# Osteocalcin-driven metabolic changes in Osteogenesis imperfecta

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## List of Abbreviations

| | |
|---|---|
| Areal bone mineral density | aBMD |
| Bisphosphonate | BP |
| Bone mineral density | BMD |
| Carboxyglutamic acid | Gla |
| Cross-linked C-telopeptide of type I collagen | CTX |
| Procollagen type I N-terminal propeptide | P1NP |
| Energy expenditure | EE |
| Heart rate | HR |

| | |
|---|---|
| Insulin receptor | InsR |
| Osteocalcin | OCN |
| Osteogenesis Imperfecta | OI |
| Oxygen consumption | $VO_2$ |
| Peripheral Quantitative Computed Tomography | pQCT |
| Six-minute walk distance | 6MWD |
| Six-minute walk test | 6MWT |
| Shriners Hospital for Children | SHC |
| Shuttle Ride Test | SRiT |
| Undercarboxylated osteocalcin | uOCN |
| Volumetric bone mineral density | vBMD |
| Wild type | WT |

## Abstract

Osteogenesis Imperfecta (OI) is a rare, genetic disorder in which the gene for a key structural molecule in bone (type I collagen) is mutated, resulting in brittle bones that fracture easily. Our clinical studies have shown that children with OI, even with the mildest forms, have slow growth and low muscle mass, which to date remains largely unexplained and untreated. It has been recently discovered that bone cells produce a hormone called osteocalcin that can affect energy balance in the whole body. We hypothesized that since individuals with OI have abnormal activity of bone cells, their osteocalcin levels may be affected. We examined if children with moderate to severe OI have differences in their metabolism compared to the control population. In this study, we observed that children with moderate to severe OI had a higher resting energy metabolism however it was not associated with abnormal activity of bone cells and increased osteocalcin levels. These results imply that the high resting energy metabolism of these children could be due to their increased heart rate or due to a deficit in the mitochondria which plays a major role in energy metabolism and oxygen consumption. We also observed that children with moderate to severe OI had a lower cardiopulmonary function than reference values. These results suggest that some muscle properties may be altered, therefore for a given effort, a child with OI will use a larger proportion of the muscle force which might lead to premature fatigue. Finally, we also observed normal serum osteocalcin levels and significantly lower P1NP and CTX serum levels in the OI group. We can infer that bisphosphonate treatment decreased abnormal bone cell activity in these children.



## Resume

L'Ostéogenèse imparfaite (OI) est une maladie génétique rare dans laquelle le gène d'une molécule structurelle clé de l'os (collagène de type I) est muté, ce qui entraîne une fragilité des os qui se fracturent facilement. Nos études cliniques ont montré que les enfants atteints d'OI, même avec les formes les plus bénignes, ont une croissance lente et une faible masse musculaire, ce qui à ce jour reste largement inexpliqué et non traité. Il a été récemment découvert que les cellules osseuses

produisent une hormone appelée ostéocalcine qui peut affecter l'équilibre énergétique dans tout le corps. Nous avons émis l'hypothèse que les personnes atteints d'OI ont une activité anormale des cellules osseuses, leurs niveaux d'ostéocalcine peuvent être affectés. Nous avons examiné si les enfants atteints d'OI modérée à sévère présentaient des différences dans leur métabolisme par rapport à la population témoin. Dans cette étude, nous avons observé que les enfants atteints d'OI modérée à sévère avaient un métabolisme énergétique au repos plus élevé, mais cela n'était pas associé à une activité anormale des cellules osseuses et à une augmentation des taux d'ostéocalcine sanguins. Ces résultats impliquent que le métabolisme énergétique élevé au repos de ces enfants pourrait être dû à une augmentation de leur fréquence cardiaque ou à un déficit des mitochondries qui jouent un rôle majeur dans le métabolisme énergétique et la consommation d'oxygène. Nous avons également observé que les enfants atteints d'OI modérée à sévère avaient une fonction cardio-pulmonaire inférieure aux valeurs de référence. Ces résultats suggèrent que certaines propriétés musculaires peuvent être affectées, donc pour un effort donné, un enfant avec l'OI utilisera une plus grande proportion de la force musculaire ce qui pourrait conduire à une fatigue prématurée. Enfin, nous avons également observé des taux sériques normaux d'ostéocalcine et des taux sériques de P1NP et de CTX significativement plus faibles dans le groupe OI. Nous pouvons en déduire que le traitement aux bisphosphonates a diminué l'activité anormale des cellules osseuses chez ces enfants.

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## Contribution of the Authors

As this is a monograph thesis, the work had two contributors. In this regard, here are the respective contributions of the student and thesis supervisor to each chapter. Please find below the list of authors and respective tasks and contribution:

- Larissa Sinkam Sieyeu Kepseu (LS), BSc and MSc (student): Recruited participants, performed all the study procedures, submitted documents to the ethical review board, managed communication with the ethical review board, collected data and conducted the statistical analysis and wrote the thesis.

- Svetlana Komarova, PhD: Was the Principal Investigator for the project and applied for the funding for the project and developed documents for the ethical review board.

- Louis-Nicolas Veilleux, PhD: Was my master's supervisor and Co-Investigator for the project, supervised LS while the study procedures were performed, in conducting the statistical analysis and in writing the thesis, edited and reviewed the thesis.

# Chapter 1 – Introduction

Osteogenesis Imperfecta (OI) is the most common heritable bone fragility disorder (prevalence 1:15,000-20,000) (1). Due to the frequent need for pharmacological treatment and surgical interventions, OI is one of the rare disorders that places the highest burden on the health system (2). The Shiners Hospital for Children Canada (SHC-Canada) follows the largest number of children with OI in North America (n = 350) and approximately 85% of these patients have dominant mutations in one of the two genes that code for collagen type I alpha chains, COL1A1 and COL1A2 (3). The main characteristics of individuals with OI are bone fractures, and skeletal deformities which result from abnormalities in bone matrix and mineral composition. In addition, my supervisor's team decreased muscle function in children with OI type I even though these individuals had similar levels of physical activity as their healthy peers (4, 5). Thus, our clinical studies have demonstrated that children with OI, including the mildest forms, have slow growth and low muscle mass, which to date remain largely unexplained and untreated (6, 7). Surgical and physical therapy approaches to treatment of severe OI are supported by pharmacological therapy which consists of cyclic bisphosphonates (BP) intravenous infusions. This approach was pioneered at the SHC in Montreal (8) and has become the standard of care worldwide (9). Bisphosphonate treatment increases bone density, reduces fracture rates, helps to reshape compressed vertebral bodies through growth and thus contributes to increase mobility (10). Nevertheless, bisphosphonate treatment is ineffective in improving overall growth or muscle mass in OI (6, 7). Thus, a better understanding of the systemic consequences of collagen type I mutations is needed to improve key aspects in the care of children with OI.

It has been recently discovered that bone cells produce a hormone called osteocalcin (OCN) that affect whole body energy homeostasis. OCN is now recognized as a bone-derived regulator of insulin sensitivity, insulin secretion and consequently glucose homeostasis (11, 12). It is produced by osteoblasts and deposited in bone matrix in a carboxylated form (13). During bone resorption, osteoclasts release and decarboxylate osteocalcin, generating an active undercarboxylated form

(uOCN) (14). Since our bone histomorphometry studies demonstrated increased bone formation and resorption (15, 16), we hypothesize that greater bone turnover in individuals with OI is associated with greater levels of uOCN and greater resting energy metabolism in comparison to controls without OI. Our primary objective is to establish the metabolic phenotype in children with OI types III and IV in comparison to controls; we will examine resting oxygen consumption ($VO_2$) and heart rate, heart rate and $VO_2$ during exercise, bone turnover (via serum levels of P1NP and CTX), volumetric bone mineral density (vBMD) via pQCT as well as serum levels of OCN and other parameters of energy metabolism. Our secondary objective will investigate if OCN contributes to the altered energy metabolism phenotype in children with OI. Lastly, we will determine if osteocalcin-driven metabolism in children with OI types III and IV differs from healthy typically developing children.

# Chapter 2 – Literature Review

## 2.1 Osteogenesis imperfecta

Osteogenesis imperfecta (OI), also known as 'brittle bone disease' is a heterogeneous, heritable, connective tissue disorder with a prevalence of 1 in 15,000 – 20,000 new births (1, 17). Its main clinical features are bone fragility, skeletal deformities, and slow growth which results in low bone mass and reduced bone material strength (1, 10, 17-19). In general, individuals with OI have low areal bone mineral density (aBMD) which is related to decreased bone size and vBMD (20). Bone histomorphometry data showed that children with OI have low bone cortical width and trabecular volume due to lower trabecular number and thickness providing evidence that children with OI have defects in the three mechanisms that lead to the accrual of bone mass. These mechanisms include production of trabeculae by endochondral ossification, bone modeling, and bone remodeling (16). Bone modeling is when either bone formation by osteoblasts or bone resorption by osteoclasts occurs on a given surface. Its main aim is to shape bone and increase bone mass and it occurs primarily in childhood but continues throughout life. However, bone remodeling is a process in which bone formation and bone resorption occurs consecutively in a combined manner on a given bone surface. Its main goal is the renewal of bone (21). Moreover, children with OI also

have a higher rate of bone cortical porosity and lower bone mechanical strength than their healthy counterparts because their bones are hypermineralized with smaller and copious mineral crystals (22).

## Classification of OI

About 85 – 90% of OI cases are caused by autosomal dominant mutations in *Col1a1 and Col1a2* (that code for collagen type I alpha chains). These mutations can either affect collagen type I structurally or reduce its amount (quantitative defects) (1, 17-19). Collagen type I is the most abundant protein found in bones, skin, muscles, and tendon extracellular matrix. It is composed of two α1 chains and one α2 chain which are encoded by *Col1a1 and Col1a2* respectively (1, 18). Type I procollagen which is produced in the endoplasmic reticulum (ER) is the precursor molecule of collagen type I (10, 18). During the post-translational modifications which are essential for proper collagen fibril formation, the procollagen undergoes changes to form the helical trimeric chain. It has been recently demonstrated that a variety of OI phenotypes results from defects in the genes involved in post-translational modification and intracellular trafficking of type I collagen or genes that are associated with the differentiation and function of osteoblasts (18). OI is now referred to as a collagen-related disorder because it can be classified in to 18 subtypes which are characterized by clinical phenotypes, bone histology, inheritance patterns and genetic causes (19). The original classification system by Sillence is still widely used to identify and categorize the OI types according to the clinical features and inheritance patterns observed in patients. These phenotypes vary in severity with milder phenotypes associated with quantitative defects and severe phenotypes associated with structural defects to collagen type I (1, 17, 18). This classification generally consists of four main clinical types; I to IV. OI type I is the mildest form of OI. Individuals with OI type I have none or a few bone deformities and have a normal or near-to-normal stature. Nevertheless, vertebral fractures which can lead to mild scoliosis are a classic feature of this type. Type II is lethal perinatally. The most common cause of death amongst affected infants is respiratory failure resulting from multiple rib fractures, a small thorax and pneumonia. Type III is the most severe non-lethal form of OI, individuals with this OI type have a very short stature as well as limb and spine deformities which are secondary to multiple fractures. Most of these individuals have triangular facies, blue-gray sclera, dentinogenesis imperfecta, vertebral compressions and scoliosis. Individuals with short stature and moderate-to-severe phenotype who

do not fit into one of the previously described categories are classified as OI type IV (1, 10, 17, 18, 23).

A few moderate to severe phenotypes have been identified which result from mutations in non-collagenous genes which are associated with the proper functioning of osteoblasts, the post-translational modification and intracellular trafficking of collagen type I (18). Some of these genes which are implicated in the pathogenesis of OI include: *Lepre1, Crtap, Ppib, Bmp1, Serpinh1, Sec24d, Creb3l1, Plod2, Fkbp10, Serpinf1, Sp7, Wnt1, Tmem38b and Ifitm5* (1, 18). OI types V and VI are classified based on the histological and phenotypic features. OI types VII to IX are caused by autosomal recessive mutations in genes that encode proteins involved in the posttranslational modification of collagen type I while autosomal recessive mutations in genes involved in the crosslinking of collagen type I cause OI types X to XII. Finally types XIII to XVIII are due to defects in osteoblast differentiation and function (1, 17, 19). The most common OI types are caused by mutations to genes that code for collagen type I alpha chains and this thesis will focus on those types rather than the rest.

## Additional characteristics of OI

In addition to skeletal fragility, individuals with OI experience muscle function and composition abnormalities. Research studies assessing muscle function using static isometric test and dynamic tests have demonstrated that OI patients have muscle function deficits (4, 24). However, they haven't been able to understand the origin of this muscle deficit. Two hypotheses have been suggested and tested thus far to account for the muscle weakness observed in this population. First, it was hypothesized that lack of physical activity in these patients could be contributing to the muscle deficits. This hypothesis was tested by comparing the type and level of physical activity performed daily by youth with OI type I to that of their healthy counterparts. In this study, the participants wore an accelerometer daily for the same period and the type of physical activity they carried out was categorized into 5 groups. These groups include sedentary, light physical activity, moderate physical activity, vigorous physical activity and moderate to vigorous physical activity. It was observed that, youth with OI type I were as active as their healthy counterparts, although both groups of youth didn't reach the daily recommendations of physical activity. According to these results, lack of physical activity in this population doesn't contribute to their muscle deficits

(5). Secondly, it was suggested that the intrinsic properties of muscle differed between OI patients and healthy controls. This hypothesis was tested by measuring the calf muscle cross-sectional area and density of OI type I patients and healthy controls between 6-21 years old using peripheral quantitative computed tomography (pQCT). In addition, each participant performed 5 different mechanography tests to assess lower limb muscle force and power. The results revealed that specific force (peak force/muscle cross-sectional area, CSA) were lower in children with OI type I compared to the healthy age- and sex-matched controls (4). These results suggest that there is something intrinsic in the muscle that is causing the muscle weakness in these population. Decrease specific force suggests presence of inter or intra-muscular fat or differences in the muscle fiber composition, amongst other possibilities.

Some other additional characteristics of OI include blue/gray sclerae, dentinogenesis imperfecta, hearing impairment, joint hypermobility, and cardiovascular and central nervous system (CNS) complications (1, 18, 19). Blue/gray sclerae is mostly found in individuals with OI type I (the mildest form) (25). Dentinogenesis imperfecta is a genetic disorder that affects the synthesis of dentin, and it can be associated with OI. It causes discoloration and translucence of teeth and they can wear off suddenly (26). Audiometric studies reported that OI patients usually experience hearing loss with an occurrence of 39% to 57.9% (27, 28). Most Individuals with OI will have hearing impairment during their adulthood. It can be of mixed type, but conductive hearing loss is predominant in children (29). About 66-70% of individuals with OI experience joint hypermobility (30, 31). This characteristic can be observed in all OI types despite their varying severities (31). Moreover, approximately 56% of individuals with OI were found to have joint dislocation and up to 39% reported having tendon ruptures (30).

There are few studies which demonstrate that individuals with OI and mouse models of OI also have altered energy metabolism. The focus of this thesis is to investigate if the altered energy metabolism could be controlled and influenced hormonally.

## Management of OI

There is no cure for OI but there are treatment options available for patients of different clinical phenotypes depending upon clinical severity, degree of impairment and the age of the individual. The goal of each treatment is to increase motor function and the independence of each patient. The

multidisciplinary approach which consists of a team of healthcare professionals (physicians, orthopaedic surgeons, dentists, physiotherapists, and occupational therapists) working together is the best approach to provide care to OI patients (1, 17, 18).

**Bisphosphonates**

Bisphosphonates (BPs) are the main pharmacological therapy used worldwide for both pediatric and adults with OI. They are analogues of pyrophosphate, and they have a high affinity for bone mineral because they bind to hydroxyapatite crystals. By binding to hydroxyapatite crystal in the bone matrix, BPs promote the inhibition of bone resorption by osteoclasts and in turn preventing the release of uOCN into the circulation. Hence the amount of uOCN available in the circulation is decreased (32, 33). BPs have been shown to increase bone volume in individuals with OI by decreasing bone resorption leading to increase in bone formation which results in higher bone mineral density (BMD) (34). In addition, BPs inhibit hydroxyapatite breakdown which also results in the suppression of bone resorption. This role of BPs has led to their use as clinical agents (35).

The most widely used bisphosphonates include pamidronate, alendronate, risedronate, and zoledronic acid. These BPs have nitrogenous side chains that impede the mevalonate pathway of sterol synthesis and interfere with post-translational modification of GTP proteins which are important for the proper function of osteoclasts. Bisphosphonates can be administered orally or intravenously. For BPs that are administered orally, only about 1% of the dose is absorbed by the gut demonstrating that oral BPs have a very low bioavailability to the circulatory system. However, patients who are given BPs intravenously have a lower risk of having gastrointestinal problems. In addition, these intravenous (IV) BPs are more readily available in blood compared to oral BPs (Brendan Lee 2016). BPs have a half-life up to a decade in the bone (36). IV BPs are more efficient than oral BPs and they have been shown to improve aBMD at almost all bone sites especially at the spine however no data from randomized controlled clinical trials have demonstrated that BPs prevent fractures, relieve pain, and improve mobility. In addition, the fracture rates and clinical status of individuals with OI has not been shown to improve upon treatment with BPs (34, 37, 38).

The recommendation to start and continue BP treatment depends on the presence of vertebral compression fractures and/or long bone fractures, and the growth potential. The most widely used IV BP treatment is zoledronate. It is easier to administer, and has a higher efficacy compared to pamidronate. Depending on the clinical response to the treatment and the LS aBMD z-score, oral

and IV BPs can be administered yearly or on as needed basis (39). If the treatment is stopped, some areas will have reduced aBMD next to previously treated areas with higher BMD at the metaphysis. This will generate stress risers and lead to increased fracture rates at the affected sites (40, 41). Regarding scoliosis experienced by individuals with OI, it is reported that BPs might help in delaying the progression of scoliosis in severe OI cases which can be evaluated by Cobb angle changes. Nevertheless, the occurrence of scoliosis in mature patients is independent of BP treatment or the age at which the BP treatment was initiated in individuals with OI types I or IV (42). Most studies have demonstrated that the effect of BP on non-vertebral fractures (NVF) is favorable but non-significant but one study by Bishop et al., demonstrated that treating OI patients with risedronate for a year significantly reduced their NVF rate (43).

In terms of complications arising from long-term treatment of BPs, atypical femoral fractures (AFF) are the most worrisome (44). Several reports have demonstrated that AFFs are more prominent in adults with OI that have been treated with BPs (45-49). Other cases reported that children with OI treated with pamidronate developed subtrochanteric fractures with characteristics of AFFs over pre-existing intramedullary rods (49). Another case of recurrent bilateral proximal femur fracture with similarities to AFFs was documented in a teenager with OI type IV that was continuously treated with pamidronate (50). Even though the occurrence and development of AFF has not been linked to BP exposure yet, the bone material and structural properties observed in OI patients together with the known effects of BP should account for the concern about continuing high dose BPs treatment in teenagers with OI (51).

**Other treatment options for OI**

Other treatment options include the use of Calcium and vitamin D, Denosumab, Anabolic therapy and TGF-β inhibition. It is recommended that individuals with OI take calcium and vitamin D to prevent adverse effects caused by bisphosphonate therapy in cases of inadequate calcium intake or vitamin D deficiency (52, 53). The optimal dose for both calcium and vitamin D intake in individuals with OI is yet to be determined but in most cases according to general guidelines the recommended calcium and vitamin D intake levels are 1300 mg and 600-800 IU per day respectively. Denosumab is a human monoclonal antibody which is primarily used for the treatment of osteoporosis. Nonetheless, several studies have demonstrated that OI patients who were given 1mg/kg subcutaneously every 3 months for 2 years didn't experience any side effects.

On the contrary, these patients had a significant increase in aBMD (54-56). Anabolic therapy consists of using teriparatide (a recombinant parathyroid hormone). It functions by promoting bone formation over bone resorption and therefore reducing the risk of vertebral and non-vertebral fractures. It is used to treat some forms of osteoporosis but its positive effect in OI type I patients was observed in a randomized placebo-controlled study in which 78 adults had a significant increase in the aBMD and vertebral BMD and bone strength after being treated with teriparatide for 18 months (57). Another novel therapeutic option is use of 1D11, a neutralizing antibody to inhibit TGF-β which is a regulator of bone remodeling. Several studies on mice models of both dominant and recessive OI have shown that using 1D11 to inhibit TGF-β leads to an increase in osteoblast numbers, a decrease in osteoclast activity and improvement in bone strength and bone mass (58-61).

**Rehabilitation and Physical Activity in OI**

Certain characteristics of individuals with OI which include altered cardiopulmonary function, motor function deficits and muscle weakness negatively impact their daily functioning. Therefore, it is extremely important to use the right therapy to help them become more independent in order to carry out their daily life activities. There are some studies which have shown that in addition to muscle weakness individuals with OI also have cardiopulmonary fitness deficits. In one of these studies, it was observed that the maximum oxygen consumption ($VO_2$peak) and the maximum oxygen consumption relative to body weight ($VO_2$peak/kg) of individuals with OI were significantly lower compared with healthy subjects. Additionally, these same patients also presented with significantly lower shoulder abductor muscle strength, grip strength, hip flexor strength and ankle dorsal flexor strength compared to reference values (62). Another study was performed to ascertain the effects of physical training on the exercise capacity, muscle strength and fatigue levels in individuals with OI types I and IV. They observed that after 12 weeks of following a graded exercise program, the $VO_2$peak, $VO_2$peak/kg, maximal working capacity (Wmax) and muscle strength of these patients were significantly improved compared with control values. So, they concluded that if individuals with OI undergo physical therapy and follow a supervised exercise program, they can improve their cardiopulmonary fitness and muscle force

(63). Considering this, individuals with OI should be encouraged to do exercise programs with minimal risk of falls and sports with limited or no contact/ collision (64, 65).

Physical therapy can be started in infants with severe OI during which the intervention focuses mainly on placing the head and spine in a position that will prevent torticollis and reduce contractures from abduction. Other interventions which include individualized muscle strengthening and aerobic conditioning (e.g., water therapy and swimming) can help children prepare for sitting, standing and be useful during protected ambulation. To follow a patient's progress, validated functional tests like the brief assessment of motor function (BAMF) are used (19). The effect of physical exercise in children or adults with severe OI with varying levels of disability has not been assessed, albeit the fact that resistance training has been shown to have a bone formation effect on the skeleton in the prepubertal period (64).

## 2.2 Altered energy metabolism in OI mice and patients

In addition to the known characteristics of OI, there are few studies that demonstrate abnormal energy metabolism in patients and mouse models of OI. A previous study performed more than 4 decades ago obtained clinical and biochemical evidence of an altered resting energy metabolism in patients with moderate to severe OI (66). They observed that the oxygen consumption, serum thyroxine levels, body temperature, heart and respiratory rates were abnormally higher in prepubertal children with OI than in healthy children of the same age group. In addition, they observed a 45% increase in the metabolic rate per unit of body weight in prepubertal children with OI. Remarkably, these metabolic changes mentioned prior didn't occur after puberty. Moreover, together with the decrease in energy metabolism observed after puberty, a significant decrease in the number of fractures was observed in individuals with OI after puberty. They were intrigued by these observations, and they questioned themselves if both changes could be due to the onset of sexual maturity during which sex hormones appear. These sex hormones could also be responsible for stopping the progression of the disease and decreasing the energy metabolism in these pubertal individuals with OI. Hence suggesting a link between an altered energy metabolism, fractures and sex hormones (66). In a recent mice study, *Col1a1*$^{Jrt/+}$ mice with a severe OI phenotype (low BMD, decreased bone volume/tissue volume, weaker and more brittle bones) were tested to identify their metabolic pattern. Young 4-week-old *Col1a1*$^{Jrt/+}$ mice portrayed age- and sex-dependent resting

metabolic changes that normalized by 8 weeks old (puberty onset). They observed elevated insulin levels in males and improved glucose tolerance in females, reduced levels of random blood glucose and a significant reduction in adipose tissues in OI mice relative to WT littermates. They also observed increased oxygen consumption ($VO_2$) and carbon dioxide production ($VCO_2$) in both male and female 4-week-old *Col1a1*$^{Jrt/+}$ mice compared to WT. Additionally, energy expenditure (EE) was higher in OI mice of both sexes compared to WT. However, at 8 weeks of age, only female mice had persistent high $VO_2$, $VCO_2$ and EE (67). More recently, the whole-body metabolism of *oim/oim* mice was assessed. They observed that this mouse model has a metabolic phenotype demonstrated by an increase in relative lean mass and decrease in fat mass. As well as a significant high resting energy expenditure (EE) regardless of reduced activity. They also observed increased $VO_2$ and $VCO_2$ in *oim/oim* mice compared to WT littermates. These metabolic changes were persistent in adult *oim/oim* mice aged between 16-19 weeks (68). Both OI mice model studies (*Col1a1*$^{Jrt/+}$ and *oim/oim)* are in line with the study on prepubertal children suggesting that energy metabolism is altered during growth in patients and mice models of OI. However, the mechanisms behind the metabolism phenotype remains largely unknown and unexplored.

## 2.3 Relationship between bone remodeling and energy metabolism

Bone remodeling is an active physiological process that is homeostatic in nature. It is essential for increasing and maintaining bone strength, regulating calcium homeostasis, and repairing microdamage caused by weight-bearing activities. It also contributes to the process of fracture healing. This process includes two cellular events that occur in series on a given bone surface. The first event is bone resorption which is mediated by bone cells called osteoclasts. During resorption, the mineralized bone matrix is destroyed. The second event is bone formation which is mediated by bone cells called osteoblasts. During bone formation, the osteoblasts deposits new matrix which eventually mineralize to form bone and fill the lacunae (11, 69-72). To estimate the amount of bone turnover which is the amount of bone that has been resorbed and formed over a period, we measure serum levels of relevant bone biomarkers. These biomarkers are Cross-linked C-telopeptide of type I collagen (CTX) and Procollagen type I N-terminal propeptide (P1NP). CTX is a product of the breakdown of type I collagen containing pyridinium cross-links and its serum

levels are associated with bone resorption. P1NP is produced from the cleavage of type I pro-collagen when it is assembled into fibrils during extracellular processes. Its serum levels are associated with bone formation (73).

Bone remodeling is an energetically costly process because it involves two distinct metabolic events, which occur constantly and simultaneously at multiple bone sites throughout the skeleton. Therefore, bone remodeling requires a fully operational energy metabolism to provide a continuous supply of energy to osteoclasts and osteoblasts for their proper function. Thus, indicating that there could be a link between bone remodeling and energy metabolism (74).

**Fat-derived endocrine control of bone remodeling**

The first hormone that was found to be implicated in the regulation of energy metabolism and bone remodeling is leptin. It is produced by adipocytes and its function is to suppress appetite and increase energy expenditure (74-77). It was demonstrated that leptin is a powerful inhibitor of bone mass accrual when leptin-deficient (*ob/ob*) mice and leptin receptor deficient (*db/db*) mice were found to have coexisting hypogonadism and high bone mass. Hence leptin was suspected to have another essential function in the regulation of bone mass. To confirm this theory, a mouse model with a partial gain of function (*l/l)* was investigated and it was observed that these mice had normal appetite when given a normal diet as well as normal energy expenditure and reproductive function. However, they had lower bone mass mostly in the trabecular bone (74, 78, 79). Then, further investigations have concluded that leptin negatively regulates bone formation via two mechanisms. The first mechanism involves a serotonin central relay during which the sympathetic signaling generated from the hypothalamus promotes osteoclast differentiation by activating *Rankl* (receptor activator of NF-kappaB ligand) gene expression. The second mechanism consists of the inhibition of *Rankl* expression through the action of cocaine- and amphetamine-regulated transcript (CART) (69, 74, 80-83). Leptin is therefore a negative regulator of bone remodeling by inhibiting the accrual of bone mass.

**Osteocalcin: a bone hormone regulating energy metabolism**

Osteocalcin (OCN) is the second most abundant protein in bone besides collagen type I. It is a vitamin-K dependent protein, and it is used clinically as a serum marker for bone turnover. Its carboxylated form is synthesized by osteoblasts and deposited in the bone matrix. Before being

secreted by the osteoblasts, it undergoes post-translational modifications. These modifications are the excision of the pre-pro peptide and carboxylation of glutamic residues in to γ-carboxyglutamic acid (Gla) residues. Due to the presence of these Gla residues, osteocalcin has a high affinity to bind to the calcium ions in the hydroxyapatite (a mineral present in the bone matrix) crystals (84-87). During bone resorption, osteoclasts decarboxylate the Gla residues of osteocalcin to produce its active form called undercarboxylated osteocalcin (uOCN) which is released from the bone matrix to the systemic circulation. To assess the role of osteocalcin, investigations on osteocalcin-deficient mice were performed. It was observed that these mice have high blood glucose, low blood levels of insulin, few pancreatic β-cells and reduced insulin sensitivity (88). Additionally, in another study WT mice were treated with continuous infusions of uOCN, they were found to have increased insulin secretion and increased glucose tolerance. When these mice were given higher doses of uOCN, their fat mass decreased significantly (89). Moreover, it was demonstrated that in non-diabetic Swedish men, and Canadian women of aboriginal and European heritage, osteocalcin levels have a negative correlation with fasting blood glucose (72). In conclusion, osteocalcin has been established as a regulator of many physiological events notably insulin secretion and sensitivity and as a result glucose homeostasis as well whole-body fat mass (72, 84, 85, 90). Some mice studies have demonstrated that undercarboxylated osteocalcin (uOCN) stimulates insulin production in the pancreas (91, 92). This, in conjunction with the role of insulin in promoting bone remodeling suggests that osteocalcin contributes to the bone-pancreas feed-forward loop regulating bone mass and insulin secretion (91, 93).

## Other effects of osteocalcin on insulin-sensitive peripheral tissues

Osteocalcin also acts on other peripheral tissues by improving their sensitivity to insulin. This was observed by assessing the hepatocytes isolated from mice infused with insulin. Increase in the phosphorylation of the insulin receptor in hepatocytes and increase in the hepatic expression of osteocalcin was observed. However, in mice which received a control vector, this phenomenon was non-existent because there was no upregulation in the phosphorylation of the insulin receptor in their hepatocytes. Additionally, they didn't have increased hepatic expression of osteocalcin (85, 94). In humans who were infused with glucose post-exercise, it was observed that uOCN serum levels were proportional to insulin sensitivity in skeletal muscle (85, 95). Lastly, a recent investigation demonstrated that the glucose transport in L6 myocytes treated with osteocalcin was

promoted whether insulin was present or not. They also observed that both carboxylated and undercarboxylated osteocalcin enhanced insulin sensitivity, glucose transport and inhibited the secretion of inflammatory markers in adipose tissue cells (85, 96).

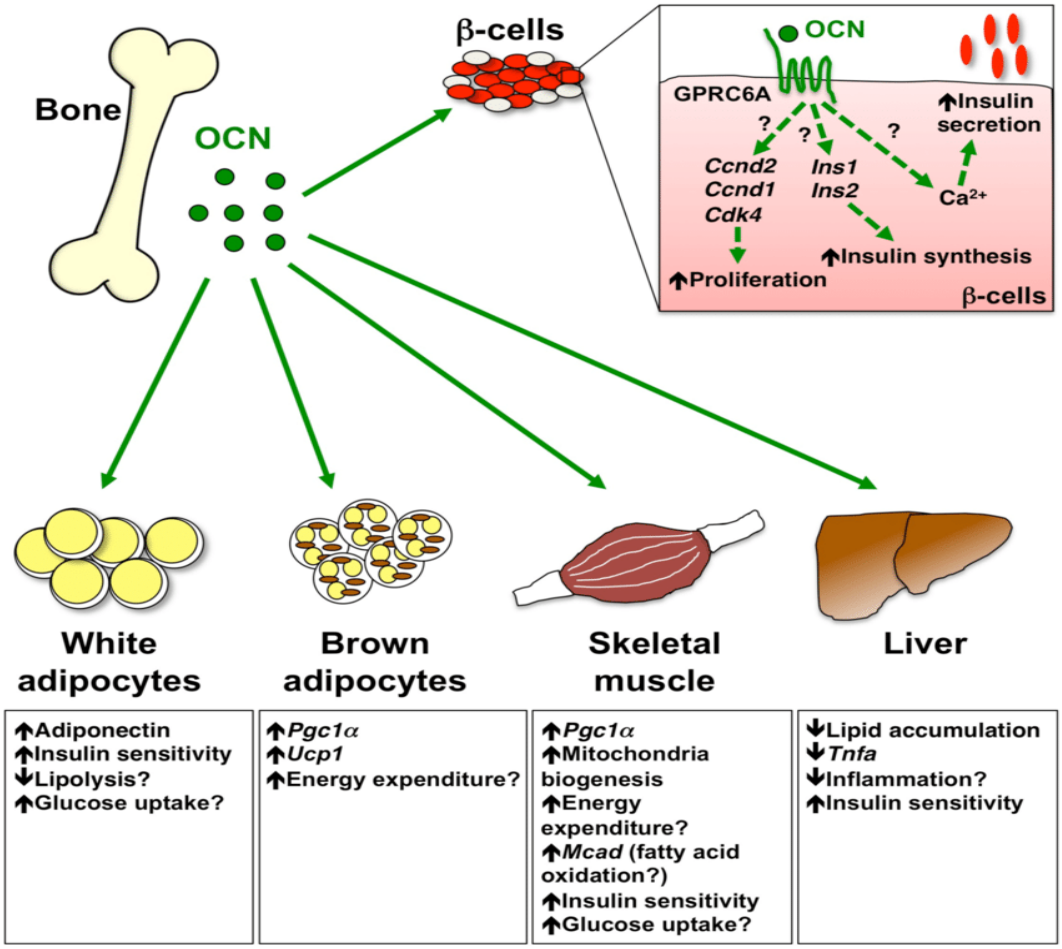


**Figure 1.** An illustration of the regulatory effects of osteocalcin (OCN) on various body tissues and organs **(86).**

### Regulation of bone formation and osteocalcin secretion by insulin

Recent evidence demonstrated that insulin mediates glucose homeostasis via a positive feedback loop by enhancing the expression of osteocalcin (a bone-derived hormone). Firstly, insulin binds to the insulin receptor (InsR) in osteoblasts resulting in the suppression of Twist2 (an inhibitor of osteoblast differentiation). Then, it leads to the expression of osteocalcin via the action of Runx2 which is a major regulator of osteocalcin expression. This was further explored by testing osteoblast specific InsR knockout mice. These mice demonstrated altered bone development and increased fat mass. They also had higher levels of blood glucose and insulin, decreased glucose tolerance and pancreatic β-cell mass. Surprisingly, they had low levels of total and undercarboxylated OCN. However, when the osteoblast specific InsR knockout mice were infused with uOCN, the metabolic changes were partially reversed by infusing the mice with uOCN (85). Additionally, the tyrosine phosphatase (OST-PTP) which is a product of the *Esp* gene dephosphorylates and deactivates the InsR in mouse osteoblasts. Hence, OST-PTP appears to have an important role in the regulation of insulin signalling in osteoblasts and as a result osteocalcin production and secretion. One of the factors that controls OST-PTP is the forkhead box protein O1 (FOXO1). It downregulates osteoprotegerin (OPG), a soluble inhibitor of RANKL-induced osteoclast differentiation and activation. This leads to osteoclasts-mediated bone resorption during which the active form of osteocalcin is released from the bone matrix into the circulation (84, 85, 92). Conclusively, insulin positively regulates bone formation by binding on InsR in osteoblasts to promote osteocalcin synthesis.

## 2.4 The potential role of osteocalcin in OI mice and patients

Given that osteocalcin has been identified as a serum marker for bone turnover, several investigations in patients and mice models of OI have been done to quantify and compare the serum levels of osteocalcin. One of the very first studies to quantify circulating osteocalcin levels demonstrated that plasma osteocalcin levels in untreated children with OI were significantly higher compared to their healthy counterparts (97). Additionally, in a previous study which was performed in several patients with mild to severe OI types, it was determined that blood osteocalcin levels were higher during childhood which is the developmental period during which bone

turnover is elevated (98). In recent studies in which the bone turnover of children with OI was assessed by histomorphometry, it was observed that bone turnover is markedly increased in these patients. This increase in bone turnover leads to low bone mass and reduced bone material strength which causes skeletal fragility and increased fracture rates in these patients (15, 16). Interestingly, both elevated blood osteocalcin levels and high fracture rates have been observed mostly during childhood in children with OI and during early developmental stages in mouse models of OI. This implies that osteocalcin hyperactivity could contribute to the altered metabolic phenotype observed in patients and mouse models of OI. Recently, a mouse model, *Col1a1*$^{Jrt/+}$ mouse was studied to elucidate its metabolic phenotype. It was demonstrated that the total OCN, undercarboxylated and carboxylated OCN levels were higher in 4-week-old *Col1a1*$^{Jrt/+}$ mice compared to WT. The *Col1a1*$^{Jrt/+}$ mice also had an increase in cross-linked C-telopeptide of type I collagen, CTX (bone resorption marker). This suggested that the collagen type I mutation in these mice stimulates and upregulates the synthesis and secretion of OCN by osteoblasts which in turn is activated by osteoclasts during bone resorption resulting in higher levels of blood uOCN. They also observed that the elevated uOCN levels were accompanied by significant changes in energy metabolism in the *Col1a1*$^{Jrt/+}$ mice compared to WT. These changes include decreased blood glucose, increased insulin levels in males and increased glucose tolerance in females, higher $VO_2$ and $VCO_2$, higher EE, and lower adipose tissues. These changes have been shown to be caused by increased levels of undercarboxylated OCN (67). It is hypothesized that high osteocalcin levels are associated with increased bone turnover and altered energy metabolism in untreated children with OI and mouse models of OI.

## 2.5 Research Question

Given the role that the skeletal system plays in regulating energy metabolism through the activity of osteocalcin, it is imperative to determine how osteocalcin activity might affect the energy metabolism of individuals with OI. This is because individuals with OI have a high bone turnover resulting in bone fragility and higher fracture rates than their healthy counterparts. This will further our understanding of the mechanisms of bone metabolism from a theoretical perspective. Additionally, we will also be able to determine if the high bone turnover in individuals with OI is associated with altered energy metabolism. There is previous evidence that shows that children with OI experience altered energy metabolism and high osteocalcin levels. And recent evidence

revealed that *Col1a1*$^{Jrt/+}$ mice had elevated uOCN levels associated with significant changes in energy homeostasis. These phenomena suggest that osteocalcin activity might have the same regulatory effect on the energy metabolism of prepubertal children with moderate to severe OI. Given the paucity of information on how the energy metabolism of individuals with OI is affected during physical activity, we can hypothesize that if serum osteocalcin levels are high then serum glucose levels will be affected due to increased insulin activity. This will lead to poor exercise performance because of the fluctuating amount of glucose taken up by the skeletal muscles. Hence, there is a need to assess the serum levels of osteocalcin and other energy metabolism markers in patients with moderate to severe OI. In addition, we will also be assessing their resting energy metabolism and energy metabolism at effort to understand if osteocalcin plays a role in altering the energy metabolism of individuals with OI. With this discovery, we can devise better exercise and physical therapy programs for individuals with OI to minimize their energy expenditure. It will also lead to the development of future studies which will aim at developing a treatment that could decrease osteocalcin levels and as a result decrease bone turnover consequently decreasing fracture rates in these patients.

This thesis will begin to provide answers the following research questions:

1. Is resting energy metabolism different between BP treated children with moderate to severe OI and healthy children?

   *Hypothesis:* We hypothesize that we will observe significantly higher resting energy metabolism (oxygen consumption and heart rate) in children with OI types III and IV because of their high bone metabolism compared to their healthy counterparts.

2. Are the fasting serum levels of osteocalcin, and other energy metabolism parameters different in treated children with moderate to severe OI (OI types III and IV) compared to healthy children?

   *Hypothesis:* We hypothesized that osteocalcin, insulin, glucose amino acids, CTX, P1NP and citric acid levels in treated children with OI types III and IV will be significantly higher than healthy children in relation to their high bone metabolism.

3. Previous studies in OI mice and in OI type I patients suggest the presence of a cardiopulmonary deficit, or stated otherwise, an altered exercise energy metabolism. One of the many potential mechanisms could be that in response to high levels of circulating osteocalcin there is a decrease in circulating glucose leading to a cardiopulmonary deficit in these patients.

   *Hypothesis:* Given that the current study aims at evaluating more severe types of OI, we hypothesize that the cardiopulmonary deficits in children with moderate to severe OI will be significantly higher than those of healthy children or norms.

4. Is cortical vBMD and the trabecular vBMD at the radius different in OI type III and IV compared to healthy controls? In a previous study, it was observed that trabecular thickening in children with OI is decreased or is completely absent which is one of the indications of increased trabecular bone modeling (16). Cortical vBMD is a parameter that is influenced by cortical porosity and the degree of mineralization of the bone material. There is evidence that children with OI have higher rates of bone cortical porosity due to higher intracortical remodeling which lowers the cortical vBMD (99).

   *Hypothesis:* We hypothesize that we will observe similar cortical vBMD between children with OI types III and IV and healthy children. We also hypothesize that the trabecular vBMD of children with OI types III and IV will be lower than those of healthy children.

# Chapter 3 – Methodology

### Study design

This was a cross-sectional study on 20 prepubertal children with OI types III and IV and 20 healthy age- and sex-matched controls. During their routine treatment visit at the Shriners Hospital for Children (SHC) Canada, participants would have an assessment of oxygen consumption, body composition, serum levels of OCN and other serum parameters of energy metabolism.

**Participants**

The inclusion and exclusion criteria for this study are as follows:

Inclusion criteria:

i) For the OI group – a clinical diagnosis of OI types III or IV with a known mutation in *Col1a1* or *Col1a2*. These OI types were chosen because previous studies have shown that children with moderate to severe OI have more severe growth deficits (6) and abnormalities in bone metabolism (16) than children with mild OI (OI type I).
ii) Pre-pubertal children older than 6 years.
iii) Medical approbation from the treating physician to initiate an evaluation
iv) English- or French-speaking

Exclusion criteria:

i) Recent fracture or surgery in the long bones of the lower limbs within the past 2 months as these stress events might affect metabolism.
ii) Any chronic health issues
iii) Stage II or above puberty using the Tanner scale (self-report or clinical evaluation) – see Appendix 1

This study was approved by the Faculty of Medicine Institutional Review Board of McGill University (IRB #A12-M36-17B). All participants provided written informed assent and their legal guardian provided informed consent before participating in the study.

**Anthropometric measurements**

Each participant's height was measured using a Harpenden stadiometer (Holtain Limited, Dyfed, UK). The weight was determined using the physician scales (Detecto Company, United States). This information was used during the oxygen consumption ($VO_2$) test.

**Biochemical analyses**

Each participant was required to be in a fasted (i.e., 12 hours without eating) state prior to blood sample collection. During their routine treatment appointment, a nurse was incharge of collecting blood and urine samples from each participant as per Table 1. The parameters of interest which were quantified from the blood samples are: osteocalcin, glucose, insulin, CTX (bone resorption marker), P1NP (bone formation marker), citric acid and amino acids.

**Table 1. Description of sample collection tubes used and the parameters and tests that were performed.**

| **Colour of collecting tube/container** | **Volume of each tube/container** | **Parameters measured per tube** | **Testing performed** |
|---|---|---|---|
| Red | 3 mL | CTX and P1NP | Chemiluminescence assay |
| Gold | 5 mL | Glucose and Insulin | Metabolomics analysis |
| Mint Green | 3 mL | Amino acid | Metabolomics analysis |
| Lavender | 2 mL | Osteocalcin | Chemiluminescence assay |
| Urine | 90 mL | Citric acid | Metabolomics analysis |

After all the samples were obtained, they were labelled and packaged with a requisition and sent to the appropriate location for processing and testing. The red, gold, and lavender tubes and the urine container were sent to the McGill University Health Center (MUHC) clinical laboratories, while the red tube was processed by the SHC-Canada laboratory. The results of the analysis for each participant were sent to the treating physician/co-investigator of the study.

**Measurement of oxygen consumption at rest**

Each participant was given a Polar® Heart rate (HR) belt to wear at the level of sternum to monitor the HR continuously. Then, the participant was asked to wear a breathing face mask which was chosen based on the participant's size. This mask was connected to a respiratory gas analyzer (Metamax®3B, Cortex Medical, Germany) composed of the Metamax®3B main unit, a flow sensor, a MaxSport belt set (S, M, L) and a sample line. Subsequently, the participant was told to sit down and stay as quiet as possible for a period of 5 minutes while breathing in and out of the mask. At the end of the 5 minutes, the mean oxygen consumption ($VO_2$) relative to body mass (ml $kg^{-1}min^{-1}$) was determined using the MetaSoft® Studio software. The test was performed at rest to to determine if resting energy metabolism is higher in individuals with OI compared to their healthy counterparts.

**Measurement of oxygen consumption during Six-minute Walk test (6MWT)**

The 6MWT was performed in a 20-m corridor outside and next to the Motion Analysis Center (MAC) at SHC-Canada. Tape was placed at two locations: at the start and at the end of the path. Participants were instructed to walk as fast as possible back and forth the 20 m path, turning around at the final mark without stopping and to cover as much ground as possible. The standardized order given to the participants was, "Walk as fast as possible for 6 minutes, but don't run or jog." Participants could stop if needed to take a break and restart later. Heart rate and oxygen saturation ($SaO_2$) was measured immediately before and after the test. Each lap covered was equivalent to 20m, so the total number of laps recorded was converted to total distance covered (in meters) (100). During the test, each participant wore the same breathing mask mentioned above connected to a respiratory gas analyzer to directly measure the maximum oxygen consumption ($VO_2max$).

**Measurement of oxygen consumption during 10m Shuttle Ride Test (SRiT)**

For patients using a propulsion wheelchair, we used the 10-m Shuttle Ride Test (SRiT) to obtain their VO2 max. This test was specifically designed and validated for out specific patient population. During the SRiT, participants propelled their wheelchair manually back and forth between two lines 10 m apart at a set incremental speed determined by an audio signal, which was played on a standard audio player on a computer at SHC-Canada. Participants stood behind one of the lines facing the second line and began riding when instructed by the audio signal. Every completed 10-m track was called a "shuttle." The initial velocity was 2.0 km/h. Participants continued riding between the two lines, turning when signaled by the audio signal. After every minute (stage), the audio signal indicated an increase in speed of 0.25 km/h. The test finished when the participant reached exhaustion or failed to reach the line (within 2 m) on two consecutive audio signals (shuttles), despite strong verbal encouragement. The achieved stage was recorded and the total distance covered was measured. This test provides valid measurement of the cardiopulmonary function only when a respiratory gas analyzer is used concurrently (101). Hence, participants wore an appropriate breathing mask connected to the above-mentioned gas analyzer to perform the test.

**Peripheral Qualitative Computed Tomography (pQCT) at the distal and proximal radius**

A pQCT was performed on the forearm to quantify the muscle and fat composition at the forearm of each participant by a trained radiology technician at the radiology department of SHC-Canada. It has been demonstrated previously that muscle and fat measurements at this site correlates strongly with total body lean and fat mass both in healthy children and OI (7, 102). The two sites of the radius that were analyzed are the metaphysis ('4% site') and the diaphysis ('65% site'). The main parameters that were measured are the trabecular volumetric bone mineral density (vBMD) at the metaphysis and cortical vBMD at the diaphysis. To obtain the trabecular vBMD, the Stratec XCT2000® equipment (Stratec Inc., Pforzheim, Germany) was positioned at the measuring site of the distal forearm with the use of coronal computed radiograph (scout view). Then a single 2.0 mm thick tomographic slice was taken at a voxel size of 0.4 x 0.4 x 2mm with a translational scan movement speed of 15mm/s. The image was acquired and processed, and the numerical values were calculated with the Stratec software package (XCT 5.40). The parameter of interest, trabecular vBMD was determined as the mean density of the 45% central are of the bone's cross-section (103).

To obtain the cortical vBMD, the scanner mentioned above was positioned on the proximal radius of the non-dominant forearm at the site whose distance from the ulnar styloid process was equivalent to 65% of the forearm length ('65% site'). Then a single 2.0 mm thick tomographic slice was taken at a voxel size of 0.4 x 0.4 x 2mm with a translational scan movement speed of 15mm/s. The same software mentioned above was used to acquire and process the image. The cortical vBMD which represents the density of the solid cortex was determined by analyzing the cortex of the radial diaphysis at a threshold of 710 mg/cm$^3$ using the software CORTBD (99).

**Statistical analysis**

The sample size suggested was 20 participants per group. In the study by Cropp et al, the $VO_2$ was 4.5 ml/kg/min (SD: 1.2) in the control group and 7.6 ml/kg/min (SD: 2.5) in the OI group (66), hence if we observe similar differences in our study, then only 9 participants per group would be needed.

The parameters that were tested to assess the basal energy metabolism which include oxygen consumption ($VO_2$) and heart rate were compared between the OI group and the control group and the OI group with the reference values (104) of healthy children with the use of a one sample t-test. To assess the energy metabolism during the 6MWT, we compared the oxygen consumption ($VO_2$) and heart rate of OI type IV participants to those of the control groups, and to the reference values of healthy children using a one-sample t-test. To assess the energy metabolism during the SRiT, we compared the average oxygen consumption ($VO_2$) and the heart rate of OI type III participants to the reference values of healthy children with a one-sample t-test. In terms of cardiopulmonary fitness, the average distance covered by OI type IV participants during the 6MWT was compared to the distance covered by the control group in the 6MWT and to the reference values of healthy children with the use of a one-sample t-test. Finally, the distance covered by OI type III participants during the SRiT was compared to the distance covered by moderate to severe participants with OI in a similar study (101) and to the reference values of healthy children performing a similar test by walking. These comparisons were done with a one-sample t-test.

The trabecular vBMD and cortical vBMD of the OI group was compared to the values of the control group and to the reference values of healthy children with a one-sample t-test. The blood parameters which include osteocalcin, glucose, insulin, CTX, P1NP, citric acid and 25

amino acids of the OI group were compared to the minimum and maximum values of the reference ranges of healthy children using a one-sample t-test. Then we assessed if the values of the parameters in the OI group fall between or out of the normal reference ranges of healthy children.

# Chapter 4 – Research Findings

### Participants

We intended to recruit 20 prepubertal children with OI types III and IV and 20 healthy control participants. However, due to the Covid-19 pandemic we had to stop recruitment because we were no longer able to perform research activities. Hence, we were only able to recruit 10 participants in total (5 OI type IV, 3 OI type III children and 2 healthy children). Three were females and seven were males. The OI type III participants' mean (SD) age was 8 (2.60) years, and their mean (SD) body mass index was 18.43 (0.48) $kg/m^2$. OI type IV participants' mean (SD) age was 9.8 (1.60) years, and the mean (SD) body mass index was 21.10 (4.77) $kg/m^2$. Control participants' mean (SD) age was 9 (2.80) years, and the mean (SD) body mass index was 15.18 (0.84) $kg/m^2$. Both The 5 OI type IV and 3 OI type III participants have been under bisphosphonate therapy, however the duration of the treatment for each participant is unknown.

**Table 2.** Summary of energy metabolic parameters of boys and girls with OI types III and IV.

| **Sex** | **Average resting HR (bpm) ± SD** | **Average $VO_2$ relative to body mass (ml/kg/min) ± SD** | **Average max HR (bpm) ± SD** | **Average $VO_2$max (L/min) ± SD** |
|---|---|---|---|---|
| Male | 103.85 ± 3.72 | 8.18 ± 2.24 | 128.05 ± 17.04 | 0.84 ± 0.28 |
| Female | 86.54 ± 4.17 | 7.25 ± 1.63 | 129.71 ± 9.65 | 0.84 ± 0.23 |

**Table 3.** Average values of biochemical parameters measured from the fasted blood samples of OI types IV and III participants relative to the pediatric reference ranges obtained from the MUHC clinical laboratories directory.

| | **Sample size (n)** | **Average values (SD)** | **Average Reference ranges** | **P-value[a] (lower limit)** | **P-value[b] (upper limit)** | **Within range** |
|---|---|---|---|---|---|---|
| Osteocalcin | 5 | 38.4 (25.64) | 24.96-171.68 | 0.153 | <0.001 | Yes |
| Glucose | 6 | 4.73 (0.24) | 3.0-5.6 | <0.001 | 0.001 | Yes |
| Insulin | 6 | 45.37 (32.09) | <161 | <0.001 | N/A | Yes |
| CTX | 4 | 0.52 (0.35) | 1.025-2.64 | **0.031** | **<0.001** | **No** |
| P1NP | 4 | 141.55 (75.07) | 390-1159 | **0.004** | **<0.001** | **No** |
| Citric acid | 6 | 2.57 (0.81) | 1.6-4.5 | 0.016 | 0.001 | Yes |

[a] P-values of one-sample T-test comparing the average value to the lower reference value; [b] P-values of one-sample T-test comparing the average value to the upper reference value.

**Biochemical results**

Amongst the parameters that were measured from the blood and urine samples of OI patients, the average serum levels for osteocalcin, glucose, insulin, and citric acid were within the reference ranges of healthy children of the same age group. However, the average serum levels for CTX and P1NP were significantly lower than the lower limit of the reference ranges of healthy children of the same age group. Therefore, CTX and P1NP serum levels were not within their respective reference ranges (Table 3).

Out of the 25 amino acids that were measured from the blood samples of OI patients, the average serum levels of 18 amino acids were within the specified reference ranges for healthy children of the same age group (Table 4).

**Table 4.** Average values of 25 amino acids measured from the fasted blood samples of OI types IV and III participants relative to the reference ranges obtained from the MUHC clinical laboratories directory.

| **Amino acids** | **Sample size (n)** | **Average value (SD)** | **Reference ranges (average between min and max value** | **P-value[a] (lower limit)** | **P-value[b] (upper limit)** | **Within range** |
|---|---|---|---|---|---|---|
| Taurine | 5 | 84.2 | 41-69 | 0.03 | 0.206 | Yes |
| Aspartic acid | 5 | 9.4 | 3-6 | 0.111 | 0.243 | No |
| Threonine | 5 | 98.8 | 65-125 | 0.046 | 0.081 | Yes |
| Serine | 5 | 128.2 | 96-155 | 0.029 | 0.047 | Yes |
| Asparagine | 5 | 49.8 | 31-67 | <0.001 | <0.001 | Yes |
| Glutamic acid | 5 | 51.2 | 13-65 | 0.002 | 0.052 | Yes |
| Glutamine | 5 | 457.8 | 493-724 | 0.203 | 0.001 | No |
| Glycine | 5 | 257 | 144-282 | 0.009 | 0.223 | Yes |
| Proline | 5 | 255.4 | 93-201 | 0.041 | 0.242 | No |
| Alanine | 5 | 320.6 | 182-319 | 0.072 | 0.492 | No |
| Valine | 5 | 229.2 | 165-234 | 0.006 | 0.379 | Yes |
| 1/2 Cystine | 5 | 31.8 | 33-54 | 0.284 | <0.001 | No |
| Methionine | 5 | 17.6 | 14-25 | 0.068 | 0.009 | Yes |
| Isoleucine | 5 | 56 | 40-69 | 0.007 | 0.014 | Yes |
| Leucine | 5 | 104.8 | 86-136 | 0.023 | 0.005 | Yes |
| Tyrosine | 5 | 56 | 39-65 | 0.009 | 0.053 | Yes |
| Phenylalanine | 5 | 43 | 40-61 | 0.178 | 0.002 | Yes |
| Ornithine | 5 | 58.2 | 25-50 | 0.015 | 0.230 | No |
| Lysine | 5 | 125.8 | 96-181 | 0.042 | 0.007 | Yes |
| Histidine | 5 | 68 | 63-93 | 0.100 | <0.001 | Yes |
| Arginine | 5 | 45.6 | 50-99 | 0.171 | <0.001 | No |
| Citrulline | 5 | 24.2 | 23-37 | 0.309 | 0.002 | Yes |

| | | | | | | |
|---|---|---|---|---|---|---|
| Homocystine | 5 | 0 | ≤ 0 | N/A | N/A | Yes |
| Allo-Isoleucine | 5 | 0 | ≤ 0 | N/A | N/A | Yes |
| Tryptophan | 5 | 44.4 | 37-76 | 0.196 | 0.007 | Yes |

[a] P-values of one-sample T-test comparing the average value to the lower reference value; [b] P-values of one-sample T-test comparing the average value to the upper reference value.

**Resting energy metabolism**

To assess resting energy metabolism, the parameters that were measured include average $VO_2$ relative to body mass and heart rate. The average $VO_2$ relative to body mass of children with OI was compared to the average value of the control group as well as to a standard reference value for children and youth of both genders aged between 2.8 years and 18.6 years (105). This analysis was done with a one-sample t-test. The average $VO_2$ relative to body mass of the OI group (7.83 ± 2.01 ml/kg/min) was not significantly different from the control group (6.75 ± 1.59 ml/kg/min). However, the average $VO_2$ relative to body mass of the OI group (7.83 ± 2.01 ml/kg/min) was 38% greater (p=0.023) than that of the reference value for children and youth of the same age (5.67 ml/kg/min) (Figure 2).

The average basal heart rate of both OI types III and IV patients were compared with that of the control group as well as to the reference basal heart rate of healthy children of the same age and gender (104) using a one-sample t-test. The results showed that the average basal heart rate of the OI group (97.36 ± 3.89 bpm) was 27% greater (p=0.003) than that of the control group (76.62 ± 2.64 bpm). In addition, the basal heart rate of the OI group (97.36 ± 3.89 bpm) was 18% greater (p=0.013) than that of the reference basal heart rate of the normal population (82.63 ± 3.11 bpm) (Figure 3).

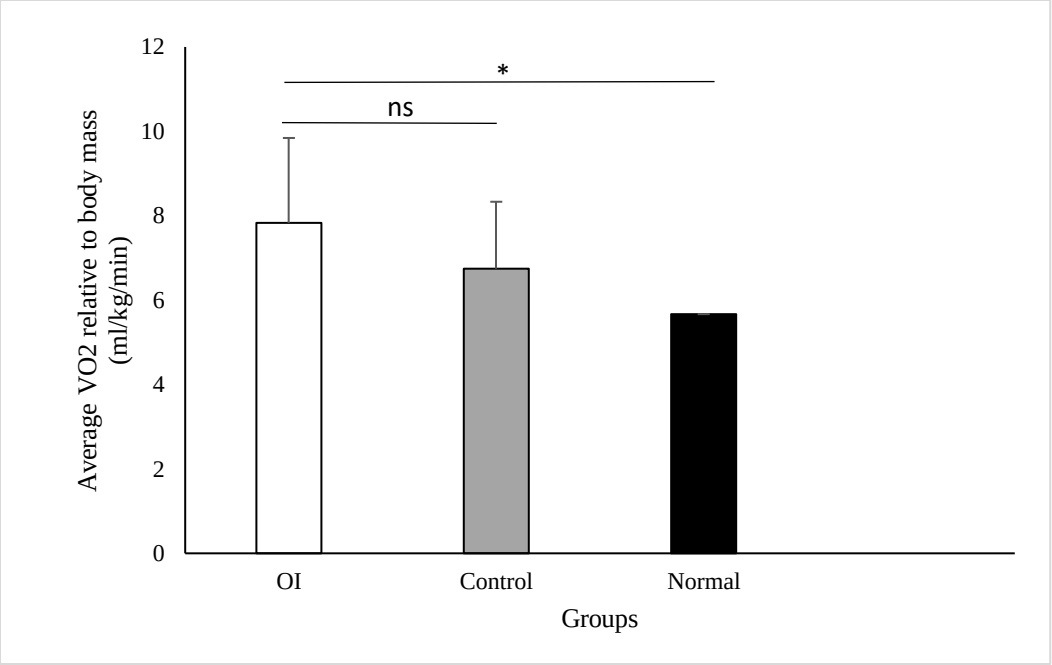


**Figure 2.** Average VO2 relative to body mass of OI type IV and III participants at rest. Results are shown for the OI type group (n= 8) relative to the average value of the control group (n=2) and the average pediatric reference values from literature (105). The level of significance of the difference between the OI group and control group is p-value 0.131 (ns: non-significant) and the level of significance of the difference between the OI group and the pediatric reference value from literature is p-value 0.023*. Data represent mean ± SD.

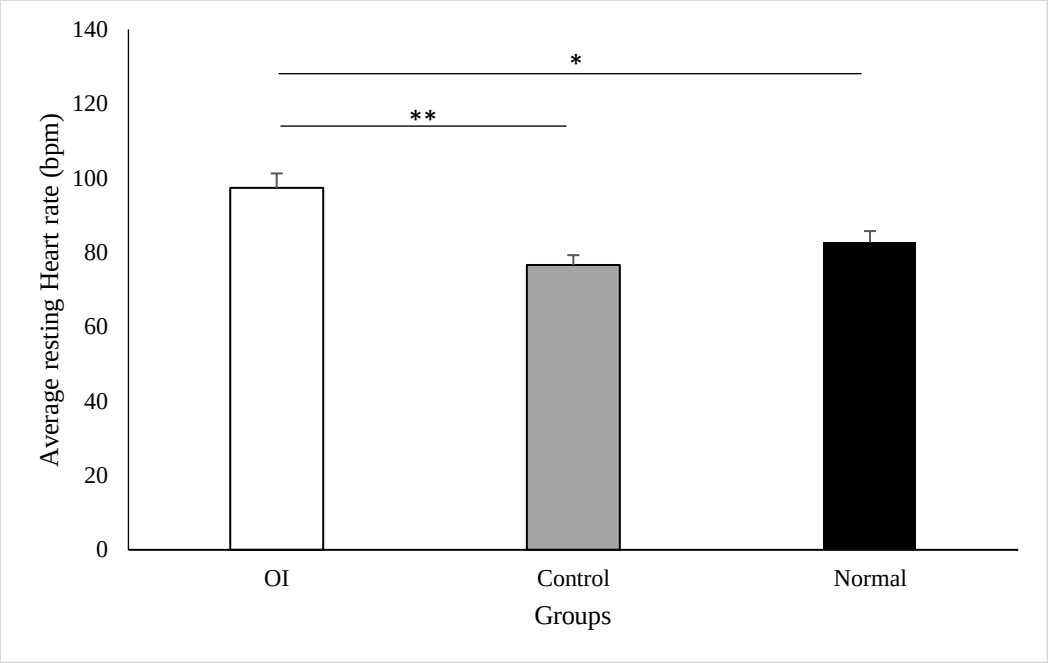


**Figure 3.** Average basal heart rate of OI type IV and III participants at rest. Results are shown for the OI type group (n= 8) relative to the average value of the control group (n=2) and the average pediatric reference values from literature (104). The level of significance of the difference between the OI group and control group is p-value 0.003** and the level of significance of the difference between the OI group and the pediatric reference value from literature is p-value 0.013*. Data represent mean ± SD.

**6MWD**

The average total 6MWD of the OI type IV group was compared to the average total 6MWD of the control group using a one-sample t-test. In addition, a one-sample t-test was also performed to compare the average total 6MWD of the OI type IV group to the average reference value of the total distance healthy children of the same age and gender would cover during a 6MWT (106). The distance covered by the OI type IV group (286.60 ± 184.01 m); was 39% lower (p=0.044) than the distance covered by the control group (471.68 ± 154.10 m). In a similar manner, the average distance covered by the OI type IV group (286.60 ± 184.01 m) was 54% lower (p=0.007) than the reference 6MWD (623.40 ± 64.80 m) (Figure 4).

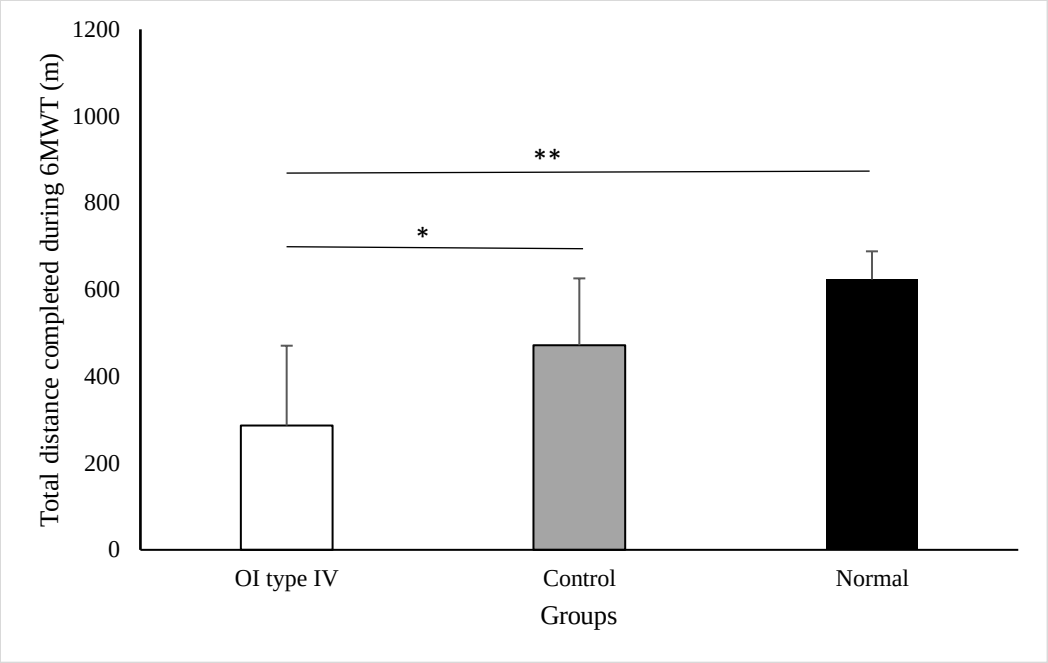


**Figure 4.** Total 6MWD (m) of OI type IV participants. Results are shown for the OI group (n= 5) relative to the control group (n=2) and the average pediatric reference values from literature (106). The level of significance of the difference between the total 6MWD of OI type IV participants and the total 6MWD of the control group is p-value 0.044*. The level of significance of the difference between the total 6MWD of OI type IV participants and the pediatric reference 6MWD of the from literature is p-value 0.007**. Data represent mean ± SD.

**Energy metabolism during 6MWT**

To assess energy metabolism during the 6MWT, the parameters that were measured are $VO_2$max (L/min) and heart rate (bpm). The average $VO_2$max of OI type IV participants was compared to the average value of the control group as well as to a standard reference value for healthy children of the same age and gender which was computed with the Wasserman equation (107). This analysis was done with a one-sample t-test. The average $VO_2$max of the OI type IV group (0.87 ± 0.28 L/min) was not significantly different from the control group (0.82 ± 0.14 L/min). However, the average $VO_2$max of the OI type IV group (0.87 ± 0.28 L/min) was 40% lower (p=0.004) than that of the reference value for children and youth with the same age (1.46 ± 0.37 L/min) (Figure 5).

The average heart rate of OI type IV participants was compared to that of the control group as well as to the average value of reference heart rates of healthy children of the same age and gender using a one-sample t-test (106). The results showed that the average heart rate of the OI type IV group (133.92 ± 6.10 bpm) was 20% higher (p=0.0006) than that of the control group (111.52 ± 12.38 bpm). In contrast, the average heart rate of the OI type IV group (133.92 ± 6.10 bpm) was not significantly different from the normal population (138.2 ± 5.76 bpm) (Figure 6).

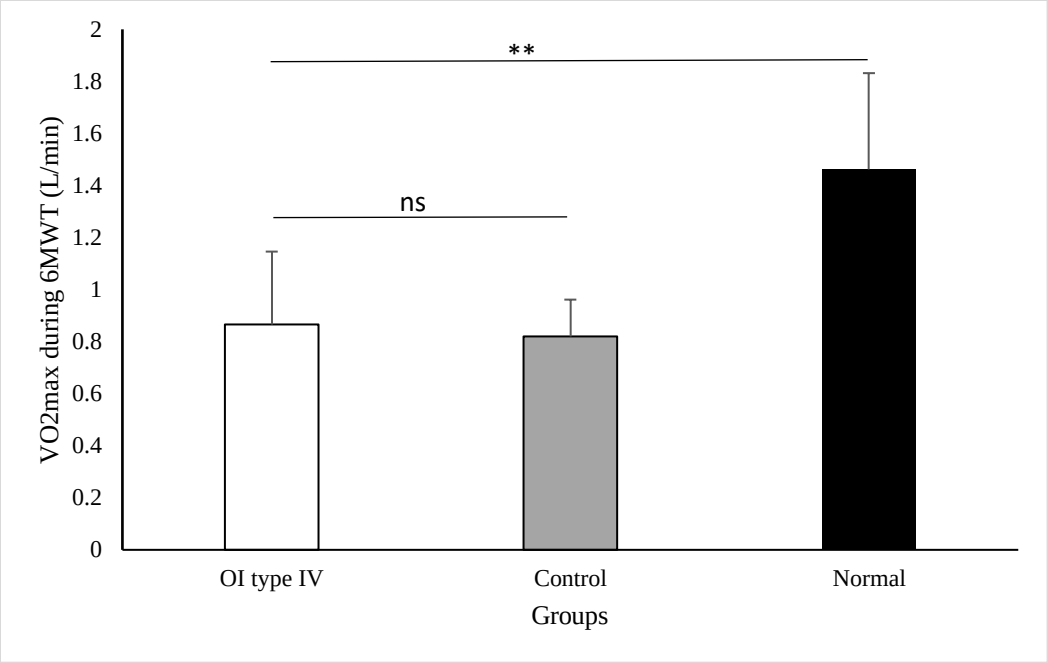


**Figure 5.** $VO_2$max (L/min) of OI type IV participants during 6MWT. Results are shown for the OI type IV group (n=5) relative to the $VO_2$max of the control group (n=2) and to the average pediatric $VO_2$max value from literature (107). The level of significance of the difference between the OI type IV group and the control group is p-value 0.412 (ns: non-significant). The level of significance of the difference between the OI type IV group and the reference $VO_2$max value of the normal population is p-value 0.004**. Data represent mean ± SD.

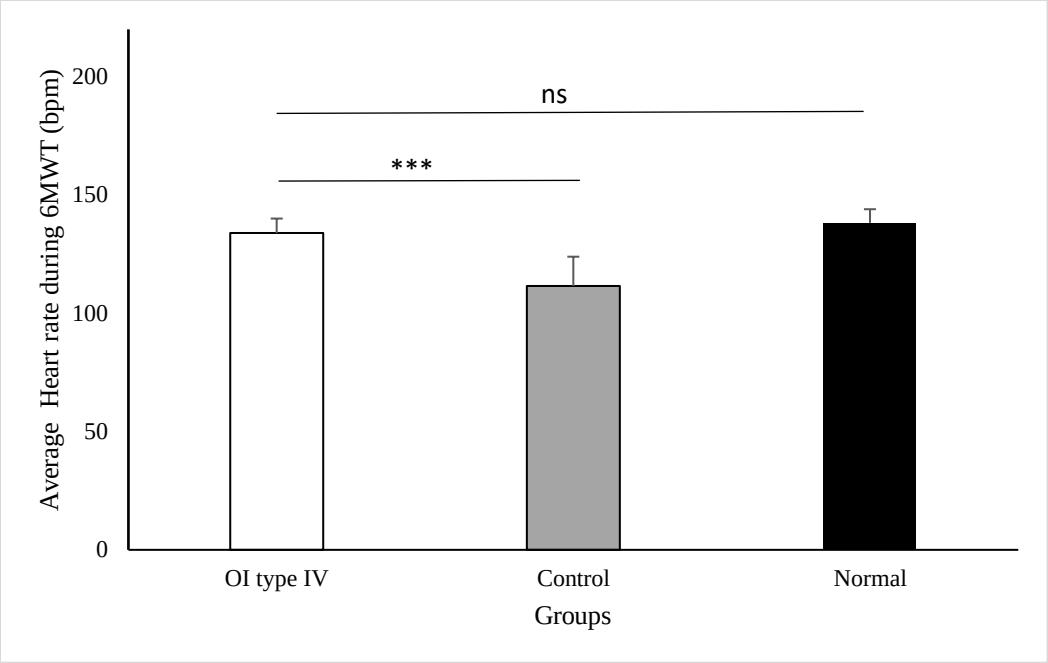


**Figure 6.** Average HR (bpm) of OI type IV participants during 6MWT. Results are shown for the OI type IV group (n=5) relative to the average HR of the control group (n=2) and to the average pediatric reference values from literature (106). The level of significance of the difference between the OI type IV group and the control group is p-value 0.0006***. The level of significance of the difference between the OI type IV group and the pediatric reference value from literature is p-value 0.096 (ns: non-significant). Data represent mean ± SD.

**Cardiopulmonary fitness during SRiT**

Due to the absence of reference data on the total distance covered by healthy children in a wheelchair while performing the SRiT, the average total distance covered by the OI type III group during the SRiT was compared to the average total distance healthy children covered while performing a walk test similar to the SRiT using a one-sample t-test (108). In addition, a one-sample t-test was also performed to compare the average total distance covered by the OI type III group in a wheelchair to the average total distance children with moderate to severe OI covered during a SRiT as a reproducibility measure (101). The OI type III group completed an average distance of (152.4 ± 47.59 m) which was 82% lower ($p=0.0007$) than the reference distance covered by the healthy children which performed a walk test (874.61 ± 185.82 m). Moreover, the average total distance covered by the OI type III group was not significantly different from the distance covered by children with moderate to severe OI (119 ± 30 m) in another study in which the SRiT was used (Figure 7).

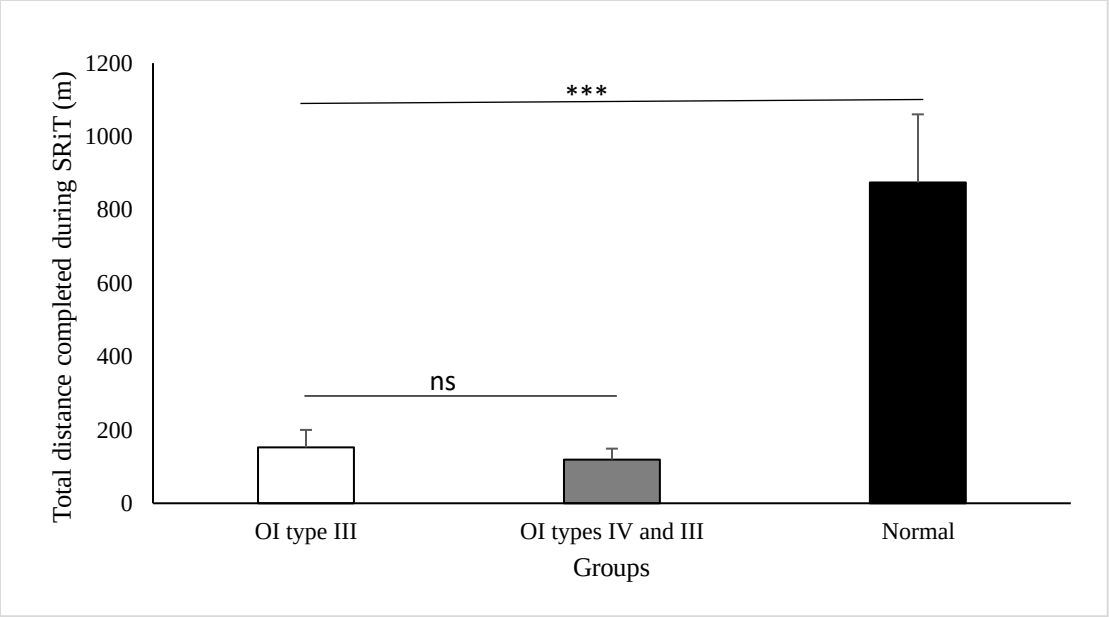


**Figure 7.** Total distance completed (m) during SRiT of OI type III participants. Results are shown for the OI type III group (n= 3) relative to the average pediatric reference values from literature (108) and to the total distance completed by OI types III and IV participants (n=13) in a similar study (101). The level of significance of the difference between the OI type III group and the pediatric reference value from literature is p-value 0.0007***. The level of significance of the difference between the OI type III group and the OI types IV and III group from *Bongers et al., 2016* is p-value 0.174 (ns: non-significant). Data represent mean ± SD.

**Energy metabolism during SRiT**

To assess energy metabolism during the SRiT, the parameters that were measured include $VO_2$max (L/min) and heart rate (bpm). The average $VO_2$max of OI type III participants were compared to the standard reference value for healthy children of the same age and gender which was computed with the Wasserman equation (107). This analysis was done with a one-sample t-test. The average $VO_2$max of the OI type III group (0.80 ± 0.22 L/min) was not significantly different from the reference value for children and youth with the same age (0.87 ± 0.44 L/min) (Figure 8).

The average heart rate of OI type III participants was compared to the average value of reference heart rates of healthy children of the same age and gender using a one-sample t-test (108). The results showed that the average heart rate of the OI type III group (115.84 ± 18.85 bpm) was not significantly different from the average heart rate of the normal population (190.7 ± 15.2 bpm) (Figure 9).

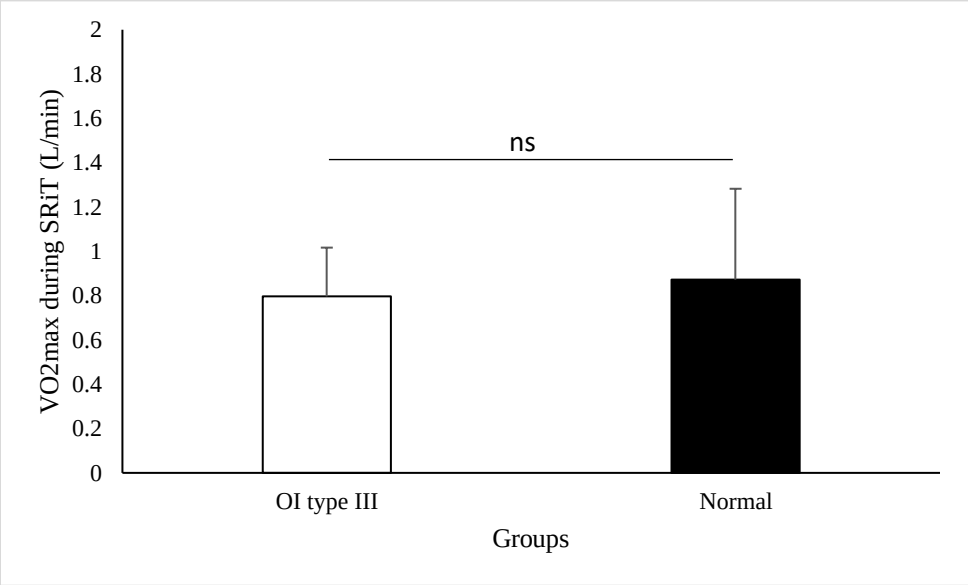


**Figure 8.** $VO_2$max OI type III participants during SRiT. Results are shown for the OI group (n= 3) relative to the average pediatric reference values from literature (107). The level of significance of the difference between the OI type III group and the pediatric reference value from literature is p-value 0.309 (ns: non-significant). Data represent mean ± SD.

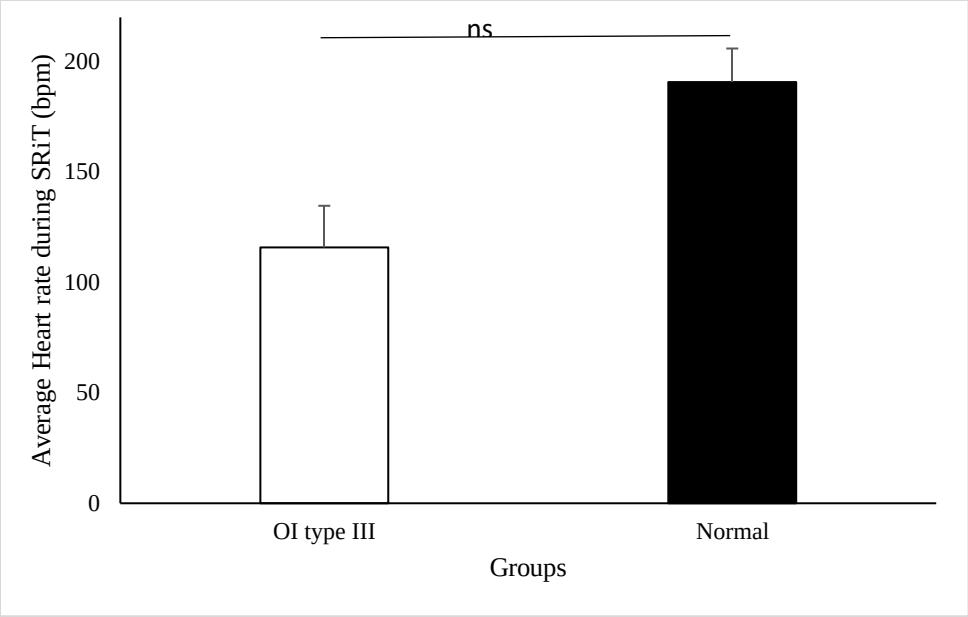


**Figure 9.** Average HR (bpm) of OI type III participants during SRiT. Results are shown for the OI type III group (n= 2) relative to the average pediatric reference values from literature (108). The level of significance of the difference between the OI type III group and the pediatric reference value from literature is p-value 0.056 (ns: non-significant). Data represent mean ± SD.

**Bone Health parameters**

The two bone parameters measured with through pQCT are trabecular vBMD at the metaphysis and cortical vBMD at the diaphysis of the radius. We observed that the average trabecular vBMD at the metaphysis of the OI group (276.85 ± 44.72 mg/cm$^3$) was 41% higher (p=0.019) than the reference value of healthy children of the same age and gender (99) (196.60 ± 6.39 mg/cm$^3$) (Figure 10).

In contrast, the average cortical vBMD at the diaphysis of the radius of the OI group (1036.63 ± 45.52 mg/cm$^3$) was not significantly different from the reference value of healthy children of the same age and gender (109) (992.78 ± 35.22 mg/cm$^3$) (Figure 11).

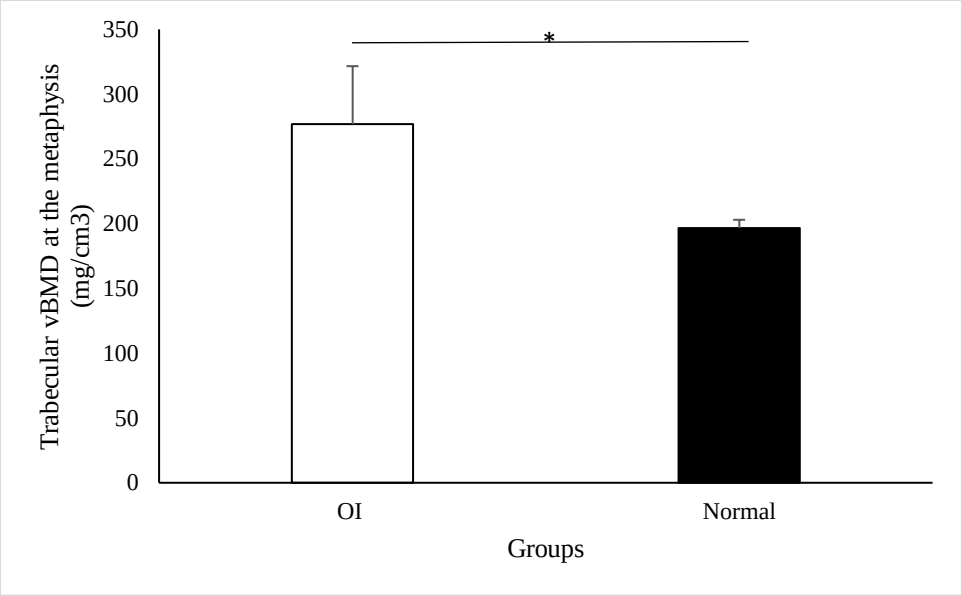


**Figure 10.** Trabecular vBMD at the metaphysis of the radius of the OI type III and IV participants. Results are shown for the OI group (n= 4) relative to the average pediatric reference values from literature (99). The level of significance of the difference between the trabecular vBMD value of the OI group and the pediatric reference value from literature is p-value 0.019*. Data represent mean ± SD.

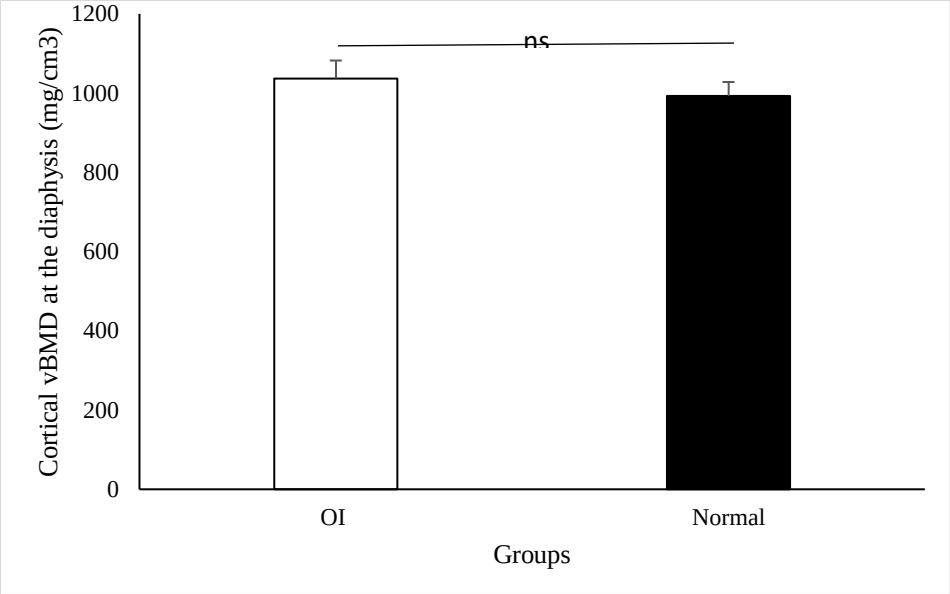


**Figure 11.** Cortical vBMD at the diaphysis of the radius of OI type III and IV participants. Results are shown for the OI group (n= 3) relative to the average pediatric reference values from literature (109). The level of significance of the difference between the cortical vBMD value of the OI group and the pediatric reference value from literature is p-value 0.119 (ns: non-significant). Data represent

# Chapter 5 - Discussion

Our overarching hypothesis was that the high bone turnover in children with OI types III and IV would cause a higher bone metabolism which will lead to an increased resting energy metabolism than in their healthy counterparts. This hypothesis was partially supported by our study results. Due to an unprecedented halt in recruitment because of the Covid-19 pandemic, we ended up with a sample size of 8 OI types III and IV participants and 2 healthy controls.

**Resting energy metabolism and bone turnover**

Our findings demonstrated that the resting energy metabolism of the OI group was significantly higher than the reference value. This is in line with previous findings that demonstrated that both the resting $VO_2$ and heart rate of prepubertal children with OI were higher than those of healthy children of the same age group (66). In opposition to our hypothesis, the fasting osteocalcin serum levels of our OI group were within normal reference ranges, suggesting that bone turnover is not elevated in these patients. The most likely explanation for this observation is that these patients were on BP treatment which promotes the inhibition of bone resorption by osteoclasts and in turn preventing the release of uOCN into the circulation (32, 33). In addition, due to normal osteocalcin serum activity, all the metabolites that are influenced by osteocalcin might maintain a normal activity. This could explain why glucose, insulin, citric acid, and majority of the amino acids were within normal reference ranges. The normal levels of osteocalcin reported in BP treated patients thus suggests that BP may lead to energy metabolism normalization.

Nevertheless, our findings suggest that during rest, children with OI types III and IV require more oxygen for energy metabolism compared to their healthy counterparts despite low bone turnover associated with BPs. This elevated resting energy metabolism could be partially due to the elevated resting heart rate observed in these patients which were 14.73 bpm higher than norms and controls. Another possibility is that similar to mice models of OI, individuals with OI may also have dysfunctional mitochondria in the skeletal muscle. In a recent study on the *oim/oim* mouse model, these mice demonstrated mitochondrial dysfunction localized in the skeletal muscle (68). Mitochondria are responsible to produce most of the human body energy. Thus, although speculative at this point, it is suggested that individuals with OI requires more oxygen to produce the same amount of energy than a typically developing child because of mitochondrial dysfunction. Obviously, more studies aiming to find an association between mitochondrial integrity and activity

in the skeletal muscle and energy metabolism and muscle weakness are needed to confirm our hypothesis.

Surprisingly, the P1NP and CTX (bone turnover markers) serum levels of our OI participants were significantly lower than the normal reference ranges. These results indicate that bone turnover hence bone metabolism was very low in these patients. It has been documented that BP treatment is associated with decreased bone remodeling (34). Therefore, we can infer that one effect of BP treatment is to decrease bone turnover leading to the reduction in serum bone turnover markers. Both findings are thus suggesting that BP treatment reduced bone turnover/bone metabolism and therefore bone metabolism is not responsible for the high resting energy metabolism in these patients.

**Cardiopulmonary function**

Given that there is a paucity of information on energy metabolism during effort/exercise we decided to assess the cardiopulmonary function of our participants during effort. Only one study in individuals with OI type I suggested that they have an absolute and relative $VO_2$max lower than their healthy counterparts (62). So, testing individuals with OI types IV and III would complement the existing literature. In terms of cardiopulmonary function, we hypothesized that the OI group would have a lower cardiopulmonary function compared to healthy controls and the reference value of children in the same age group. We assessed the cardiopulmonary function of OI type IV participants with a 6MWT and given the severe clinical presentation of OI type III, their cardiopulmonary function was assessed with the use of a Shuttle Ride test (SRiT). We observed that the average 6MWD of the OI type IV group was significantly lower than that of the control group and the reference value. Moreover, the average $VO_2$max of the OI type IV group was not significantly lower than the control group but significantly lower than the reference value. In addition, the average heart rate of the OI type IV group during effort was higher than the control group, but no different from the reference values. When we divided the $VO_2$max with the 6MWD of the OI type IV group and compared to the reference value. We noted that $VO_2$max/distance for the OI type IV group is 0.0030L/min/m while the reference value is 0.0023L/min/m. This suggests that the cardiopulmonary function of the OI type IV group is lower than that of healthy children because OI type IV patients consume more oxygen to cover a 1 m distance than their healthy

counterparts. Therefore, OI type IV participants are less efficient at using oxygen than their healthy counterparts.

For the OI type III group, the cardiopulmonary fitness during effort was not significantly different from the reference values. However, this comparison was not equitable, so we decided to compare the distance covered by the OI type III group during the SRiT to the distance covered by patients with moderate to severe OI in another study in which the same method was used (101). We observed that the average distance covered by our OI type III participants was similar to the average distance covered by the moderate to severe participants in the other study. In addition, the distance covered by our OI type III group was lower than the distance covered by healthy children who performed a similar test to the SRiT in another study (101). Given the small number of OI type III participants we had, we cannot conclude strongly whether our hypothesis is true or false. However, these observations taken together suggest that OI type III participants might have a lower cardiopulmonary function compared to healthy children.

The results of the current study are in line with those of a previous one in which participants with OI type I showed cardiopulmonary deficits during physical exertion (62). There are multiple hypotheses to explain these observed deficits. Firstly, previous studies from our research group have shown that individuals with OI have decreased specific force (lower force per unit of muscle area), suggesting that intrinsic muscle properties may be altered in OI. Some of the intrinsic muscle properties include muscle fiber composition, inter and intra- muscle fat. In turn, this suggest that for a given effort, a patient with OI will use a larger proportion of their muscle maximal force which may lead to premature muscle fatigue than their healthy counterparts (4). Secondly, previous mice studies suggest that tendon material properties might be lower in OI than in WT mice (110). This is because tendons are packed with collagen type I. Therefore, lower material properties would lead to less efficient force transmission, i.e., energy will be dissipated in the tendon rather than being transferred to the bone to generate movement. Again, this may lead to early fatigue. Finally, recent mice studies suggest that there might be mitochondrial dysfunction in the skeletal muscle associated with OI mice with a collagen type I mutation (68, 111). Since the mitochondria play an important role in energy production and oxygen consumption, it would be interesting to investigate if deficits in this structure might cause the lower cardiopulmonary function in individuals with OI.

**Bone Health parameters**

It is well documented that OI patients have low bone cortical width and trabecular volume due to reduced trabecular number and thickness showing that children with OI have low bone mass (16). This low bone mass is caused by abnormalities in bone modeling, production of trabeculae by endochondral ossification and bone remodeling. Hence, we hypothesized that we would observe a significantly lower trabecular vBMD and cortical vBMD in OI type III and IV patients compared to healthy children. Surprisingly, we observed that the trabecular vBMD at the metaphysis was significantly higher than the reference value. However, the cortical vBMD at the diaphysis was not significantly different from the reference value for healthy children. These results could have been influenced by the effect of bisphosphonate treatment which is known to reduce osteoclast activity resulting in the inhibition of bone resorption, thereby promoting bone formation from the inside which results in higher BMD (34). In a previous study, zoledronate was found to increase total BMD as well as reduce bone turnover in children with secondary osteoporosis with over 2 years of BP treatment (112). In addition, another study reported that zoledronate increased volumetric BMD in children who had sustained a spinal cord injury (113). Hence demonstrating that the effect of BPs could account for the increase in trabecular vBMD in the metaphysis in our patients. Another beneficial effect of bisphosphonates is the increase in bone mineralization (34). Therefore, an increase in bone mineralization will lead to an increase in cortical vBMD in individuals with OI because cortical vBMD is mainly impacted by the degree of matrix mineralization. So, this could explain that the cortical vBMD of the OI types III and IV participants was non significantly different from the reference value for healthy children.

**Supplementary analyses**

Our secondary analyses demonstrated that there are no significant differences in the resting energy metabolism between boys and girls in the OI group. There is a lack of studies in humans with OI that compare the differences in resting energy metabolism between prepubertal boys and girls. However, it was demonstrated that 4-week-old male and female *Col1a1*$^{Jrt/+}$ mice consume more oxygen and produce more $CO_2$ per body weight compared to WT during low movement. Hence there are no differences in resting energy metabolism between both sexes in this mice study (67). It is well documented that fat body composition is a significant predictor of resting metabolic rate (114). Our findings could be explained by the fact that the average BMI of our female and

male participants is considered normal. Therefore, both boys and girls did not have a high body fat content, so comparatively both resting energy metabolisms were not significantly different. Moreover, for both OI types, the energy metabolism during effort was not significantly different between boys and girls. This observation is in line with the study on *Col1a1*$^{Jrt/+}$ mice, in which energy expenditure at 4 weeks old was the same in both sexes (67). Therefore, further investigations with a reasonable sample size should be developed to measure the resting energy metabolism and energy metabolism during effort of children with OI without BP treatment and compare it to those under BP treatment. Moreover, the body fat composition of prepubertal children with OI should be evaluated to investigate if it could explain the hypermetabolic state observed in this population.

**Limitations of the study**

The main limitation of this study was the small sample size. The results we obtained from 5 OI type IV and 3 OI type III children cannot be generalized to all pre-pubertal children with OI types III and IV. In addition, the small sample size of controls allowed for very little comparison to be made between the OI type III and IV participants and healthy children. Moreover, we compared the results we obtained from the OI group to the reference values of healthy children of the same age group in replacement of a true control group because our control group was made up of only 2 participants. Furthermore, the collection of several blood samples from children who might fear needles was one of the main reasons 33.33% of the children we approached refused to participate in our study. Many participants did not appreciate having to stay fasted after receiving their bisphosphonate treatment to perform the resting oxygen consumption test. Some participants were unable to have a pQCT performed on them due to their small size. Therefore, to overcome this challenge we should have used another method to assess volumetric bone density (bone turnover) such as dual energy x-ray absorptiometry (DEXA). Another limitation was the use of a manual wheelchair by OI type III patients during the SRiT. For participants who use an electric wheelchair, the time needed to adjust to having to propel themselves on the manual wheelchair might have slightly affected their performance on the SRiT. For future studies, it will be beneficial to buy electric wheelchairs for OI type III participants so that we can reduce the time needed for the participant to adjust to a new wheelchair. Furthermore, we compared the distance covered by OI type III participants with the reference distance covered by healthy children performing a similar

test while walking. This might have caused us to underestimate the performance of OI type III participants. We also used reference values because we didn't find data on healthy children who had performed this test with a wheelchair.

# Chapter 6 - Conclusion

The results of the current study suggest that the high resting energy metabolism observed in OI type III and IV patients is not associated with high bone turnover and high osteocalcin levels. However, their high resting energy metabolism could be due to their elevated heart rate or because of a deficit in the mitochondria which plays a major role in energy metabolism and oxygen consumption. Nevertheless, the latter explanation remains to be assessed in humans. Our patients also had lower cardiopulmonary function during the 6MWT and SRiT, demonstrating that muscle weakness which is due to deficits in the intrinsic properties of the muscle such as a dysfunctional mitochondrion might lower their cardiopulmonary function. In addition, given that we observed normal serum osteocalcin levels and significantly lower P1NP and CTX serum levels between the OI group and reference values, therefore we can infer that BP treatment decreases bone turnover in these patients.

**Future directions**

Firstly, future studies need to be developed with a larger sample size to assess the resting energy metabolism, energy metabolism during physical activity in untreated and untrained prepubertal children with OI types III and IV. This will be to confirm if there is truly a hypermetabolic state in these patients. Secondly, other studies should be developed to investigate if the muscle weakness these patients experience is associated with the altered resting energy metabolic state observed in these patients. This study will help us begin to decipher if there is a connection between muscle weakness and energy metabolism. Lastly, studies with a larger sample size should be performed to measure osteocalcin levels and all the other energy metabolism parameters in untreated OI patients and compare it to those in treated OI patients. This study will determine if osteocalcin levels are high in untreated individuals with OI and if it is associated with a high energy metabolic state. These studies are important to help us gain more insight on the pathophysiology of OI.

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