal study conducted with 8 to 14 year old children showed that peak lean body mass preceded peak BMD in both sexes [133]. This reinstates the functional relationship shared between muscle development and bone development, where the latter is most likely driven by the former [133]. Moreover, functional capabilities are affected by the loss of muscle and bone mass and might cause further immobilization [130]. Children with OI are thus recommended to participate in physical activity programs to optimize muscle strength and consequently bone strength [132].

1.5.2 Vitamin D and muscle interaction

The relationship between vitamin D and muscle metabolism has yet to be clearly defined. Vitamin D's active metabolite (1,25OH₂D) has been widely shown to act upon target tissues such as the intestine, the skeleton and parathyroid glands through activation of vitamin D receptors (VDR) [33, 125, 127]. However, researchers debate whether vitamin D affects muscle through direct (VDR receptor) or indirect mechanisms since contradictory results have surfaced regarding the presence of VDR in muscle cells [33, 125].

Whether through direct or indirect mechanisms, proof surrounding vitamin D's impact upon muscle function and development is quite strong. *In vivo* studies have been conducted in VDR gene-null mutant mice (VDR ^{-/-}) and muscle abnormalities were present with a significant 20% decrease in muscle cell diameter compared to that of

wild-type mice [134]. Independent of secondary mineral abnormalities, VDR (-/-) mice have smaller muscle fibers than VDR (+/+) mice at 3 weeks old and this abnormality progresses as studied until 8 weeks old [134]. Human studies assessing vitamin D deficiencies in youth have shown impaired motor function and development [135]. For example, in type I vitamin D resistant rickets distinct muscle weakness and delayed growth are present [135]. These patients are unable to convert vitamin D into its metabolically active form because of a mutation in the renal enzyme 25OHD-1- α -hydroxylase [135]. In these children, muscle weakness is readily reversed following supplementation with 1,25(OH)₂D, proving that this form of vitamin D is essential to adequate muscle function and development [135]. Moreover, a 1 year randomized controlled trial with vitamin D supplements showed a significant impact ($p \leq 0.05$) on lean body mass, obtained through DXA, in a group of 168 girls [34]. Serum concentrations of 25OHD have also been shown to positively correlate to musculoskeletal factors such as jumping height, muscle power and muscle force in adolescent girls [35].

1.5.3 Jumping mechanography

Clinical evaluation of muscle function is limited. Isometric force can be accessed through dynamometry yet this test is restricted to upper body strength and is representative of a muscle contraction seldom performed in everyday life [136, 137]. Isometric force is conducted when a muscle is activated but held at a constant length such as when carrying an object with arms stretched out [136]. As lean body mass and BMD are known to correlate, kinetic muscle analysis could help further investigate the physiological implications of muscle and bone interactions.

Jumping mechanography consists of a new clinical assessment tool aimed towards evaluating an individual's muscle function through ground reaction forces [35, 137]. A force plate is thus used to measure the forces involved in movement [138]. Jumping mechanography is based on Newton's third law of motion stating that for every action there is an equal and opposite reaction [138]. Therefore, ground reaction force is

the opposite and equal force the ground or force plate produces in response to the vector of force applied upon it [138]. Notably, we apply different degrees of force to the ground based on the impact of our exercise [138]. Hence, jumping mechanography enables more relevant measures of dynamic muscle force and power in comparison to isometric tests currently used [35, 137].

Force platforms are used to conduct jumping mechanography tests. Typical force platforms consist of two symmetrical plates allowing for distinct assessments of the right and left side [137]. The platform contains eight sensors, four under each plate, used to measure the vertical ground reaction forces [137]. The sensors' measurements are transferred for analysis by computerized software where muscle function can be determined according to force, power and velocity yielded per jump [137].

1.5.3.1 Assessment of muscle power in children with OI

A comparison study conducted with women suffering from hypovitaminosis D myopathy found the most affected muscles to be in the lower limbs [139]. Consequently, jumping mechanography provides sound clinical evaluation of vitamin D's impact on muscles required for locomotion [35]. Jumping mechanography has also been found to evaluate a broader range of individuals with lesser physical capabilities [140]. Remarkably, elderly subjects whom were unable to conduct the chair rise test were able to complete jumping mechanography [140]. Jumping mechanography therefore allows for muscle function assessments to be conducted in more severely affected individuals such as is the case in children with OI.

Prior to establishing jumping mechanography for clinical use, reproducibility of measures was assessed in healthy youth and adults during an test-retest evaluation conducted seven days apart [137]. Moreover, specific test procedures were detailed and described for forthcoming research implementation [137]. In one particular study, five mechanographic tests were described including *Multiple Two-Legged Hopping*,

Multiple One-Legged Hopping, Single Two-Legged Jump, Heel Rise Test and Chair Rise Test [137]. The coefficient of variation was used to measure variability of the jumps performed. *Multiple Two-Legged Hopping's* and *Multiple One-Legged Hopping's* main outcome parameter was force measurements and were found to produce a coefficient of variation between 4.2% and 6.4% for children conducting inter-day jumping mechanography [137]. On the other hand, tests aimed towards evaluating power (*Single Two-Legged Jump, Heel Rise Test and Chair Rise Test*) rendered coefficients of variation between 3.4% and 15.6%. Conclusively, the results obtained in this reproducibility study showed low variability between inter-day test-retest therefore proving the described procedure is appropriate for muscle assessments in clinical settings [137].

Jumping mechanography can be utilized to assess the impact of vitamin D supplementation on muscle in children affected by varying degrees of OI. Since hypovitaminosis D myopathy has been found to affected muscles in the lower limbs, jumping mechanography can be conducted to clinically evaluate vitamin D's impact on muscles [139]. Objective analysis of changes in individual's muscle power can be measured at baseline and at the end of the study through use of the *Heel Rise Test* and the *Single Two-Legged Jump*.

Tables and Figures

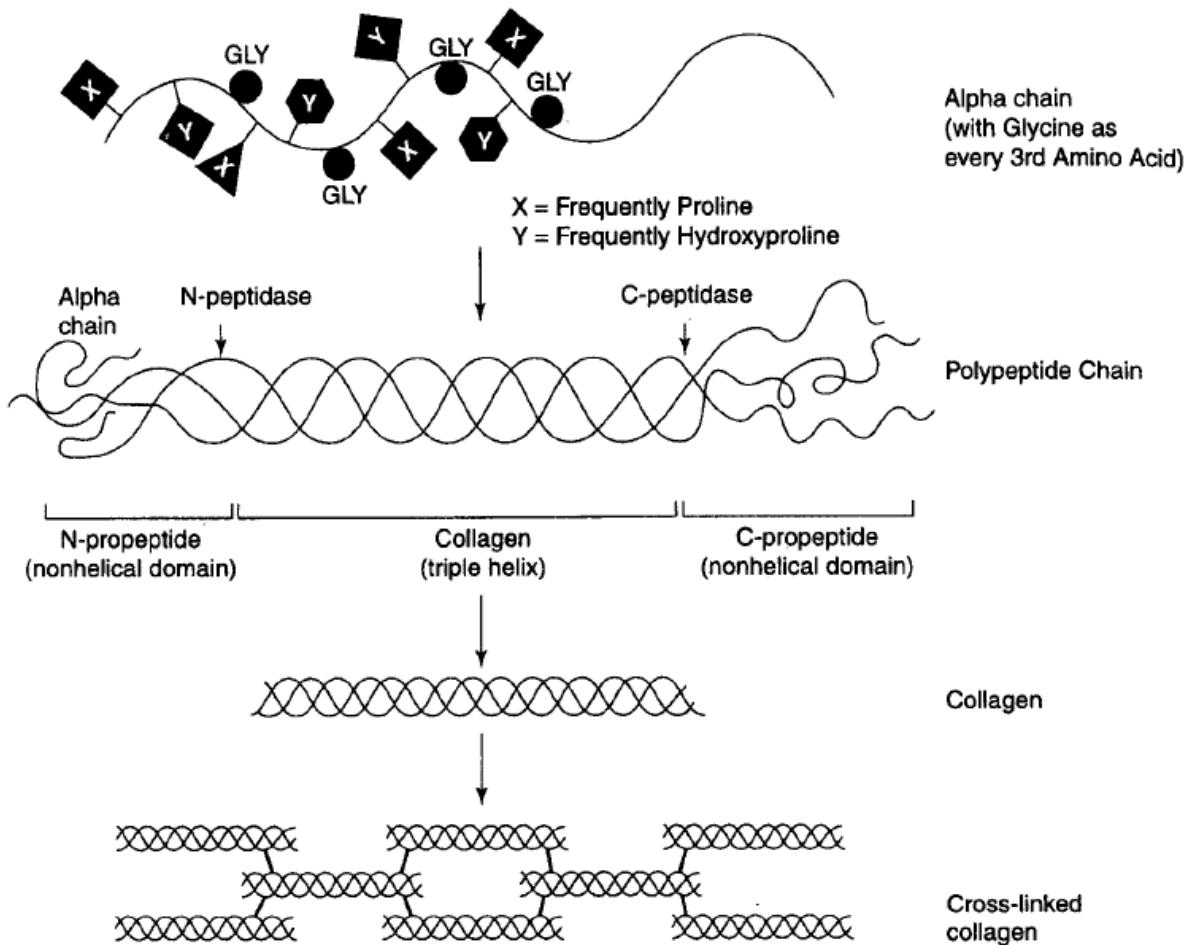


Figure 1. Formation of type I collagen matrix. The α chains form individually; they then aggregate into triple helices with propeptide chains on either end. During triple helix formation, the collagen is postranslationally modified, the propeptide chains are cleaved and the fully formed triple helix is incorporated into the collagen matrix by cross-linking with other collagen triple helices. It is the regularity of the helices and their placement with cross-linking into the matrix that gives the tissues their strength. Reproduced, with permission, from [141]

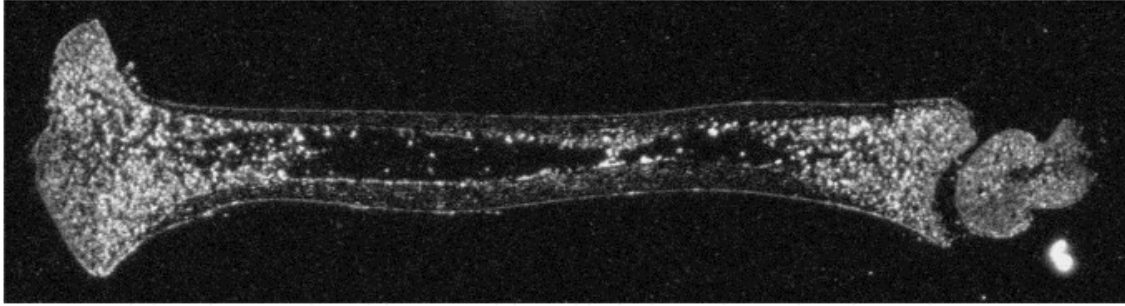


Figure 2. Affinity of bisphosphonates for trabecular bone as they attach to bone mineral. Autoradioluminograph of the tibia (longitudinal section) at 96 h after a 15 min IV infusion of 0.15mg/kg zoledronic acid to skeletally mature male dogs; whiter area corresponds to higher levels of radioactivity. Reproduced, with permission, from [55]

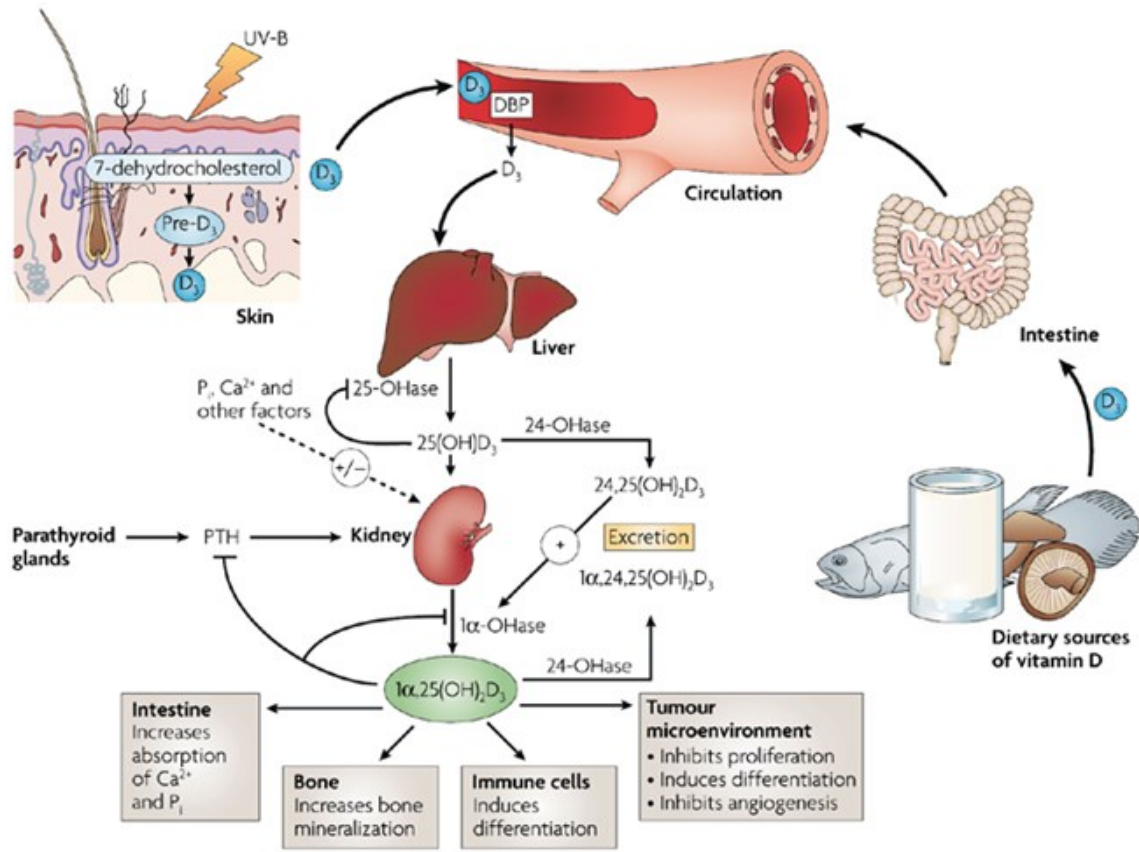


Figure 3. Endogenous and exogenous synthesis of vitamin D₃. Endogenous synthesis of vitamin D₃ (cholecalciferol, D₃) occurs cutaneously where pro-vitamin D₃ (7-dehydrocholesterol) is converted to pre-vitamin D₃ (pre-D₃) in response to ultraviolet B (sunlight) exposure. Vitamin D₃, obtained from the isomerization of pre-vitamin D₃ in the epidermal basal layers or intestinal absorption of natural and fortified foods and supplements, binds to vitamin D-binding protein (DBP) in the bloodstream, and is transported to the liver. D₃ is hydroxylated by liver 25-hydroxylases (25-OHase). The resultant 25-hydroxycholecalciferol (25(OH)D₃) is 1α-hydroxylated in the kidney by 25-hydroxyvitamin D₃-1α-hydroxylase (1α-OHase). This yields the active 1α,25(OH)₂D₃ (calcitriol), which has different effects on various target tissues. The synthesis of 1α,25(OH)₂D₃ from 25(OH)D₃ is stimulated by parathyroid hormone (PTH) and suppressed by Ca²⁺, P_i and 1α,25(OH)₂D₃ itself. Reproduced, with rights, from [142]

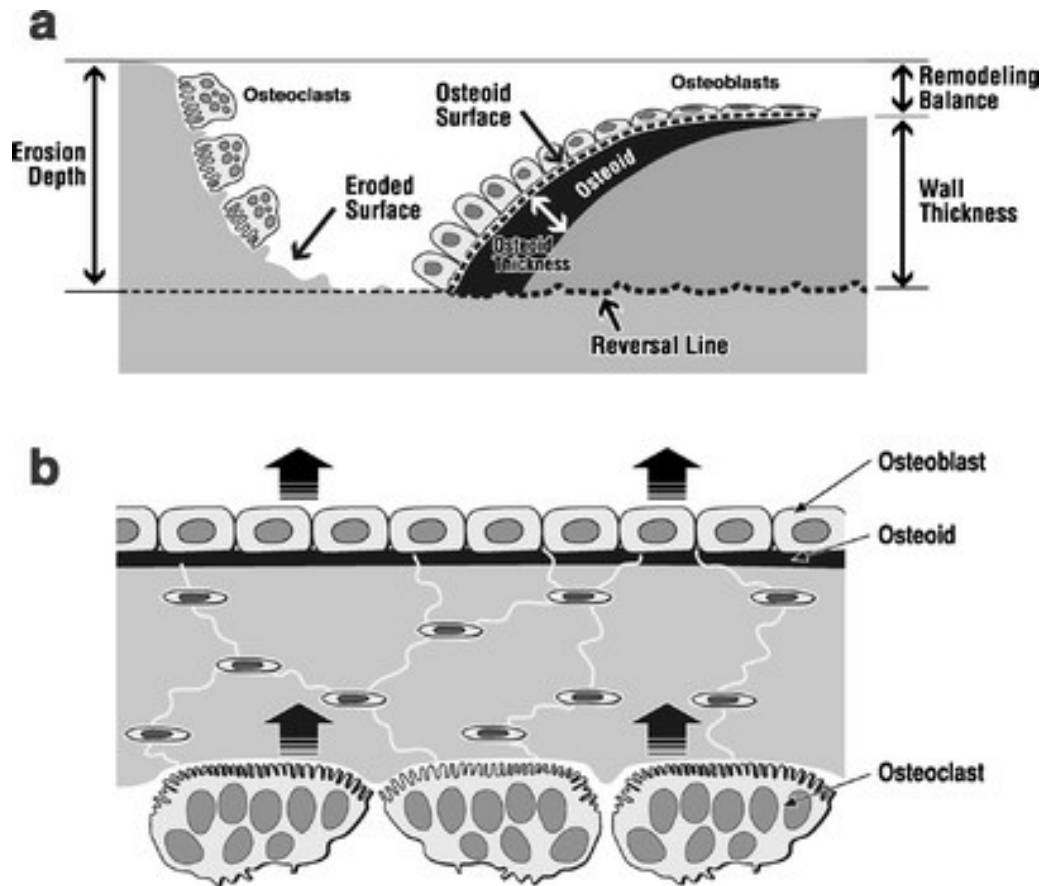


Figure 4. **a** Lateral view of a remodeling site in trabecular bone. Osteoclasts in the front dig a trench across the bone surface, which is then refilled by a team of osteoblasts. **b** Modeling site. Osteoblasts and osteoclasts are located on opposite sides of a bone cortex. As indicated by the *arrows*, osteoblasts add bone to the upper surface, whereas osteoclasts remove bone from the lower surface. Thus, the piece of bone in this example is moving upwards. The thickness of the cortex will increase if osteoblasts add more bone than the osteoclasts remove. Reproduced, with permission, from [143]

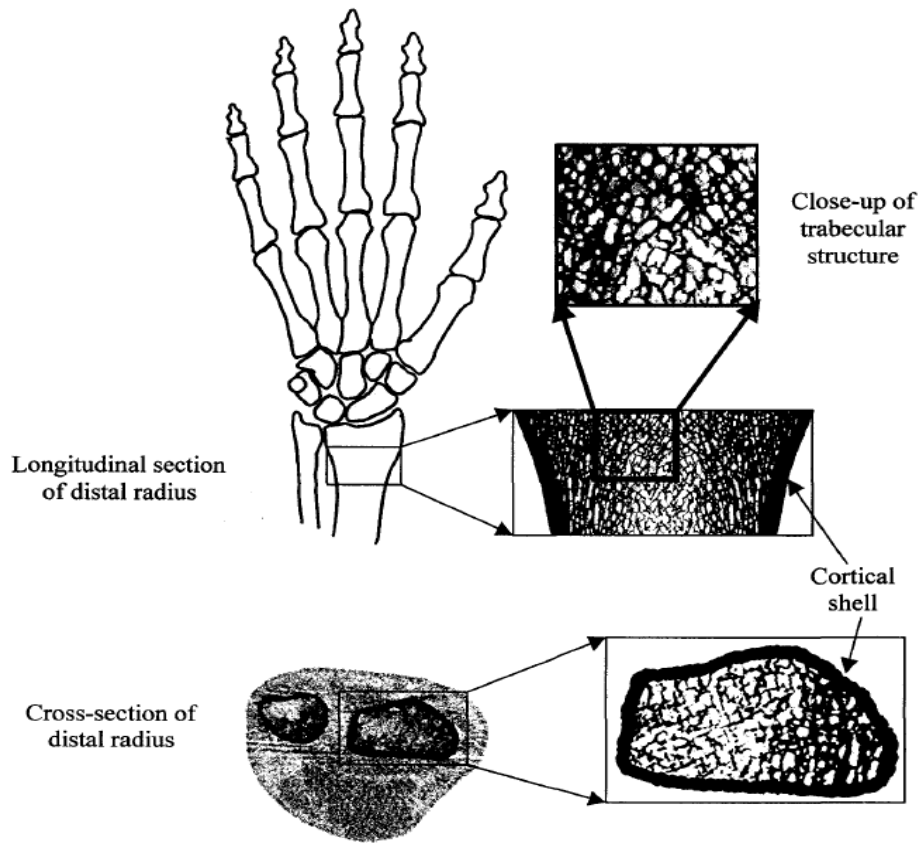


Figure 5. Cortical and trabecular bone at the distal radius in longitudinal and cross-sections. Reproduced, with permission, from [94]

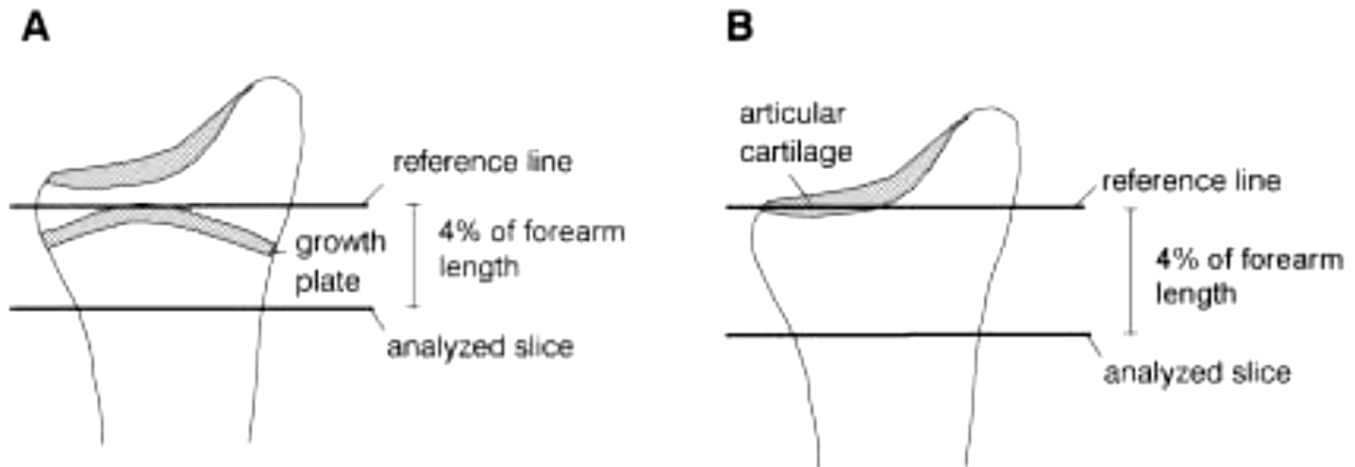


Figure 6. Schematic representation of the distal radius. PQCT is performed at a site whose distance to the "reference line" corresponds either to 4% or 66% of forearm length. The reference line is selected as follows. A. When the growth plate is still open, the reference line is drawn through the most distal portion of the growth plate. B. When the growth plate is no longer visible, the reference line is drawn through the middle of the ulnar border of the articular cartilage. Reproduced, with permission, from [121]

2. RATIONALE AND OBJECTIVES

Osteogenesis imperfecta (OI) is a genetic disease characterized by low bone mineral density (BMD) and increased risk of fractures. Since vitamin D is essential to bone mineralization, examining the impact of serum 25-hydroxyvitamin D (25OHD) concentrations on BMD in children with OI is highly relevant. Rationale for this study stems from the clinical need to address nutritional recommendations for children and adolescents with OI particularly in regard to their vitamin D status. Studies evaluating the impact of vitamin D supplementation on BMD have shown significant change in lumbar spine areal BMD (LS-aBMD) in healthy children with baseline 25OHD concentrations below 50 nmol/L. Since patients with OI have been found to present with suboptimal serum 25OHD concentrations, the potential for increase in BMD could be significant. Higher BMD helps reduce the incidence of fractures in patients and in mouse models with OI. To date, studies examining vitamin D status in children with OI have been retrospective or cross-sectional only. In addition to its impact on bone, vitamin D also has a positive influence on muscle mass which may improve postural stability therefore reducing the risk of fractures. A high dose of 2000 international units (IU) of vitamin D is safe and has been shown to help reduce fracture risk in adults whereas 400 IU is the standard dose used by the study population.

The objectives of this one-year randomized, double-blind study are to: 1) assess the difference between a supplementation of 2000 and 400 IU of vitamin D on LS-aBMD in young patients with OI, 2) assess trabecular and cortical changes in BMD at the radius, and 3) evaluate changes in neuromuscular function through jumping mechanography after one-year of vitamin D supplementation.

The results gathered from this research will help determine optimal serum 25OHD concentrations for children with OI in regard to BMD accrual. This nutritional intervention, if proven effective, would provide a lifelong low-cost and highly accessible treatment plan for patients living with OI. Lastly, this study could serve as a foundation for optimal serum 25OHD concentrations in related bone disorders.

3. MANUSCRIPT

Effect of High-Dose Vitamin D Supplementation on Bone Density in Youth with Osteogenesis Imperfecta: A Randomized Controlled Trial

Laura Plante¹, Louis-Nicolas Veilleux², Hope Weiler¹, Frank Rauch²

¹School of Dietetics and Human Nutrition, McGill University, Montreal

²Shriners Hospital for Children and Department of Pediatrics, McGill University,
Montreal, Quebec, Canada.

3.1 Abstract

Objectives: To assess whether high-dose vitamin D supplementation will result in significantly higher lumbar spine areal bone mineral density (LS-aBMD) z-scores in youth with osteogenesis imperfect (OI) after one-year; and to evaluate the effect of vitamin D supplementation on lower limb muscle power assessed through jumping mechanography.

Methods: Patients were randomized in equal number to receive either 400 or 2000 international units (IU) of vitamin D supplements and stratified according to baseline bisphosphonate treatment status and pubertal stage.

Study design: A one-year double blind randomized controlled trial conducted at the Shriners Hospital for Children in Montreal.

Results: At baseline, average serum 25-hydroxyvitamin D (25OHD) concentration was 65.6 nmol/L (SD 20.4) with no difference seen between treatment groups ($p=0.77$). Deficient serum 25OHD concentrations (<50 nmol/L) were measured in only 21% of patients at baseline. Supplementation resulted in higher serum 25OHD concentrations in almost all participants (90%) with significantly higher increases seen with 2000 IU (mean [95% C.I.] = 30.5 nmol/L [21.3; 39.6] vs 15.2 nmol/L [6.4; 24.1], $p= 0.02$). No significant changes were detected in BMD measurements or in lower limb muscle power between treatment groups from baseline to final visit.

Conclusions: Supplementation with either 400 or 2000 IU of vitamin D translates into significant increases in serum 25OHD concentrations in children with OI. However, increases in baseline serum 25OHD concentrations already within a healthy range (≥ 50 nmol/L) do not translate into increases in BMD z-scores in children with OI.

Keywords: Osteogenesis imperfecta, vitamin D, bone mineral density

Potential conflicts of interest: None

Trial Registration: This trial is registered at the U.S. National Institute of Health (ClinicalTrials.gov) #NCT01713231 and Health Canada approved protocol # A02-M14-12A and control #155695

Funding: Funding for this study was provided by The Shriners Hospital as well as the Network for Oral and Health Bone Research

3.2 Introduction

Osteogenesis imperfecta (OI) is the most common primary bone fragility disorder affecting youth [1]. This genetic disease is characterized by low bone mineral density (BMD) and an increased risk of fractures [144]. Previous studies have shown widespread deficiencies in serum 25-hydroxyvitamin D (25OHD) concentrations in this population [24, 25, 27, 30, 32]; thus the importance of examining the impact of serum 25OHD concentrations on BMD in children with OI.

It is well established that vitamin D plays an essential role in achieving adequate bone mineralization namely through its central role in calcium homeostasis [75]. Vitamin D status is best measured by the serum concentration of 25OHD which accounts for both endogenous and exogenous sources of the vitamin [20]. Although vitamin D is an important marker of bone health, no clear recommendations have been made regarding ideal serum 25OHD concentrations in children with bone fragility disorders. General recommendations for optimal serum 25OHD concentrations range from 50 to 75 nmol/L but concentrations between 75 and 110 nmol/L have been shown to best prevent fractures in adults [21-23, 145]. A high dose of 2000 international units (IU) of vitamin D per day is required to meet the latter serum 25OHD concentrations and has been shown to be safe [34, 146]. The standard dose taken by the study population is 400 IU per day.

Randomized controlled trials of vitamin D supplementation in healthy youth with serum 25OHD concentrations above 50 nmol/L have not shown significant improvements on bone density outcomes [28]. However, it is speculated that vitamin D supplementation may be clinically warranted in children with deficient serum 25OHD concentrations [28, 29]. However, previous OI population based retrospective studies have failed to report a relationship between serum 25OHD concentrations and histomorphometric measures of bone mass, metabolism and mineralization [24]. More recently however, serum 25OHD concentrations in children with OI have been positively correlated with lumbar spine areal BMD (LS-aBMD) z-scores [32]. Retrospective data collection showed that for

every 1 nmol/L increase in serum 25OHD concentration, LS-aBMD z-score increased by 0.008 in children with mild to severe OI types (I, III and IV) [32].

The purpose of this study was to evaluate the efficacy of high-dose vitamin D supplementation on bone BMD measures in children with OI through a one-year randomized, double-blind study. The primary objective of the study was to investigate the changes in LS-aBMD z-scores; the secondary objective was to investigate changes in cortical and trabecular BMD z-scores as well as evaluate the effect of a yearly vitamin D supplementation on lower limb muscle power assessed through jumping mechanography.

3.3 Materials and methods

3.3.1 Subjects

Study participants were recruited from the Shriners Hospital for Children in Montreal between September 2012 and June 2013 for this one-year randomized, double blind study. Patients of both sexes were eligible if they were between 6 and 19 years of age and were genetically diagnosed with OI of any type (I, III, IV, V or VI). Patients were ineligible if BMD measurement of the lumbar spine was impossible and if receiving bisphosphonate treatments for less than two years. Pregnancy at any time during the study was also exclusionary. Any disease, disorder or medication use known to interfere with 25OHD metabolism was also an exclusion factor. Such medications include active vitamin D metabolites, anti-epileptics, corticosteroids and thyroid hormones. Moreover, renal and liver diseases known to interfere with vitamin D metabolism as well as any known calcium or phosphate metabolism disorder were grounds for exclusion. Of the 89 patients assessed, 11 did not meet inclusion criteria and 18 declined participation (Figure 1; p. 61). In the end, 60 patients were randomized. The study was pre-approved by McGill University's Institutional Review Board. Signed informed consents were obtained from parents of children below 18 years of age, and assents were obtained from children over 8 years of age.

3.3.2 Treatment protocol and follow-up

Patients were randomized in equal number to receive either 400 IU per day or 2000 IU per day of vitamin D (in the form of D₃) supplements and stratified according to baseline bisphosphonate treatment status and pubertal stage. A laboratory employee, not otherwise involved in the study, used a computerized randomization list to generate patient allocation. This employee then provided the study coordinator with the fitting vitamin D pills to be given to each participant resulting in a double blind study. Tablet form supplements were of similar size, taste and appearance and were provided by the same manufacturer (Jamieson Inc.). In-person study visits occurred at baseline and at final visit (Appendix 1; p.76) and phone follow-ups were conducted every 3 months collecting information on adverse events, concomitant medication and fractures. Unused vitamin D pills were collected at final visit allowing for assessment of compliance. Compliance rate was calculated by dividing the number of vitamin D pills consumed by the total number of days in between study visits. All patients received standard medical care by means of clinical assessments including radiographic and physical examinations during the study. Patients taking other forms of vitamin D supplements were asked to stop for the study period. Any additional intake of vitamin D through multivitamin use was accounted for in our intake assessments.

The primary efficacy variable was the change in lumbar spine (L₁-L₄) areal BMD z-score. Secondary efficacy variables were changes in cortical and trabecular BMD z-scores at the radius, as well as changes in serum 25OHD, PTH and collagen type I C-telopeptide (CTx) relative to baseline. As an exploratory outcome, percent change in lower extremity muscle power per body mass was also assessed. A variety of clinical and biochemical variables were also measured.

3.3.3 Anthropometric measurements

Each visit included anthropometric measurements. Height was measured with a Harpenden stadiometer (Holtain). Height and weight measurements were converted to age- and sex- specific z-scores based on published reference data by the Center for Disease Control and Prevention [147].

3.3.4 Radiological studies

Quality control on the DXA is performed daily with the use of *spine phantom* provided by the manufacturer prior to conducting any measurement. Bone densitometry was performed in the antero-posterior direction at the lumbar spine (L₁-L₄) by DXA at baseline and at final visit (QDR Discovery, Hologic Inc., Waltham, MA, USA, software version 12.3). Areal BMD results were converted to age- and sex- specific z-scores using data provided by the manufacturer. These were based on the studies of Glastre et al. [148] and Southard et al. [149] comprising a total of 353 children and adolescents.

Measurements of cortical and trabecular bone mass were obtained through pQCT of the nondominant forearm (XCT2000, Stratec Inc., Pforzheim, Germany). Again, quality control is conducted daily on the pQCT machine through the use of a phantom forearm prior to conducting any measurements. The dominant forearm was measured if the patient had intramedullary rodding on the nondominant side. Two measurement sites were assessed representing metaphyseal (4% site) and diaphyseal (65% site) bone. Measurements were converted to age- and sex- specific z-scores based on pediatric reference data published by Rauch and Schönau [121, 150]. pQCT scans were analyzed by study PI and eliminated from analysis if movement artifacts were believed to impact measurement values.

3.3.5 Biochemical analyses

Blood samples were collected between 7:30 and 10 am at baseline and every 6 months thereafter following an overnight fast. Serum bone markers were measured in the Bone Marker Laboratory of the Shriners hospital for Children in Montreal. Serum concentrations of pro collagen type I N-terminal propeptide (PINP), CTX and serum 25OHD concentrations were analysed by the IDS-iSYS automated analyser at the Shriners hospital for Children in Montreal (Immunodiagnostics Systems, Scottsdale, AZ). Quality control kits are supplied by manufacturer and run before and after every measurement with low, medium and high standards for each blood parameter. Also, internal pool controls are created and run similarly for which results are tracked for changes over time and adjustments are sought when needed. Serum PTH was determined by RIA (Diasorin, Stillwater, MN). Serum phosphorus, creatinine, bone specific alkaline phosphatase as well as ionized and total calcium values were determined by standard methods at St-Mary's Hospital Laboratory which performs routine laboratory work for the Shriners hospital for Children in Montreal. Biochemical measurements ensured no serious adverse event, although unlikely at vitamin D dosage provided, of hypercalcemia or hypercalciuria occurred (Table 2 and 3, p. 64-65) [145]. In some cases, blood samples were not obtained or were insufficient given the challenge of obtaining such samples in this population. This explains the differences in the final number of patients analysed per biochemical outcome (Table 3; p.65).

3.3.6 Dietary assessments

Dietary intake assessments were conducted with the parent and child helped by standardised food portions (Nasco, US) at the initial visit. Total intake of vitamin D was assessed by a registered dietitian (L.P.) completing validated food frequency questionnaires (FFQ) at baseline and every 6 months thereafter [151]. Additional vitamin D supplements were accounted for in dietary analyses. Also, 24 hour diet recalls were completed over the phone at 3 and 9 months according to the multiple pass method shown to be the most accurate method to estimate children's total energy intake

[152]. Nutrient analyses were estimated using the Nutritionist Pro Software (Axxya systems, Stafford, TX) based on either the Canadian Nutrient File 2010b database or the United States Department of Agriculture database according to each participant's country of residence. This distinction is essential since vitamin D fortification regulations differ between both countries.

3.3.7 Mechanography

A ground reaction force plate (Leonardo Mechanograph Ground Reaction Force Plate, Novotec Medical GmbH, Pforzheim, Germany) was utilized to assess muscle power. A *Single Two-Legged Jump* and a *Heel Rise Test* were conducted with all able study participants. Patients having suffered lower limb fractures in the past 6 months were excluded from conducting these tests (n= 15 at baseline and n= 12 at final visit). The primary outcome parameter for both jumps was peak power relative to body mass. Each jump was repeated three times and the most successful try, thus the jump with the highest peak power measured, was analysed by computerized software (Leonardo Mechanography GRFP Research Edition software, version 4.2-b05.53-RES, Novotec Medical Inc.).

3.3.8 Statistical analysis

The required size of the study population was calculated on the hypothesis that supplementation with oral vitamin D₃ would render lumbar spine areal BMD z-score changes equivalent to one fifth of those seen with oral bisphosphonate treatment (SD 1)[15]. Assuming a 20% dropout rate, an alpha error of 5% and a power of 80%, 60 patients were required to detect changes in lumbar spine areal BMD z-scores of 0.2 (SD 0.2) between treatment groups.

Comparisons between treatment groups were based on an intent-to-treat analysis. In case of missing data, the last observation was carried forward. In comparing groups at

baseline, two-sample *t*-tests and χ^2 tests were used. Change from baseline in LS-aBMD z-score between treatment groups was obtained by an analysis of covariance model with treatment as a factor and baseline result as a covariate. All changes in z-score radiology measures were assessed by ANOVA with baseline result as a covariate. The percent changes for biochemical values from baseline to 1 year were obtained through ANOVA tests with sex, age and baseline result as covariates. A 5% significance level was maintained throughout analyses and all tests were two-sided. All data was verified validated prior to analysis and screened for outliers using the outlier labelling rule [153]. No data was deemed a valid deletion. Statistical calculations were conducted using SPSS software (version 22.0, SPSS Inc., Chicago, IL, USA). Adverse events and concomitant medications taken during study period were tabulated.

3.4 Results

Sixty patients were randomized (Figure 1; p.61). Most clinical characteristics at baseline were similar between groups (Tables 1 & 2; p.63-64). Overall, 72 % of participants were Canadian citizens. However, a higher proportion of female patients were randomized to the 400 IU treatment group ($p=0.07$). Sex was thus used as a covariate in order to control for the difference in distribution found between treatment groups. Compliance rates of 63% and 71% were observed in 400 IU treatment group and 2000 IU treatment group, respectively. Both treatment groups saw two patients withdraw from the study due to loss at follow up ($n=1$ in the 2000 IU group, $n=2$ in the 400 IU group) or consent withdrawal ($n=1$ in the 2000 IU group) (Figure 1; p.61).

3.4.1 Biochemistry

Almost all study participants (90%) had higher 25OHD serum concentrations after one-year of treatment (Figure 2; p.62). However, mean increases of serum 25OHD concentrations were twice as high in the 2000 IU group (mean [95% C.I.] = 30.5 nmol/L [21.3; 39.6]) compared to the 400 IU group (15.2 nmol/L [6.4; 24.1]) ($p=0.02$). No other

serum bone markers showed significant changes with regard to the different treatment groups (Table 3; p.65).

3.4.2 Radiology

Mean increases in lumbar spine areal BMD z-scores were nil in the 400 IU treatment group but increased by 0.1 in the 2000 IU treatment group ($p=0.63$). No significant difference between the 2000 IU and the 400 IU groups were detected for changes in DXA parameters for the lumbar spine, as well as results for pQCT at the radial metaphysis and diaphysis (Table 3; p.65). Cortical bone cross-sectional area and cortical thickness z-scores decreased less in the 2000 IU group (mean -0.1) than they did in the 400 IU group (mean -0.4). Similarly, total bone mineral content at the diaphysis showed no mean z-score changes in the 2000 IU group opposing the negative mean changes seen in the 400 IU group ($p=0.07$).

3.4.3 Dietary analyses

At baseline total vitamin D intake, including diet and supplementation, was similar between treatment groups (Table 2; p.64). After treatment, as expected, 95% of the third tertile for change in total vitamin D intake was made up of children in the 2000 IU group (Table 3; p.65). Also, positive change in total vitamin D intake (FFQ) was significantly greater in the 2000 IU group than in the 400 IU group amongst children in the second tertile ($p=0.04$). The Bland-Altman plot compared dietary vitamin D intake agreement between FFQ and 24 hour recalls showing a positive mean difference indicating an overestimation by FFQ (Figure 3; p.66).

Dietary assessments through 24 hour recalls showed similar results for vitamin D intake at 3 months (361 ± 219 in the 2000 IU group $n=24$ and 303 ± 206 in the 400 IU group $n=28$, $p=0.33$) and at 9 months (273 ± 191 for the 2000 IU group $n=25$ and 328 ± 279 for the 400 IU group $n=28$, $p=0.40$) (Appendix 2; p.77). As for calcium, mean intakes

were estimated at 917 mg daily as calculated through 24 hour recalls 901 ± 363 for the 2000 IU group (n=26) and 930 ± 346 for the 400 IU group (n=30) ($p=0.77$).

3.4.4 Mechanography

Treatment differences in the changes of jumping power were not statistically significant nor were changes in muscle cross-sectional area (Table 3; p.65).

3.4.5 Clinical adverse events

No cases of hypercalcemia or hypercalciuria occurred. No other serious adverse events related to treatment were noted. The most common adverse event reported was headaches and frequency was similar in both treatment groups (n=2 taking 2000 IU, n=3 taking 400 IU, $p=0.64$). Some patients reported a noticeable change in common cold frequency throughout the treatment year in comparison to previous years (n=2 taking 2000 IU, n=3 taking 400 IU, $p=0.64$). Twelve patients in the 2000 IU group and fifteen patients in the 400 IU group sustained at least one fracture during the study period. The total number of long-bone fractures was of 18 and 17 in groups of 2000 IU and 400 IU respectively. The number of long-bone fractures per patient ranged from 0 to 3 in the 2000 IU group and from 0 to 4 in the 400 IU group. No significant differences were found between treatment groups in regard to rate of radiographically confirmed long-bone fractures ($p=0.26$).

3.5 Discussion

In this study on children with moderate to severe OI, we found that daily oral vitamin D supplementation significantly increased serum 25OHD concentrations whether the supplement was of 400 IU or of 2000 IU ($p<0.001$). On the other hand, there was no significant effect of vitamin D supplementation on our primary outcome measure of LS-aBMD z-score. No changes were found in all other bone density measures explored. Similarly, randomized controlled trials of vitamin D supplementation conducted in

healthy youth have not shown increases in bone density measures [29, 34]. Nevertheless, additional observations can be drawn.

Overall, 21% of our patients had deficient (<50nmol/L) serum 25OHD concentrations at baseline, similar to previous findings in our study population (27%) [32]. Given that most baseline serum 25OHD concentrations were above 50 nmol/L, this study was conducted mainly in children with healthy vitamin D status. This may in part explain the lack of apparent effect of vitamin D supplementation on bone health parameters.

Separate analysis was completed on patients with inadequate baseline serum 25OHD concentrations (≤ 50 nmol/L) (n=6 in 2000 IU group, n=6 in 400 IU group). The sample size being too small to warrant a statistical analysis, the following results are based on descriptive analyses only. Significantly higher baseline PTH levels were observed (mean \pm SD: 2.9 ± 1.4) compared to the serum 25OHD sufficient patients (mean \pm SD: 2.0 ± 0.9) as is expected of the inverse relationship between these bone metabolism parameters (p=0.01). However, no significant changes in LS-aBMD z-score were found between treatments in the subgroup with inadequate baseline serum 25OHD concentrations (mean \pm SD: 1.1 ± 1.1 for the 2000 IU group vs -0.2 ± 1.1 in the 400 IU group, p=0.07). Previous randomized controlled trials have shown contradicting results with regard to the effect of vitamin D supplementation on changes in LS-aBMD in female children with mean baseline 25OHD concentrations below 50 nmol/L [154, 155]. Our participants with baseline deficient 25OHD concentrations showed significantly lower compliance (50%) compared to participants with sufficient baseline 25OHD concentrations (71%) (p=0.02). Nevertheless, mean increases in serum 25OHD concentrations were similar between baseline inadequate and sufficient serum 25OHD status patients. The lack of significant effect on BMD z-scores may be attributable to the low compliance rate or to the small sample size of the inadequate serum 25OHD concentrations subgroup.

Vitamin D consumption assessed through 24 hour recalls suggested averages above national intakes by children at both 3 month (330 ± 212 IU) and 9 month (302 ± 241 IU) collections. This dietary intake is significantly greater than that of healthy Canadian and

American youth shown to consume averages of 252 IU and 192 IU of vitamin D respectively [156, 157]. Data obtained through FFQ is known to overestimate intakes and was thus expressed as tertiles [151]. Nevertheless, the proportion of supplementation with vitamin D in our population is 23 % which is similar to national Canadian and American youth averages of respectively 23% and 25% [158, 159]. The discrepancy in regard to total dietary intake of vitamin D in this population compared to national averages is thus most likely attributable to the increased awareness of the importance of consuming daily dietary vitamin D sources in patients with a bone fragility disorder such as OI. According to dietary assessments of Canadians and Americans aged 2 and up, milk was shown to contribute to 44% of vitamin D's total daily intake [160]. Moreover, frequency of milk consumption has been shown to positively correlate with serum 25OHD concentrations in these populations as was seen in ours ($p=0.03$) [161, 162]. On average, 68% of our population consumed more than 1 portion of milk daily compared to 59% of Canadians doing so within the same age group [161]. Mean daily calcium intake in our population was 986 mg which falls within averages seen in Canadians of the same age (913 to 1287 mg) [163]. Adequate intakes of calcium were met by 67% of younger children and 17% of children older than 9 years of age. Large discrepancies in the percent of children meeting the recommended intakes are attributable to needs increasing from 800 mg to 1300 mg of calcium required to support bone growth in healthy adolescents [21]. Comparable intakes have been documented in Canadian children with the exception of males 9 to 18 years of age out of which 37-43% met their adequate intakes [163]. However, since children with OI are significantly smaller in size, calcium needs established for healthy children may not be suitable references.

The increased dietary intake of vitamin D does not translate into higher serum 25OHD concentrations. Our population had mean serum 25OHD concentrations (65.6 nmol/L) similar to averages found in the same age group in both Canadian (71.6 nmol/L) and American (67.0 nmol/L) national assessments [161, 162]. A possible explanation for the lack of higher serum 25OHD concentration in our study population is that endogenous synthesis of vitamin D is not contributing as much as it is in healthy children. Previous

studies have suggested this be due to our population's reduced sun exposure secondary to their restricted mobility [32]. Moreover, no differences in serum 25OHD concentrations were found between samples collected during or outside of endogenous vitamin D synthesising periods [164].

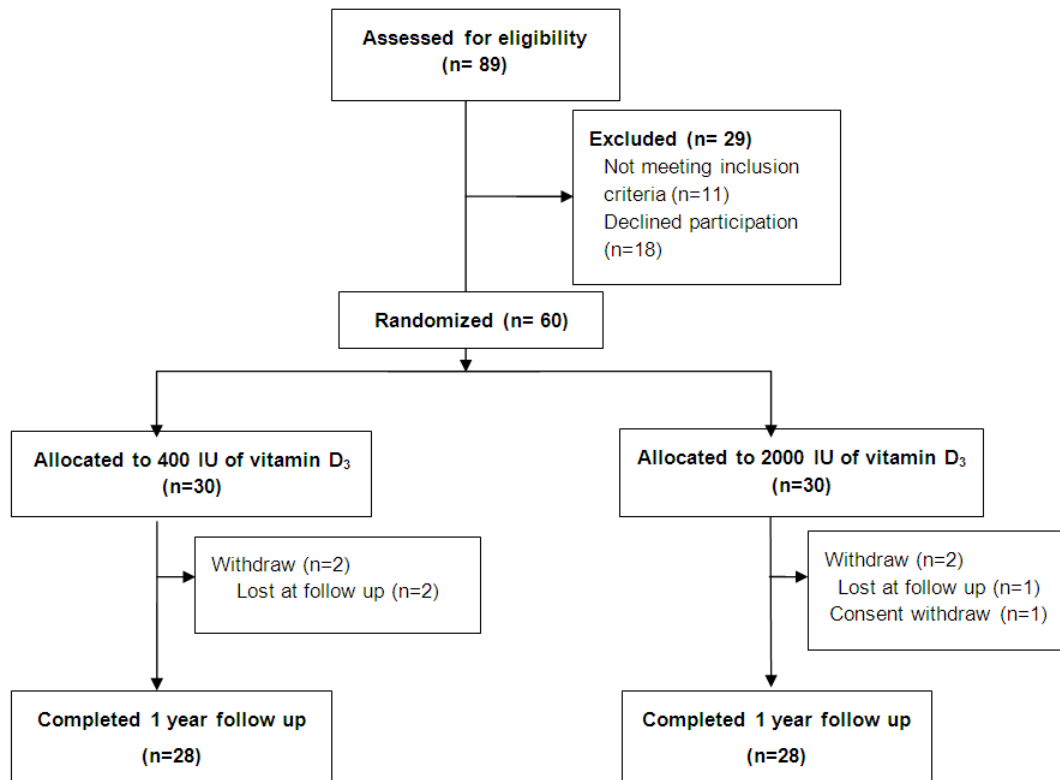
For this study, vitamin D was given in the form of D₃ and not D₂ since some studies suggest the former more effectively increases serum 25OHD concentrations [165, 166]. Previous research assessed the average increments on serum 25OHD concentrations in healthy children taking supplemental amounts of vitamin D over 3 months [167]. Baseline serum 25OHD concentrations were similar to our populations', yet healthy children taking 400 IU of vitamin D saw their serum 25OHD concentrations increase by a mean of only 5.5 nmol/L compared to our population's increase of 15.2 nmol/L. On the other hand, supplementation of 2000 IU saw an increment in serum 25OHD concentrations of 37.6 nmol/L compared to our population's 30.5 nmol/L increase. Overall compliance in this study averaged 52% compared to 67% in our study population. This leads us to question whether the underlying metabolism of 25OHD differs in children with OI compared to healthy children secondary to the disorder.

This randomized controlled trial was the first of its kind to assess the impact of high-dose vitamin D supplementation in children with OI. Very few adverse events were reported and supplementation of 2000 IU of daily vitamin D proved to be safe with no cases of hypercalcemia reported.

In conclusion, this one-year randomized controlled trial shows that supplementation of either 400 IU or 2000 IU of vitamin D translates into significant increases in serum 25OHD concentrations in children with OI. However, increases in baseline serum 25OHD concentrations already within a healthy range do not translate into improvements in BMD z-scores in children with OI.

Tables and Figures

Figure 1. CONSORT flow chart



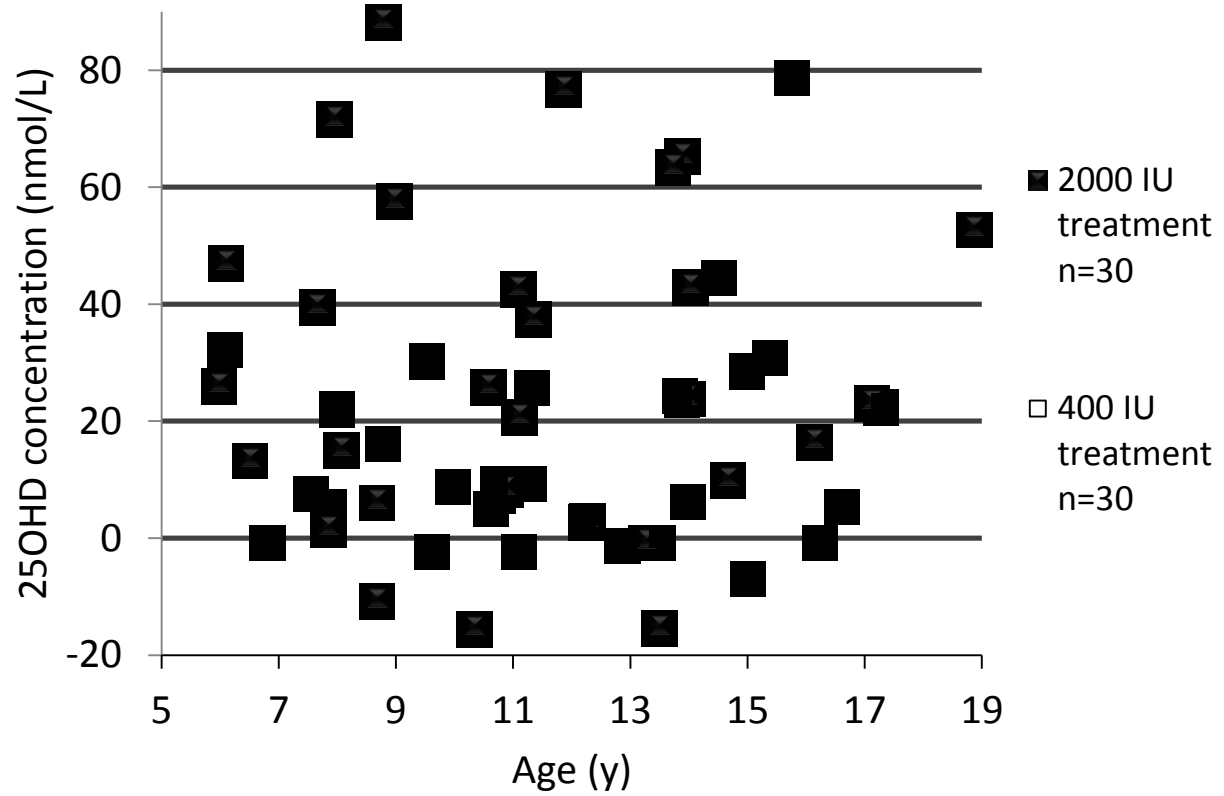


Figure 2. Changes in serum 25OHD concentrations from baseline to final visit in study population according to age.

Table 1. Clinical characteristics of population at baseline

Variable	400 IU (n=30)	2000 IU (n=30)	<i>p</i>
Male/Female	9/21	16/14	0.07
Pubertal stage (prepubertal/others)	12/18	13/17	0.79
Bisphosphonate status (treated/not treated)	22/8	21/9	0.77
OI type (I//IV/III,V&VI)	(12/11/7)	(11/14/5)	0.62
Age (y)	11.7 (3.3)	11.6 (3.3)	0.86
Height (z-score)	-3.0 (2.5)	-2.4 (2.3)	0.39
Weight (z-score)	-1.5 (1.5)	-0.7 (1.7)	0.07

Data are mean (SD)

Table 2. Baseline characteristics by treatment allocation

Variable	n	400 IU	n	2000 IU	<i>p</i>
DXA lumbar spine (L₁-L₄)					
aBMD (z-score)	30	-2.0 (1.1)	30	-2.1 (1.2)	0.64
BMC (z-score)	27	-1.3 (0.9)	28	-1.3 (1.1)	0.88
pQCT radial metaphysis (4%)					
Total cross-sectional area (z-score)	27	-0.8 (1.6)	26	-0.7 (1.6)	0.92
Total vBMD (z-score)	27	0.0 (1.9)	26	0.3 (1.8)	0.56
Total BMC (z-score)	27	-0.7 (1.5)	26	-0.5 (1.7)	0.50
Trabecular BMD (z-score)	27	-0.6 (1.9)	26	-0.2 (1.9)	0.44
pQCT radial diaphysis (65%)					
Total cross-sectional area (z-score)	25	-3.0 (1.4)	25	-2.9 (1.5)	0.92
Cortical cross-sectional area (z-score)	25	-1.1 (1.3)	25	-1.3 (1.4)	0.67
Cortical vBMD (z-score)	25	0.9 (1.6)	25	0.9 (1.5)	0.95
Total BMC (z-score)	25	-1.6 (1.3)	25	-1.6 (1.4)	0.92
Muscle cross-sectional area (z-score)	25	-1.4 (1.3)	25	-1.3 (1.7)	0.80
Serum biochemistry					
Ca (mmol/L)	30	2.38 (0.08)	29	2.42 (0.08)	0.08
Phosphorus (mmol/L)	30	1.56 (0.19)	29	1.57 (0.2)	0.81
Alkaline phosphatase (U/L)	30	189 (97)	29	205 (102)	0.52
25OHD (nmol/L)	30	66.4 (21.9)	28	64.7 (19.1)	0.77
PINP (µg/L)	29	200 (147)	29	266 (189)	0.14
CTX (ng/mL)	29	0.90 (0.53)	28	0.98 (0.52)	0.57
PTH (pmol/L)	27	2.3 (1)	25	2.1 (1.1)	0.70
Dietary analysis (Food and supplemental sources)					
Total vitamin D intake by FFQ (range), (IU/d)					
Tertile 1 (78-257)	10	183 (59)	10	185 (39)	0.92
Tertile 2 (287-528)	8	409 (74)	12	424 (88)	0.69
Tertile 3 (542-1190)	12	734 (179)	8	793 (170)	0.47
Mechanography tests (peak power per body mass, W/kg)					
Heel rise test	16	4.44 (2.16)	18	5.31 (2.16)	0.25
Single two-legged jump	11	27.3 (7.1)	11	34.3 (10.1)	0.07

Results are mean (SD). *P* values represent the significance of the difference between both groups (independent sample t-test)

Table 3. Changes after one-year of treatment

Variable	n	400 IU	n	2000 IU	<i>p</i>
Clinical					
Height (z-score)	30	0.2 (-0.1; 0.5)	30	0.0 (-0.3; 0.2)	0.33
Weight (z-score)	30	0.3 (-0.1; 0.6)	30	0.2 (-0.1; 0.6)	0.83
DXA lumbar spine (L₁-L₄)					
aBMD (z-score) ^a	30	0.0 (-0.1; 0.2)	30	0.1 (-0.1; 0.2)	0.63
BMC (z-score) ^a	27	0.0 (-0.2; 0.1)	27	0.0 (-0.1; 0.1)	0.70
pQCT radial metaphysis (4%)					
Total cross-sectional area (z-score) ^a	27	0.0 (-0.4; 0.4)	26	-0.2 (-0.6; 0.2)	0.57
Total vBMD (z-score) ^a	27	-0.4 (-0.8; 0.0)	26	-0.1 (-0.5; 0.3)	0.38
Total BMC (z-score) ^a	27	-0.3 (-0.7; 0.2)	26	-0.2 (-0.6; 0.2)	0.73
Trabecular vBMD (z-score) ^a	27	-1.0 (-1.7; -0.3)	26	-0.4 (-1.0; 0.3)	0.22
pQCT radial diaphysis (65%)					
Total cross-sectional area (z-score) ^a	25	0.1 (-0.2; 0.4)	25	0.1 (-0.2; 0.4)	0.98
Cortical cross-sectional area (z-score) ^a	25	-0.4 (-0.6; -0.1)	25	-0.1 (-0.3; 0.2)	0.07
Cortical vBMD (z-score) ^a	25	-0.2 (-0.6; 0.2)	25	0.0 (-0.4; 0.4)	0.53
Total BMC (z-score) ^a	25	-0.3 (-0.5; -0.0)	25	0.0 (-0.2; 0.3)	0.07
Muscle cross-sectional area (z-score) ^a	25	0.1 (-0.1; 0.4)	25	0.1 (-0.2; 0.4)	0.93
Serum biochemistry					
Ca (mmol/L) ^b	30	0.00 (-0.03; 0.03)	29	0.01 (-0.02; 0.04)	0.84
Phosphorus (mmol/L) ^b	30	-0.01 (-0.06; 0.03)	29	-0.02 (-0.07; 0.03)	0.83
Alkaline phosphatase (U/Liter) ^b	30	-4 (-15; 7)	29	-12 (-23; -1)	0.32
25OHD (nmol/L) ^b	30	15.2 (6.4; 24.1)	28	30.5 (21.3; 39.6)	0.02
PINP (µg/L) ^b	27	-4 (-38; 31)	26	-24 (-59; 11)	0.43
CTX (ng/mL) ^b	29	-0.03 (-0.14; 0.08)	28	0.02 (-0.10; 0.13)	0.58
PTH (pmol/L) ^b	27	0.0 (-0.3; 0.2)	25	-0.2 (-0.4; 0.1)	0.39
Dietary analysis (Food & supplemental sources)					
Total vitamin D intake by FFQ (range), (IU/d) ^b					
Tertile 1 (-1000-134)	13	-130 (-269; -1)	7	-240 (-430; -49)	0.40
Tertile 2 (167-900)	16	412 (288; 535)	4	700 (442; 959)	0.05
Tertile 3 (944-1902)	1	1070 (409; 1731)	19	1444 (1302; 1585)	0.26
Mechanography tests (peak power per body mass)					
Heel rise test (%) ^b	16	0.92 (-9.4; 11.2)	18	0.76 (-8.9; 10.4)	0.98
Single two-legged jump (%) ^b	11	6.1 (-0.5; 12.7)	11	-2.9 (-9.5 3.7)	0.07

Data are mean change with 95% CIs.

P values represent the significance of the difference between both groups (ANCOVA).

^aAdjusted for baseline result; ^badjusted for age, sex, and baseline result.

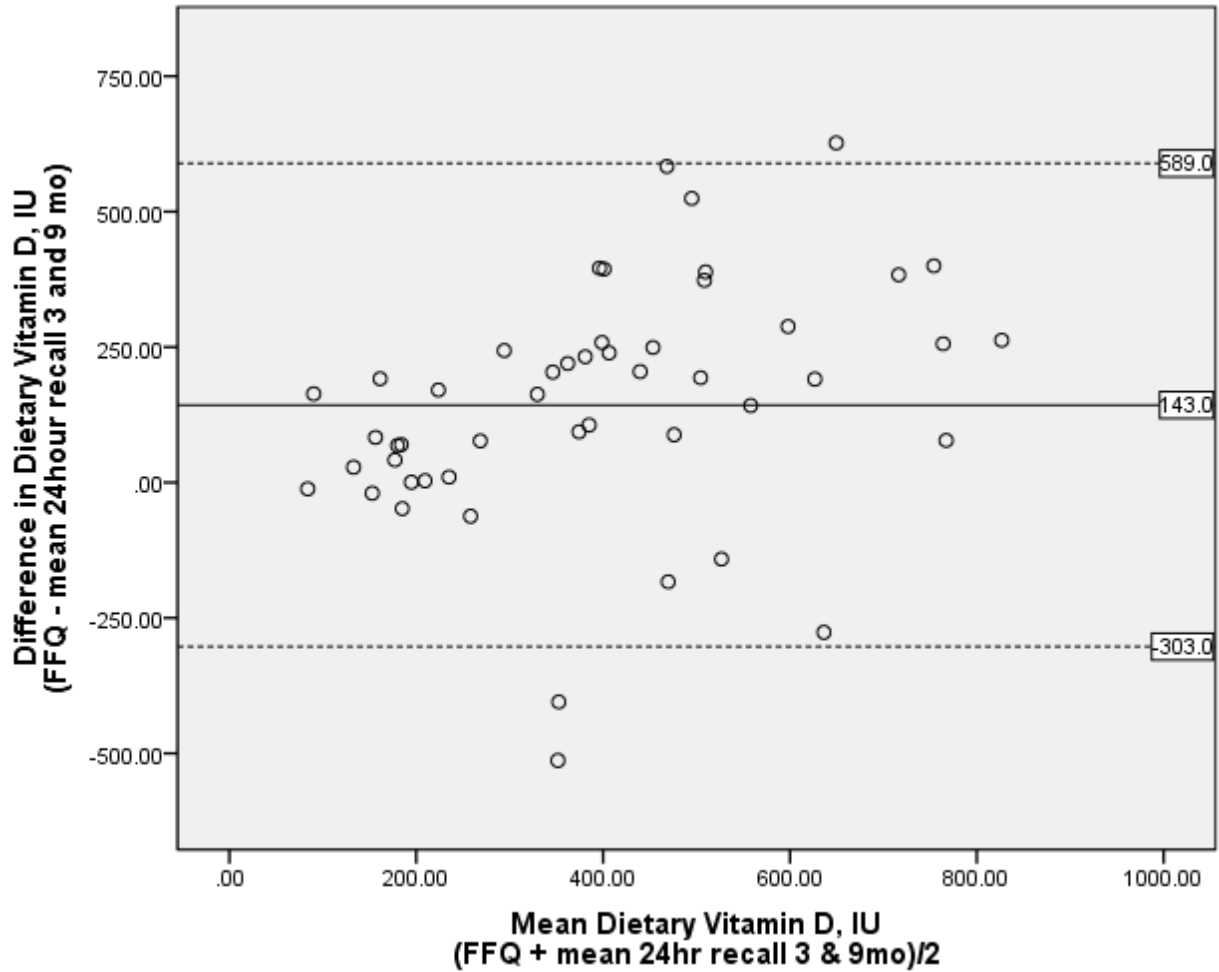


Figure 3: Bland-Altman plot showing agreement between FFQ and mean 24-h recalls at 3 and 9 months for dietary vitamin D intake. Solid line represents the mean difference; dashed lines are plus or minus 2 SD. Negative mean difference indicates overall underestimation by FFQ and positive mean difference indicates overall overestimation by FFQ, n= 49.

4. EXTENDED DISCUSSION

4.1 Findings/summary

A retrospective study conducted in this population showed increases in serum 25OHD concentration of 1 nmol/L to be correlated with increments of 0.008 in LS-aBMD z-score values [32]. To clarify these descriptive observations, the primary objective of this trial was to determine whether a high-dose supplement of oral vitamin D given over one year would have a significant impact on LS-aBMD in children with OI. It was hypothesised that increases in serum 25OHD concentrations would positively correlate with increases in z-score values of LS-aBMD. The secondary objectives of this thesis were to investigate changes in cortical and trabecular BMD z-scores as well as evaluate the effect of a yearly vitamin D supplementation on lower limb muscle power assessed through jumping mechanography.

Results of the present study showed average baseline serum 25OHD concentrations were above the recommended 50 nmol/L in both treatment groups (2000 IU: 66.4 ± 21.9 , 400 IU: 64.7 ± 19.1 nmol/L, $p=0.77$). After one year of supplementation, serum 25OHD concentrations showed significantly greater increases in the high-dose group compared to the low-dose group (mean [95% C.I.], 30.5 [21.3; 39.6] versus 15.2 [6.4; 24.1] nmol/L, $p=0.02$). Had the retrospective study's hypothesis held true, such increases in serum 25OHD concentrations would have yielded average increases in LS-aBMD z-scores of 0.24 in the 2000 IU group versus 0.12 in the 400 IU group. However, increases in serum 25OHD concentrations did not translate into the postulated changes in LS-aBMD z-scores in either treatment group (mean [95% C.I.], 0.1 [-0.1; 0.2] in the 2000 IU group versus 0.0 [-0.1; 0.2] in the 400 IU group, $p=0.63$). Furthermore, no significant changes were detected in cortical or trabecular BMD z-scores. Muscle power assessments showed no significant difference between treatment groups.

4.2 Sample size

The sample size used for this study was based on the knowledge provided by a retrospective study in this population showing that for every 1 nmol/L increase in serum 25OHD concentrations, LS-aBMD z-score values increase by 0.008. On average, supplementation with high-doses of vitamin D (2000 IU) in healthy children has resulted in increments of serum 25OHD concentrations ranging from 37 to 52 nmol/L [146, 167]. Assuming supplementation with high-dose vitamin D will have a similar effect on children with OI, an estimated change in LS-aBMD of 0.2 is achievable. This study was thus powered to detect a change in LS-aBMD z-score of 0.2 which is the equivalent to one fifth of the changes occasioned by bisphosphonate treatments [15].

In order to assess standard deviation of the z-score change, data from 110 OI patients meeting eligibility criteria from this study were analysed. Results showed standard deviation of z-score changes in LS-aBMD measurements over one year to be of 0.28. The power of this test was set to be at 80% and the alpha error at 0.05.

Therefore sample size calculation for desired margin of error was as follows,

$$n = \left(\frac{Z_{1-\alpha/2}}{2d} \right)^2 \frac{1}{p^*(1-p^*)}$$

However, p^* is a guessed value for the true proportion and chosen to be 0.5.

$$n = \left(\frac{Z_{1-\alpha/2}}{2d} \right)^2$$

with margin of error **d** to detect LS-aBMD z-score change difference of **0.2** between two groups

$$\alpha = 0.05$$

$$n = \left(\frac{1.96}{2 \cdot 0.2} \right)^2 = 24.01$$

Hence, 25 participants should be chosen in each group at an alpha error of 0.05 and a power of 80%. Study dropouts were accounted for at a rate of 20%, thus 30 participants were recruited for each treatment group in order to be able to detect significant effects of treatment and discrepancies between treatment groups.

4.3 Strengths and limitations

The randomized, double blind experimental design of this study ensured the highest level of evidence by controlling for potential biases throughout data collection and analysis. The Shriners Hospital for Children in Montreal follows the largest population of children diagnosed with OI in North America in part due to the initiation and discovery of successful bisphosphonate treatments to help increase BMD [10]. Therefore, the study setting enabled the recruitment of a large sample size of children with OI further strengthening this study.

The IDS-iSYS automated assay analyser is a suitable and accurate tool to measure serum 25OHD concentrations [168], and this important measurement being conducted in-house ensures adequate quality control measures were taken throughout the study and adjustments were sought as needed. The supplemental form of vitamin D₃ is fully detected by this assay, thus ensuring changes in serum 25OHD concentrations occasioned by our study treatment were detected.

The use of pQCT for volumetric BMD measurement analyzes also gives strength to this study by enabling the distinction between cortical and trabecular bone compartments. PQCT measurements allowed independent evaluations of bone size, shape as well as mineral density for which the impact of vitamin D has yet to be studied in this

population. Moreover, pQCT provided data on fat percentage of cross-sectional area measured shown to strongly correlation with total body percent fat [169]. This data therefore provided good estimates for total body composition in our study population.

Study results were evaluated with the intent to treat population which is a suitable application in pragmatic trials meaning that the effectiveness of vitamin D treatment was assessed in conjunction with routine clinical practice [170, 171]. Compliance was objectively assessed by means of pill count however some participants did not return bottles and thus data on compliance was missing (n=9). Similar studies have shown higher compliance rates ranging from 77% to 89% [29, 34, 154, 155]. Overall compliance did not differ between treatment groups (mean \pm SD, 2000 IU: 71 \pm 20% versus 400 IU: 63 \pm 30%, p=0.28), yet separate analysis showed compliance was significantly lower in patients with baseline serum 25OHD concentrations below 50 nmol/L (mean \pm SD, serum 25OHD \geq 50 nmol/L: 71 \pm 24% versus \leq 50 nmol/L: 50 \pm 30%, p=0.02). Exploring the subgroup with baseline inadequate 25OHD concentrations, we found that when controlling for compliance, age, gender and baseline values, changes in LS-aBDM z-scores were of -0.7 (-1.2; -0.1) in the 2000 IU group compared to 0.3 (0.0; 0.6) in the 400 IU group (p=0.03). This significant difference between groups is difficult to explain in such a small sample size (n=12). However, the fact that there were 2.5 times more females in the 400 IU subgroup (p=0.09) may have impacted the results since peak bone mass is attained in females and mean age was similar between groups (mean \pm SD, 2000 IU: 11.9 \pm 2.5 versus 400 IU: 12.9 \pm 1.8 years, p=0.45) [172]. The study was not powered to enable a subset gender analysis resulting in an inherent study weakness. Moreover, patients deficient in serum 25OHD concentrations have the potential to reap the most benefit from vitamin D supplementation on BMD outcomes as has been shown in healthy children [34, 154]. Future research could focus on patients with OI in whom serum 25OHD concentrations are below the recommended 50 nmol/L and stratify randomization based on sex as well as age.

The study treatment of two supplemental doses of vitamin D (2000 IU high-dose versus 400 IU low-dose) may have prevented the observation of significant changes between treatment groups. Since the low-dose treatment group saw no changes in our primary outcome, we can assume that the 400 IU supplementation merely helped maintain baseline values of LS-aBMD z-score. Perhaps the use of a placebo instead of the low-dose vitamin D would have resulted in a significant difference in our primary outcome of LS-aBMD z-score. The use of a high dose of 2000 IU of vitamin D was based on the fact that this dose had been shown to help prevent fractures in adults while showing no adverse events when taken by children [34, 145, 146]. It is important to note that the relationship between vitamin D intake and serum 25OHD concentrations is highly dependent on height and weight of individuals [146]. Thus, OI patients being typically smaller and lighter than their healthy counterparts, it was deemed safest not to exceed 2000 IU per day [173].

Future research could also focus on children during peak bone accrual years in order to evaluate the impact on changes in LS-aBMD z-scores as they are most significant during pubertal growth. Recruitment should thus be restricted to children in pubertal stages II to IV according to established Tanner stages [174-176]. Notably, statistically significant differences were found in changes of LS-aBMD z-scores when comparing prepubertal (Tanner stage I) to pubertal children (Tanner stages II-V) in our study ($p=0.02$). Mean (95% C.I.) changes in the primary outcome measure were of -0.2 (-0.4; 0.0) z-score in the prepubertal group compared to of 0.2 (0.1; 0.4) z-score in the pubertal group. No significant differences in BMD outcomes were found between treatment groups when looking only at pubertal children in this study. Nevertheless, restricting recruitment to pubertal children could help better distinguish differences in treatment outcomes.

4.3.1 Assessment of dietary intake

Utilizing the validated FFQ in conjunction with 24 hour dietary recalls to assess vitamin D intake further strengthened this study [151]. When completing dietary assessments, distinctions were made between Canadian and American children and calculations were made in accordance with each country's vitamin D fortified foods. The inclusion of 24 hour dietary recalls allowed us to corroborate the information gathered from FFQ and multiple assessments throughout the study allowed for tracking of seasonal dietary changes.

However, no information was gathered in regard to endogenous synthesis of vitamin D. Analyses did not show differences in average serum 25OHD concentrations between samples taken during or outside of endogenous vitamin D synthesizing periods. Data could have been collected with regard to time spent outdoors and skin exposure in order to obtain average estimates of vitamin D synthesis in our study population. Moreover, use of a reflectance spectrophotometer could further contribute to the assessment of endogenous vitamin D synthesis based on changes in skin color. Nonetheless, the study was planned over one year in order to control for each individual's endogenous synthesis of vitamin D contribution to serum 25OHD concentrations. While UVB exposure was not quantified, each participant acted as their own control with regard to seasonal effects on vitamin D status. Secondary to reduced mobility, it is hypothesised that children with OI spend less time outdoors than their healthy counterparts [32]. Fittingly, no difference was detected between serum 25OHD concentrations collected during or outside of endogenous synthesising periods (Appendix 1; p.76). Since endogenous synthesis of vitamin D may be limited in children with OI, intake through foods and supplements is fundamental in maintaining healthy serum 25OHD concentrations.

4.4 Relevance to the field of research

This high level of evidence study is the first to assess the impact of high-dose vitamin D supplementation on BMD in patients with OI. Although numerous studies report high prevalence of serum 25OHD concentrations below the recommended 50 nmol/L in children with OI [25, 27, 30, 32], only one study has looked at dietary intake of vitamin D in this population [27]. This study was cross-sectional and included only a total of 26 participants with OI (type I and III) whereas our study had a larger population size (n=60) and includes a broad array of OI types (I, III, IV, V and VI). Moreover, this study is the first to provide in depth dietary assessments of children with OI followed over one year. Although endogenous synthesis of vitamin D was not assessed, this study has shed light on the importance of diet and supplemental sources of vitamin D in order for children with OI to meet their daily needs.

4.5 Future research

This thesis has provided insight into the impact of high-dose vitamin D supplementation on measures of BMD in children with OI. Baseline serum 25OHD concentrations above 50 nmol/L followed by either 400 or 2000 IU of vitamin D supplementation for a year do not result in increased BMD measures in this population. Future research in this population should focus on children with serum 25OHD concentrations below the recommended 50 nmol/L at baseline. This could help set recommendations for target serum 25OHD concentrations in order to optimize BMD outcomes for children with OI. Longer studies could also help detect whether sustained higher serum 25OHD concentrations help improve BMD z-scores in this population. Since no adverse effects were seen in the high-dose vitamin D treatment group, higher supplementation could also be considered. Overall, this work supports clinical practice to achieve and maintain vitamin D status above 50 nmol/L of 25OHD in accordance with IOM recommendations.

The potential for future research in the field of nutrition in this population is great. Recently, significant differences in total body composition were found in type III OI patients whom showcase greater incidence of fat compared to other types of OI. Dietary assessments could help justify whether diet plays a role in the matter. According to dietary intake assessments conducted within this study, total caloric intake is similar between OI types (I, III and V) yet macronutrient distribution differs (Appendix 3;p.78). Further exploring dietary differences could help shed light on whether the physiological discrepancies between OI types can be partly attributed to dietary intake. In collaboration with body fat measurements through the pQCT, future studies could determine whether a correlation exists between dietary intake and total body fat in children with OI.

5. CONCLUSION

Vitamin D inadequacy, as defined by a serum 25OHD concentration below 50 nmol/L, was not found to be prevalent in our population of children with OI. Supplementation with either 400 or 2000 IU of vitamin D both resulted in significant increases in serum 25OHD concentrations; and high-dose vitamin D (2000 IU) was shown to be safe with no serious adverse events reported. Although high-dose vitamin D supplementation resulted in significantly higher serum 25OHD concentrations, it did not translate into greater increases in BMD measurements.

Nonetheless, serum 25OHD concentrations above 50 nmol/L are optimal for bone health in healthy children and should be maintained in youth with OI as well. Preventing vitamin D inadequacy in children with OI should be addressed through both dietary and supplemental intake.

6. APPENDIX

Appendix 1. Data collection throughout study

Data	Baseline	3 months	6 months	9 months	12 months
Biochemical (Serum and urine collection)	✓				✓
BMD (DXA and pQCT)	✓				✓
Jumping mechanography	✓				✓
Dietary intake (FFQ)	✓		✓		✓
Dietary intake (24 hour recall)		✓		✓	✓
Adverse events and concomitant medications		✓	✓	✓	✓
Compliance assessment through pill count					✓

Appendix 2. Daily macronutrient intake assessed through 24 hour dietary recall

	OI types			<i>p</i>
	I	III	IV	
3 month intake	(n=23)	(n=9)	(n=18)	
Total calories (kcal)	1682 (1473; 1890)	1310 (973; 1648)	1457 (1221; 1693)	0.13
Carbohydrate (%)	55.1 (50.6; 60.0)	43.4 (36.2; 50.7)	51.6 (46.5; 56.6)	0.03
Protein (%)	15.9 (14.3; 17.5)*	18.5 (15.9; 21.1)	18.4 (16.6; 20.3)*	0.08
Fat (%)	29.8 (26.3; 33.2)	38.6 (33.0; 44.3)	31.3 (27.4; 35.3)	0.03
9 month intake	(n=22)	(n=8)	(n=19)	
Total calories (kcal)	1783 (1558; 2009)	1472 (1094; 1850)	1598 (1356; 1840)	0.30
Carbohydrate (%)	55.6 (52.4; 58.8)*	51.1 (45.7; 56.5)	50.4 (46.9; 53.8)*	0.07
Protein (%)	16.0 (14.1; 18.0)	19.6 (16.3; 22.8)	19.5 (17.4; 21.6)	0.04
Fat (%)	29.1 (26.4; 31.9)	30.0 (25.4; 34.5)	31.2 (28.2; 34.1)	0.60

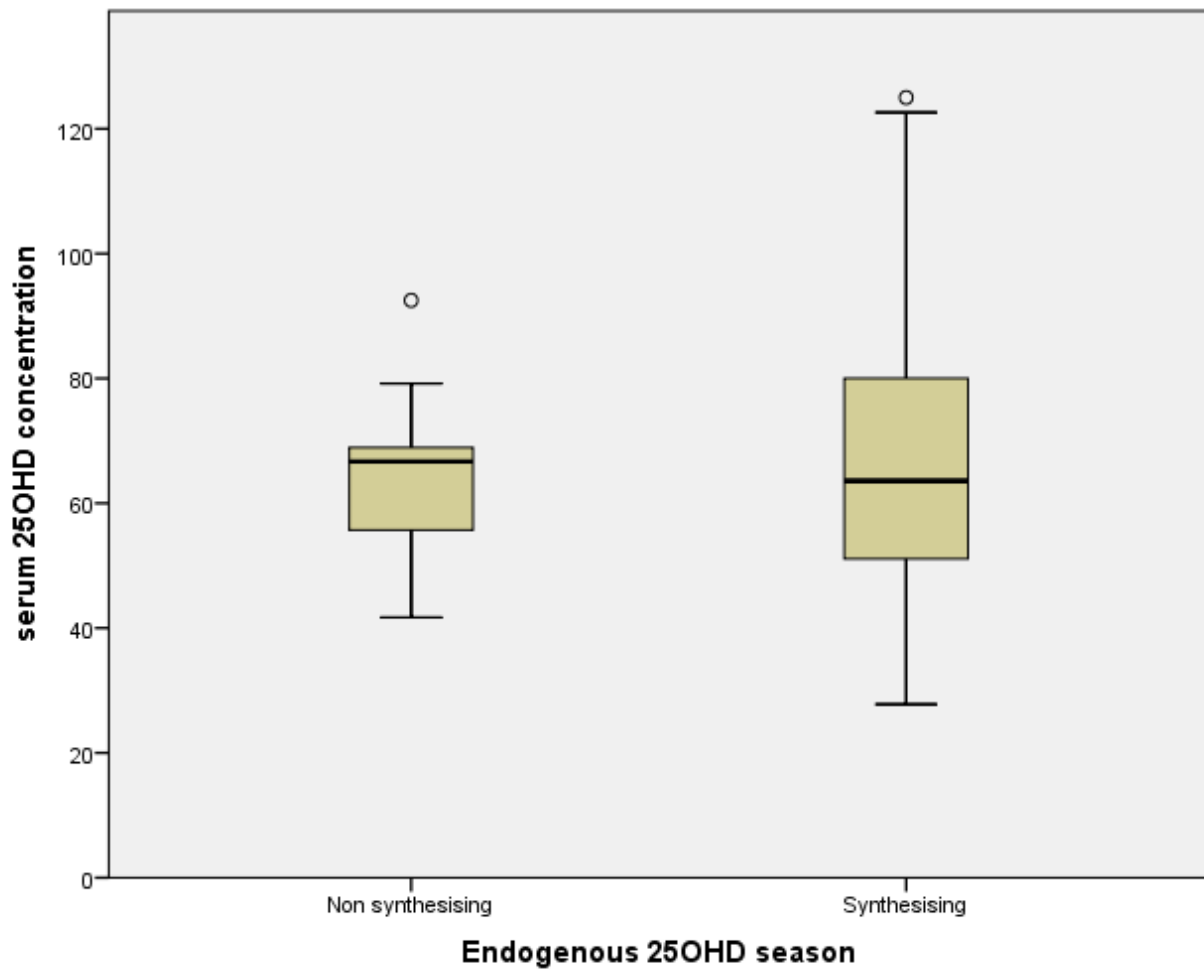
Data are mean with 95% CIs.

P values represent the significance of the difference between all three groups (ANCOVA).

^a Adjusted for age and sex.

*Statistically significant difference using alpha 0.05

Appendix 3. Box plot depicting serum 25OHD concentrations collecting during or outside of vitamin D endogenous synthesising period



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