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# Effect of Bisphosphonates on the response to mechanical stimulation in children with osteogenesis imperfecta

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By

Sivagamy Sithambaram

A thesis submitted in partial fulfilment of the requirements for the degree of  
Doctor of Medicine

The University of Sheffield  
Academic Unit of Child Health  
Department of Oncology and Metabolism

January 2024

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## Statement of attribution:

Study inception: Professor Nick Bishop and Sivagamy Sithambaram

Study design: Professor Nick Bishop, Sivagamy Sithambaram and Sheffield Children’s Clinical Research Facility (CRF) Team

Ethics: Professor Nick Bishop, Sivagamy Sithambaram and Sheffield Children’s Clinical Research Facility (CRF) Team

Patient Visits: Sivagamy Sithambaram and Research Nurse

Result analysis: Professor Alan Rigby and Sivagamy Sithambaram

## List of presentations and publications:

1. International Conference on Children's Bone Health 2020 – electronic poster (E-poster) presentation
2. International Conference on Osteogenesis Imperfecta 2017 – E- poster presentation
3. Sithambaram S, Harrison R, Gopal-Kothandapandi S, Rigby A, Bishop N. Bisphosphonate Treatment Alters the Skeletal Response to Mechanical Stimulation in Children With Osteogenesis Imperfecta: A Pilot Study. *JBMR Plus*. 2022 Mar 1;6(3):e10592. doi: 10.1002/jbm4.10592. PMID: 35309861; PMCID: PMC8914162.

## Summary

Children with osteogenesis imperfecta (OI) are commonly treated with bisphosphonates. We investigated the skeletal response to mechanical stimulation in children with osteogenesis imperfecta (OI) before and after bisphosphonate treatment. Twelve children (5 boys; 7 girls), aged 4.5-14.9 years with mild OI and naïve to bisphosphonate treatment were recruited. They stood on a high-frequency (30 Hz), low-amplitude (50 to 200  $\mu$ ) vibrating platform (Marodyne LivMD) for 10 minutes daily (2.5 minutes X 4 with interspersed 1-minute rest periods) for 7 days (whole body vibration [WBV] 1; day (D 1–7), followed successively by 5 weeks' monitoring without intervention, 6 weeks' risedronate treatment, 1 week of WBV (WBV2; D85–91), and 1 week without intervention (D92–98). Procollagen type I N-terminal propeptide (P1NP), bone-specific alkaline phosphatase (BSALP), and carboxy-terminal telopeptide of type I collagen cross-link (CTX) were measured at baseline and intervals bracketing periods of vibration and risedronate treatment. Both P1NP and CTX rose to D8 (18.4%, 13.8%,  $p < 0.05$ , respectively), plateaued, then rose again at D43 (19.8%, 19.2%, respectively,  $p < 0.05$  versus baseline). At D85 (after risedronate) both P1NP and CTX had fallen to pre-WBV1 levels. A significantly smaller increase in P1NP was found after WBV2 (D85–91) at D92 (3.5%, 9.2%, respectively) and D99 versus after WBV1 (both  $p < 0.05$ ). BSALP

changed little after WBV1, fell during risedronate, and rose toward baseline after WBV2. We thus showed that WBV increased bone formation and resorption; that increase was attenuated after risedronate. The early increase in P1NP and CTX (D8) after WBV1 suggests increased osteoid formation within existing remodeling units but not increased mineralization. Later increases in P1NP/CTX (D42) suggesting creased remodeling cycle initiation after WBV. Risedronate suppressed both biomarkers. The lower increase in P1NP/CTX after WBV2 suggests limited capacity to increase osteoid formation from existing “early stage” osteoblasts and a possible “hangover” effect of risedronate on remodeling activation. These results provide insights into both the response to WBV, ie,mechanical stimulation, and the effect of antiresorptive therapy in children with OI.

## Acknowledgements

First of all, I would like to thank my supervisors Nick Bishop and Amaka Offiah for all your support. Thank you, Nick, for all your help, support, time and motivation. I could not have completed this without you. Alan Rigby, thank you very much for your help and support with the results and statistics.

Thank you to Jill Clayton-Smith for overall support with the writing up of the thesis. Fatma Gossiel, thank you for all your help with the analysis of bone biomarkers. A huge thanks to PhD students, Nicki Barker, Rachel Harrison and Sujatha Gopal-Kothandapani for all your advice with the study overall.

I am very grateful to the staff at the Clinical Research Facility at Sheffield Children’s Hospital who have worked together with me in some of the study visits and blood sampling. Thank you to all the participants and their families who have kindly welcomed me into their homes early in the mornings allowing me to conduct the study visits.

Thank you to my parents and family for ongoing help, support and encouragement. I could not have done this without you.

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## 1. Literature Review

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### 1.1 Introduction

Osteogenesis Imperfecta (OI) is a spectrum of genetic disorders associated with decreased bone density and increased bone fragility with an estimated prevalence of 7.4/100000 (Lindahl et al 2015 )– see Table 1. It is characterised by fractures associated with minimal or absent trauma, dentinogenesis imperfecta, blue sclerae and progressive hearing loss (Basel and Steiner, 2009). The fracture risk is greatest during childhood. Osteogenesis imperfecta has been classified into different subtypes with varying severity. It has a genetic basis, being inherited in an autosomal dominant, recessive or X-linked fashion and to date 20 different genes giving rise to OI have been identified. Individuals affected by OI, particularly the more severe types have a number of significant health problems impacting on their quality of life and the condition requires life-long multidisciplinary management.

The aim of treatment and management is to maximise function and wellbeing of children and young people in order to live as independently as possible. Bisphosphonates are a group of drugs that slow down bone loss by suppressing osteoclast activity, inhibiting bone remodelling and osteoclastic modelling activity. They are extensively used in children and young people with OI. It is important to understand if bisphosphonate treatment influences the normal response to mechanical stimulation in children with osteogenesis imperfecta, as physical activity is known to be a major determinant of bone accrual and maintenance during this period of life.

Whole body vibration provides a method of applying standardised mechanical stimulation to bone that is then reflected in bone biomarker changes (Gopal-Kothandapani et al 2020, Harrison et al 2015). It is a type of passive exercise training that uses high-frequency mechanical stimuli generated by a vibrating platform and transmitted through the body (Küçükdeveci et al 2019). Low intensity whole body vibration (0.4g, 30Hz) is considered safe and suitable to be used as a standardized form of physical exercise in research setting to assess the skeletal response to mechanical loading/stimulation in individuals with low bone mass.

I will provide a detailed review of OI, bone modelling and remodelling and the influence of mechanical stimulation.



Table 1 OI prevalence worldwide

1 Prevalence rates are per 10,000 population

Reference	Gimeno-Martos et al., 2017	Lindahl et al., 2015	Orioli et al., 1986	Stoll et al., 1989	Andersen and Hauge, 1989	Moffitt et al., 2011	Orioli et al., 1986
Region	Spain (Valencia region)	Sweden (paediatric population)	Italy		Denmark (Fyn region)	Texas, USA	South America
Collection Year(s)	2004-2014	2005-2014	1978-1981		1970-1983		1978-1983
Case definition (main method)	Clinical diagnosis	Diagnostic testing	Clinical diagnosis		Clinical and radiological diagnosis		Clinical diagnosis
Data collection method	Survey (patient records)	Survey (patient records, family interviews)	Birth Defects Registry		Survey (patient records)	Birth Defects Registry	Birth Defects Registry
Design	Observational study (cross-sectional)	Observational study (cross-sectional and longitudinal)	Observational study (cross-sectional)		Observational study (cross-sectional and longitudinal)		Observational study (cross-sectional)
Reference population size	5.0 m (2014)	1.9 m (2010)	0.22 m births		0.45 m (1983)		0.35 m births
Calculated prevalence <sup>1</sup>	0.29	0.74	0.37	0.64	1.06		0.43

## 1.2 Classification of OI

OI was originally classified into 4 types based on clinical and radiological features (Sillence et al, 1979). Table 2 shows the original Sillence classification. The original Sillence classification was revised by Sillence and van Dijk in 2014 incorporating the known genes (van Dijk and Sillence, 2014). Advances in genomic technology has led to a better understanding of the causative genes in OI and the genes involved in bone metabolism.

Alternative classification of OI is a functional one based on the compromised metabolic pathways as proposed by Forlino and Marini (Forlino and Marini, 2016). These include:

- A) collagen synthesis, structure defects (*COL1A1* and *COL1A2*)
- B) collagen modification defects (*CRTAP*, *LEPRE1* and *PP1B*)
- C) collagen processing and cross-linking defects (*SERPINH1*, *FKBP10*, *KDEL2*, *PLOD2* and *BMP1*)
- D) mineralization defects (*IFITM5* and *SERPINF1*)
- E) osteoblast differentiation defects (*WNT1*, *CREB3L1*, *SP7*, *TMEM3B*, *SPARC*, *MBTPS2*, *MESD* and *TENT5A*).

I have not included the *PLS3* gene variants as a cause of OI. We know that PLS3 protein is a regulator bone of development with the available in vivo data. Immunohistochemical colocalization experiments confirmed a distinct actin-bundling function of PLS3 in developing bone structure. It is hypothesized that *PLS3* variants lead to decreased mechanosensing of osteocytes, with subsequent dysregulation of bone modeling or remodeling, which results in osteoporosis and fractures (Van Dijk et al 2013).

Individuals with *PLS3* gene variants have early onset osteoporosis with increased risk of fractures. They do not have any other features of osteogenesis imperfecta. Some of them are reported to have joint hypermobility. Strictly speaking, individuals with osteogenesis imperfecta have a collagen defect that causes osteoporosis and other associated features. Therefore, *PLS3* pathogenic variants is not a cause of OI but a cause of osteoporosis.

Table 2 Original Silience classification of Osteogenesis Imperfecta

OI type	Clinical and Radiological Features	Severity	Inheritance pattern
I	Blue sclera Minimal deformities Hearing loss in adulthood	Mild	AD
II	Multiple intrauterine fractures Beaded ribs Crumpled femora	Perinatally lethal	AD, AR
III	Normal sclera Frequent fractures Severe deformities of the long bones and spine Short stature	Severe deforming	AD, AR
IV	Normal sclera Moderate deformities of long bones and spine	Moderate	AD

AD autosomal dominant inheritance

AR autosomal recessive inheritance

Table 3 Functional metabolic classification of Osteogenesis Imperfecta

Metabolic pathway	Gene	Protein	Bone deformity	Typical characteristics	Inheritance
A. Collagen synthesis, structure	<i>COL1A1</i> quantitative defect	Collagen type I, alpha 1	Mild	Grey/blue sclera. Dentinogenesis imperfecta may be present Adult onset hearing loss in about 50%	AD
	<i>COL1A1/COL1A2</i> qualitative defect	Collagen type I, alpha 2	Variable	Normal/grey/blue sclera. Dentinogenesis imperfecta and hearing loss may be absent/present A specific variant in <i>COL1A1</i> – c.3040C>T causes infantile cortical hyperostosis – Caffey disease (Gensure et al 2005)  C-propeptide cleavage site mutations in either <i>COL1A1</i> or 2 cause a high bone mass phenotype similar to that seen with BMP1 (Cundy et al 2018)	AD
B. Collagen modification defects	<i>CRTAP</i>	Cartilage associated Protein	Severe rhizomelia	Normal/grey sclera. No dentinogenesis imperfecta/hearing loss	AR
	<i>LEPRE1</i>	Leucine proline enriched proteoglycan1 /prolyl 3-hydroxylase1	Severe rhizomelia	Normal sclera No dentinogenesis imperfecta/hearing loss	AR
	<i>PPIB</i>	Peptidylprolyl isomerase B/ cyclophilin B	Severe	Grey sclera No dentinogenesis imperfecta/hearing loss	AR
C. Collagen	<i>SERPINH1</i>	Serpin	Severe	Skin blisters and bullae at birth, inguinal hernia	AR

processing and cross-linking defects		peptidase inhibitor, clade H, member 1/heat shock protein 47			
	<i>FKBP10</i>	FK506 binding protein 65	Mild to severe	Variable congenital contractures, encompasses Bruck and Kuskokwim syndromes	AR
	<i>KDEL2</i>	KDEL endoplasmic reticulum protein retention receptor 2	Moderate to severe	No hearing loss Muscular hypotonia Wheel-chair dependence in most patients	AR
	<i>PLOD2</i>	Procollagen lysine, 2 oxoglutarat 5-dioxygenase 2	Moderate to severe	Progressive joint contractures	AR
	<i>BMP1</i>	Bone morphogenic protein1/procollagen C proteinase	Variable	A very high bone mass phenotype Normal sclera. No hearing loss/dentinogenesis imperfecta C-propeptide cleavage site variants in <i>COL1A1/COL1A2</i> cause a similar a very high bone mass phenotype	AR

D. Mineralisation defects	<i>IFITM5</i>	Interferon-induced transmembrane protein 5	Variable	Ossification of the forearm interosseous membrane, radial head dislocation, subepiphyseal metaphyseal radiodense band	AD
	<i>SERPINF1</i>	Pigment epithelium-derived factor	Moderate to severe	Normal at birth, unmineralised osteoid, fish scale appearance of lamellar bone pattern, raised ALP, loss of serum PEDF	AR
E. Osteoblast differentiation defects	<i>SP7</i>	Transcription factor 7 /osterix	Severe	Delayed tooth eruption, midface hypoplasia	AR
	<i>WNT1</i>	Wingless-type MMTV integration site family, member 1	Severe	Possible neurological defects	AR
	<i>CREB3L1</i>	cAMP responsive element binding protein 3 like 1	Severe	Multiple fractures of extremities been described in a Turkish family. Blue sclera.	AR
	<i>TMEM38B</i>	Transmembrane protein 38 B	Severe	Normal to blue sclera. No hearing loss/ dentinogenesis imperfecta	AR
	<i>SPARC</i>	SPARC (Osteonectin)	Severe	Severe early onset scoliosis	AR

	<i>MBTPS2</i>	S2P	Moderate to severe	Affected males with pre and postnatal long bone fractures, scoliosis, thoracic deformity	XR
	<i>MESD</i>	Mesoderm Development LRP Chaperone	Severe	Normal sclera. Disorganised dentition and/or oligodontia Global developmental delay/intellectual disability No hearing loss/dentinogenesis imperfecta	AR
	<i>TENT5A</i>	Terminal nucleotidyltransferase 5A	Severe	Congenital bowing of lower limbs Clinical features similar to Stüve Wiedemann syndrome	AR

AD autosomal dominant inheritance AR autosomal recessive inheritance

### 1.3 Clinical Features of OI

The primary clinical feature of OI is the bone fragility, which means that OI bone fractures with minimal trauma or with no obvious trauma. The secondary features can be divided further into sections. Here is an overview of the different clinical features. It is important to recognize that the features can be very variable even among those with the same gene variant of OI. It is unclear why this is the case. There are likely other as yet undiscovered modifying factors.

#### 1.3.1 Skeletal features

OI mainly affects the skeletal system. The main features apart from bone fragility are spine deformities, long bone deformities and joint hypermobility. Spine deformities include scoliosis, abnormalities of the lumbar spine (spondylolysis and spondylolisthesis) and craniocervical junction anomalies. Among these, scoliosis is probably the commonest spine deformity with an overall prevalence ranging from 26 – 78% (Gang Liu et al, 2017; Castelein et al, 2019). Scoliosis is a result of the vertebral fragility, ligamentous laxity and muscle weakness. The prevalence and the severity of scoliosis depends on the type of OI. Anissipour et al 2014, studied 316 children with OI and found that 157 of them had scoliosis with a prevalence of 50%. Out of these, the highest prevalence was seen in Sillence OI type III (68%), followed by Sillence OI type IV (54%) and Sillence OI type I (39%). The same study showed that the progression of scoliosis is highest in Sillence OI type III with a rate of 6° per year, followed by Sillence OI type IV with a rate of 4° per year and Sillence OI type I with 1° per year. (Anissipour et al, 2014)

Spondylolysis is a stress fracture of pars interarticularis. Spondylolisthesis occurs when the vertebra shifts out of place due to the instability from the stress fracture. Hatz et al, 2022 studied the radiographs of 110 children with OI and found that the incidence of spondylolysis is about 5.2% and the spondylolisthesis is about 4.2% (Hatz et, 2014). Craniocervical junction anomalies including basilar invagination, basilar impression and platybasia are uncommon but serious complications of OI. Basilar invagination occurs when the odontoid process of the cervical vertebra prolapses into the foramen magnum. Basilar impression is similar to basilar invagination but it is not the same. Basilar impression can be



defined as secondary basilar invagination because it occurs due to softening of the bone in bone pathologies. It is defined by Arponen et al, 2012 as relative lowering of the cranial base with the odontoid process or the topmost vertebra positioned above the skull caudal border. Platybasia is flattening of the bone and it is usually associated with basilar invagination and/or impression (Arponen et al, 2012). Platybasia on its own is unlikely to cause any problems (Pinter et al 2016) Arponen and colleagues studied images of 31 patients with OI from 0 to 39 years and found that the incidence of basilar invagination and impression was 13% and 15% respectively; platybasia was seen in 29%.

Long bone deformities (bowing) are usually the consequence of multiple fractures and inadequate healing. However, mild bowing is seen in some cases without any fracture. Joint hypermobility is a common finding in individuals with OI, particularly in children. It is seen in about 70% of the pediatric OI population (Arponen et al 2014).

### 1.3.2 Growth

Individuals with OI tend to have short stature with absolute or relative macrocephaly. Individuals with Sillence type I OI follow the normal growth curve but the final height achieved is a few inches shorter than an ordinary individual (Marini J and Dang D 2020). The other types of OI usually have a much shorter final height. Lund et al, 1999 studied 86 individuals including children and adults (age range from 0-60 years) and found that the head circumference is 2 standard deviations above the standing height in individuals with Sillence type III/IV. This is likely due to the poorly mineralized soft calvarial bone. The same study showed that the truncal height is shorter in Sillence type III/IV compared to Sillence type I OI. Short stature is likely due to the defective osteoblastic response to the growth hormone-IGF1 axis (Marini et al, 1993).

### 1.3.3 Dental anomalies

Dental abnormalities include dentinogenesis imperfecta, hypodontia, ectopic tooth, microdontia, dental malocclusion and crowding. Dentinogenesis imperfecta

(DI) is the commonest with a prevalence of 75% followed by hypodontia with a prevalence of 70% (Marcal et al 2019). DI is characterized by teeth that are discoloured (yellowish brown/pinkish brown/gray) and translucent. Dentin is a second layer under the enamel that is composed of type 1 collagen (90%) and non-collagenous protein (10%). Dentin is severely hypomineralised and altered in DI.

#### 1.3.4 Hearing loss

Hearing loss is a common feature with prevalence ranging from 50 – 92% (Pillion et al 2011). It is usually bilateral with onset around 20 years but may be as early as 4 years (Carre et al 2019). Both conductive and sensorineural hearing loss are seen and can be variable from mild to profound. It is most commonly seen in Sillence type I OI. Conductive hearing loss is commoner in the younger population whereas sensorineural hearing loss is more commonly seen in older individuals. Abnormal remodeling of the temporal bone causes fixation of the stapes footplate resulting in conductive hearing loss (Joseph et, 2021). We do not yet know the cause of sensorineural hearing loss but is thought to be also due abnormal temporal bone remodeling and cochlear hair cells atrophy (Hermie et al, 2017).

#### 1.3.5 Cardiovascular and Skin manifestations

Cardiovascular findings are uncommon and include valvular insufficiency, atrial septal defect and left ventricular wall thickening. Zhao et al, 2022 studied 69 OI individuals with OI and showed that mild regurgitation of the tricuspid or mitral valve is seen in 26 out of the 69 study participants. Thiele et al, 2012 studied 46 individuals with Sillence type III and type IV OI. The group showed that valvular regurgitation is seen in 70% and mild tricuspid regurgitation is seen more commonly than mitral, aortic and pulmonary valve regurgitation. Cardiac extracellular matrix is composed of mainly type 1 collagen (80%) and type 3 collagen. Skin is also composed mainly of type 1 collagen. Individuals with OI have impaired elasticity that are not usually obvious on clinical examination. Hansen and colleagues studied the skin mechanics of 10 patients with OI and

compared them with age matched controls. They found that the patient with OI have significantly less elasticity and distensibility compared to controls (Hansen et al, 2002). Therefore, the skin is stiffer similar to the bone properties seen in individuals with OI.

Table 4 Comparing the clinical features in the different Sillence OI types

Sillence OI type	Clinical features							
	Skeletal features				Growth	Dental	Hearing Loss	Cardiovascular and Skin features
	Scoliosis	Bowing	Hypermobility	Skull abnormalities				
Type I	Rare	Rare	Common		Normal growth curve but slightly shorter final height	Uncommon	Common Conductive in children Sensorineural in adults	Mild valvular regurgitation and Impaired skin elasticity in all types
Type III	Common	Common	Common	Rare	Short with relative macrocephaly	Dentinogenesis Imperfecta	Rare	
Type IV	Common	Common	Common		Short with relative macrocephaly	Dentinogenesis Imperfecta	Rare	

## Radiological features of OI

### 1.4.1 Prenatal

Generally, prenatal ultrasound would be helpful for the more severe types of OI including Sillence OI type II and type III. The other types of OI may not have any prenatal manifestations and therefore cannot be diagnosed accurately on ultrasound scan imaging. The features include decreased echogenicity of the skeletal structures suggestive of defective mineralization, thin skull vault, limb bowing and/or angulation, limb shortening and fractures. It can be difficult to differentiate severe OI and lethal skeletal dysplasia. In such circumstances, low dose CT scan with 3D reconstructions can be used to aid in the diagnosis (Renaud et al, 2013).

### 1.4.2. Infancy and childhood

The radiological features are variable depending on the severity of OI. The main features include osteopenia, bone deformities and fractures. Osteopenia which means reduced bone mineral density can only be identified on a radiograph when there is 30-50% loss of bone mineral. The radiographic clues include picture frame like appearance of the vertebral bodies, increased lucency of the medullary space of the long bones and thin cortical bones. The trabecular bone has a high turnover rate compared to the cortical bone and therefore, the first bones to manifest osteopenia is usually the vertebral bodies especially the thoracic vertebrae. When the osteopenia is severe, cortical thinning is also seen. Apart from the main features, scoliosis and skull base abnormalities can be seen in those with moderate to severe OI.

OI could present itself as an unexplained fracture in infancy. It would be important to exclude non accidental injury in such cases.

### 1.4.3 Adulthood

Generally, the frequency of fractures declines from about 20 to 50 years. Roberts et al, 2016 showed that about a quarter of OI related fractures occur in adulthood and they are commonly fractures of the vertebrae followed by the hips and feet (Roberts et, 2016).

## 1.5 Pathology/histopathology of OI

### 1.5.1 Normal bone tissue

Bone is a connective tissue that consists of cells surrounded by extracellular matrix. The principal bone cells include osteocytes, osteoblast and osteoclasts in addition to others including adipocytes, macrophages and T cells.

Osteocytes are the most abundant cell type in bone and accounts for about 90% of the total bone; they are osteoblasts that are embedded uniformly in the extracellular matrix (ECM) within a space known as lacuna-canalicular system. It is stellate shaped with long cytoplasmic appendages known as cytoplasmic processes. The unique shape and structure allow osteocytes to connect to neighbouring osteocytes, pericellular matrix which is a layer surrounding the osteocyte cell body in the lacuna and to the matrix directly through 'collagen hillocks'. The distance between osteocytes is about 20-30 micrometers (Sugawara et al, 2005) Osteocytes are the main mechanosensory cell in addition to osteoblasts, osteoclasts and the mesenchymal stem cells (Bonewald, 2011).

Osteoblasts are derived from mesenchymal stem cells. The differentiation to osteoblasts is under the control of various factors like hormones (IGF1, growth hormone, parathyroid hormone) and transcription factors SOX9, RUNX2 and Osterix. Active osteoblasts have large Golgi apparatus and endoplasmic reticulum. Golgi apparatus is where the collagenous and non-collagenous protein received from endoplasmic reticulum are further processed. Osteoblasts secrete the unmineralized organic matrix, osteoid composed mainly of collagen type I. Osteoid then undergo mineralization. Osteoblasts that get entrapped in the mineralized matrix become osteocyte and the rest become lining cells or die by apoptosis. Osteoblastogenesis is

regulated by the canonical WNT signalling pathway and several others including Hedgehog protein signalling, PTHrP, NOTCH, FGF and BMP signalling. Sclerostin and DKK1, inhibit WNT signalling and therefore inhibit osteoblastogenesis. We know that WNT and PTHrP are two of the well-studied pathways involved in exercise induced osteogenesis. WNT binds to its receptor and co-receptors, Frizzled and LDL receptor -related protein 5 or 6, which results in an increase in the B-catenin that translocates to the nucleus activating the downstream target genes for osteoblastogenesis.

Osteoclasts are derived from the monocyte/macrophage lineage of the hematopoietic stem cells. Several cytokines and transcription factors regulate the differentiation to osteoclasts. These include PU.1, NF- $\kappa$ B, M-CSF, NFATc1 and RANKL. Osteoclastogenesis is promoted by of Receptor activator of nuclear factor kappa-B ligand (RANKL) binding to the Receptor activator of nuclear kappa B (RANK) receptor on the osteoclasts. Osteocytes is the main source of soluble RANKL for osteoclastogenesis. The binding of RANKL to RANK receptor is inhibited by osteoprotegerin (OPG), which is a decoy receptor that binds to RANKL and therefore blocks the binding to RANK. Mechanical loading increases the expression RANKL on pre-osteoblasts; RANKL binds to Receptor activator of nuclear kappa B (RANK) on the pre-osteoclasts.

Therefore, RANKL expression on preosteoblasts is the initial step controlling osteoclasts development, ensuring bone formation follows bone resorption in each cycle. Most studies have shown mechanical loading and exercise increases OPG levels and decreased RANKL levels. However, there are some studies that showed no significant differences in the levels of OPG and RANKL following exercise. This could be related to the timing when the levels were taken. We also know that mechanical loading suppresses the secretion of SOS and DKK-I, promoting bone formation (Moester et al, 2010).

### **1.5.2 Bone extracellular matrix**

Extracellular matrix is composed of mainly type I collagen and mineral. Mineralised collagen fibrils are basic building blocks of bone. Mineral contributes to the hardness

and collagen contributes to the tensile strength of bone; together the collagen-mineral composite provides rigidity and fracture resistance. Mineral [mainly calcium phosphate] accounts for 65-70% of bone mass, water [~10%] and the rest are matrix proteins (predominantly type I collagen), and a small amount of citrate [~2%] (Davies et al., 2014). It is believed that small mineral platelets are arranged along the long axis of collagen fibrils (Landis et al., 1996).

Type I collagen is a trimer formed by 1 alpha-2 polypeptide and 2 alpha-1 polypeptide chains intertwined together in a triple helix structure (Gelse et al., 2003). Polypeptide chains are converted to collagen fibrils in a multistep process. First step is formation of procollagen from polypeptide chains. In endoplasmic reticulum, polypeptide chains undergo post translational modifications including hydroxylation, glycosylation, trimerisation, disulphide bonding, folding, prolyl cis trans isomerisation and triple helix formation (Hulmes, 2008). This triple helix structure formed is called procollagen. Procollagen is then packaged in Golgi and excreted to the extracellular space (Hulmes, 2008). Tropocollagen is formed when amino and carboxy terminal propeptides are cleaved from procollagen molecules during or shortly after excretion into the extracellular space (Hulmes, 2008). Collagen fibril is formed by a polymer of these tropocollagen molecules connected by intermolecular enzymatic and non-enzymatic crosslinks (Bishop, 2016). Fig. 1 shows the multistep process involved in collagen synthesis.



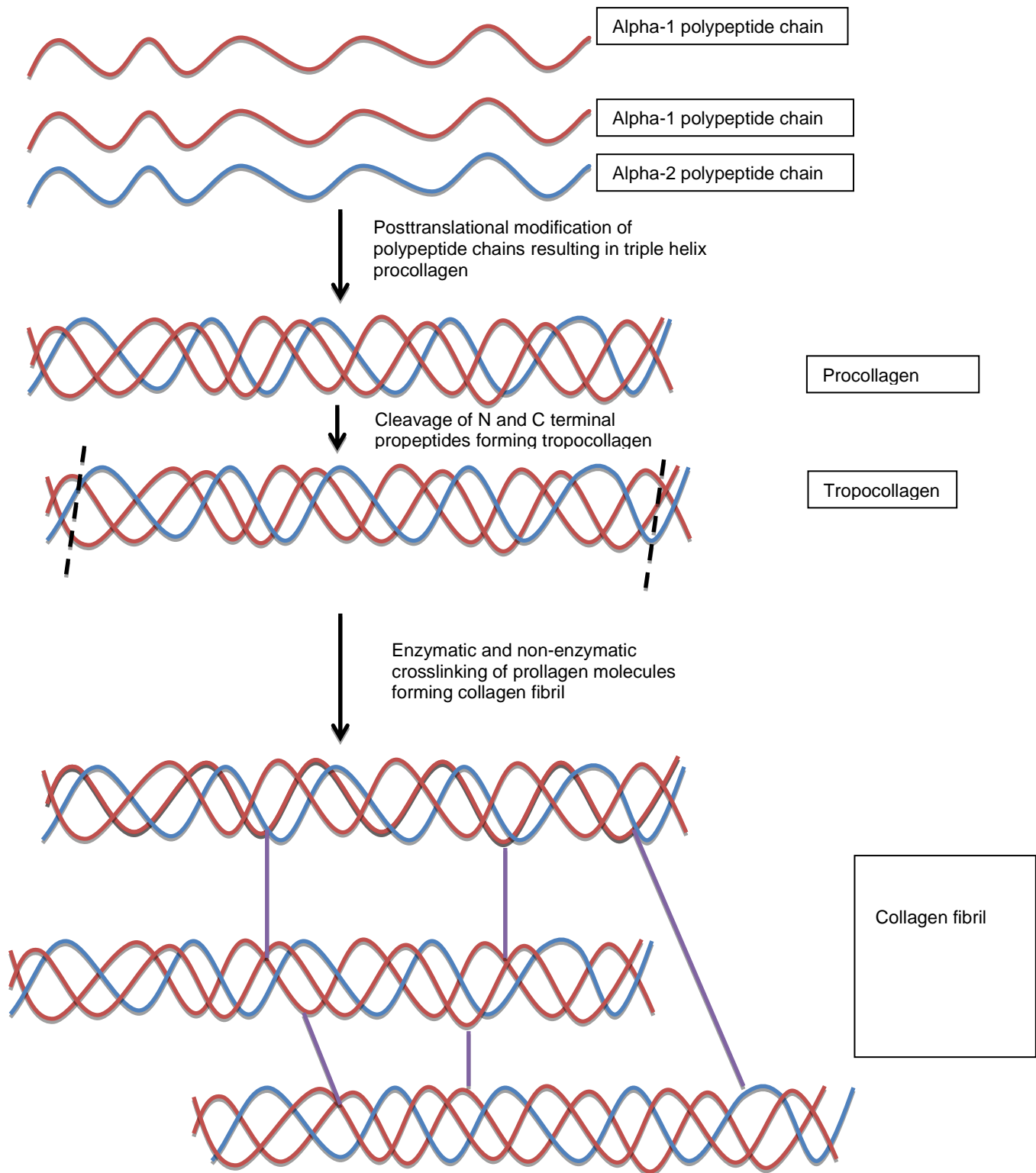


Figure 1 Collagen Fibril Synthesis

Enzymes and molecules released during bone formation and collagen degradation products produced during bone resorption can be measured for evaluation of bone formation and resorption (Seibel, 2005). The most widely used bone turnover markers for bone formation are serum bone specific alkaline phosphatase [ALP] and procollagen type 1 N propeptide [P1NP] (Seibel, 2005; Rauchenzauner et al 2007; Wheeler et al., 2013; Schini et al 2023).

Bone specific ALP is one type of tissue nonspecific ALP along with kidney and liver type ALP. It hydrolyses the pyrophosphate (PPi) which is the local inhibitor of mineralisation. This would result in the release of phosphate that is used for mineralization. Therefore, bone ALP levels reflect the bone mineralization activity, which is the later stage of bone formation. Its production is positively correlated with bone formation rate as measured by histomorphometry (Parfitt et al 1987).

Using current immunoassays, it is hard to identify bone specific from liver type ALP because they share the same amino acid sequences. However, the application and interpretation of bone ALP as a bone turnover marker should be unaffected by liver diseases because bone specific ALP is normally cleared from the serum by liver. Tariq et al 2019 conducted a study to determine whether serum calcium, phosphate and alkaline phosphatase (ALP) are predictors of bone mineral density (BMD) in postmenopausal non-osteoporotic, osteopenic, and osteoporotic females. The study included a total of 168 postmenopausal females and found that ALP was the strongest predictor of T-score in post menopausal osteopenic females.

P1NP and P1CP (Procollagen type 1 C-terminal propeptides are the by-products of collagen neosynthesis (Seibel, 2005). P1NP and P1CP levels reflect the osteoid formation activity, which is the early stage of bone formation. P1NP is a more sensitive marker than P1CP (Crofton et al 2004). P1NP is very stable in serum after venepuncture, freezing and thawing (Hlaing et al 2014) and is not affected by food intake. Moreover, it has very low biological and circadian variation (Gillett et al, 2021). Iftikhar et al 2020 conducted a study of 267 post-menopausal women and showed that the sensitivity and specificity of P1NP values for the diagnosis of osteoporosis were 83.3% and 70.8% respectively. The study was conducted by comparing Dual energy x-ray absorptiometry (DEXA) scan t-score with P1NP values. The study concluded that P1NP is a reliable marker to predict spinal osteoporosis. A

Chinese study concluded by Liu Gang et al, 2018 revealed diagnostic sensitivity of P1NP 65.9% and specificity of 85.7% .

The most useful markers for bone resorption are urinary NTX (N-terminal crosslinked telopeptide of type I collagen) and serum CTX (carboxyterminal telopeptide of type I collagen) (Seibel, 2005, Wheater et al, 2013). NTX and CTX are collagen breakdown products that reflect bone resorption activity (Seibel, 2005). CTX has been evaluated in many studies to be useful for osteoporosis evaluation and treatment (Qu et al 2020, Vasikaran et al, 2011, Dobnig et al 2007, Sornay-Rendu et al 2005, Garnero et al 2001).

Bone formation can be simply divided into 2 major steps. Firstly, organic matrix is synthesised and laid at specific sites and secondly, mineralisation of the matrix. Mineralisation occurs in 2 successive stages, a rapid primary mineralisation (within a few days) followed by slow secondary mineralisation (within a few years). Bone mineralisation can only occur in the presence of collagen fibrils and is a non-uniform process. It occurs on specific nucleation and scaffolding sites of collagen fibrils (Saratchandra et al., 2000). Primary and secondary mineralisation occurs at different sites at different times (Fratzl et al., 2004). As a result, there are packets of bone with differing mineral content at any one time (Fratz et al., 2004). Conditions with high bone turnover rates disrupt the secondary mineralisation as the bone is removed before secondary mineralisation can take place. It is hypothesised that the disruptions of bone matrix mineralisation could possibly contribute to crack initiation and propagation (Akkus et al., 2003; Fratzl et al., 2004).

### **1.5.3 Modelling and Remodelling of Bone**

Bone is a dynamic tissue continually undergoing modeling and remodeling. Modeling is a process by which bone changes its overall shape and size in response to mechanical loading and during skeletal growth (Dempster and Raisz, 2015). It is an uncoupled process where osteoblasts' and osteoclasts' activity are independent of

each other (Dempster and Raisz, 2015). It occurs primarily in childhood but continues throughout life (Dempster and Raisz, 2015).

Bone remodeling is a process by which bone matrix is replaced and small defects [microdamage] are repaired. Its primary function is renewal of bone (Dempster and Raisz, 2015). Bone remodeling involves sequential osteoclast-mediated bone resorption and osteoblast-mediated bone formation. It occurs throughout life and is the predominant process in adulthood. During remodelling in adults, bone resorption occurs at a rate equal to that of bone formation, in a process referred to as coupling; in children, more bone is formed than is removed (approximately 4% more per remodelling cycle), allowing for bone mass accretion during growth (Parfitt et al, 2000).

Mechanical loading is a major regulator of bone remodelling. The key steps in bone remodelling which include activation, resorption, reversal, formation and termination (Hadjidakis et al 2006). Bone remodelling takes about 120-200 days in the cortical and trabecular bone respectively. Activation is when the osteoclast progenitor cells are recruited and activated. The lining bone cells separate from the bone surface and form a raised canopy or tent of cells, marking the site for resorption. Osteoclast precursor cells arrive at the resorption site. Then, multinucleated osteoclasts are formed, which are the mature activated osteoclasts.. The next step is the resorption phase that takes about 2 weeks. Mature activated osteoclasts form a ruffled border made of podosomes and attaches to the resorption site. It dissolves the matrix by pumping protons generated by carbonic anhydrase II, which is subsequently degraded by metalloproteinases and cathepsin K. We used to think that the osteoclasts undergo apoptosis once resorption is completed. However, new evidence shows that the osteoclasts are split into smaller motile cells (> 5 micrometres in diameter) known as osteomorphs (McDonald et al, 2021). The resorption phase is closely followed by reversal which takes about 4-5 weeks. This is when bone gets ready for the next phase which is bone formation. A threshold of osteoprogenitor cell density is necessary in order for bone formation to take place. This is a tightly coupled process; the exact signals involved in not well understood yet. It is thought that the osteoclasts are the cells initiating osteoprogenitor cell recruitment and expansion. Bone repair and formation is dependant on the magnitude of resorption and the rate of progenitor cell

recruitment and expansion. Bone formation takes about 120 days (4 months) to complete. Osteoid starts to mineralise after about 15 days it is laid. Bone mineralisation is complex and is regulated systemically and locally. The bone remodelling is terminated once the mineralisation is complete. (Ott, 2004).

Targeted bone modeling and remodelling activity in response to local stress occurs at various locations at different stages at any one time. Remodelling is a renewal process where old bone is replaced by new bone. This requires tight coordination of resorption and formation through basic multicellular units (BMUs). Therefore, resorption and formation are always 'coupled' in remodelling. Bone loss occurs when there is an imbalance with reduced bone formation compared to resorption due to various factors involved in bone remodelling.

The knowledge of bone biology is rapidly advancing and now we know how bone actually converts the mechanical loading into biochemical signals which then causes downstream activation of the signalling pathways. This process is known as mechanotransduction which can be divided into 3 main phases; mechanocoupling, biochemical coupling and signalling pathways.

Mechanocoupling simply describes the cell deformation at the cellular level, caused by mechanical stimulation. Mechanical stimulation causes various biophysical forces including shear, strain, fluid flow and pressure which result in cell deformation. Among these, shear stress from the fluid flow has been recognised as the main force applied to osteocytes (Jacobs et al, 2010).

The biophysical stimuli cause osteocyte cell deformation which induces biochemical coupling through several mechanosensors or receptors. Well studied mechanosensors include Integrin containing focal adhesions complexes, gap junctions, hemichannels, primary cilium, glycocalyx and mechanical sensitive ion channels. Integrin containing focal adhesion complexes connect the cell to the extracellular matrix, gap junctions connect neighbouring cells and hemichannels at the cell membrane connect the cell to the environment. Primary cilium is a hair-like thin organelle protruding from almost all cell types. It responds to mechanical and chemical stimuli. Primary cilia mediates the osteocyte responses by regulating the calcium entry into the cells and intracellular cAMP levels (Kwon et al, 2010). Glycocalyx is a specialised layer of extracellular matrix surrounding the osteocytes at the space within the lacuno-canalicular system.

Mechanical sensitive ion channels (MSIC) are stretch/strain sensitive ion channels that open allowing calcium influx following membrane tension differences sensed from mechanical loading. Piezo1 is a well-studied MSIC which is a large, curved ion channel expressed in osteocytes and osteoblasts (Sun et al, 2019). These mechanosensors will signal an influx of  $\text{Ca}^{2+}$  which would result in the release of proteins like RANKL, sclerostin, DKK1 and osteoprotegerin through structures known as extracellular vesicles (Bolamperti et al, 2022). This will then activate the signalling pathways involved in bone formation and resorption. It is also known that osteocyte apoptosis from microcracks releases that the microcracks from mechanical loading cause osteocyte apoptosis which release damage-associated molecular pattern (DAMP) proteins. These proteins cause the release of pro inflammatory cytokines that initiates osteoclastogenesis (Komori T, 2016).

One of the well-studied pathways for mechanical loading induced osteogenesis is the WNT signalling pathway. There are  $\beta$ -catenin independent and  $\beta$ -catenin dependent (canonical) pathways. Target genes for osteogenesis are activated through these pathways. Osteocytes is the main source of soluble RANKL for osteoclastogenesis. Osteoprotegerin binds to RANKL preventing excessive resorption. On the other hand, sclerostin and DKK1, inhibit WNT signalling and therefore inhibit osteoblastogenesis. We know that mechanical loading suppresses the secretion of sclerostin and DKK-I, therefore promotes bone formation (Moester et al, 2010).

#### 1.5.4 Pathogenesis of OI

Defective collagen and mineralisation together contribute to poor intrinsic quality of bone in people with OI. There is altered size and structure of collagen (Bart et al., 2014) with increased non-enzymatic crosslinking in OI bone (Carrierro et al., 2014) contributing to reduced toughness and plasticity. There is abnormally high bone matrix mineralisation with altered mineral ultrastructure contributing to brittleness (Bishop, 2016). The mineral platelets are disorderly arranged, with higher packing density and thinner in size (Fratzl et al., 1996; Fratzl-Zetman et al., 2014; Imbert et al.,

2014). The altered mineral ultrastructure is likely due to abnormal fibrillogenesis that provides the template for normal mineralisation (Saratchandra et al., 2000). Disorganised bone matrix results in bone that is less able to absorb and dissipate energy and hence, easily susceptible to fractures (Bishop, 2016).

Micro architecturally, there is reduced volume density of cortical bones with increased porosity and reduced volume of trabecular bone with reduced connectivity (Bishop, 2016). Macroscopically, the width of tubular long bones is reduced (Bishop, 2016).

In children with OI, bone turnover is increased with defective modelling and remodelling. There is an imbalance of remodelling activity favouring osteoclast activity (Rauch et al., 2000). There is increased recruitment of remodelling teams but the performance of both osteoblasts and osteoclasts teams are decreased (Rauch et al., 2000). Replacement of resorbed bone is defective compared to normal, and the number of osteocytes is increased (Rauch et al., 2000).

## 1.6 Genetic Aetiology of OI

The majority of OI [85-90%] is caused by autosomal dominant pathogenic variants in *COL1A1* or *COL1A2* genes of type I collagen (Forlini and Marini, 2016).

A small minority of OI is caused by autosomal recessive mutations in collagen-related genes. First recessive gene, the *CRTAP* gene known to cause lethal and severe OI was discovered in 2006 (Barnes et al.) Over the last decade with rapid advances in genetic technology, multiple additional OI genes have been identified. Very recently, a first X-linked recessive OI has been described (Kang et al, 2017). Thornley et al, 2021 studied 100 children from the OI highly specialized service (HSS) and found that about 20% of those with severe form of OI were identified to have pathogenic variants in the non-*COL1A1/COL1A2* origin. Among the 20% with non-*COL1A1/COL1A2* genes, the commonest affected gene was *IFITM5* (22), followed by *LEPRE1*(12), *SERPINF1*(8) and *BMP1*(6) genes (Thornley et al, 2021).

### 1.6.1 Collagen synthesis and structure defects

This group is the commonest type of OI representing 85-90% of all OI and it can be further subdivided into quantitative and qualitative defects. It is caused by autosomal dominant mutation in *COL1A1* or *COL1A2* gene. The two genes are very similar in structure with 51 and 52 exons respectively and the mRNAs transcribed are about the same length.

Each of the genes encode an alpha chain with 1,014 amino acids, composed of Gly-X-Y tripeptide repeats, flanked by amino- and carboxyl-terminal propeptides. Type 1 collagen is a trimer made of 2 alpha-1 and 1 alpha-2 polypeptide chains. The chains first come together in the carboxy-terminal end and progress toward the amino-terminal end. Glycine is essential at every third residue of the triple helical domain for proper folding due to the steric effects of glycine. The triple helix formed in the absence of glycine at the 3rd residue, is abnormal and defective functionally.

A quantitative defect resulting in reduced synthesis of structurally normal collagen is caused by mutations in *COL1A1* allele due to premature termination codons. The premature termination codon causes nonsense mediated decay of the mRNA message. Therefore, there is reduced amount of alpha 1 polypeptide chains resulting in about half the amount of structurally normal trimer. Clinically, this results in a milder form of OI characterized usually by fractures that tend to reduce after adolescence, blue sclerae, hearing loss and near normal stature.

A qualitative defect resulting in structurally defective type 1 collagen is caused usually by mutations in either *COL1A1*/*COL1A2* genes due to substitution of the glycine residue (Basel and Steiner, 2009) in the triple helical domain of either alpha 1 or alpha 2 polypeptide chain. Glycine substitutions in either chain lead to a delay in helix folding, causing post-translational overmodification. Marini et al 2007 studied the genotype phenotype relationship caused by *COL1A1*/*COL1A2* genes and found that about 80% of the *COL1A2* variants are non-lethal and about 33% of the *COL1A1* variants with glycine substitution are lethal. Substitutions in the alpha 1 chain by arginine, valine, glutamic acid, aspartic acid, and tryptophan



are usually lethal if they occur near the carboxyl-terminal end of the triple helix and have a moderately severe form if they occur in the remainder of the chain. The study also showed that specific regions of the *COL1A1* gene are associated with lethality better known as hotspots; they are the 2 major ligand binding regions (MLBR) known as MLBR2 and MLBR3. The clinical presentation is more variable with mutations that affect glycine residues in the alpha 2 chain (Marini et al 2007). Another study by Campanini et al 2021 showed that the specific C-propeptide variant in the *COL1A1* gene appear to have high bone mass phenotype. C-propeptide variants account for about ~5% of the variants that have been identified in type I collagen (Symoens et al., 2014). Type I procollagen C-propeptides are involved in the intracellular procollagen assembly and the extracellular assembly of collagen fibrils (Symoens et al., 2014). It is also needed for the regulation of bone mineralisation (Syx et al., 2015). A specific variant in the *COL1A1* gene – c.3040C>T causes infantile cortical hyperostosis known as Caffey disease (Gensure et al 2005).

### 1.6.2 Collagen modification defects

This group of OI is caused by recessive mutations in the *CRTAP*, *LEPRE1* and *PPIB* genes. The assembled polypeptide chains undergo post translational modification and folding in the endoplasmic reticulum resulting in procollagens. The post translational modification and folding require the hydroxylation complex composed of Prolyl 3-hydroxylase 1 (P3H1), cartilage-associated protein (CRTAP) and cyclophilin B (CyPB). P3H1, CRTAP and CyPB proteins are encoded by *LEPRE1*, *CRTAP* and *PPIB* genes respectively. A study by Homan et al 2014, showed that the main action of the complex is to fold the triple helix as the absence of P3H1 result in abnormal collagen folding and over modification.

### 1.6.3 Collagen processing and cross-linking defects

This type of OI is caused by recessive mutations in the *SERPINH1*, *FKBP10*, *KDEL2*, *PLOD2* and *BMP1* genes. *SERPINH1* and *FKB10* encode collagen chaperones HSP47 and FKB65 respectively. HSP47 and FKB65 fold and stabilise the newly formed triple helix (procollagen). Christiansen et al, 2010 showed that the absence of HSP47 caused increased transit from the endoplasmic reticulum to the Golgi apparatus resulting compromised triple helix structure.

Retrograde traffic from the Golgi apparatus to the endoplasmic reticulum are thought to be regulated by the KDEL receptors. BMPI (bone morphogenetic protein1) and mTLD are two of the four proteins (the others are mTLL1 and mTLL2) encoded by *BMP1* gene that cleaves off the C- terminal to form mature type 1 tropocollagen. Of these 4 proteins, BMP1 was found to have the greatest cleavage activity (Sangsin et al, 2017). It also affects regulation of TGF $\beta$  and activins which impact osteoclastogenesis. Variants in the *BMP1* gene causes a high bone mass phenotype similar to the C-propeptide variants of the *COL1A1* gene (Campanini et al 2021).

The mature type 1 tropocollagen (without the terminal propeptides) will form crosslinks with one another producing collagen fibrils. *PLOD2* is an enzyme within the endoplasmic reticulum cisternae which is encoded by the *PLOD2* gene. They are important for the stability of intermolecular crosslinks.

#### 1.6.4 Mineralisation defects

Mineralisation defects are caused by mutations in 2 genes; *SERPINF1* and *IFITM5*. *SERPINF1* encodes PEDF (pigment-epithelium derived factor) which also interacts with RANKL. PEDF is a factor that affects vasculogenesis and interacts primarily with VEGF; it may also interact with IFITM5. PEDF probably has some role in osteoid mineralization (Land et al, 2007) This type of OI has a characteristic bone histomorphometry with increased amounts of broad osteoid and a fish-scale pattern under polarized light.

Pathogenic variants in the *IFITM5* gene cause autosomal dominant OI. *IFITM5* encodes BRIL (bone-restricted interferon-induced transmembrane protein-like protein). The variant is upstream of exon 1 and results in a new protein – MALEP-BRIL, whose function is unknown. Individuals with *IFITM5* pathogenic variants

have characteristic radiological features with hyperplastic callus, interosseous membrane calcification of the radius/ulna and radial head dislocation. Histologically, mesh-like lamellar pattern is evident under polarised light.

### 1.6.5 Osteoblasts differentiation defects

This is the most recently added group of OI with new gene discoveries. The exact underlying pathogenesis is not crystal clear as the other groups. So far, there are 6 genes (*WNT1*, *CREB3L1*, *SP7*, *TMEM3B*, *SPARC*, *MBTPS2*) in this groups which are involved in the osteoblast differentiation and function. The most recently discovered *MBTPS2* is the first X-linked OI gene and the rest of the genes in this group cause recessive OI.

## 1.7 Management of OI

### 1.7.1 General

OI is best managed by a patient-centred multidisciplinary approach. The multidisciplinary team includes medical, surgical, orthopaedics, genetics, nursing, physiotherapy and occupational therapy, psychology, social work expertise, pain management and dentistry (Arundel, 2015).

Major components of management are medical management with bisphosphonates treatment together with physiotherapy and occupational therapy (Forlino and Marini, 2016). Physiotherapy interventions include muscle strengthening and prevention of joint contractures. Occupational therapy interventions mainly focus on improvement in function including activities of daily living, ambulation and modification or adjustments for daily tasks. Physiotherapists and occupational therapist work very closely with each other (Marr et al 2017). Basilar impression is an uncommon but serious complication of OI resulting in brain stem compression and hydrocephalus (Forlino and Marini, 2016; Arundel 2015). Neurosurgical intervention may be required in those with basilar impression depending on the individual signs and symptoms. Orthopaedic surgeons' input is required for insertion of intramedullary

rods or nails to stabilise recurrent fractures and correct bony deformities (Arundel, 2015). Scoliosis progression requires the expertise of spinal surgeons. Children with dentinogenesis imperfecta will require regular review by a dentist and may need specialised treatment at some point. Pain management team offers specialised pain relief measures while, psychologists and social workers provide the overall support necessary for children and families with OI. Fig. 2 shows the multidisciplinary team involved in OI.

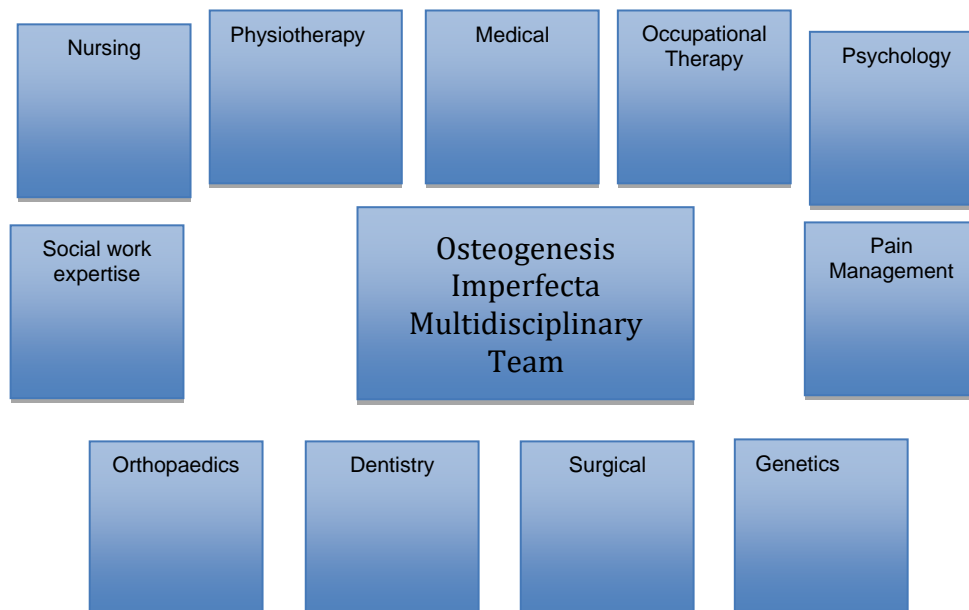


Figure 2 Multidisciplinary team in Osteogenesis Imperfecta

### 1.7.2 Growth and general health

Growth and general health in children are looked after by the metabolic bone disease consultant. The consultant monitors skeletal growth by regular anthropometric growth measurements, evaluates the response to medical treatment, monitors the calcium and

vitamin D levels and the overall management. The consultant will also keep an eye on potential complications and refer to the appropriate specialties including the orthopedic surgeons and the neurosurgeons. The specialist nurse will help to coordinate the care with other specialties and ensures the best possible experience for the patient and the family.

### 1.7.3 Hearing

Hearing loss is seen in some types of OI. It is recommended that every child with OI should have a formal hearing assessment before starting school (Carre et al 2019). Hearing tests should be repeated at 3 yearly intervals. Adults who have concerns with their hearing should be assessed and followed up by the audiologist on a regular basis.

### 1.7.4 Dental

Dental evaluation is important in all patients with OI. It has been suggested (personal communication with the dental team) that a child with OI should see a dentist by 6 months after the eruption of the first baby tooth. At about the age of 7 years, an assessment for Class III malocclusion is advised. All patients regardless of whether they have dentinogenesis imperfecta or not, should have regular dental checkup. This can usually be done at the local dentist. However, in individuals with more complex dental problems, the care should be at the dental hospital.

### 1.7.6 Medical management with bisphosphonates

Bisphosphonates are a group of potent anti-resorptive agents that inhibit osteoclast function. They are adopted as a standard of care in the treatment of children with OI (Forlino and Marini, 2016) and are also used in other diseases with increased bone turnover rate to reduce resorption (Russell, 2011).

Bisphosphonates are structurally similar to pyrophosphate [PPi], a by-product of cellular metabolism (Russell, 2011). PPi is a major inhibitor of physiologic and pathologic calcification, bone mineralization and resorption. (Fleisch, 1981; Ho et al., 2000). PPi is derived from ADP/ATP through the action of ENPP1 [Ectonucleotide pyrophosphatase/phosphodiesterase1], and exported from cells through ANK channels [non-enzymatic plasma membrane channel] (Russell, 2011).

Bisphosphonates have an inhibitory effect on osteoclast activity. They attach to hydroxyapatite binding sites on naked bone surfaces and are endocytosed by osteoclasts during resorption (Russell, 2011). Nitrogen-containing bisphosphonates inhibit the enzyme farnesyl pyrophosphate synthase (FPPS) in the mevalonate pathway (Russell, 2011). Inhibition of FPPS impairs the prenylation process resulting in altered osteoclast function, which include altered cytoskeletal arrangement, membrane ruffling, intracellular vesicle trafficking and apoptosis (Coxon and Rogers, 2003; Soares et al., 2016). FPPS is required for biosynthesis of isoprenoid lipids that are essential for posttranslational modification of GTPases (Russell, 2011; Soares et al., 2016). GTPases are enzymes essential for intracellular signalling within osteoclasts (Russell, 2011; Soares et al., 2016).

Recent studies by Bellido and Plotkin (2011) have shown that it also has a separate anti-apoptotic effect on both osteoclasts and osteoblasts. Anti apoptotic effect on osteoblasts is exerted at a lower constant dosage of bisphosphonates compared to the one required for the inhibitory effect on osteoclasts and the effect is lost at higher concentrations of bisphosphonates (Bellido and Plotkin, 2011). Activation of ERKs (extracellular-signal related kinases), which are survival kinases are believed to cause the prosurvival effect on osteoblasts (Bellido and Plotkin, 2011).

Bisphosphonates have been widely used in the treatment of children with OI over the last 20 years. Recent Cochrane review has synthesized that bisphosphonates increase bone mineral density in children with OI (Dwan et al., 2016). Microarchitecturally, the changes seen in children with OI treated with bisphosphonates include an increase in trabecular number, thickness and connectivity, reduced intracortical porosity and increased cortical thickness (Bishop, 2016). Inhibiting remodeling and reducing bone turnover rate results in mineralisation uniformity and increased intracortical anabolic modeling activity (Roschger et al., 2001). Macroscopically, restoration of vertebral shape and size (Gatti et al., 2005; Letocha, 2005; Munns et al., 2005; Land, 2006) has been reported. Overall, it decreases pain and improves physical mobility (Feehan et al, 2018; Montpetit et al 2015). However, recent Cochrane review highlighted that despite these observed changes, the effects of such therapy on fracture frequency are equivocal, though multiple studies (RCTs and observational cohorts) report this independently and no studies report increased fracture rate with treatment (Dwan et al., 2016). It is safe and well tolerated in the short term as evidenced by a recent systematic review (Rijks et al., 2015).

There are a number of controversies/debate in the use of bisphosphonates in children. These include issues around the incidence atypical femoral fractures, osteonecrosis of the jaw, long-term safety and optimal dose and duration of treatment. Theoretically, by reducing bone resorption, bone formation is also reduced to a certain extent as the process of bone remodeling is disrupted. Bisphosphonates increase the volume of bone but not the quality (the poor quality of bone remain).

Atypical femoral fractures are fractures that are located anywhere along the femur from just distal to the lesser trochanter to just proximal to the supracondylar flare associated with minimal or no trauma. To date, there has been one reported case of possible atypical femoral fracture (Vasanwala et al 2016).

A recent study by Vuorimies et al, 2017 showed that the femur fractures in children with OI were not related to bisphosphonate treatment rather they are related to the OI type. This study looked at 3 different treatment strategies and compared the femur fractures among all 3 groups. There was no significant difference in the incidence of the fractures in each of the 3 different treatment groups. Osteonecrosis of the jaw is another debate often talked about, however there are no reported cases of ONJ in children with OI, to date (Hennedige et al, 2013)

Vital questions regarding the long-term safety and optimal dose and duration of treatment remain unanswered. Bisphosphonates affect bone mass and architecture rather than bone quality. Inhibiting resorption and hence remodeling could result in microdamage accumulation; however, there is currently little evidence to indicate that this is causing problems. Bisphosphonates also have a variable residence time in bone, depending on the individual bisphosphonate's mineral binding avidity. It can persist for years after treatment cessation.

Bisphosphonates can be administered intravenously or orally. In the UK, cyclical intravenous pamidronate or zoledronate is the treatment of choice in children with moderate to severe OI. The IV pamidronate dose should not exceed 12 mg/kg/year (Arundel, 2015) and the usual IV Zoledronate dose is 0.1mg/kg/year.

Oral risedronate is considered in children with mild OI. Oral risedronate has been shown to reduce fracture rates in randomised controlled trials (Bishop et al., 2013).

Currently, there is no clear international consensus regarding the optimal dose and duration of treatment (Arundel, 2015). Studies have suggested that the maximum benefit of age-related areal bone density is obtained by 2-3 years of treatment (Rauch et al., 2002; Letocha et al., 2005).

### **1.7.7 Mechanical stimulation – whole body vibration**

Bone is highly responsive to mechanical stimulation. Bone adapts by changing and adjusting its architecture to changing mechanical forces. Bone structure is compromised by disuse and enhanced by exercise. The skeleton is most responsive to physical activity during prepubertal and peripubertal years (Bass, 2000). During growth, there is increased body weight, muscle strength & longitudinal bone growth resulting in increased load on bone. The skeleton adapts to these loads by increasing its strength (“mechanostat” theory). Periods of growth are thought to be the best time to influence bone through increased loading due to the high rates of bone modeling & remodeling. Specker and colleagues performed a systematic review of 22 trials and found that prepubertal children randomized to exercise had greater increases in bone mineral content and areal bone mineral density than control children (Specker et al, 2015) The exercise used in the trials include high impact exercises, resistance training, weight bearing activities, aerobic sessions and jumping activities.

Whole Body Vibration [WBV] is a form of mechanical stimulation that provides physical oscillation. It is also a suitable standardised form of mechanical stimulation than exercise in research settings. WBV can be delivered in different amplitudes (peak to peak displacement in mm) and different frequencies (rate of oscillation) using a device called vibration platform (Cardinale and Wakeling, 2005). The user stands or sits on the vibration platform for a set time [usually 10-20 minutes] with rest periods in between. Vibrations can be delivered over a range of frequencies [15–60 Hz] and amplitudes from less than 1 mm to 10 mm (Cardinale and Wakeling, 2005). The acceleration delivered can reach up to 15 g [where 1 g acceleration due to the Earth’s gravitational field] (Cardinale and Wakeling, 2005). There are 2 categories of vibration; one is pivotal (side alternating) and the other is lineal (vertical) (Cardinale and Wakeling, 2005). Pivotal vibration provides reciprocal vertical displacement



about the fulcrum while lineal vibration provides uniform up and down vertical oscillation (Cardinale and Wakeling, 2005; Rauch, 2009). Various different protocols of vibration have been studied with different combinations of frequencies and accelerations/ magnitudes. Low magnitude is  $<1g$  and high magnitude is  $>1g$ . Low frequency is  $<20\text{Hz}$  and high frequency is  $>20\text{Hz}$  (Oliveira et al 2016).

The exact mechanism of action of WBV is unknown. It involves the transfer of energy from the machine to the body (Jordan et al., 2005, Erceg et al., 2015). The immediate effect is muscle activation through stimulation of muscle spindles and alpha motor neurons (Jordan et al., 2005; Rauch 2009). The longer-term effect on bone formation is hypothesised to be a result of the interaction between fluid shear forces and cellular mechanics (Roelants et al., 2004; Hsieh et al., 2001; Erceg et al., 2015). Animal studies have shown that bone remodelling is sensitive to strain magnitude, frequency, rate and rest periods between stimulation (Hsieh et al., 2001, Srinivasan et al., 2002, Warden et al., 2004, LaMothe et al., 2005, Nagasawa et al., 2008, Vanleene et al., 2013). Vanleene et al., 2013 showed that young (3-8 weeks' old) growing OI bone of mice are most responsive to low amplitude/acceleration, high frequency WBV (0.3g, 45Hz) (Vanleene et al., 2013). The study showed that there was a significant increase in trabecular bone volume fraction, cortical area and thickness following vibration. Gnyubkin et al, 2016 showed that high acceleration, high frequency WBV (2g, 90Hz) increases the mineralisation process during skeletal growth resulting in higher peak mineral density in mature skeleton of healthy mice (Gnyubkin et al., 2016).

WBV therapy is targeted at musculoskeletal strengthening clinically and has been trialled in a variety of conditions. It has been shown to have therapeutic advantage (improved mobility and balance, muscle strength, postural strength and bone density) in various osteopenic populations such as post-menopausal women and adults (Roelants et al., 2004; Verschueren et al., 2004; Pitukcheewanont et al., 2006; Gilsanz et al., 2006; Eek et al., 2008; Ruan et al., 2008; Semler et al., 2008; Mikhael et al., 2010; Olama et al., 2010; Wren et al., 2010; El-Shamy et al., 2013; Katusic et al., 2013; Lee et al., 2013; Soderpalm et al., 2013; Stark et al., 2013; Unger et al., 2013; Vry et al., 2013; Kilebrant et al., 2015; Myung-Sook et al., 2015; Ibrahim et al., 2014; Cheng et al., 2015(a); Cheng et al., 2015(b); Tupimai et al., 2016). A systematic review and meta-analysis on the effect of WBV (frequencies ranging from 12-90Hz

and acceleration of < 1g and >1g) on bone mineral density in 2010 showed that a small but significant increase in bone mineral density in children [spine and tibia] and post menopausal women [hip] but no effect in young adults (Slatkovska et al., 2010). Another recent systematic review and meta-analysis on WBV (frequencies ranging from 12-90Hz and acceleration ranging from 0.1g to 10.9g) in post menopausal women showed that the most responsive area to WBV is the lumbar spine area (Oliviera et al., 2016). A recent study of WBV in healthy boys showed that even a short period of exposure could increase the bone formation marker in excess of one reflecting bone resorption (Harrison et al., 2015). Harrison et al, 2015 compared high frequency high magnitude (20Hz, 6.4g) platform with high frequency low magnitude platform (32-37Hz, 0.3g). The study showed that bone in a healthy growing skeleton does have the capacity to respond quickly to WBV irrespective of the magnitude of that vibration. In children with reduced bone density, low intensity vibration has been used with variable success. It has been shown to improve bone density in individuals with Duchenne Muscular Dystrophy (Bianchi et al 2013), cerebral palsy (Ward et al, 2004) and idiopathic scoliosis (Lam et al, 2013). However, it has not been shown to improve bone density in children with OI (Hogler et al 2017). Hogler et al, 2017 looked at the effect of whole-body vibration (using 3 different amplitudes of 2mm, 4mm and 6mm with a frequency of 20-25Hz) in 24 children with OI types I, III and IV over a 5 month period. The study showed that there is an increase in lean mass without any increase in bone mass or muscle function. The study concludes that this could be due to the reduced responsiveness of the OI bone-muscle unit.

Although high frequency low magnitude WBV may not increase bone mass in children with OI, it does engender changes in bone biomarkers over short periods of time making it suitable and appropriate in the context of this study to assess the effects of a bone-directed therapy on bone formation and resorption (Gopal-Kothandapani et al 2020, Harrison et al 2015).

### **1.7.8 Combined effect of Bisphosphonates and mechanical stimulation**

Previous studies evaluating the combined effect of bisphosphonates and mechanical stimulation have shown inconsistent results. Some showed additive effect whilst the

majority of studies showed no interaction between mechanical stimulation and bisphosphonates. To date, this relationship between bisphosphonates and mechanical stimulation has only been studied in ovariectomised rats and osteoporotic women; no reported studies in OI. Table 5 details the studies evaluating the combined effect of bisphosphonates and mechanical stimulation.

Table 5. Studies evaluating the combined effect of bisphosphonates and mechanical stimulation.

(OVX – ovariectomised rats ALN - alendronate, Z – Zoledronic acid, ETD- Etidronate, WBV – Whole Body Vibration, Sh – Sham, E – exercise,  
BMC – Bone Mineral Content, BMD – Bone Mineral Density, BP – bisphosphonate, L4 – 4<sup>th</sup> lumbar vertebra, vs – versus)

Trial name	Year	Study subjects	Mechanical stimulation method	Bisphosphonate regime	Main Outcomes
STUDIES WITHOUT AN ADDITIVE OUTCOME					
Hatori et al	2015	84 rats divided into 3 subgroups (sh OVX, OVX, ALN). Each subgroups were divided into WBV vs sham-WBV	WBV loading 10 min/day using 10 consecutive steps (5Hz increase every step) from 130--150 Hz) Duration : 4 days vs 14 days	ALN: Alendronate 2mg/kg 3 days/week SC injection (starting 5 days post ovariectomy) Control groups given Saline Duration: till the end of study	OVX+WBV group: increased cortical thickness and reduced medullar area of tibia ALN+WBV and ALN+shWBV= no significant difference between these 2 groups on bone microstructural parameters, no additive effect of WBV
Iwamoto et al	2012	54 postmenopausal osteoporotic women (age 51-91) divided into	WBV 4 min/day, frequency 20 Hz, 2 days /week Duration: 6 months Supervised and performed in clinic	All subjects: Alendronate 35 mg weekly orally Duration: till the end of study	ALN+WBV and ALN+Control: No significant difference on serum ALP or urinary NTX in these groups ALN+WBV: improved body balance, flexibility and walking velocity

		2 groups: WBV vs control			
Iwamoto et al	2005	50 postmenopausal osteoporotic women (age 55-88) divided into 2 groups: WBV vs placebo	WBV 4 min/day, Frequency 20 Hz, 1 day/week Duration: 12 months	All subjects: Alendronate 5 mg/day orally Duration: till the end of study	ALN+WBV and ALN+placebo: No significant difference on lumbar spine BMD, serum ALP and urinary NTX. ALN+WBV: reduction in chronic back pain
Lespessailles et al	2009	60 rats divided into 5 groups: OVX, shOVX, OVX+E, OVX+Z, OVX+E, OVX+Z+E	Treadmill exercise: 15m/min, 60 min/day, 5 days/week Duration: 12 weeks	Z: Zoledronic acid single dose 20 µ/kg IV injected 2 days before ovariectomy surgery. Control groups given saline	OVX+Z and OVX+Z+E: No significant difference in BMC, bone strength and trabecular bone architecture
Uusi-Rasi et al	2003	159 post menopausal women divided into 4 groups ALN+E, ALN, Placebo+E, Placebo	Exercise session: 15 min of warm up, 20 min of jumping and 15 min of stretching and calisthenics (non impact exercise) and 10 min of cool down. 3 sessions/week Duration: 12 months Supervised by exercise leaders	ALN: Alendronate 5mg/day orally Placebo: no details	ALN and ALN+E: No significant difference in bone mass. ALN vs Placebo: ALN had significant increase in lumbar spine and femoral neck bone mass compared to placebo. Alendronate had no effect on physical performance. Exercise: Improved physical performance (jumping exercise improved leg extensor power, dynamic balance and cardiorespiratory fitness)

Chilibeck et al	2002	57 postmenopausal women divided into 4 groups ETD, ETD+E, E+Placebo, Placebo	Exercise session: Strength training: a warm up (5 min of cycling followed by stretching) followed by 2 sets of 8-10 repetitions on each of the following: bench press, latissimus dorsi pull down, shoulder press, biceps curl, back and hip extension, knee flexion, knee extension and leg press 3 sessions/week Duration: 12 months	ETD: Etidronate 400 mg/day for 14 days of 500mg/day of calcium carbonate in a cycle repeated 4 times over 12 months Placebo: no details	ETD and ETD+E: No differences on BMD
Jagger et al	1996	35 rats divided into 5 groups 1- unpinned,+vehicle 2-pinned,+not loaded+vehicle 3 – pinned,+not loaded+BP 4- pinned, loaded+vehicle	Invasive loading – 1Hz, load of 150 N Pins inserted into the 7 <sup>th</sup> and 9 <sup>th</sup> caudal vertebra	BP: A single dose of 3-amino-1-hydroxypropylidene-1-bisphosphonate 0.3 mg/kg subcutaneous injection 24 hours before loading Vehicle : no details given	Loaded group vs non loaded group(4 vs 2): Loaded bone showed 16 fold increase in bone formation rate of the 8 <sup>th</sup> caudal vertebra BP vs vehicle gp (5 vs 4): No significant effect of BP on bone formation induced by loading on the vertebral bone. Bone formation rate in the tibia is suppressed in the BP treated group(5) compared to bisphosphonates untreated group(4)

		5-pinned, loaded+BP			
STUDIES WITH AN ADDITIVE OUTCOME					
Camargos et al	2015	34 rats divided into 5 groups shOVX, OVX+shWBV, OVX+WBV, ALN+shWBV and ALN+WBV	WBV loading 10 min/day using 10 consecutive steps (5Hz increase every step) from 130--150 Hz) Duration: 14 days	ALN: Alendronate 2mg/kg 3 days/week SC injection (starting 5 days post ovariectomy) Control groups given Saline Duration: till the end of study	ALN+shWBV vs ALN+WBV: WBV vs ALN+WBV: ALN+WBV had significant increase in cortical thickness but no significant difference in bone stiffness and strength.
Chen et al	2014	128 ovariectomised rats divided into 5 groups ShOVX, OVX+Vehicle, OVX+WBV, OVX + ALN, OVX+WBV+ALN)	WBV at 45–55 Hz 20 min/day, 5 day/week Duration: 3 months.	ALN: Alendronate 1 mg/week once/week subcutaneous injection Vehicle: phosphate buffered saline	OVX+ALN vs OVX+ALN+WBV: OVX+WBV+ALN had improved trabecular architecture, but no significant difference in bone turnover markers and biomechanical testing
Sugiyama et al	2011	60 mice divided into 5 groups 1-saline	External loading of right tibia 3 alternate days/week 7 minutes/day Left bones: control	Risedronate increasing doses as described already in the study subject details	Risedronate +loading had additive effect on trabecular bone volume at 15 µ/kg/day and 150 µ/kg/day compared to risedronate + unloaded bone. No differences noted in the

		2-RSD 0.15 $\mu$ /kg 3- Risedronate 1.5 $\mu$ /kg 4-Risedronate 15 $\mu$ /kg 5-Risedronate 150 $\mu$ /kg	Duration: 17 days	Group 1 is given saline	cortical bone.
Fuchs et al	2007	344 rats divided into 5 groups shOVX, OVX+ALN, OVX+vehicle, OVX+vehicle+E OVX+ALN+E	Exercise session: running on a motorized treadmill at a 5% incline for 60 min/day, 22–24 m/min, 5 days/week.  Duration: 14 weeks	ALN: Alendronate 0.015 mg/kg 2 times/week Vehicle: sterile water  Duration: till the end of study	ALN+E: additive benefits on total and mid-shaft femur BMC, L4 vertebrae BMC, and mid-shaft femur cortical thickness and area.



Three studies have shown that bisphosphonates inhibit the normal anabolic response to mechanical stimulation (Iwamoto et al., 2005, Iwamoto et al., 2012, Hatori et al., 2015). Hatori et al., (2015) showed there was a lack of WBV effect on the bisphosphonates treated group. The bone microarchitecture of bisphosphonates treated ovariectomised rats was similar with or without WBV whereas the bone microarchitecture of untreated bone had an improvement with WBV compared to those without WBV (Hatori et al., 2015). Likewise, there was no additive effect of WBV on bone density or bone turnover markers on osteoporotic women treated with alendronate (Iwamoto et al., 2005; Iwamoto et al., 2012). Similarly, exercise and bisphosphonate treatment did not show additive effects on bone mass in postmenopausal women (Uusi-Rasi et al., 2003; Chilibeck et al., 2002) or bone architecture in ovariectomised rats (Lespessailles et al., 2009). Jagger et al., (1996) reported that bisphosphonate treatment did not have additive or inhibitory effect on rat vertebral bone loaded invasively. However, there was an inhibitory effect on the tibial bone with suppression of bone formation rate in this study (Jagger et al., 1996). Jagger et al., (1996) used a single dose of 0.3 mg/kg of 3-amino-1-hydroxypropylidene-1-bisphosphonate before invasive loading of rats.

In contrast, four studies in ovariectomised rats have shown additive effect of mechanical stimulation and bisphosphonates on bone density (Fuchs et al., 2007), cortical thickness (Camargos et al., 2015) and trabecular architecture (Sugiyama et al., 2011; Chen et al., 2014). However, there was no significant additive effect on bone turnover markers [osteocalcin and CTX] in the study by Chen et al., (2014).

It is unclear as to why the study by Camargos et al., (2015) showed an additive effect on cortical thickness whilst no additive effect was shown in the study by Hatori et al., (2015). Both the studies by Camargos et al., (2015) and Hatori et al., (2015) had similar study designs except Hatori et al., (2015) had a larger sample of 84 compared to 34. The study by Fuchs et al., (2007) and Chen et al., (2014) used a lower dose of alendronate (0.015 mg/kg body weight 2 days/week and 1mg/kg body weight once/week respectively) than the study by Hatori et al., (2015) [2mg/kg body weight 3 days/week]. It could be that the smaller dose of bisphosphonate has allowed rather than suppress the normal anabolic response to mechanical loading. Sugiyama et al., (2011) used risedronate, which is a different type of bisphosphonate compared to alendronate used in other studies. Previous studies have shown that postmenopausal

women treated with risedronate had a greater anabolic response to teriparatide than alendronate (Miller et al., 2008, Chevalier et al., 2010).

Taking all the above together, we can conclude that the combined effect of whole-body vibration and bisphosphonate on bone is unclear. The studies have used imaging studies and/or bone turnover markers to assess the bone's response to mechanical loading. It would be more informative to do a longitudinal study looking at a specific reliable variable over an extended period. It might be useful to assess the short-term response using bone turnover markers and long-term response using imaging in addition to bone turnover markers.

### 1.7.9 Skeletal response to mechanical stimulation

It is unclear to what extent the skeleton in children with OI is responsive to mechanical stimulation; it may be that it is normally responsive, but the osteoblastic activity that occurs in response to mechanical stimulation is defective. It could be that the higher intrinsic levels of bone turnover in OI reduce the capacity of bone to further respond by increasing bone formation in excess of bone resorption. Age and genetic variations are known to influence the skeletal responsiveness to mechanical stimuli. (Rubin et al., 1992; Judex et al., 2002). Bone mass is regulated by canonical wnt-signalling pathway through mechanisms that include stem cell renewal, preosteoblast replication, stimulation of osteoblastogenesis and inhibition of osteoblast and osteocyte apoptosis (Krishnan et al, 2006).

It might also be the case that the use of bisphosphonates abrogates the ability of bone tissue and cells to respond to mechanical stimulation. As bisphosphonates inhibit bone resorption mainly, bone formation is also inhibited in remodelling as the osteoblasts and osteoclasts are coupled with resorption preceding formation. Mechanical stimulation could still stimulate bone formation independently in modelling as then the osteoblasts and osteoclasts are not coupled.

In an ideal situation, we should aim to increase the bone volume and strength by reducing bone resorption whilst also promoting new bone formation. A better understanding of the influence of bisphosphonates on the responsiveness to mechanical stimulation is crucial to tailor management accordingly. If bisphosphonate treatment is proven to alter the normal response of skeleton to mechanical stimulation, then the bisphosphonate type, dose, frequency, duration and the timing of bisphosphonate administration in relation to mechanical stimulation may need to be optimised to prevent bone breakdown but at the same time allow adequate bone formation.

### 1.9 Aims and Hypothesis of the current study

Bisphosphonates and physiotherapy are arguably the main pillars of treatment in OI. Uncertainties remain regarding the interaction between bisphosphonates and mechanical stimulation. Do bisphosphonates inhibit or enhance the normal response to mechanical stimulation? No studies have looked at this relationship in children with OI so far. Understanding the influence of bisphosphonates on OI skeleton is crucial for optimisation of current available treatment and before any further interventional studies.

The aims of our current study were therefore:

1. To study if bisphosphonate treatment influences the normal response to mechanical stimulation in children with osteogenesis imperfecta, by looking at serial bone turnover markers
2. To study the effects of age, gender and weight on the response to mechanical stimulation in children with osteogenesis imperfecta

Our hypothesis:

1. Bisphosphonate will inhibit the normal response to mechanical stimulation in children with osteogenesis imperfecta
2. Age will affect the response to mechanical stimulation in children with osteogenesis imperfecta

## 2 Methods

This single-center interventional trial was approved by the South Sheffield Research Ethics Committee (ref 17/YH/0018) and registered with Clinicaltrials.gov, identifier NCT03208582. Participants attending the OI service at Sheffield Children's Hospital were approached regarding the study. The eligibility criteria were:

- Ages 4.5 to 15.9 years
- Able to speak fluent English
- Diagnosed with osteogenesis imperfecta
- Able to stand
- Naïve to treatment with bisphosphonates.

OI diagnosis was made using clinical and radiological features by the OI team at Sheffield Children's Hospital. We chose this specific age group because we wanted to assess the skeletal response in children generally from pre-schoolers till teenagers. We chose children above 4.5 years because those younger than 4 are unlikely to tolerate regular blood sampling and stand on the platform as instructed. They are also unlikely to take tablets. We wanted to see if there is any gender influence in childhood, therefore we recruited both boys and girls.

Children with OI in our clinic are recommended to take a vitamin D supplement; those with a low calcium intake (based on reported low dairy product intake) are asked to take a calcium supplement. All the children in this study had previously received vitamin D; none had had additional calcium. Children were excluded if one or more of the following criteria were met:

- presence of other chronic illnesses including renal failure likely to affect bone metabolism
- balance problems
- recent fracture (in the last 6 months)
- recent (last 12 months) or current treatment likely to affect bone (excluding inhaled or intermittent oral therapy with steroids for asthma)
- involvement in another interventional research project hypocalcemia

- pregnancy or lactation
- known hypersensitivity to risedronate or any of the excipients

Participants stood on a high-frequency (30 Hz), low-amplitude (50–200  $\mu$ ) vibrating platform (Marodyne LivMD, BTT Health, Inning am Ammersee, Germany) for 10 minutes daily (2.5 minutes  $\times$  4 with interspersed 1-minute rest periods) (WBV1) for 7 days (D1–7), followed successively by 5 weeks without further intervention, 6 weeks' oral risedronate treatment with accompanying calcium and vitamin D, 1 week WBV (WBV2, D85–91), and 1 further week without intervention (D92–98). The first WBV exposure was at Sheffield Children's Hospital Clinical Research Facility; this is when I run through the study again. I introduce the WBV platform and demonstrate the way it works. This is also an opportunity for the participant and the family to ask any questions related to the study. We requested parents to keep a diary to assess compliance to the vibration performed during the period of the study.

We chose whole body vibration because it provides a consistent, standardized form of mechanical stimulation known to elicit changes in bone turnover markers in children. We specifically used high-frequency (30 Hz), low-amplitude (50–200  $\mu$ ) vibrating platform because our own previous study (Harrison et al 2015) showed that bone in a healthy growing skeleton does have the capacity to respond quickly to WBV irrespective of the magnitude of that vibration. Therefore, we felt that high frequency low magnitude WBV would be a safe and effective approach for children with OI who may be unable to perform high impact exercises.

The reason why we used the duration of 5 weeks for WBV1 intervention free period is because we were expecting the effect to be principally on modelling, which would have returned to normal by 5 weeks (Harrison et al 2015). The timing of WBV2 in relation to the risedronate treatment is simply because we wanted to see the 'immediate' effect.

Risedronate, calcium, and vitamin D were given during the 6-week period from D43 to D84. The dose of risedronate administered (parentally supervised) was 1mg/kg/week rounded to the nearest 5mg using film-coated tablets of 5mg and 35 mg. Risedronate was administered first thing in the morning as per manufacturer's instructions in the fasting state with a large glass of water, with nothing further to eat or drink other than tap water for the next 30 minutes. Due to its low permeability, it often interacts with food present in the digestive medium and leads to poor bioavailability of less than 1%. Bone turnover markers CTX and ALP are affected by food intake. In addition, all three bone turnover markers P1NP, CTX and ALP have a circadian rhythm with peaks in the early morning and a trough during the day.

Therefore, it should be taken in the fasting state for optimum absorption and to minimize inter and intra individual variations in bone turnover markers. We also controlled other sources of variability including age, pubertal status, OI type and physical activity. We only recruited children aged 4.5-15.9 and these children were all in prepubertal stage or early puberty except 1. The participants in our study were all diagnosed with type 1 OI and they were advised to do the same type of physical activity during the study period.

We chose a duration of 6 weeks for Risedronate treatment. There isn't a paper looking at the speed, duration and/ or efficacy of Risedronate in children with osteogenesis imperfecta. Naylor et al 2016 showed that significant reductions ( $P < 0.001$ ) in bone resorption markers (CTX and NTX) by week 4 and significant reductions in bone formation markers (P1NP and bone ALP) by 12 weeks. A review paper by Giljevic et al 2006 shows that decreases in biochemical markers of bone turnover were observed as soon as within 1 month and reached a maximum in 3-6 months of Actonel 35 mg application once a week or 5 mg a day in women with osteoporosis. Therefore, we felt that 6 weeks of treatment is not unreasonable and would have an effect on the P1NP, ALP and CTX. We also did not want to expose children to a treatment that was not expected to have clear clinical benefit for longer than necessary.

Vitamin D and calcium were given daily in the evening as Calcichew 500mg/200IU tablets (Takeda Ltd, High Wycombe, UK), 1 tablet for participants weighing less than 30kg and 2 tablets for participants weighing 30kg or more. Risedronate and Calcichew tablets were prescribed through the Sheffield Children's Hospital

pharmacy; any unused medication was returned and checked in order to ascertain compliance.

Blood samples were taken at baseline on D1, and on D8, D15, D43, D85, D92, and D99, bracketing the periods of vibration and drug treatment. All samples were taken in the fasting state before 9:00 a.m. The blood samples at D8, D15, D43, D85, D92 and D99 were taken by myself and the research nurses at the participants' homes. Samples were spun at 2500 rpm for 10 minutes at 4 °C and centrifuged samples stored at -80 °C. The diagram (figure 3) below shows the flow chart of study processes.

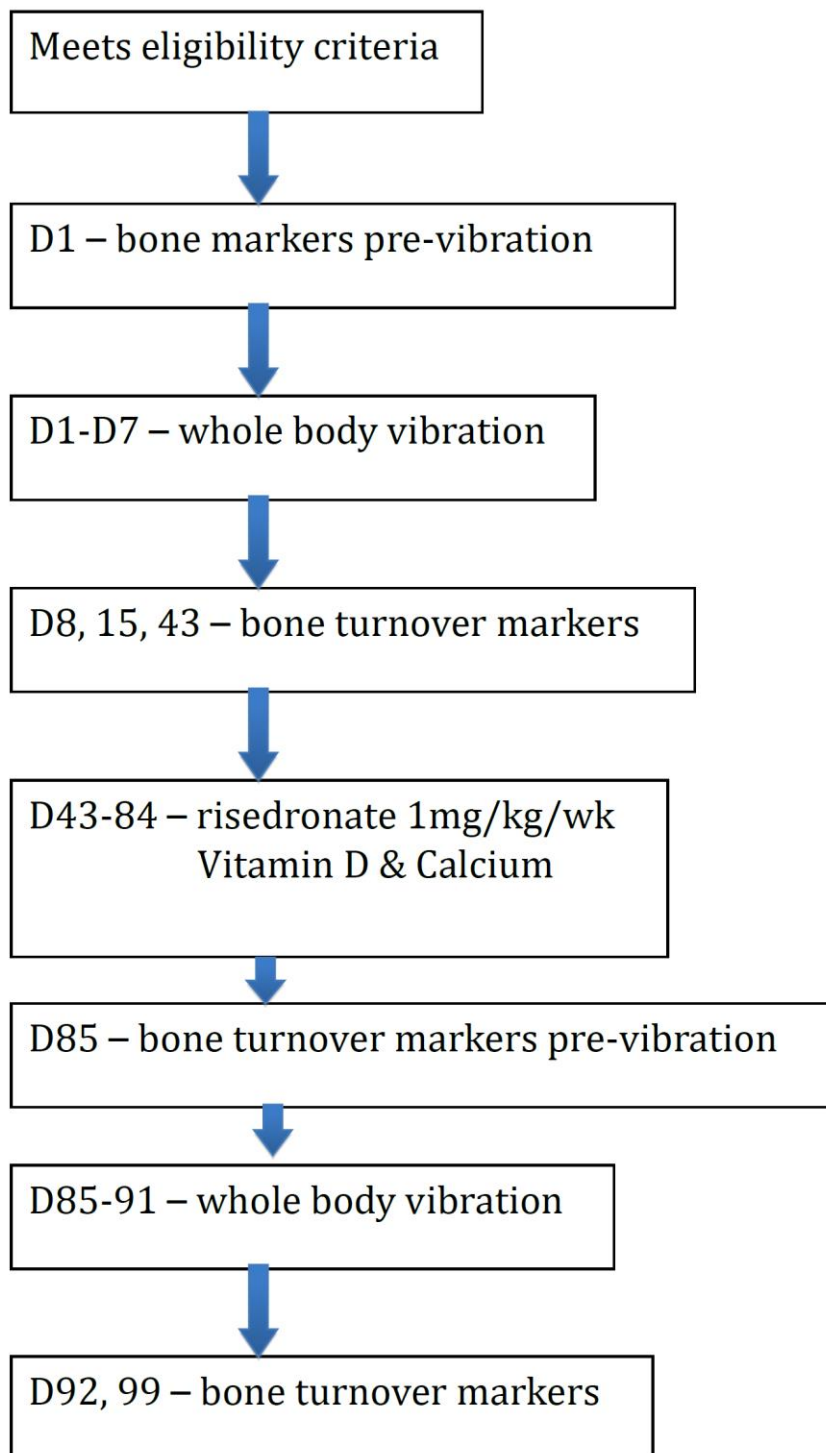


Figure 3 Flow chart of study processes



Samples were analyzed by a senior technician in the Bone Biochemistry Laboratory, University of Sheffield. The samples were analysed in a single batch for bone-specific alkaline phosphatase (BSALP), procollagen type 1 N-terminal propeptide (P1NP), and C-terminal telopeptide of type 1 collagen (CTX), using an Elecsys Cobas E411 automated immunoassay system interassay coefficients of variation (CVs) 2.8% to 8.4% for CTX and <1.7% for P1NP. BSALP was measured on an iSYS automated immunoassay system (Immunodiagnosics Ltd., Boldon, UK) with interassay CV 4.2%.

Pubertal status of every patient was self-assessed using a sex appropriate pictorial scale. Pregnancy testing was carried out in the Sheffield Children's Hospital Clinical Research Facility before risedronate was given to any female subject aged 10 years or older.

Our choice of sample size is pragmatic and not informed by power. A literature review of sample size for pilot and feasibility studies found a few articles with recommendations for two arm trials but nothing of good quality for single-arm interventions (Billingham et al, 2013). The smallest number suggested for a two-arm trial was 12 patients per group (Hertzog, 2008), whereas others have suggested larger numbers (Lancaster et al, 2004).

Data were analyzed using Stata v16 (StataCorp LLC, College Station, TX, USA). Data are presented as tables of raw data and figures showing adjusted (for age, sex, and weight) means from Analysis of variance (ANOVA) models. ANOVA is a linear regression method for comparing group means. It was pioneered by Professor Sir RA Fisher in the 1920s. Here the groups are the days when measurements were taken (days 1, 8, 15, 43, 85, 92, 99). A key statistical assumption underpinning ANOVA is independence of observations. This is violated under a repeated measures structure (same person measured on each day). The repeated measures ANOVA allows for correlation within individual subjects across days. If the correlation is not taken into account ANOVA means are unreliable (95% confidence intervals are too narrow [optimistic in statistical parlance]). ANOVA is flexible to include covariate adjustment (age, weight, sex) where these variables may confound (distort) relationships. Adjustment makes allowance for possible "confounders" that would otherwise obscure the relationships between the bone biomarkers with intervention

and time; adjustment for these factors was undertaken based on consistent patterns of higher values in older, heavier girls.

Linear regression methods assume that data follow a normal probability distribution. When plotted the normal distribution is a bell-shaped curve. It is described by its mean and standard deviation (SD). Under a perfect normal distribution, the following relationships hold true: 68% of observations are within 1 SD of the mean; 95% within 2 SDs; 99% within 3 SDs. Real data is less than perfect. Hence, means are said to be 'predicted' from an exact normal distribution. Predicted means from the repeated measures ANOVA were plotted along with 95% confidence intervals.

Pairwise comparisons (n=21) between days were calculated unadjusted for multiple comparisons (Rothman, 1990). Rothman contended '... scientists should not be so reluctant to explore leads that may turn out to be wrong that they penalize themselves by missing possibly important findings' sentiments with which I agree (Rothman 1990). Any p values were based upon differences in adjusted means.

Continuously distributed data was summarized by median (25<sup>th</sup>/75<sup>th</sup> centiles). Box and whisker plots represent a visual representation of P1NP, CTX and ALP over the different days (1,8,15,43,85,92,99). The median (50<sup>th</sup> centile) of each bone marker is indicated by a horizontal line in the middle of the rectangular box. The two ends of the box are named lower and upper quartiles (25<sup>th</sup> and 75<sup>th</sup> centiles) respectively. The difference between the upper and lower quartiles is called the interquartile range (IQR). The 'whiskers' on either side of the box are calculated according to a formula. The upper whisker is calculated as upper quartile + (1.5 x IQR); the lower whisker is calculated as lower quartile - (1.5 x IQR). Whiskers may or may not be capped by a small horizontal line depending on the values. The dots beyond the whiskers are extreme values of the distribution.

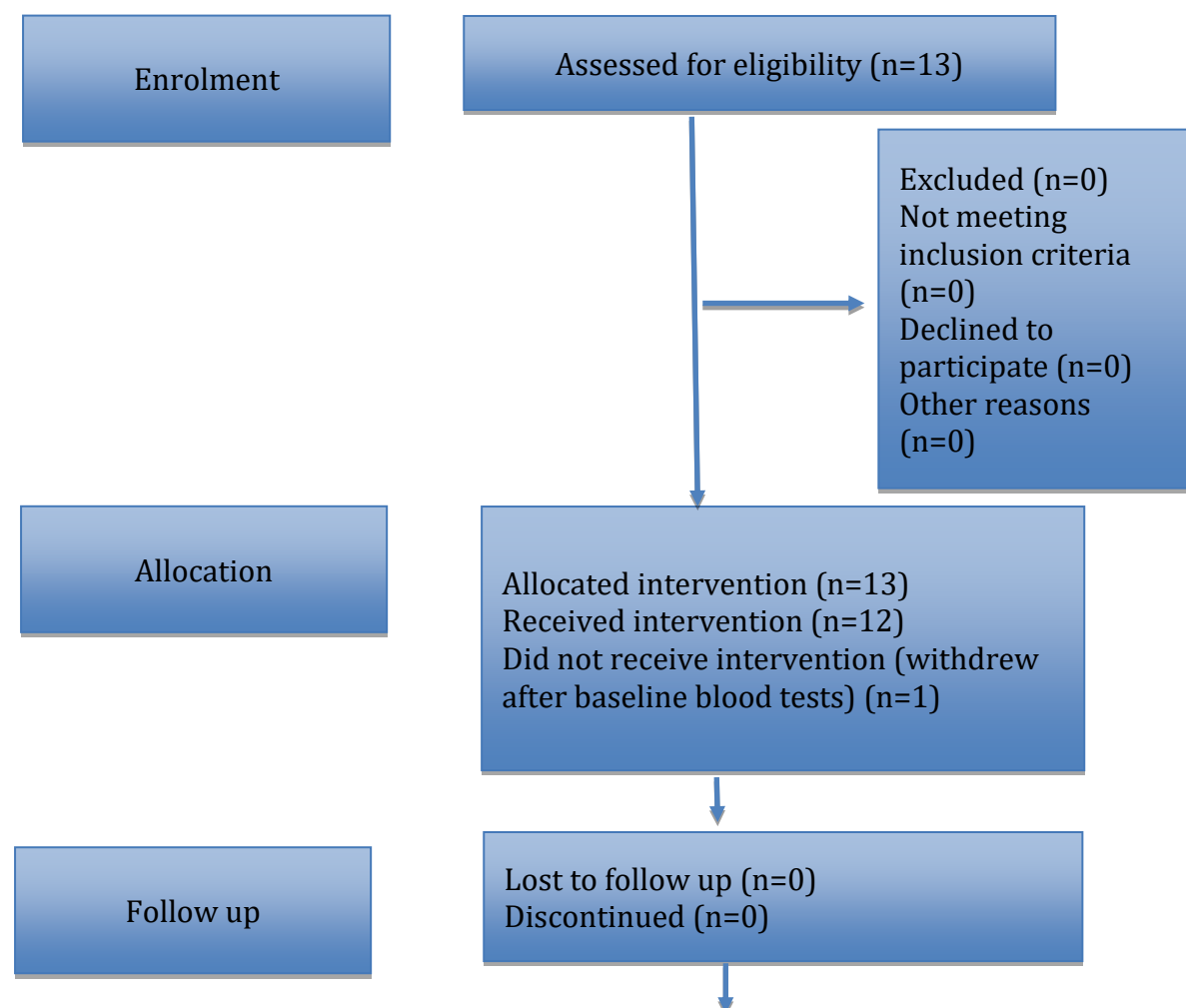
We examined for possible interactions between sex, age, weight and height with day of study. Age, weight and height were dichotomized at the median. Cut-off points as follows: age ( $\leq 9$  years vs  $\geq 9$  years); weight ( $\leq 26$  kg vs  $\geq 26$  kg); height ( $\leq 132$  cm vs  $\geq 132$  cm). We are aware that dichotomizing continuous distributed variable is not best statistical practice (Altman and Royston, 2006) but our data was not strong

enough for three group classifications. Interactions are exploratory only and are represented graphically. Non-parallel lines provide evidence of an interaction between two or more variables. Interactions are not shown for height separately because height and age were completely tied-up with each other (the same children  $\leq 9$  years were also classified  $\leq 132$  cm tall).

### 3 Results

#### 3.1 Demographics

Thirteen children with OI, naïve to bisphosphonate treatment, were enrolled into and participated in the study between May 5, 2017, and February 8, 2018. One child withdrew after baseline blood tests were taken and before any WBV was performed; their data have been excluded. The CONSORT diagram showing the flow of patients is provided in Fig. 3.



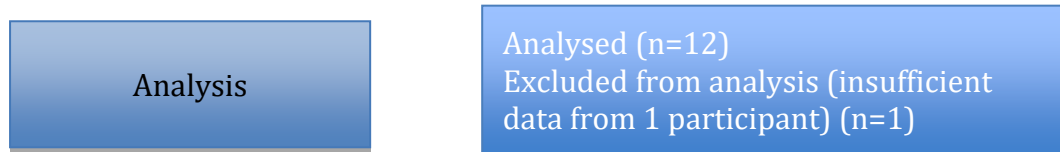


Figure 4 Consort Diagram

The baseline demographics are shown in Table 6. All participants but one were either prepubertal or in early puberty (Tanner stage I or II), and there was a slight preponderance of girls (7/12).

Table 6 Age, gender, height, weight and pubertal status of the 12 study participants

ID	Age (y)	Gender	Weight (kg)	Height (cm)	Pubertal status
1	5.8	M	16.0	108.0	Stage 1
2	11.3	F	36.4	140.4	Breasts Stage II, Pubic hair Stage I
3	9.9	M	26.0	137.5	Stage II
5 <sup>a</sup>	5.6	M	21.7	101.0	Stage I
6	14.9	M	49.8	167.1	Stage IV
7	9.8	F	54.8	147.4	Breast Stage II, Pubic hair Stage II
8	6.9	M	17.5	116.0	Stage I
9	6.1	F	30.8	125.7	Stage I
10	5.8	F	18.6	113.0	Stage I
11	9.4	F	42.0	147.7	Breast Stage II, Pubic hair Stage I
12	4.5	F	16.9	95.2	Stage I
14 <sup>b</sup>	9.7	F	35.4	141.7	Breast stage II, Pubic hair stage I

<sup>a</sup>Number 4 withdrew

<sup>b</sup>Number 13 not assigned

### 3.2 Biomarkers across the whole study period

The main results for each of the biomarkers across the whole study are presented first, followed by each marker one by one. The summary unadjusted data for each biomarker at each time period are shown in Table 6. The unadjusted data for each biomarker are shown separately in Tables 7a-7c.

Intervention	Time point (days)	Serum P1NP, ng/mL (mean, 95% CI)	Serum CTX, ng/L (mean, 95% CI)	Serum BSALP, ng/mL (mean, 95% CI)
WBV1	1	464.6 (417.8, 511.5)	1.30 (1.20, 1.41)	89.8 (86.2, 93.4)
	8	550.0 (499.3, 602.5)	1.48 (1.37, 1.60)	90.1 (86.3, 94.0)
No intervention	15	509.5 (458.0, 561.1)	1.45 (1.34, 1.57)	90.2 (86.2, 94.2)
Risedronate treatment	43	556.7 (509.8, 603.6)	1.55 (1.44, 1.66)	89.0 (85.3, 94.8)
	85	460.1 (411.2, 508.9)	1.30 (1.19, 1.41)	85.9 (82.1, 89.8)
WBV2	92	476.0 (424.4, 527.6)	1.42 (1.31, 1.54)	87.9 (84.0, 91.7)
No intervention	99	480.5 (433.6, 527.3)	1.40 (1.30, 1.51)	88.4 (84.3, 92.3)

Table 7 Raw Data Means (95% CIs) for P1NP, CTX and BSALP

ID	PINP (ng/ml)						
	WBV1		No Intervention	Risedronate treatment		WBV2	No intervention
	Day 1	Day 8	Day 15	Day 43	Day 85	Day 92	Day 99
1	223.7	220.0	NS	209.6	203.0	272.4	226.8
2	301.5	373	338.6	358.5	267.6	310.8	304.9
3	417.9	NS	529.2	662.6	374.8	412.8	452.8
5	531.9	887.5	610.5	987.9	653.2	561.7	789.9
6	366.9	401.6	329.2	455.8	310.3	268.1	284.1
7	173.1	289.1	180.5	259.6	204.3	129.0	203.1
8	212.0	211.3	162.0	198.0	178.4	IS	257.1
9	595.5	IS	IS	339.9	IS	IS	463.4
10	424.3	479.6	615.9	470.0	481.1	460.2	458.9
11	1083.0	1122.0	1136.0	1200.0	1200.0	1200.0	1115.0
12	498.1	578.0	561.4	520.8	322.7	432.1	382.6
14	644.4	894.3	835.5	914.6	796.3	944.7	723.8

Table 8a – PINP

ID	CTX (ng/ml)						
	WBV1		No intervention	Risedronate treatment		WBV2	No intervention
	Day 1	Day 8	Day 15	Day 43	Day 85	Day 92	Day 99
1	0.723	1.000	NS	1.030	0.931	1.080	1.120
2	1.230	1.440	1.380	1.330	1.120	1.300	1.190
3	1.460	NS	2.030	2.020	1.830	1.760	2.000
5	1.170	1.580	1.420	1.450	1.270	1.140	1.430
6	1.370	1.420	1.320	1.520	1.440	1.290	1.450
7	1.170	1.400	1.380	1.540	1.090	1.090	1.400
8	1.110	0.986	1.070	1.060	1.050	IS	1.120
9	1.010	IS	IS	IS	IS	IS	1.060
10	1.180	1.390	1.380	1.480	1.480	1.260	1.290
11	2.370	2.110	1.900	2.240	1.790	2.530	1.610
12	1.420	1.330	1.400	1.490	0.888	1.130	1.070
14	1.180	1.810	1.790	2.010	1.490	2.080	1.850

Table 8b -CTX

ID	ALP (ng/ml)				
	WBV1		No intervention	Risedronate treatment	WBV2

	Day 1	Day 8	Day 15	Day 43	Day 85	Day 92	Day 99
1	88.504	87.264	NS	76.443	IS	84.531	88.038
2	91.688	89.804	90.819	91.202	87.896	88.968	86.682
3	95.418	NS	96.603	89.378	91.015	90.99	94.394
5	92.973	93.618	92.086	91.177	86.424	86.788	87.861
6	92.759	94.943	93.929	97.035	85.896	91.151	87.237
7	85.916	89.136	90.969	88.603	94.165	97.576	90.219
8	90.948	IS	IS	IS	IS	IS	IS
9	75.8	IS	IS	IS	IS	IS	IS
10	84.126	82.197	IS	80.573	76.253	74.325	IS
11	92.422	95.755	92.413	92.911	95.203	89.938	91.236
12	93.803	87.725	91.084	93.556	75.745	IS	88.839
14	93.538	95.836	94.943	98.712	93.567	96.026	98.946

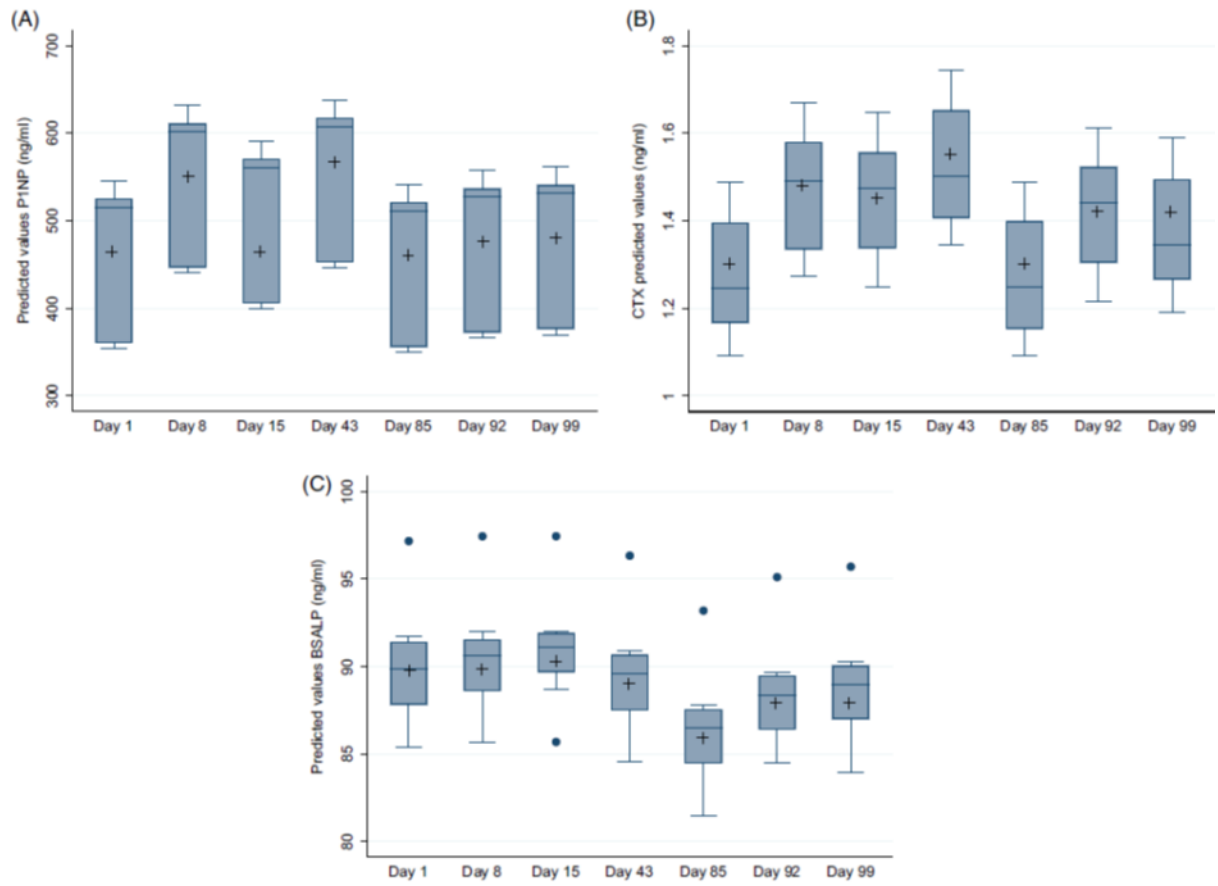
Table 8c - BSALP

Tables 8a-8c shows the data for every study participant over the different days (1,8,15,43, 85, 92, 99) for each biomarkers P1NP, CTX and BSALP respectively. NS- no sample received; IS – insufficient sample received.

The baseline mean absolute values obtained for P1NP and CTX were around 65% of the mean values we obtained in healthy prepubertal boys (Harrison et al, 2015) and 80% of those in our studies of younger children (Gopal-Kothandapani et al, 2020). We did not measure BSALP in our previous studies.

OI is a high bone turnover disease; however, OI type 1 is caused by haploinsufficiency variants. The P1NP and CTX baseline values are as above because haploinsufficiency variants are known to have lower bone turnover markers compared to healthy controls or OI types III and IV caused by helical domain alterations (Garnero et al 2009, Braga et al 2004 and Minisola et al 1994). This is likely because haploinsufficiency variants produce a decreased amount of collagen.

The percentage changes in the unadjusted means from D1–8 for P1NP and CTX, were thus an increase of 18.4% and 13.8%, respectively; both markers then fell slightly (7.3% and 2.0%, respectively) from D8 to D15 and were again increased with respect to baseline at D43, 19.8% and 19.2%, respectively. The change from D43–85 across the period of risedronate therapy was effectively a return to pre-vibration baseline levels. The percentage changes from D85–92 across the second period of vibration for P1NP and CTX, respectively, were an increase of 3.4% and 9.2%.





### Figure 5 A-C Changes in P1NP, CTX and BSALP

Fig. 5. A) Change in adjusted P1NP (ng/mL) across the study period. Boxes represent second and third quartiles with the internal horizontal line showing the median; whiskers are 1.5 times the interquartile range. + shows arithmetic mean; ● shows outliers more than 3 times the interquartile range from the median. B) Change in adjusted CTX (ng/mL) across the study period. Boxes represent second and third quartiles with the internal horizontal line showing the median; whiskers are 1.5 times the interquartile range. + shows arithmetic mean; ● shows outliers more than 3 times the interquartile range from the median. C) Change in adjusted BSALP (ng/mL) across the study period. Boxes represent second and third quartiles with the internal horizontal line showing the median; whiskers are 1.5 times the interquartile range. + shows arithmetic mean; ● shows outliers more than 3 times the interquartile range from the median.

Figures 5A–C shows the change in each of the bone biomarkers assessed at each time point in graphical form, with the means (from ANOVAs, see methods section) adjusted for age, sex, and weight. For P1NP and CTX, the pattern of change is similar. For P1NP, there was a significant initial increase from D1–8 ( $p = 0.010$ ), a plateau or slight decrement from D8–15, and then a second peak on D43 ( $p = 0.03$  versus D1) before the period during which risedronate, calcium, and vitamin D were administered.

For CTX, there was a significant initial increase from D1–8 ( $p = 0.014$ ), a plateau or slight decrement from D8–15, and then a second peak on D43 ( $p < 0.001$  versus D1). After the administration of the medication between D43 and D85, P1NP fell significantly ( $p = 0.016$ ). The change in mean adjusted CTX did not reach statistical significance ( $p = 0.086$ ). The subsequent percentage change from D85–92 in P1NP was less than it had been from D1–8 (3.7% versus 19.5%;  $p < 0.05$ ) but the difference in percentage change in CTX (9.8% versus 14.4%) did not reach significance; Fig. 6).

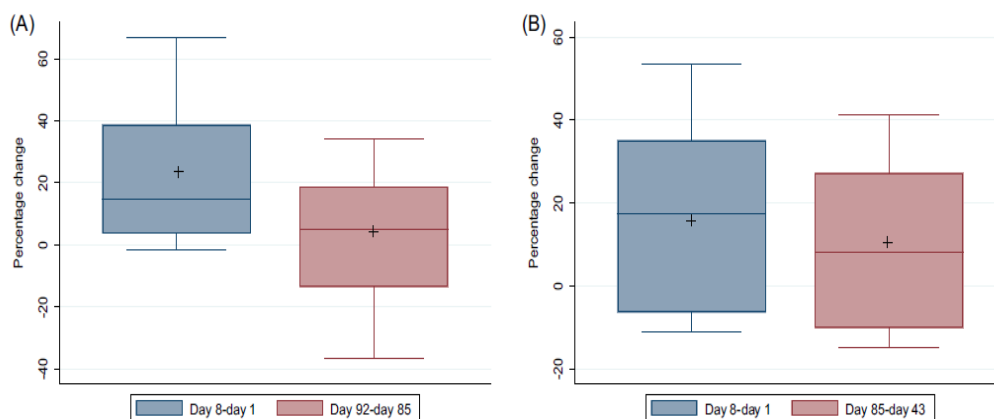


Figure 6 A-B – Box plots comparisons for P1NP and CTX

A) Box plots showing comparison of P1NP percentage changes after whole body vibration period 1 (day 8-day1) and period 2 (day 92 – day 85). Boxes represent second and third quartiles with the internal horizontal line showing the median; whiskers are 1.5 times the interquartile range. + shows arithmetic mean. B) Box plots showing comparison of CTX percentage changes after whole body vibration period 1 (day 8-day1) and period 2 (day 92 – day 85). Boxes represent second and third quartiles with the internal horizontal line showing the median; whiskers are 1.5 times the interquartile range. + shows arithmetic mean.

The pattern of change in BSALP was different from that of P1NP and CTX. There was little change after the first period of whole-body vibration from D1–42; BSALP appeared to fall from D42–85 during the period of treatment with risedronate and Calcichew and rose back toward the pre-vibration baseline at the end of the study. The absolute changes in BSALP did not, however, reach significance at any time point (D1 versus D8  $p = 0.822$ ; D1 versus D43  $p = 0.621$ ; D43 versus D85  $p = 0.451$ ). One child suffered a fractured forearm (radius and ulna) 2 days into the period of bisphosphonate administration. This was the only radiologically confirmed fracture among participants during the study.

The graphs (Fig 7a-7c) below show the individual study participants' data across the whole study period for each P1NP, CTX and BSALP respectively. The data clearly shows the heterogeneity of bone turnover markers. Sources contributing to bone turnover markers' variability include uncontrollable (eg, age, gender, ethnicity) and controllable factors, particularly relating to collection conditions (eg, fasting/feeding state, and timing relative to circadian rhythms, menstrual cycling, and exercise). Pregnancy, season, drugs, and recent fracture/ fractures can also affect bone turnover

markers. In our study, the specific reason for the heterogeneity is likely due to the differences in age, gender and ethnicity. Although, we have tried to reduce the controllable factors by standardizing the fasting time, blood collection time and the physical activities during the ‘no intervention’ period, we cannot eliminate this completely.

ID 11 appears to be an obvious outlier with a different P1NP pattern altogether. This is a 9 yr and 5-month old female at pubertal stage 1-II who developed ulna/radius fracture on day 50. P1NP has a higher baseline of 1083 ng/ml that increased following vibration and remained higher than baseline at 4 weeks following vibration despite no interventions. However, it has remained the same (1200 ng/ml) following Risedronate treatment and following vibration post Risedronate treatment. The same participant ID 11 has a higher baseline for CTX but seem to follow a generally similar pattern to the rest.

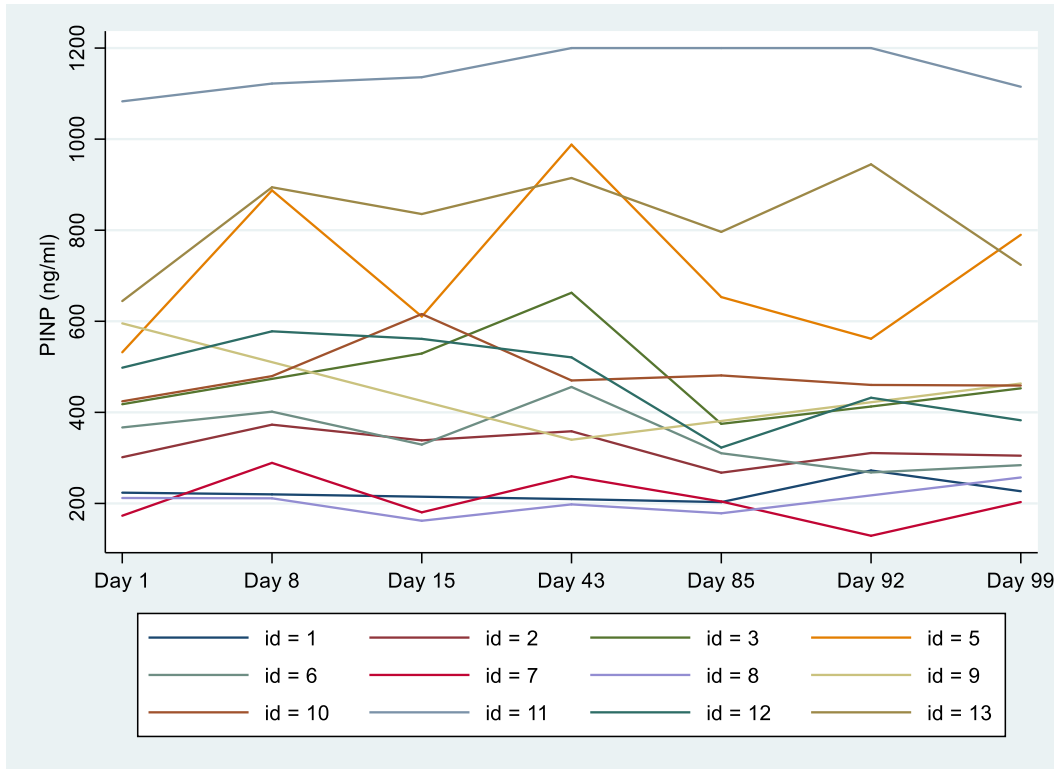


Figure 7a

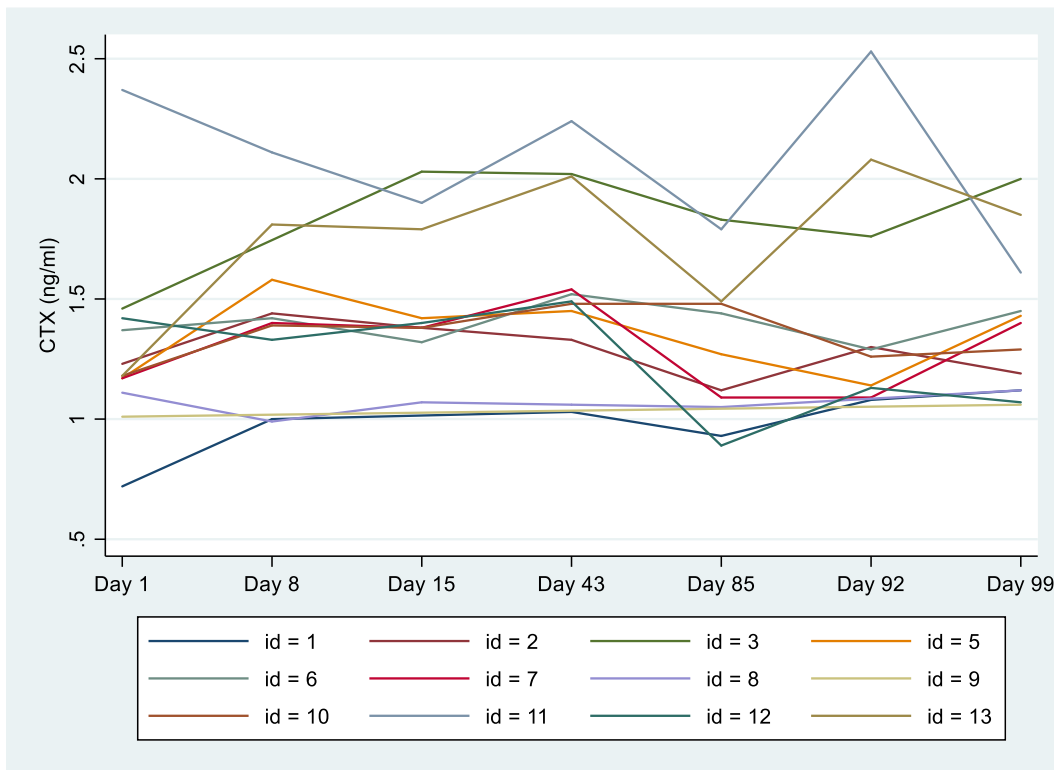


Figure 7b

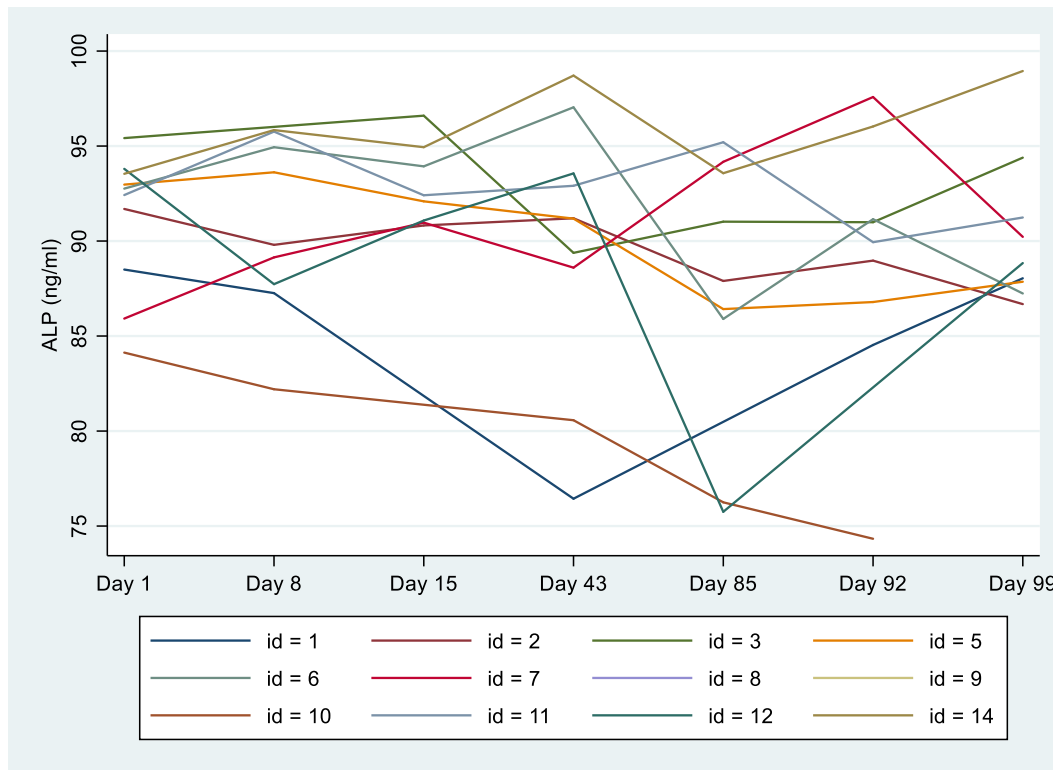


Figure 7c

Figure 7a-c Individual study participants' data across the whole study period for each biomarkers P1NP, CTX and BSALP respectively.

### 3.3 Interactions between gender, age and weight for each of the biomarkers across the whole study period

#### 3.3.1 Interactions between P1NP, gender, age and weight

Figures 8a – 8e show the P1NP interaction between age, gender and weight.

Figure 8a shows the interaction between P1NP and gender, figure 8b shows the interaction between P1NP and age, figure 8c shows the interaction between P1NP and weight, figure 8d shows the interaction between P1NP, gender and age, figure 8e shows the interaction between P1NP, gender, age and weight.

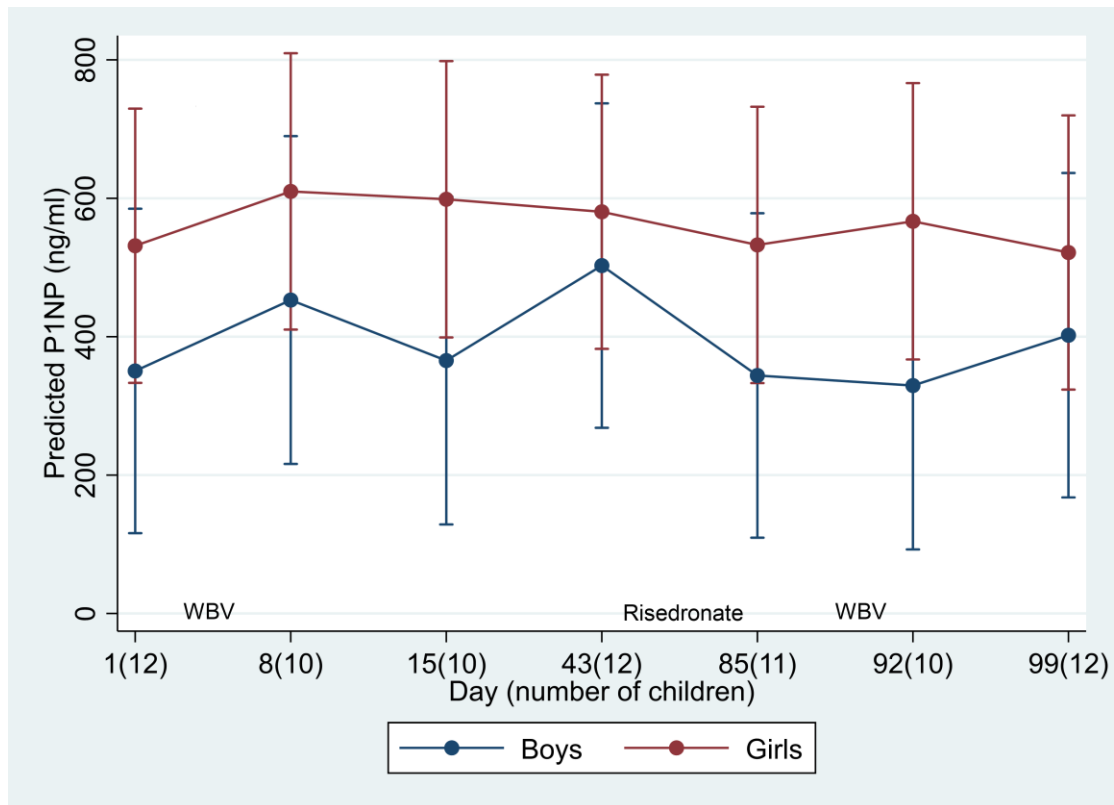


Figure 8a. Graph showing the interaction between P1NP and gender across the whole period of study

Overall, girls have higher mean P1NP values than boys. Between Day 1-8, the parallel lines show that effect of mechanical stimulation is not dependent on gender; both boys and girls show an increase of P1NP at D8 from D1.

Between D8- D15, there is an interaction, which means that the difference in P1NP may be dependent on gender.

Between D15-D43, where participants are advised to maintain their activities to those that they will normally undertake, again there is an interaction. Boys show an increase at D43 (5 weeks following mechanical stimulation) whereas girls continue to show a decrease of P1NP at D43. Looking at D8-D43, it is likely that boys have a biphasic response (rebound effect) to the initial vibration with a decrease at D15 followed by an increase at D43. Girls show a gradual decline at D43 from the initial vibration (D1-D8), but the P1NP at D43 is still higher than baseline.

Between D43-D85, the P1NP decreased generally at D85 (6 weeks following Risedronate treatment).

Between D85-D92, the P1NP response may be dependent on gender. Girls show an increase at 92 (1 week following mechanical stimulation post Risedronate treatment) whereas boys show a decrease.

Between D92-D99 (2 weeks following mechanical stimulation post Risedronate treatment), P1NP response is again dependent on gender. Boys show an increase at D99 compared to D92 while girls show a decrease. It appears that boys have a later rise (1 week later) on P1NP compared to girls post Risedronate treatment.

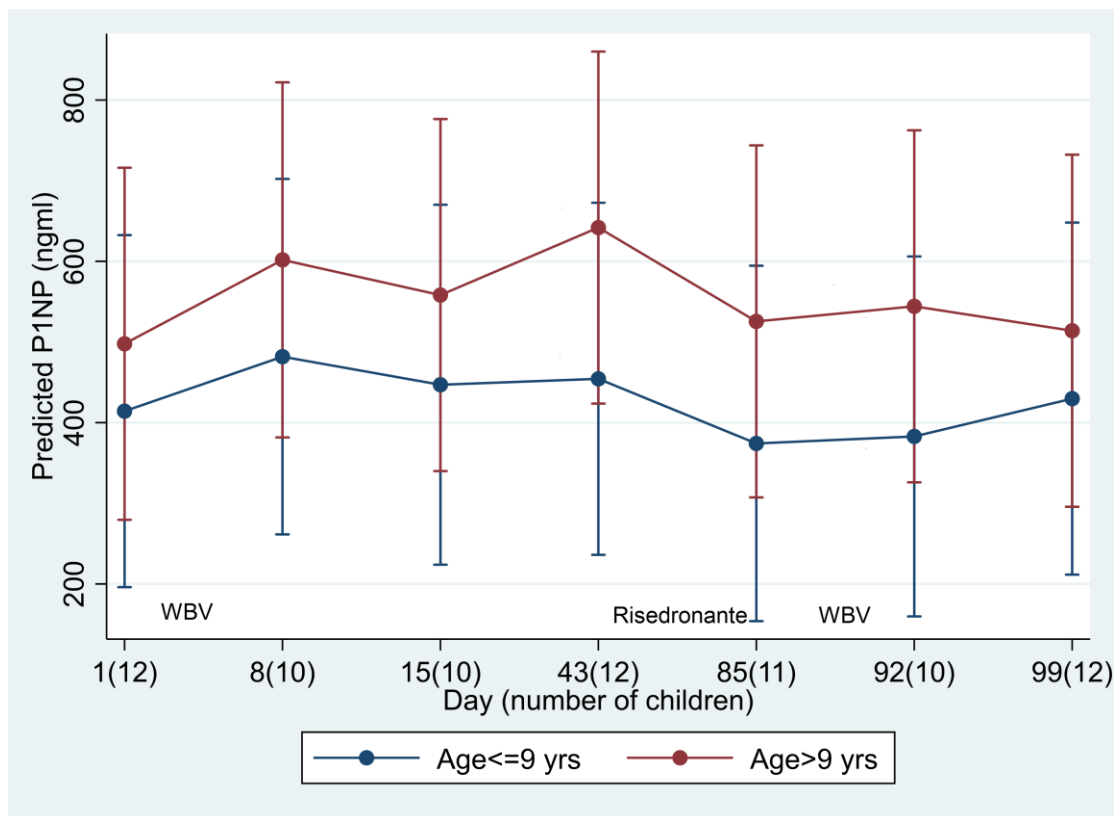


Figure 8b. Graph showing the interaction between age and day

Overall, participants > 9 years have higher mean P1NP values than participants < 9 years.

Between Day 1-8, the nearly parallel lines show that the effect of mechanical stimulation is not dependent on age; both groups show an increase of P1NP at D8 from D1.

Between D8- D15 again the lines are nearly parallel, which means that the P1NP decrease at D15 (2 weeks following mechanical stimulation), is not dependent on age groups.

Between D43-D85 the nearly parallel lines mean that the P1NP decrease at D85 (following 6 weeks treatment with Risedronate), is not dependent on age.

Between D85-D92, the P1NP lines for both groups are nearly parallel again, not dependent on age. Both groups show a rise at D92 (1 week following mechanical stimulation post Risedronate treatment).

Between D92-D99, the P1NP response is dependent on age. Participants >9 years show a decrease at D99 (2 weeks following mechanical stimulation post Risedronate treatment) whereas those <9 years continue to increase. Younger group appear to have a longer lasting effect of vibration post Risedronate treatment compared to the older group.



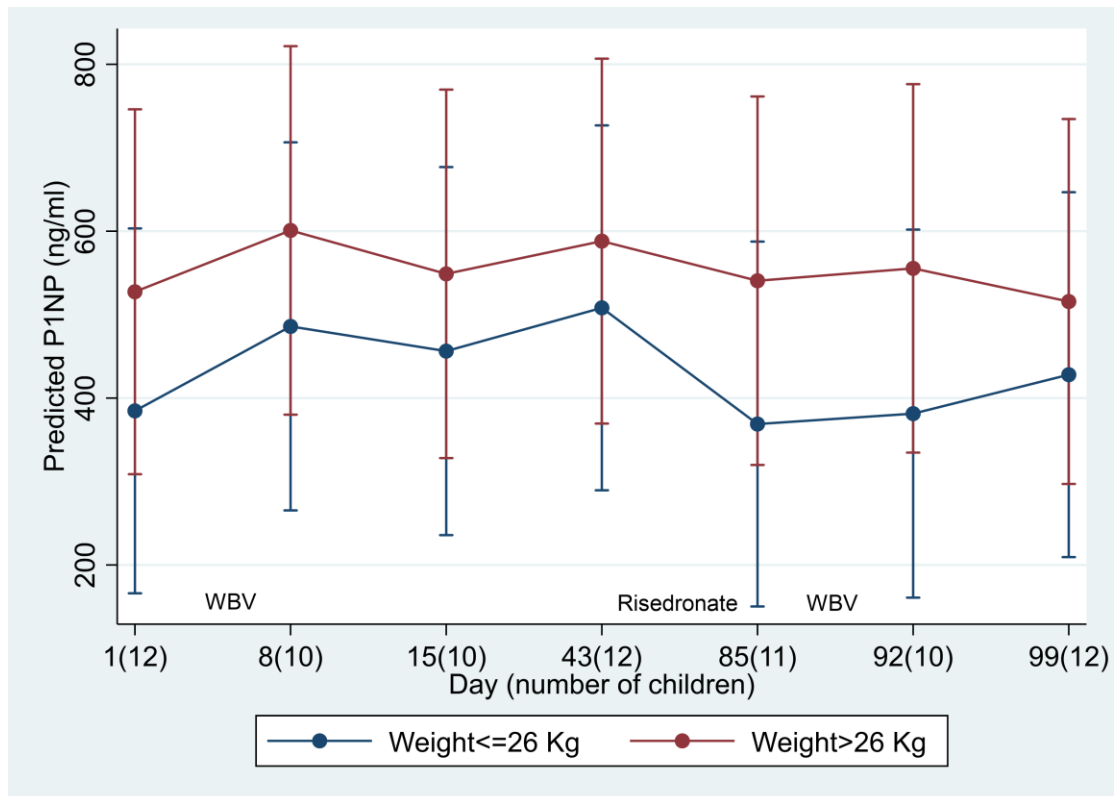


Figure 8c. Graph showing interaction between P1NP and weight

Overall, participants with a weight > 26 kg have higher mean P1NP values than participants with a weight < 26 kg.

Between Day 1-8, the nearly parallel lines show that effect of mechanical stimulation is not dependent on weight; both groups show an increase of P1NP at D8 from D1.

Between D8- D15 again the lines are again nearly parallel, which means that the P1NP decrease at D15 (2 weeks following mechanical stimulation), is not dependent on weight. Similarly, between D15-D43, (when participants are advised to undertake their normal daily activities), the nearly parallel lines mean that the increase in P1NP is not dependent on weight.

Looking at D8-D43, both groups show a biphasic response to the initial vibration with a decrease at D15 followed by an increase at D43.

Between D85-D92, the nearly parallel lines mean that the P1NP response is not dependent on weight; both groups show an increase at D92 (1 week following mechanical stimulation post Risedronate treatment).

Between D92-D99, the P1NP response is dependent on age. Participants with a weight >26 kg years show a decrease at D99 (2 weeks following mechanical stimulation post Risedronate treatment) whereas those with a weight < 26 kg continue to increase. Lighter participants appear to have a longer lasting effect to vibration post Risedronate treatment compared to heavier participants.

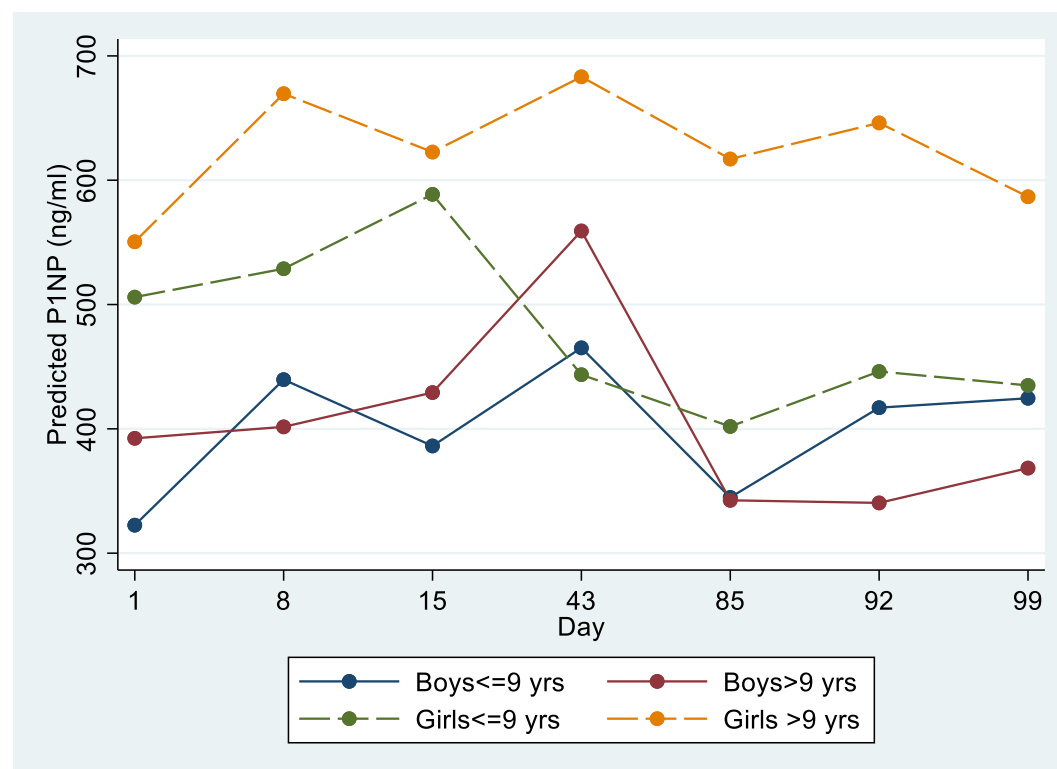


Figure 8d. Graph showing the interaction between P1NP, gender, age and day across the whole study period.

Overall, girls > 9 years have a higher P1NP than all other groups.

Between Day 1-8, girls < 9 years and boys > 9 years have nearly parallel lines. Nearly parallel lines are also seen in girls > 9 years and boys < 9 years.

Between D15-D43 (when participants are advised to undertake their normal daily activities), boys < 9 years and girls > 9 years show a similar response with nearly parallel lines. Both these groups (younger boys and older girls) show an increase of

P1NP at D43. These 2 groups show a similar biphasic response at D43 to initial vibration.

Between D92-D99, the P1NP response in girls is nearly similar in both groups with parallel lines showing a decrease at D99 (2 weeks following mechanical stimulation post Risedronate treatment). Parallel lines showing an increase are seen in boys from both older and younger groups. This shows that the response to vibration post Risedronate treatment may be dependent on gender but not age.

Vibration appears to have a longer lasting effect on boys, particularly younger boys following Risedronate treatment.

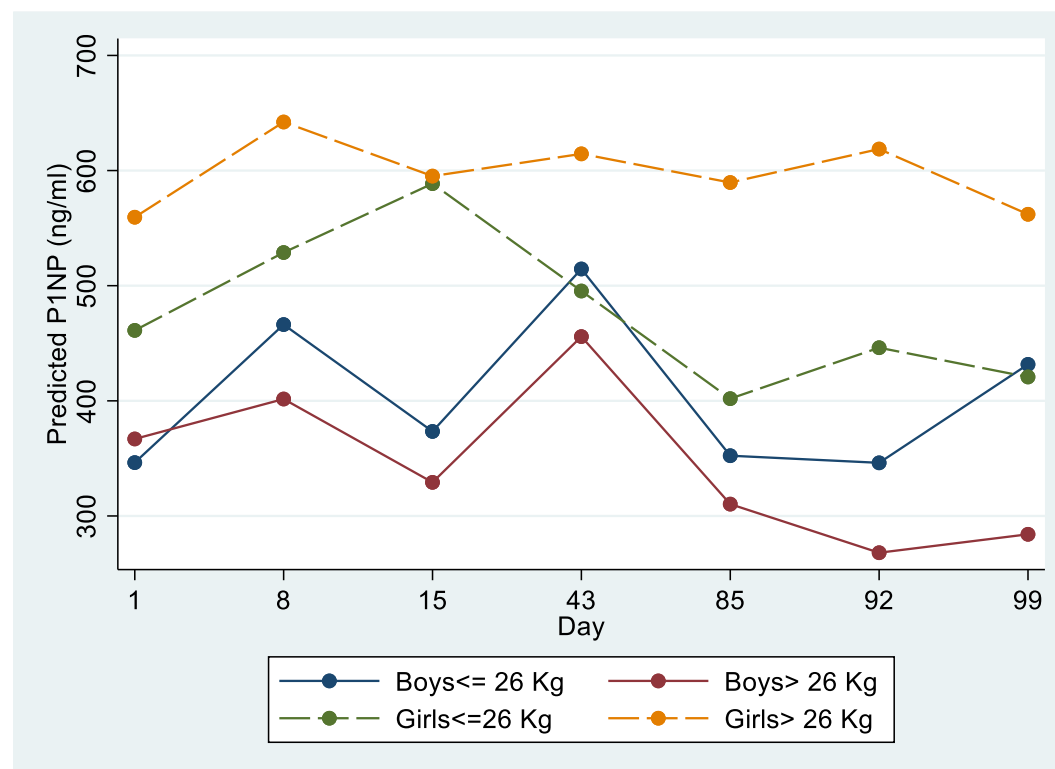


Figure 8e. Graph showing interaction between P1NP, gender and weight.

Figure 8 a-e - interaction between P1NP, gender and weight

Overall, girls with a weight > 26 kg have a higher P1NP than all other groups.

Between Day 1-8, girls < 26 kg and >26 kg has nearly parallel lines. Boys generally show an increase at D8.

Between D92-D99 (2 weeks following mechanical stimulation post Risedronate treatment), the P1NP response in girls is nearly similar in both groups with parallel

lines showing a decrease. Both groups of boys regardless of weight show a main effect of increase. Vibration seems to have a longer lasting effect on boys, particularly lighter boys following Risedronate treatment.

### **3.3.2 Interactions between CTX, gender, age and weight**

Figures 9a – 9e show the interaction between CTX, age, gender and weight.

Figure 9a shows the interaction between CTX and gender, figure 9b shows the interaction between CTX and age, figure 9c shows the interaction between CTX and weight, figure 9d shows the interaction between CTX, gender and age and figure 8e shows the interaction between CTX, gender, age and weight.

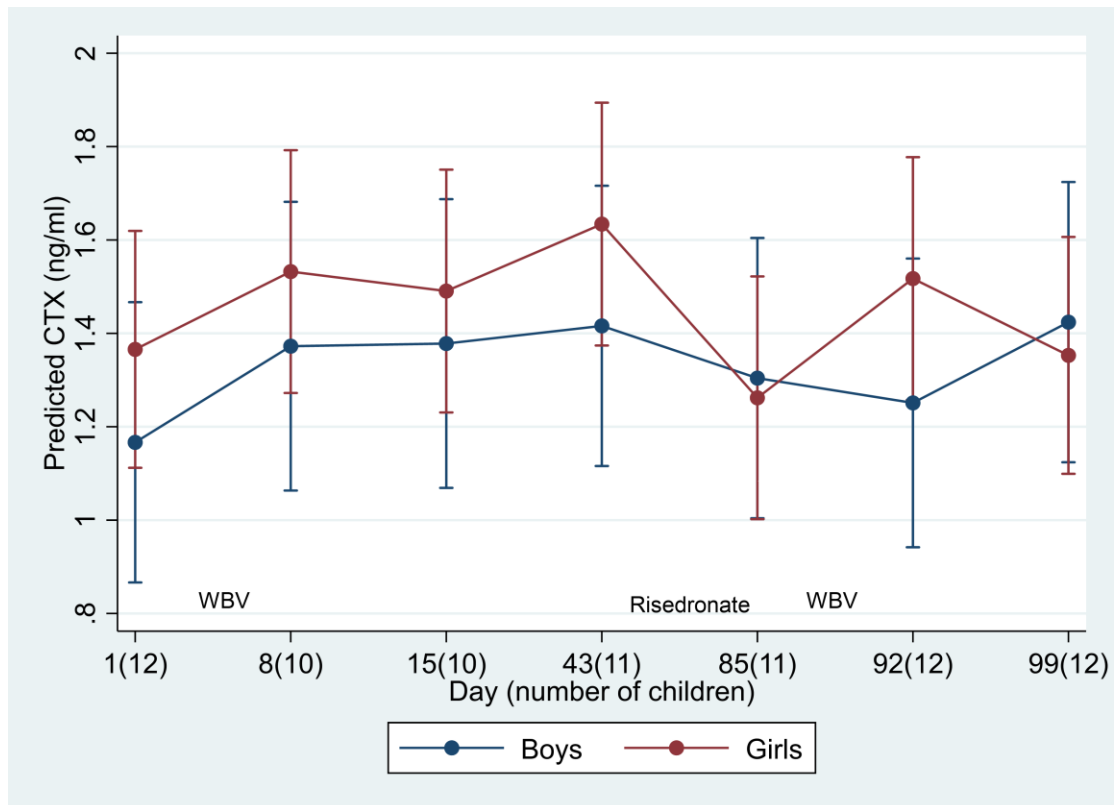


Figure 9a. Graph showing the interaction between CTX and gender

Overall, girls have higher mean CTX values than boys. Between Day 1-8, the parallel lines show that effect of mechanical stimulation is not dependent on gender; both boys and girls show an increase of CTX at D8 following 1 week of vibration.

The CTX is higher at D43 than baseline in both boys and girls.

Between D85-D92, the CTX response appears to be dependent on gender. Girls show a sharp increase at 92 (1 week following mechanical stimulation post Risedronate treatment) whereas boys show a decrease.

Between D92-D99 (2 weeks following mechanical stimulation post Risedronate treatment), CTX response is again dependent on gender. Boys show an increase at D99 compared to D92 while girls show a decrease. It appears that boys have a longer lasting effect of vibration post Risedronate treatment compared to girls.

The rate of rise in CTX pre (D1 - D8) and post Risedronate treatment (D85-D92) appears to be nearly the same in girls. The rate of rise in boys is also nearly the same pre and post Risedronate treatment but the post treatment rise seems to have kicked in a week later in boys (D92-D99) compared to girls (D85-D92).

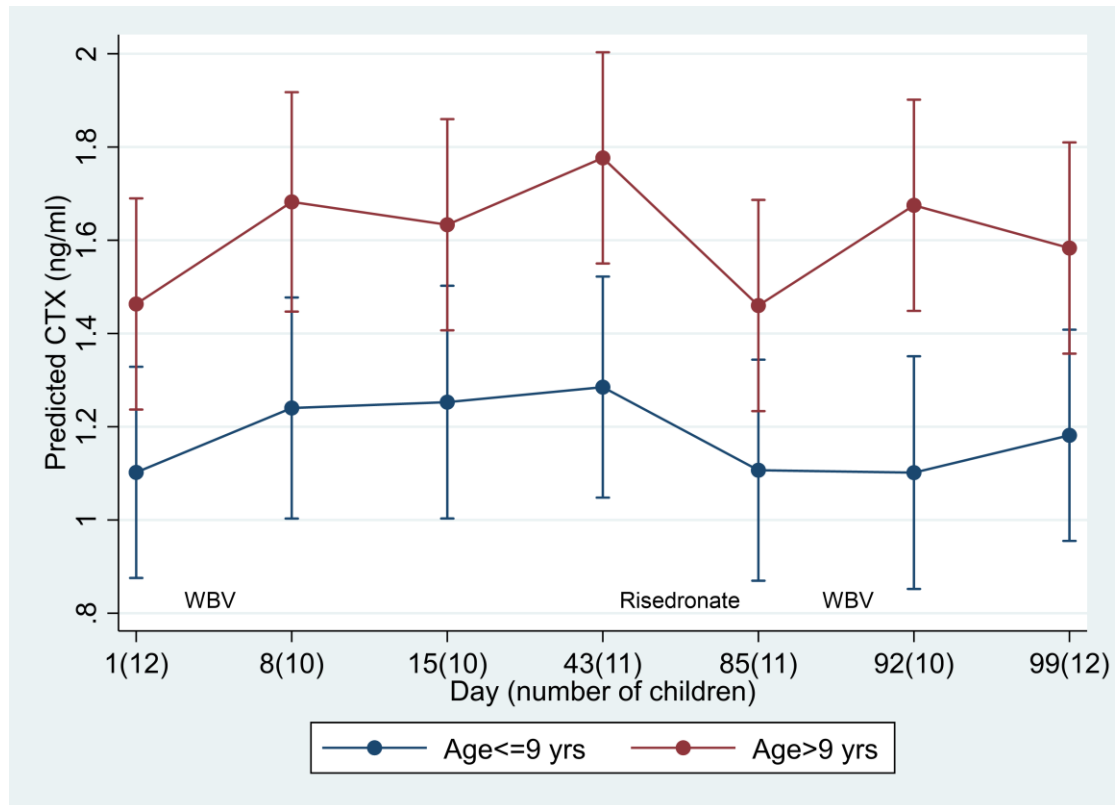


Figure 9b. Graph showing the interaction between CTX and age.

Overall, participants > 9 years have a higher CTX than participants < 9 years.

Between Day 1-8, the nearly parallel lines show that the effect of mechanical stimulation is not dependent on age; both groups show an increase of P1NP at D8 from D1.

Both younger and older participants have a higher CTX at D43 compared to baseline. Between D43-D85, older participants show a sharper decrease at D85 (following 6 weeks treatment with Risedronate) than younger participants.

Between D85-D92, older participants show a rise at D92 (1 week following mechanical stimulation post Risedronate treatment), whereas younger participants show a nearly plateau/subtle decrease.

Between D92-D99, the CTX response is dependent on age. Participants >9 years show a decrease at D99 (2 weeks following mechanical stimulation post Risedronate treatment) whereas those <9 years show an increase.

The rate of rise in CTX pre (D1-D8) and post Risedronate treatment (D85-D92) is nearly the same in the older participants. The younger participants also have a nearly same rate of rise pre and post Risedronate treatment but they appear to have a delayed rise (1 week later) to vibration post Risedronate treatment compared to older participants.

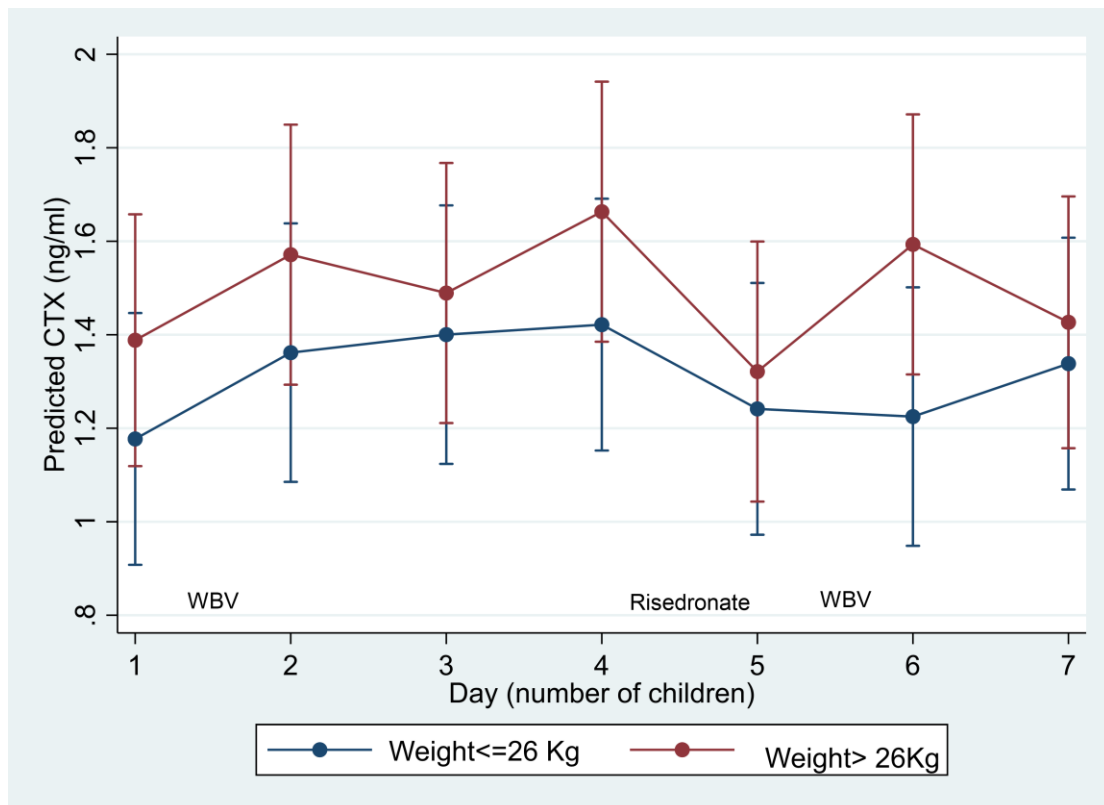


Figure 9c. Graph showing the interaction between CTX and weight

Overall, participants with a weight > 26 kg have a higher CTX than participants with a weight < 26 kg.

Between Day 1-8, the nearly parallel lines show that effect of mechanical stimulation is not dependent on weight; both groups show an increase of CTX at D8 from D1.

Between D92-D99, the CTX response is dependent on age. Participants with a weight >26 kg years show a decrease at D99 (2 weeks following mechanical stimulation post Risedronate treatment) whereas those with a weight < 26 kg continue to increase. Lighter participants appear to have a delayed increase (1 week later) in CTX compared to heavier participants.

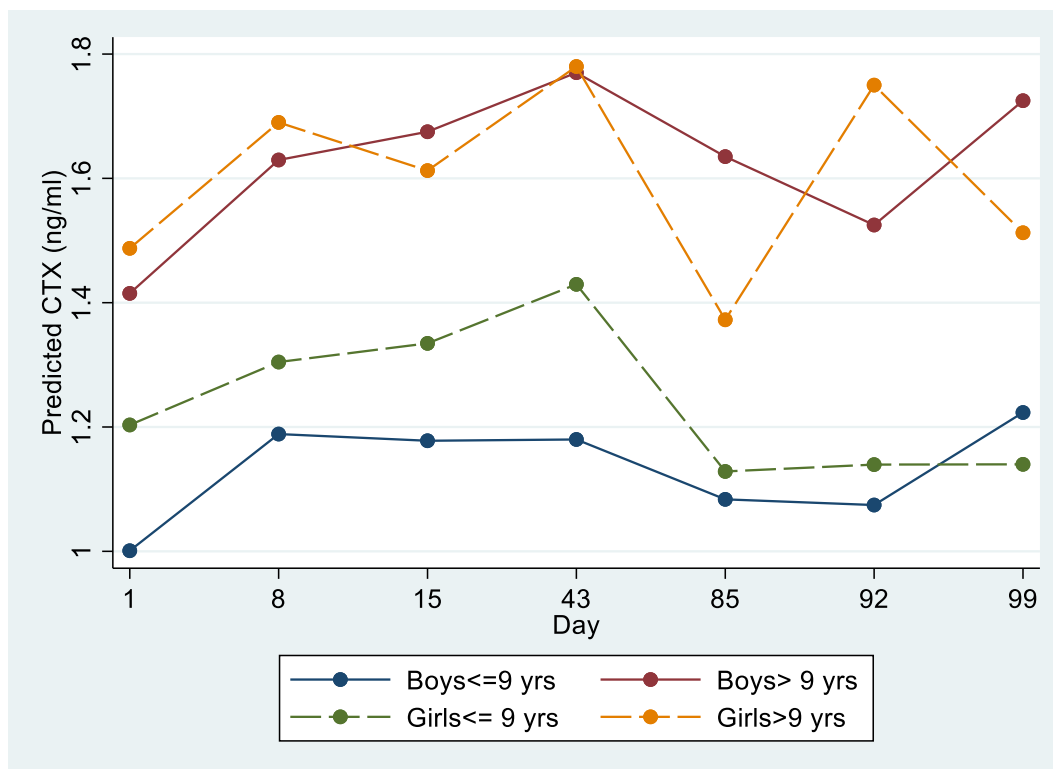


Figure 9d. Graph showing the interaction between CTX, gender and age

Overall, girls > 9 years have higher mean CTX values than all other groups.

Between Day 1-8, boys >9 years, boys < 9yrs and girls >9 years all have nearly parallel lines.

Between D15-D43 (when participants are advised to undertake their normal daily activities), girls <9 years and boys >9 years show a similar response with nearly parallel lines with an increase at D43.



Overall at D43, all groups (older girls and boys and younger girls and boys) show an increase of CTX at D43 compared to baseline.

Between D43-D85, girls (both < 9 years and 9 years) show nearly parallel lines with a decrease at D85 (following 6 weeks treatment with Risedronate). Boys both > 9 years and > 9years show nearly parallel lines.

Between D85-D92, both girls < 9 years and >9 years show an increase at D92 (1 week following mechanical stimulation post Risedronate treatment).

Between D92-D99, the CTX response in boys is nearly similar in both groups with parallel lines showing an increase at D99 (2 weeks following mechanical stimulation post Risedronate treatment). Parallel lines showing a decrease are seen in girls from both older and younger groups. This shows that the response to vibration post Risedronate treatment is dependent on gender but not age. There seems to be a delayed response (1 week later) in boys compared to girls.

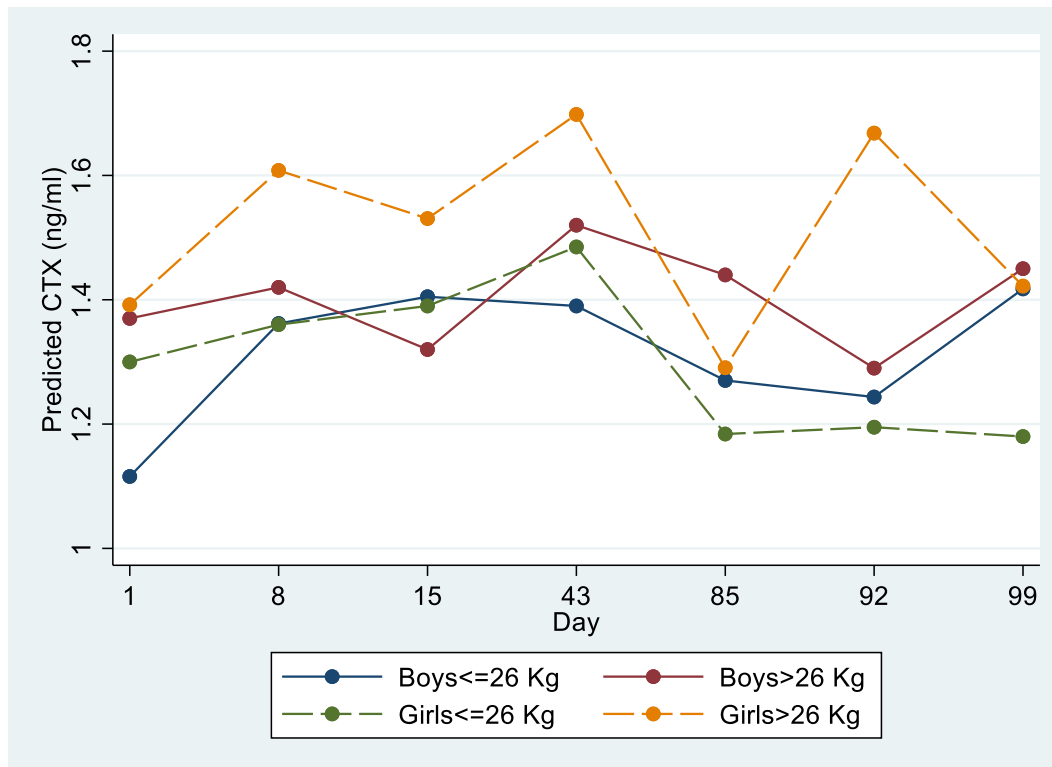


Figure 9e. Graph showing the interaction between CTX, gender and weight  
 Figure 9 a-e – interaction between CTX , age, gender and weight

Overall, girls with a weight > 26 kg have a higher mean CTX values than all other groups.

Between Day 1-8, girls >26 kg and boys <26kg have nearly parallel lines. Boys >26 kg and girls <26 kg have nearly parallel lines. Generally, all groups show an increased CTX at D8 following 1 week of vibration.

Between D8- D15, heavier participants show similar response with a decrease of CTX at D15 (2 weeks following vibration). Lighter participants show a similar response with a continued increase at D15.

Between D85-D92, both boys < 26 kg and >26 kg has nearly parallel lines with a decrease at D92 (1 week following mechanical stimulation post Risedronate treatment). Both girls show an increase at D92.

Between D92-D99, both groups of girls show a decrease at D99 (2 weeks following mechanical stimulation post Risedronate). Both groups of boys show a very similar response with an increase at D99.

### 3.3.3 Interactions between ALP, gender, age and weight

Figures 9a – 9e show the ALP interaction between age, gender, weight and day. Figure 9a shows the ALP interaction between ALP and gender, figure 9b shows the interaction between ALP and age, figure 9c shows the interaction between ALP and weight, figure 9d shows the interaction between ALP, gender and age and figure 9e shows the interaction between ALP, gender, age and weight.

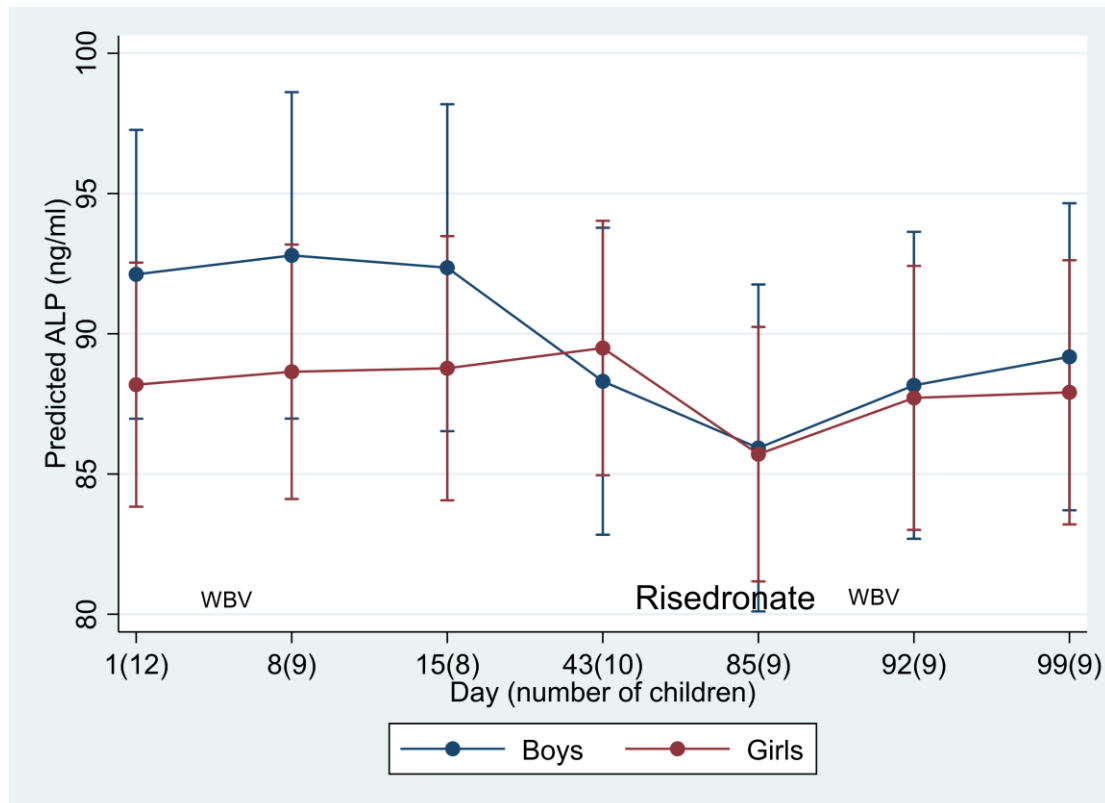


Figure 10a. Graph showing the interaction between ALP and gender.

Overall, boys have higher mean ALP values than girls. Between Day 1-8, the parallel lines show that the effect of mechanical stimulation is not dependent on gender; both boys and girls show an increase of ALP at D8 following 1 week of vibration.

Between D8- D15, boys show a decrease at D15 (2 weeks following mechanical stimulation) whereas girls show a very subtle increase.

Between D15-D43 (where participants are advised to maintain their activities to those that they will normally undertake), girls show a continued rise at D43 whereas boys show a sharp decrease.

Between D43-D85, both boys and girls show a decrease of ALP following 6 weeks of treatment with Risedronate.

Between D85-D92, both boys and girls show a similar response at D92 (1 week following vibration post Risedronate treatment).

Between D92-D99, both boys and girls show a continued increase at D99 (2 weeks following vibration post Risedronate treatment) although the increase is very subtle in girls compared to boys.

The rate of rise in ALP post Risedronate (D85-D92) is steeper than pre Risedronate (D1-D8). This pattern is very different to CTX and ALP.

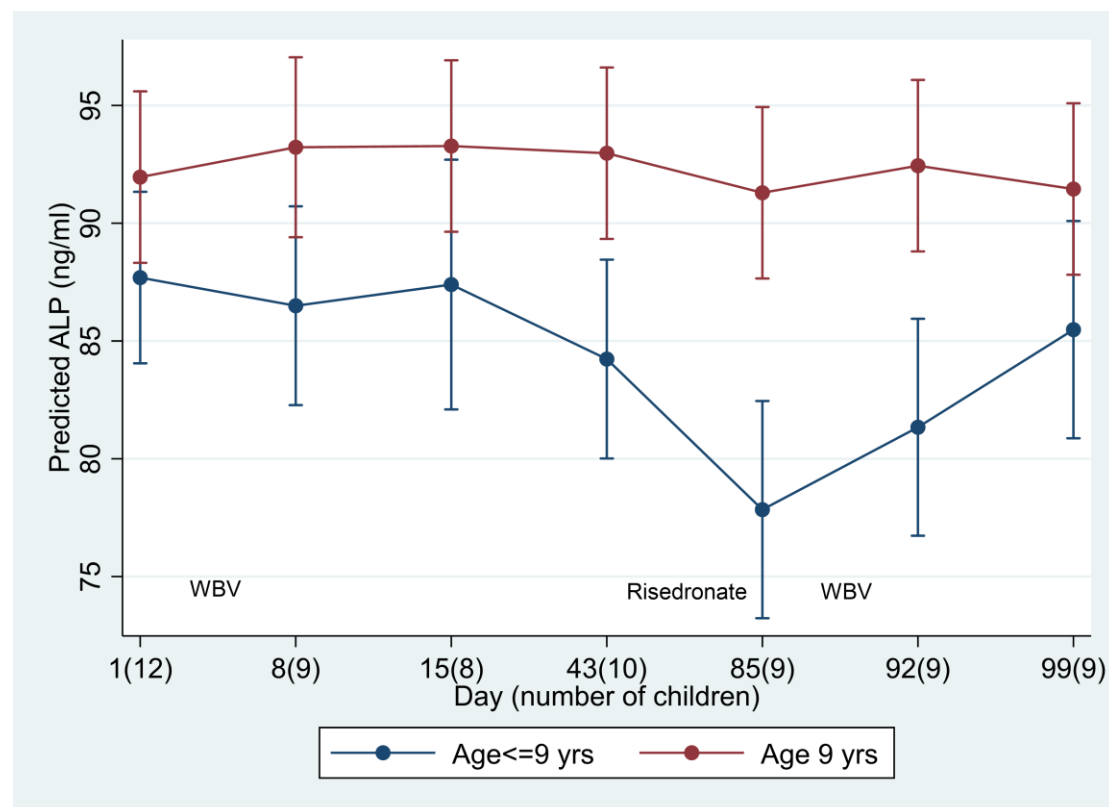


Figure 10b. Graph shows the interaction between ALP and age

Overall, participants > 9 years have higher mean ALP values than participants < 9 years.

Between D1-D8 (post vibration), participants >9 years show an increase of ALP but participants <9 years show a decrease.

Between D8- D15 (1 week following vibration), participants >9 years and participants <9 years show increasing trend. Participants > 9 years show continued increase but at a slower rate than day 1-8. Participants < 9 years show an increasing trend.

Between D15-D43 (when participants are advised to undertake their normal daily activities), participants <9 years and participants >9 years both show a decreasing trend.

Overall, at D43, participants > 9 years have a nearly same ALP as baseline whereas those < 9 years show a lower ALP compared to baseline.

Between D43-D85, both groups show a decreasing trend with nearly parallel lines at D85 (following 6 weeks treatment with Risedronate).

Between D85-D92, both groups show an increasing trend with nearly parallel lines at D92 (1 week following mechanical stimulation post Risedronate treatment).

Between D92-D99, the ALP responses are different in the 2 groups (2 weeks following mechanical stimulation post Risedronate treatment). Participants > 9 years show a decrease whereas those < 9 years show an increase.

The pattern generally shows that there isn't much difference at all to ALP at day 99 compared to baseline for both the groups. In fact, participants < 9 years have a lower ALP than baseline.

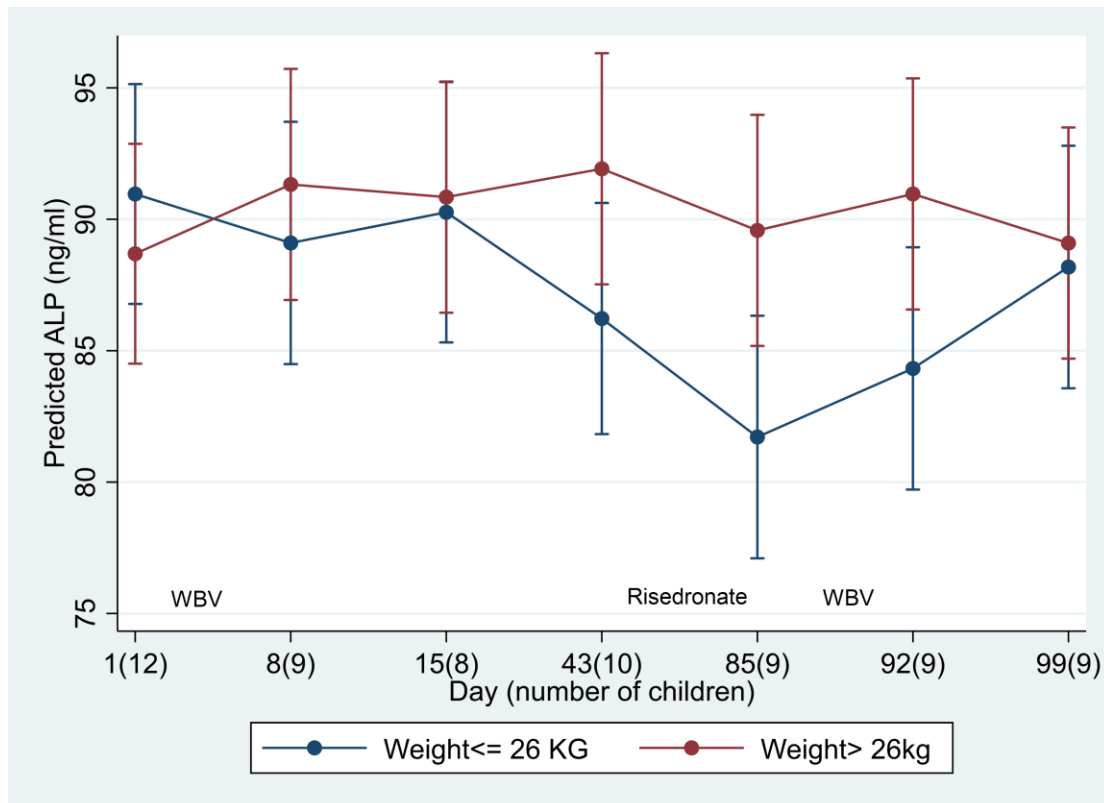


Figure 10c. Graph showing the relationship between ALP and weight

Between Day 1-8 (post vibration), participants > 26 kg show a decrease of ALP but participants <26 kg show an increase.

Between D8- D15 (1 week following vibration), the participants show different responses with participants >26 kg showing a decrease whereas participants < 26 years show an increase.

Between D15-D43 (when participants are advised to undertake their normal daily activities), the participants again show different responses. Participants > 26 kg show and increase whereas those < 26 kg show a decrease.

Overall at D43, participants > 26 kg have a higher ALP compared to baseline but those < 26 kg have lower ALP than baseline. Participants > 26 kg have a nearly same ALP as baseline whereas those < 26 kg show a lower ALP compared to baseline.

Between D85-D92, both groups show an increasing trend with nearly parallel lines at D92 (1 week following mechanical stimulation post Risedronate treatment).

Between D92-D99, the ALP responses are different in the 2 groups (2 weeks following mechanical stimulation post Risedronate treatment). Participants > 26 kg years show a decrease whereas those < 26 kg show an increase.

The pattern generally shows that there isn't much difference at all to ALP at day 99 compared to baseline for both the groups. In fact, participants < 26 kg have a lower ALP than baseline.

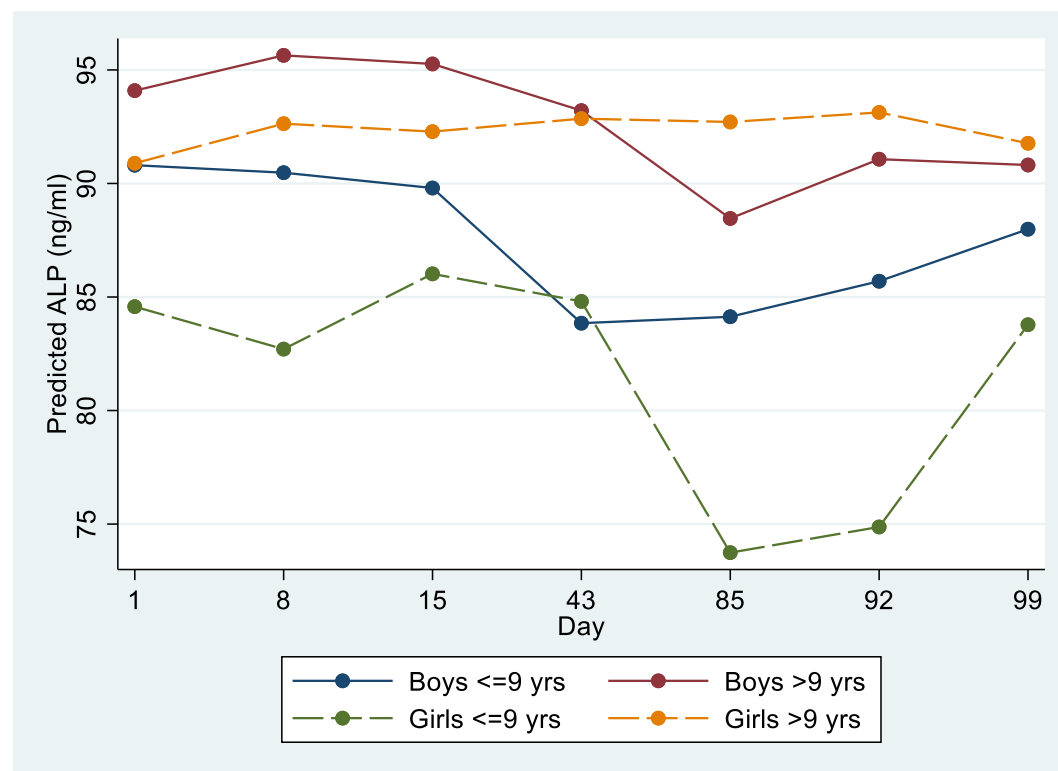


Figure 10d. Graph showing the interaction between BSALP gender and age

Overall, boys > 9 years have a higher ALP than all other groups.

Between Day 1-8, girls and boys >9 years have nearly parallel lines with an increasing trend; girls and boys < 9 years also have nearly parallel lines but with a decreasing trend.

Between D8- D15, boys in both groups and girls >9 years show a decreasing trend in ALP (1 week following vibration) with nearly parallel lines.

Overall, at D43, boys < 9 years show a decrease compared to baseline but all other groups did not show much difference compared to baseline.

Between D85-D92, all groups show increasing trend.

Between D92-D99, boys and girls > 9 years show a decreasing trend while boys and girls < 9 years show an increasing trend (2 weeks following mechanical stimulation post Risedronate treatment). There isn't much difference in all the groups with nearly the same value at D99 compared to baseline.



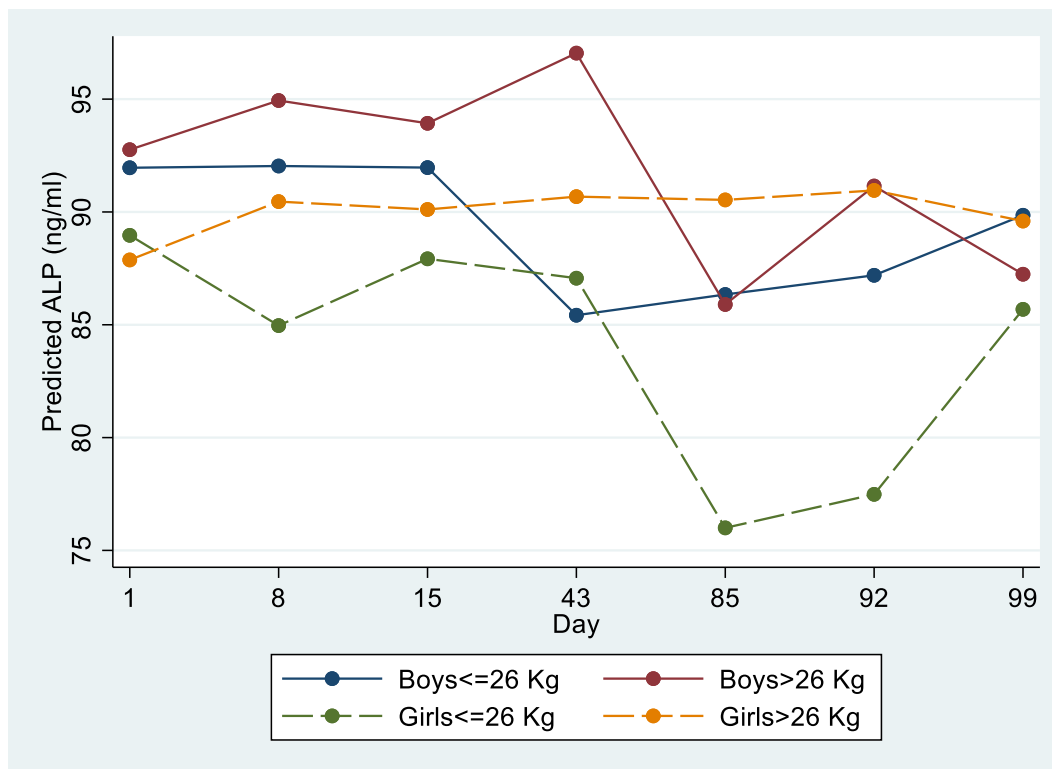


Figure 10e. Graph showing the interaction between gender and weight  
 Figure 10 a-e – interaction between ALP, age, gender and weight

Boys > 26 kg have higher mean baseline values than all other groups.

Between Day 1-8, heavier girls and boys show increasing trend with nearly parallel lines. Lighter boys and girls show decreasing trend with nearly parallel lines.

Between D8- D15, heavier participants show similar response with a decrease of ALP at D15 (2 weeks following vibration). Lighter participants show a similar response with increasing trend at D15.

Between D15-D43, (when participants are advised to limit their activities to only daily activities), again heavier participants show a similar response with an increasing trend and the lighter participants show decreasing trend.

Between D85-D92, all groups show increasing trend.

Between D92-D99, lighter girls and boys show increasing trend whereas heavier boys and girls show decreasing trend. Overall, all groups do not show much difference at all at D99 compared to baseline.

## 4. Discussion

We wished to determine whether a short period of bisphosphonate exposure influenced the bone response to a standardised form of mechanical stimulation. We showed that bisphosphonates attenuated the normal bone response to mechanical stimulation in children with osteogenesis imperfecta.

The osteocytes and osteoblasts in children with osteogenesis imperfecta have altered number and function (Zimmerman et al, 2019; Rauch et al, 2000).

Children with OI have increased lacune density, hypermineralised tissue and overall reduced amount of matrix per osteocyte. All of these together, affect the ability of the osteocyte as a mechanosensory cell. Zimmerman et al in 2018 showed that osteocytes in OI bone secrete increased levels of RANKL and the Wnt/ $\beta$ -catenin signalling pathway is dysregulated. There is also increased sclerostin and DKK1 expression by the osteocytes inhibiting the Wnt/ $\beta$ -catenin signalling pathway.

Bone modelling and remodelling is a highly dynamic activity which can be captured by bone turnover markers at several time points. This is probably the most accurate way of assessing the bone's response to mechanical stimulation. In our study, we used the bone formation markers P1NP and ALP and bone resorption marker CTX. We know that P1NP and CTX are early markers of bone formation and resorption while ALP is a late marker of bone formation reflecting the mineralisation phase of bone formation.

### 4.1 Biomarkers P1NP, CTX and ALP response across the whole study period

In our study, we found that P1NP and CTX show a similar pattern of response to mechanical stimulation whereas ALP shows a different pattern altogether.

There is a significant change in P1NP and CTX following 1 week of mechanical stimulation in children with osteogenesis imperfecta naïve to bisphosphonates treatment. We showed a higher rise in P1NP compared to CTX although not statistically significant.

Mechanical stimulation is sensed by osteocytes which stimulate resident osteoblasts and osteoclasts to increase their activity releasing P1NP and CTX respectively. The reason for a higher rise in P1NP compared to CTX is likely because of uncoupled modeling activity with net increased osteoid formation at the cellular level. To our knowledge, there are no other studies looking at bone turnover markers and mechanical stimulation or physical activity in children with osteoporosis. However, there are several studies looking at bone turnover markers in healthy prepubertal children and adults with osteoporosis. Our study findings are consistent with a recent study by Harrison et al 2015 that showed a 25.1% increase of P1NP and 10.9% of CTX at D8 in healthy pre-pubertal boys. In this study, 36 pre-pubertal boys stood on vibration platform for 10 minutes for either 1(group 1), 3(group 2) or 5 days (group 3). Combining the groups, there was a significant increase of P1NP and CTX at D8. A number of studies (Pasqualini et al 2019, Adami et al 2008, Mosti et al 2013) in osteoporotic individuals have shown an increase of bone formation without bone resorption in response to physical activity. Pasqualini et al, 2019 showed that, after 3 months of a standardized training programme on 33 individuals with osteoporosis, P1NP and osteogenic cells were significantly increased. However, there were no significant changes observed for CTX and sclerostin. The training session was twice a week with a duration of 45 minutes for each session that includes aerobic and resistance training. Adami et al, showed that there is a significant change in P1NP and osteocalcin following 1 month of exercise training in 24 individuals with osteoporosis. The exercise here was a 90-minute session 3 or 4 days per week which includes walking, running, step ups, stair climbing; these are alternated with spine flexion and extension exercises, and mobility exercise of the upper and lower trunk. Mosti et al, 2013 showed that the ratio of P1NP/CTX tended to increase (21.5%) following a 12-week period of squat exercise 3 times per week, in a randomized controlled study of individuals with osteoporosis. These studies show that a mature bone with osteoporosis does respond with increased bone formation at the cellular level. It is reasonable to speculate that a growing skeleton with osteoporosis is likely to respond better than a mature bone, based on the known effects of exercise in an adult skeleton. Tan et al, 2014 published a systematic review of 37 studies and found that 26 out of the 37 studies (70%) showed a positive relationship between bone strength and mechanical loading in growing skeleton. This included 6 randomised

controlled trials, 7 recreational physical activity studies and 13 organised sports studies.

Our study also showed that there is a biphasic increase in P1NP and CTX following 1 week of mechanical stimulation and the effect appears to have lasted for 5 weeks. There is an initial increase at the end of 1 week of WBV. This is followed by a decrease at the end of the 2nd week and a net increase at the end of the 5<sup>th</sup> week (Day 43). This is an interesting finding that possibly means that mechanical stimulation not only increases the activity of resident osteoblasts and osteoclasts but also stimulates additional remodeling activities, recruiting more osteoblasts and osteoclasts.

Another possible explanation is that the WBV also stimulates the stem cells to increase the osteocyte/osteoblast lineage cells and monocyte/macrophage lineage that may then be released to the blood stream. These cells may then differentiate to osteoblasts and osteoclasts which will then release P1NP and CTX and is probably the reason for the later second increase at day 43. Future studies could look at the biomarkers specific to osteoblast and osteoclast progenitors.

Previous in vitro and in vivo studies have shown that mechanical stimulation induces the bone marrow stem cells to commit to osteoblast lineage cells. (Sittichokechaiwut et al, 2010; Sarraf et al, 2011; Chen et al, 2016; Gao et al, 2017)

Luu et al, 2019 showed that the mice treated with whole body vibration for 12 weeks showed mesenchymal stem cell proliferation and upregulation of RUNX2. Luu and colleagues studied the effects of whole-body vibration on the osteogenic and adipogenic differentiation of mesenchymal stem cells in obesity (diet – induced) mouse models.

There is also some evidence that mechanical stimulation also inhibits osteoclast formation. Kulkarni et al 2013 applied whole body vibration one hour each day for 3 days on osteoclast like cells and found that the mRNA and the biomarkers for osteoclast fusion were reduced. The study showed that there is inhibition of osteoclast fusion following whole body vibration.

Although we know that mechanical stimulation or physical activity causes increased bone strength either directly or indirectly through skeletal muscles, there is lack of

evidence regarding the exact duration, intensity and frequency of training in individuals with osteoporosis (Kistler-Fischbacher et al 2021). In our study, the effect of mechanical stimulation appears to have lasted for 5 weeks.

Following 6 weeks of Risedronate treatment, both P1NP and CTX decreased to baseline level at D85.

There was no significant increase at D92; the increase that was seen in CTX and P1NP from D85-D92 was significantly reduced compared to that seen from D1-D8.

The P1NP and CTX response to vibration appears to be different when the bone is naïve to treatment compared to post treatment. We show that the slower rise of P1NP and CTX at day 85 is likely due to the effect of Risedronate treatment. We know that the primary effect of bisphosphonate is inhibition of resorption which then lead to inhibition of bone formation since resorption and formation are coupled. New evidence suggests that there may be additional mechanism for reduced bone formation. Bisphosphonates may also delay or slow down the recruitment of the osteoprogenitor cells required to initiate bone formation at remodelling sites. (P.R. Jensen et al., 2021). Xenia et al, 2022 showed that Alendronate suppressed bone formation in cortical bone remodelling and the effects on bone were significant after 3 years of treatment (Borggaard et al, 2022).

Bisphosphonate has also been shown to blunt PTH induced bone formation (Black et al, 2003; Finkelstein et al, 2003).

We know from previous studies that exercise induced bone adaptation is through PTH or PTHrP mediated signaling. PTHrP is produced locally by osteoprogenitor cells and plays a key role in bone remodelling. It promotes the differentiation of mature osteoblasts responsible for bone formation and also stimulates the differentiation of osteoclasts for bone resorption indirectly through osteoblasts (Brescia et al 2022). Fan et al 2017 showed that the deletion of the *PTH1R* in bone marrow mesenchymal progenitor cells results in low bone mass, high bone marrow adiposity and increased bone resorption. When PTH is used therapeutically as an anabolic agent, bone formation markers increase early and bone resorption markers rise slowly (Black et al. 2003, 2008; Frolik et al. 2003; Neer et al. 2001; Suva et al 2020). It may be that bisphosphonates attenuate bone formation directly by delaying the recruitment of

osteoprogenitor cells and indirectly through PTH. This is a speculation that needs to be confirmed by future studies.

Although we think that the smaller response to mechanical stimulation is likely due to the effect of Risedronate, it could also be due to ‘bone adaptation’. This is when the bone has adapted to such a mechanical stimulus and therefore the response following a second exposure to the same stimulus produced a less marked response. It is a negative feedback loop that adapts or adjusts the mechanical strain level in the bone tissue. Mechanical loading stimulates bone formation modeling and remodeling (repair and renewal) which adjusts the bone structure and reduce the mechanical strain. When a similar or a reduced mechanical strain is repeated, the bone is considered completely adapted and bone formation is reduced (Guerriere et al 2022). Bone response is also different if the stimulus is continuous or intermittent with rest in between. This has been shown in previous studies (Yang et al 2017, Saxon et al 2005). As discussed before, the mechanosensitivity of bone decreases after a few loading cycles – the osteogenic window is short. Saxon et al 2005 showed that bone formation is improved when 15 weeks of loading is intermittent with 5 weeks rest in between, compared to continuous loading. However, the bone formation rate at 15 weeks is smaller than at 5 weeks. In order to confirm whether the effect is due to Risedronate or ‘bone adaptation’, we need look at 2 groups, one with Risedronate and one without Risedronate in the future.

The likelihood that there would be a significant reduction in bone turnover purely due to the time since the first WBV intervention is small given the short duration and magnitude of the original stimulus, compared to normal day-to-day activity and there isn’t a clear biological explanation for why this might happen. We do know that the skeleton is designed to respond to mechanical loading on a day-to-day basis.

In this study, ALP has a different pattern on the response to vibration compared to PINP and CTX. ALP increased 0.33% at D8 from D1. There is a small, continued increase at D15 from D8. The ALP rise seen at D8 and D15 was not sustained at D43 different to PINP/CTX pattern. ALP decreased significantly following 6 weeks treatment with Risedronate, which is expected. ALP is seen to rise in response to vibration following Risedronate treatment and it continues to rise 1 week later without

any interventions. ALP increased more (2.33%) from D85-D92 compared to D1-D8 (0.33%)[P=NS].

Although ALP appears to have increased more at D92 following Risedronate treatment compared to pre- Risedronate treatment, the rise at D92 likely represents the mineralization activity of the initial osteoid laid down pre-Risedronate treatment. Individuals with OI have aberrant mineralization activity and the mineral apposition rate is reduced in individuals with OI (Rauch et al, 2000). This may be the reason why the ALP rise isn't statistically significant. It might also be related to the timing of the sampling as the ALP is continuing to rise at D99, the last day of the study. We speculate that ALP may have continued to rise should we continue to monitor for a longer duration. This is because ALP rises during the mineralization phase which can be spread over many weeks.

The acute exercise effects on bone specific ALP in growing children is unclear. Previous studies in men reported no change in bone ALP following running, cycling or resistance exercise (Guillemant et al. 2004, Scott et al. 2010, 2011, 2013). The bone ALP levels in all the trials above were taken within a duration range from immediately post exercise to about 1 week post exercise. Sherk et al. 2013, studied the bone turnover marker responses to resistance exercise alone and resistance exercise combined with a 5 minute bout of WBV in 10 young women. Bone turnover markers were obtained immediately pre and post exercise and 30 minutes post exercise. No change was observed in bone ALP.

Long-term participation (>1 month) in high-impact exercise results in elevated levels of ALP among young adults (Erickson and Vukovich 2010; Shibata et al. 2003; Woitge et al. 1998). ALP levels reflect mineralisation activity and is likely to show a change after at least 1 month, in healthy individuals. Alp et al 2015 states that ALP change can be appreciated after about 1 month of exercise.

However, a study by Kish et al 2015 showed that ALP increased significantly 24 hours post exercise in boys and young men. This study examined the effect of high mechanical loading (144 jumps). It may be that the bone of the boys and young men are in active remodelling phase with millions of osteoid sites and therefore the ALP levels may reflect mineralisation of the already available osteoid. This study also

showed that the response was greater in boys than men (Kish et al, 2015) which suggests that the growing skeleton is more responsive than the mature adult skeleton.

## 4.2 Individual study participants line graphs

Looking at the individual study participants' line graphs, ID 11 seems to be an outlier with a higher baseline. There are a number of possible explanations. Firstly, it might suggest non-adherence to treatment, alternatively this could be due to the fact that the bone requires longer period of treatment before any change in the biomarkers. Or does this mean that the bone cells have reached its maximum potential and will not respond to any further interventions?

## 4.3 Interactions between each of the biomarkers with gender, age and weight

### 4.3.1 Interactions between biomarkers P1NP and CTX with gender

We further analysed the data by looking into the pattern of P1NP and CTX change between boys and girls. Both boys and girls show increased P1NP and CTX at Day 43 (5 weeks post WBV). However, boys show a clear biphasic P1NP response and a higher increase from baseline compared to girls. 2 weeks following WBV post Risedronate, girls show minimal/ no difference but boys show an attenuated response. Overall, it appears that boys are still able to show some response in spite of the effect of Risedronate. This is in agreement with a previous study by P Ferrer et al, 2022 where 295 Spanish children were followed up from 1 year of age till 7 years to see the effect of physical activity of bone health. In this study, boys were shown to have higher bone mineral content and density compared to girls in response to physical activity. Haapasalo et al in 1996 showed that young male players' skeleton was more responsive compared to young female players following similar mechanical loading. The humeral length difference between the playing and the non-playing arm for females was +1.1% compared to +1.4% in males.

Males generally have larger bone size and therefore stronger skeletal frame compared to females. Males have increased bone mineral content within the larger periosteal



envelope of the long bones. In addition, males also have taller and wider vertebral bodies compared to females. It is tempting to speculate that the gender differences in the response to physical activity is likely secondary to the sex hormones. It was previously thought that androgen causes increased periosteal bone expansion in males whereas estrogen has an inhibitory action in females. New evidence suggests that both androgens and estrogens are required for periosteal bone expansion. In both males and females, androgens induce periosteal bone formation, but the low estrogen levels influence the periosteal mechanical sensitivity. Low levels of estrogen promotes periosteal expansion whereas high levels inhibit periosteal expansion directly or indirectly through IGF-1 regulation (Vanderschueren et al, 2006). Males have high levels of androgen and low levels of estrogen and therefore better response to mechanical stimuli (mechanoresponse) compared to females. Males and females also respond differently to growth hormone replacement therapy. A study by White et al, 2003 showed that the 24 -h mean CTX increased significantly following 1 month of treatment in men compared to 3 months in women. P1NP increased significantly at 1 month of treatment in men compared to 12 months in women.

#### **4.3.2 Interactions between biomarkers P1NP and CTX with age**

Looking at the graphs showing the P1NP and CTX change for age, participants older than 9 years respond better compared to the younger children pre-Risedronate. This is in agreement with a previous study by Rempe et al 2022, that showed that daily physical activity in the late prepubertal and early pubertal children is associated with higher bone formation and lower bone resorption than physical activity 1-2 times/week. No differences were seen in bone formation and resorption in late pubertal and post pubertal children.

This could be explained by the fact that children older than 9 years are more likely to be in the pre-pubertal stage. Puberty is associated with growth spurt and increased levels of growth hormone, IGF-1 and sex hormones. At pre-puberty, there is a slow rise in these hormones followed by a steeper rise in puberty. GH and IGF-1 are pivotal in bone homeostasis. They are not only responsible for longitudinal bone growth but also play an important role in the regulation of osteoblastogenesis, bone modelling and remodelling directly and indirectly through signalling molecules.

Bravenboer et al, 1996 showed that bone biopsies of male adult patients with growth hormone deficiency have decreased bone formation with decreased osteoid and mineralising surfaces. GH affects the fate of mesenchymal stem cells favouring osteoblastogenesis and opposing adipogenesis (Gevers et al 2002). It also stimulates the differentiated function of mature osteoblasts through IGF-1.

In addition, GH inhibits osteoclastogenesis by stimulating osteoprotegerin which binds to RANK-L (Mrak et al 2007). GH also stimulates and modulates osteoblastogenesis through the expression of bone morphogenetic proteins (Canalis et al 2003) and runt-related transcription factor-2, the master regulator of osteoblastogenesis (Ziros et al, 2004, Giustina A et al 2008)

Those more than 9 years appeared to have lesser response following WBV post risedronate compared to the younger children. This may be related to the blunted effect of PTH in Risedronate treated children more than 9 years. We already know that the anabolic actions of parathyroid hormone are mediated through IGF-1 (Bikle et al 2002).

Patients with growth hormone deficiency have altered circadian PTH rhythm which may affect bone remodelling. It may be that the blunted effect of PTH is more pronounced at the prepubertal stage when the IGF levels are higher compared to the younger children. (White et al 2007).

#### **4.3.3 Interactions between biomarkers P1NP and CTX with weight**

Looking at graphs showing P1NP and CTX change for weight, the response to WBV pre Risedronate is the same for both groups regardless of weight. There is a slight difference in the pattern of P1NP and CTX following Risedronate treatment; P1NP rise appears to be similar at the end of 2 weeks regardless of weight but CTX levels drop after 1 week for the heavier children. Overall, weight does not appear to be a major influencing factor in the response to mechanical stimuli in this study. It is important to note that none of the children in our study cohort were obese.

There isn't any previous study looking at the relationship between weight and mechanical response using bone turnover markers in humans. However, there are

studies looking at the relationship between obesity and mechanical response. Adipose tissue secretes cytokines known as adipokines which affect bone turnover systemically or locally. One previous study by Lecka-Czernik et al, 2015 using mouse model, showed that obesity is probably biphasic, with increased bone formation initially due to increased mechanical load followed by decreased bone formation due to pro-inflammatory molecules. The pro-inflammatory molecules increase osteoclasts activity and decreased osteoblasts activity. (Fintini et al, 2020). Dmitri et al 2010 studied the effects of bone turnover in obese children by looking at levels of leptin, adiponectin, osteoprotegerin, RANKL, P1NP, CTX and DKK1. The study showed that obese children have lower leptin levels compared to non-obese children, and the leptin levels were inversely related to osteoprotegerin levels. The ratio of CTX/P1NP was higher in obese children suggestive of higher bone resorption relative to formation.

A recent review paper highlights that the relationship between obesity and skeletal response is complex and is dependent on multiple factors like gender, age, bone site, mechanical load by the weight, duration of obesity and the adipose tissue site. (Hou et al, 2020)

#### 4.4 Limitations of study

This work has a number of limitations. The study was undertaken on a small group of children with mild OI and a wide age range with different pubertal status. Therefore, the results cannot be generalised to all forms of OI, especially those with non-collagen defects. The ideal would be to do a larger study, with more numbers at each age/stage of puberty. However, it would be difficult to recruit more severely affected children who had not received bisphosphonate treatment already. One child suffered a fracture during the period of bisphosphonate administration; that event may have impacted on subsequent bone marker measurements, although the individual data for that patient post-fracture are similar to those of the other children. We did not measure factors likely to reflect osteocyte activity or osteoblast–osteoclast cross-talk; in our previous studies, we found no acute change in circulating sclerostin or osteoprotegerin in response to WBV (Harrison et al, 2015) We did not check serum vitamin D and PTH during the study; however, all children under our care with osteogenesis imperfecta are advised to take regular daily vitamin D supplements, and

we also give calcium supplements to those who report low dairy product intake. The administration of calcium and vitamin D in combination with the risedronate (following the manufacturer's recommendation) may have also impacted on short-term bone turnover. In our previous studies of children receiving risedronate in a placebo-controlled trial (Bishop et al, 2013) however, we saw no change in urinary crosslinked N-telopeptide of type 1 collagen at 3 months for those receiving calcium and vitamin D as placebo, in contrast to a significant decline for those also receiving risedronate. We cannot exclude the possibility that exposure to two successive episodes of WBV at this interval would result in altered bone turnover marker responses in the absence of risedronate, calcium, and vitamin D. The children in this study included girls; we saw similar percentage increases in P1NP and CTX in girls whose mothers had received supplemental vitamin D during pregnancy in the placebo-controlled MAVIDOS study (Gopal-Kothandapani et al, 2020).

#### 4.5 Future Work

The study raises the question about how we should be balancing the effect of Risedronate and the normal response to mechanical stimulation. We should aim to reduce bone resorption but optimise bone formation. Future studies should look into ways or strategies using bouts of mechanical stimulation in between Risedronate treatment.

Our study has also showed that WBV can provide a standardized way to measure and assess the response of bone to loading in children with mild OI. WBV was also used in the MAVIDOS study (Gopal-Kothandapani et al 2020) looking at the postnatal response in children at 4-5 years following antenatal Vitamin D supplementation in the pregnancy. It can be used in individuals with diabetes, neuromuscular conditions and those on treatment with drugs (eg. Steroid) that increase the risk of fractures. WBV could be used as a tool to assess skeletal health, rather than as a therapeutic intervention, specifically in those individuals where they are unable to perform high intensity exercises including low bone density disorders and/ or increased risk of fracture.

## 5. Conclusion

We have shown that the response of bone biomarkers in children with OI to a defined period of mechanical stimulation in the form of whole-body vibration is similar initially, to that of normal children and significantly reduced after bisphosphonate treatment. Our interpretation of the current data set and the patterns of the response is that whole body vibration both increases the activity of osteoblasts already forming osteoid and stimulates additional bone remodeling cycles. Further work in children both with and without OI is clearly required to establish the robustness of these interpretations and determine the magnitude and duration of any such responses.

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## 7 Appendices

### 7.1 Study paper

#### CLINICAL TRIAL



# Bisphosphonate Treatment Alters the Skeletal Response to Mechanical Stimulation in Children With Osteogenesis Imperfecta: A Pilot Study

Siva Sithambaran,<sup>1</sup> Rachel Harrison,<sup>2</sup> Sujatha Gopal-Kothandapandi,<sup>1</sup> Alan Rigby,<sup>3,4</sup> and Nick Bishop<sup>1,2</sup>

<sup>1</sup>Department of Oncology and Metabolism, University of Sheffield, Sheffield, UK

<sup>2</sup>Sheffield Children's NHS FT, Sheffield, UK

<sup>3</sup>University of Hull, Hull, UK

<sup>4</sup>Hull York Medical School, Hull, UK

#### ABSTRACT

Children with osteogenesis imperfecta (OI) are commonly treated with bisphosphonates. We investigated the skeletal response to mechanical stimulation in children with OI before and after bisphosphonate treatment. Twelve children with OI, naive to bisphosphonate treatment, stood on a high-frequency (30 Hz), low-amplitude (50 to 200  $\mu$ ) vibrating platform (Marodyne LivMD) for 10 minutes daily (2.5 minutes  $\times$  4 with interspersed 1-minute rest periods) for 7 days (whole body vibration [WBV] 1; day (D) 1–7), followed successively by 5 weeks' monitoring without intervention, 6 weeks' risedronate treatment, 1 week of WBV (WBV2; D85–91), and 1 week without intervention (D92–98). Procollagen type I N-terminal propeptide (P1NP), bone-specific alkaline phosphatase (BSALP), and carboxy-terminal telopeptide of type I collagen cross-link (CTX) were measured at baseline and intervals bracketing periods of vibration and risedronate treatment. Both P1NP and CTX rose to D8 (18.4%, 13.8%,  $p < 0.05$ , respectively), plateaued, then rose again at D43 (19.8%, 19.2%, respectively,  $p < 0.05$  versus baseline). At D85 (after risedronate) both P1NP and CTX had fallen to pre-WBV1 levels. A significantly smaller increase in P1NP was found after WBV2 (D85–91) at D92 (3.5%, 9.2%, respectively) and D99 versus after WBV1 (both  $p < 0.05$ ). BSALP changed little after WBV1, fell during risedronate, and rose toward baseline after WBV2. We thus showed that WBV increased bone formation and resorption; that increase was attenuated after risedronate. The early increase in P1NP and CTX (D8) after WBV1 suggests increased osteoid formation within existing remodeling units but not increased mineralization. Later increases in P1NP/CTX (D42) suggest increased remodeling cycle initiation after WBV. Risedronate suppressed both biomarkers. The lower increase in P1NP/CTX after WBV2 suggests limited capacity to increase osteoid formation from existing "early stage" osteoblasts and a possible "hangover" effect of risedronate on remodeling activation. These results provide insights into both the response to WBV, ie, mechanical stimulation, and the effect of antiresorptive therapy in children with OI. © 2021 The Authors. *JBMR Plus* published by Wiley Periodicals LLC. on behalf of American Society for Bone and Mineral Research.

**KEY WORDS:** ANALYSIS/QUANTITATION OF BONE; ANTIRESORPTIVES; BIOCHEMICAL MARKERS OF BONE TURNOVER; CLINICAL TRIAL; OSTEOGENESIS IMPERFECTA

#### 1. Introduction

Osteogenesis Imperfecta (OI) is a spectrum of genetic disorders with decreased bone mass and increased bone fragility. It is characterized by fractures associated with minimal or absent trauma, dentinogenesis imperfecta, blue sclerae, and progressive hearing loss. OI has a prevalence of approximately 11/100,000,<sup>(1)</sup> and the fracture risk is greatest during childhood.<sup>(2)</sup>

At a tissue level, the characteristic feature of bone in OI is its brittle nature.<sup>(3)</sup> This is largely because of an excess in the

amount of mineral present relative to the fiber content of the bone material and also the altered 3-dimensional structure of the fibrous component of the matrix. At a microscopic level, there is a reduced amount of bone tissue, with both the cortical and trabecular bone being affected. The cortices are narrower, with large intracortical pores, and trabecular connectivity is disrupted. The overall width of tubular long bones is reduced. This combination of material brittleness, disrupted microarchitecture and reduced bone size all combine to reduce fracture resistance and increase bone fragility. In children with OI, bone turnover is increased; the replacement of resorbed bone is defective

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Received in original form October 11, 2021; accepted November 28, 2021.

Address correspondence to: Nick Bishop, MD, Sheffield Children's Hospital, Western Bank, Sheffield, S10 2TH, UK. E-mail: nj.bishop@shef.ac.uk

*JBMR*<sup>®</sup> Plus (WOA), Vol. 6, No. 3, March 2022, e10592.

DOI: 10.1002/jbm4.10592

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compared with normal, and the density of osteocytes (terminally differentiated osteoblasts that are embedded within the bone substance and that form part of the pressure-sensing network in bone) is increased.<sup>(4)</sup>

Bisphosphonates are potent antiresorptive agents that inhibit osteoclast function; the nitrogen-containing bisphosphonates act on specific enzymes in the mevalonate pathway, reducing prenylation activity and effectively poisoning the osteoclasts, resulting in reduced function and apoptosis.<sup>(5)</sup>

Bisphosphonates have been widely used in the treatment of children with OI over the last 25 to 30 years. At a tissue level, the changes found in children with OI treated with bisphosphonates include an increase in trabecular number, thickness, and connectivity, reduced intracortical porosity, and increased cortical thickness.<sup>(6)</sup> At a whole bone level, restoration of vertebral shape and size as well as increased bone width in long bones and reduced long bone bowing deformity have been reported.<sup>(7)</sup> However, two recent reviews have highlighted that despite these observed changes, the effects of such therapy on fracture frequency are equivocal, though multiple studies (randomized controlled trials and observational cohorts) report this independently, and no studies report an increased fracture rate with treatment.<sup>(8,9)</sup>

Whole body vibration (WBV) therapy is targeted at musculo-skeletal strengthening and has been trialed in a variety of conditions. WBV has been shown to have therapeutic advantage in various osteopenic preclinical models and populations such as postmenopausal women (improved mobility, muscle strength, postural strength, and bone density) and children with osteogenesis imperfecta (improved mobility).<sup>(10–15)</sup>

An alternative approach to the use of WBV is to regard it as a means to provide a standardized dose of mechanical stimulation to the skeleton. Our studies of WBV in healthy boys showed that a short period of exposure—5 days of 10 minutes WBV per day—could increase a bone formation marker in excess of one reflecting bone resorption.<sup>(16)</sup> We have also used the same short period of WBV stimulation to demonstrate differences in the skeletal response of children whose mothers were enrolled in the MAVI-DOS study and received vitamin D supplementation as opposed to placebo during pregnancy.<sup>(17)</sup> The bone turnover markers assessed reflect type 1 collagen formation (procollagen type 1 N-terminal propeptide), the mineralization of bone matrix (bone-specific alkaline phosphatase) and type 1 collagen destruction (C-terminal telopeptide of type 1 collagen).

It is unclear to what extent the skeleton in children with OI is responsive to mechanical stimulation; it may be that it is normally responsive, but the osteoblastic activity that occurs in response to mechanical stimulation is defective. It could be that the higher intrinsic levels of bone turnover in OI reduce the capacity of bone to further respond by increasing bone formation in excess of bone resorption. It might also be the case that the use of bisphosphonates alters the ability of bone tissue and cells to respond to mechanical stimulation. As bisphosphonates inhibit bone resorption, bone formation is also inhibited as a result of reduced remodeling; reduced osteoblast surface has been shown in the bone biopsies of OI children receiving bisphosphonates.<sup>(6)</sup>

We undertook this study to test the hypothesis that bisphosphonate treatment would reduce the response of the skeleton, as measured by changes in biomarkers reflecting bone formation and resorption, to whole body vibration in children with OI.

## 2. Materials and Methods

This single-center interventional trial was approved by the South Sheffield Research Ethics Committee (ref 17/YH/0018) and registered with Clinicaltrials.gov, identifier NCT03208582. Participants attending the OI service at Sheffield Children's Hospital were approached regarding the study. The eligibility criteria were: ages 4 to 16 years; able to speak fluent English; diagnosed with osteogenesis imperfecta; able to stand; and naïve to treatment with bisphosphonates. Children with OI in our clinic are recommended to take a vitamin D supplement; those with a low calcium intake (based on reported low dairy product intake) are asked to take a calcium supplement. All the children in this study had previously received vitamin D; none had had additional calcium. Children were excluded if one or more of the following criteria were met: presence of other chronic illnesses including renal failure likely to affect bone metabolism; balance problems; recent fracture (in the last 6 months); recent (last 12 months) or current treatment likely to affect bone (excluding inhaled or intermittent oral therapy with steroids for asthma); involvement in another interventional research project; hypocalcemia; pregnancy or lactation; or known hypersensitivity to risedronate or any of the excipients.

Participants stood on a high-frequency (30 Hz), low-amplitude (50–200  $\mu$ ) vibrating platform (Marodyne LivMD, BTT Health, Inning am Ammersee, Germany) for 10 minutes daily (2.5 minutes  $\times$  4 with interspersed 1-minute rest periods) (WBV1) for 7 days (D1–7), followed successively by 5 weeks without further intervention, 6 weeks' oral risedronate treatment with accompanying calcium and vitamin D, 1 week WBV (WBV2, D85–91), and 1 further week without intervention (D92–98).

Risedronate, calcium, and vitamin D were given during the 6-week period from D43 to D84.

The dose of risedronate administered (parentally supervised) was 1 mg/kg/week rounded to the nearest 5 mg using film-coated tablets of 5 mg and 35 mg. Risedronate was administered first thing in the morning as per manufacturer's instructions in the fasting state with a large glass of water, with nothing further to eat or drink other than tap water for the next 30 minutes. Vitamin D and calcium were given daily in the evening as Calcichew 500 mg/200 IU tablets (Takeda Ltd, High Wycombe, UK), 1 tablet for participants weighing less than 30 kg and 2 tablets for participants weighing 30 kg or more. Risedronate and Calcichew tablets were prescribed through the Sheffield Children's Hospital pharmacy; any unused medication was returned and checked in order to ascertain compliance.

Blood samples were taken at baseline on D1, and on D8, D15, D43, D85, D92, and D99, bracketing the periods of vibration and drug treatment. All samples were taken in the fasting state before 9:00 a.m. Samples were spun at 2500 rpm for 10 minutes at 4°C and centrifuged samples stored at –80°C. Samples were analyzed in a single batch for bone-specific alkaline phosphatase (BSALP), procollagen type 1 N-terminal propeptide (P1NP), and C-terminal telopeptide of type 1 collagen (CTX) in the Bone Biochemistry Laboratory, University of Sheffield, using an Elecsys Cobas E411 automated immunoassay system interassay coefficients of variation (CVs) 2.8% to 8.4% for CTX and <1.7% for P1NP. BSALP was measured on an iSYS automated immunoassay system (Immunodiagnosics Ltd., Boldon, UK) with interassay CV 4.2%.

Pubertal status of every patient was assessed using a sex-appropriate pictorial scale. Pregnancy testing was carried out in the Sheffield Children's Hospital Clinical Research Facility before risedronate was given to any female subject aged 10 years or older.

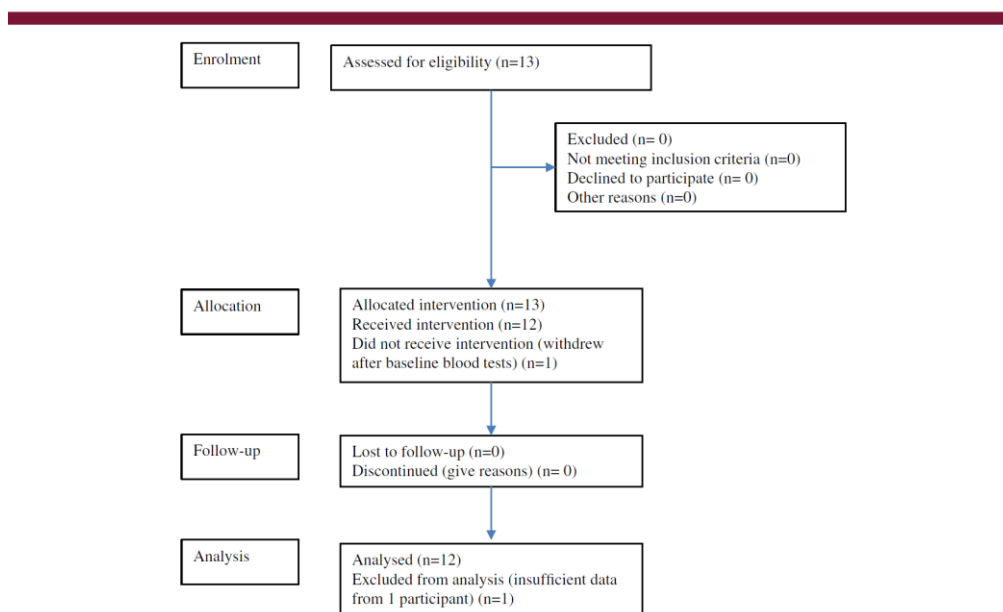


Fig. 1. CONSORT diagram.

Our choice of sample size is pragmatic and not informed by power. A literature review of sample size for pilot and feasibility studies found a few articles with recommendations for two-arm trials but nothing of good quality for single-arm interventions.<sup>(18)</sup> The smallest number suggested for a two-arm trial was 12 patients per group,<sup>(19)</sup> whereas others have suggested larger numbers.<sup>(20)</sup>

Data were analyzed using Stata v16 (StataCorp LLC, College Station, TX, USA).

Data are presented as tables of raw data and figures showing adjusted (for age, sex, and weight) means from ANOVA models. Adjustment makes allowance for possible “confounders” that

would otherwise obscure the relationships between the bone biomarkers with intervention and time; adjustment for these factors was undertaken based on consistent patterns of higher values in older, heavier girls. Any *p* values were based upon differences in adjusted means.

### 3. Results

Thirteen children with OI, naïve to bisphosphonate treatment, were enrolled into and participated in the study between May 5, 2017, and February 8, 2018. One child withdrew after

Table 1. Study Participants’ Demographic Data

ID	Age (years)	Sex	Weight (kg)	Height (cm)	Pubertal status
1	5.8	M	16.0	108.0	Stage I
2	11.3	F	36.4	140.4	Breast stage II, pubic hair stage I
3	9.9	M	26.0	137.5	Stage II
5 <sup>a</sup>	5.6	M	21.7	101.0	Stage I
6	14.9	M	49.8	167.1	Stage IV
7	9.8	F	54.8	147.4	Breast stage II, pubic hair stage II
8	6.9	M	17.5	116.0	Stage I
9	6.1	F	30.8	125.7	Stage I
10	5.8	F	18.6	113.0	Stage I
11	9.4	F	42.0	147.7	Breast stage II, pubic hair stage I
12	4.5	F	16.9	95.2	Stage I
14 <sup>b</sup>	9.7	F	35.4	141.7	Breast stage II, pubic hair stage I

<sup>a</sup>Number 4 withdrew.

<sup>b</sup>Number 13 not assigned.

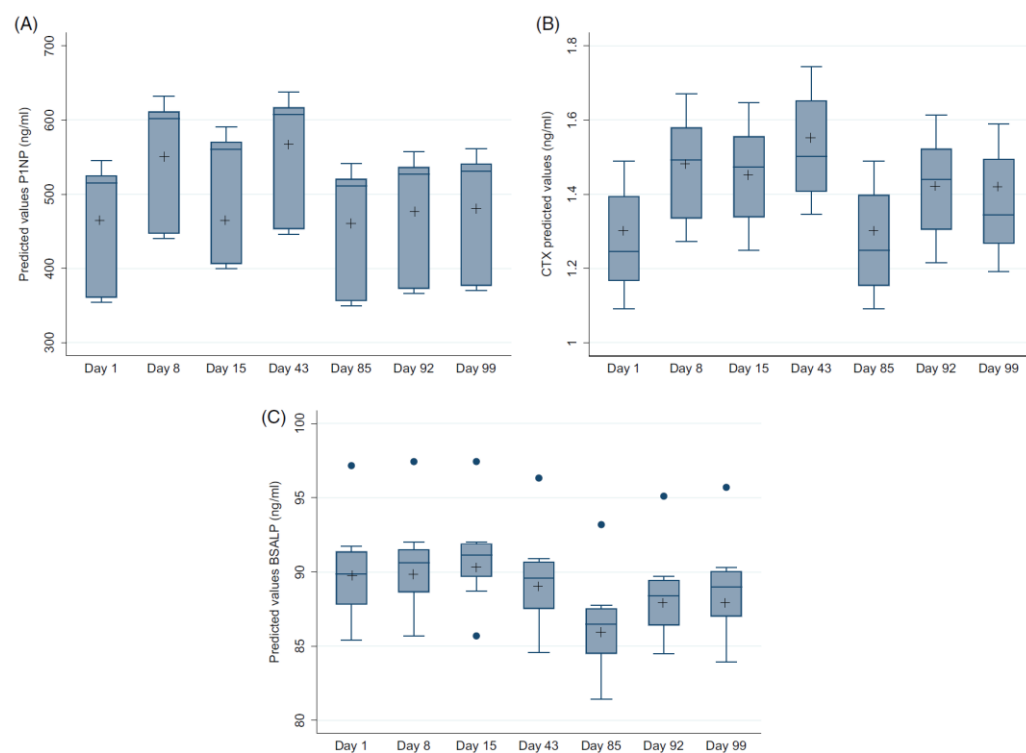
**Table 2.** Raw Data Means (95% CIs) for P1NP, CTX, and BSALP

Time point (days)	Serum P1NP, ng/mL (mean, 95% CI)	Serum CTX, ng/L (mean, 95% CI)	Serum BSALP, ng/mL (mean, 95% CI)
1	464.6 (417.8, 511.5)	1.30 (1.20, 1.41)	89.8 (86.2, 93.4)
8	550.0 (499.3, 602.5)	1.48 (1.37, 1.60)	90.1 (86.3, 94.0)
15	509.5 (458.0, 561.1)	1.45 (1.34, 1.57)	90.2 (86.2, 94.2)
43	556.7 (509.8, 603.6)	1.55 (1.44, 1.66)	89.0 (85.3, 94.8)
85	460.1 (411.2, 508.9)	1.30 (1.19, 1.41)	85.9 (82.1, 89.8)
92	476.0 (424.4, 527.6)	1.42 (1.31, 1.54)	87.9 (84.0, 91.7)
99	480.5 (433.6, 527.3)	1.40 (1.30, 1.51)	88.4 (84.3, 92.3)

CI = confidence interval; P1NP = procollagen type I N-terminal propeptide; CTX = carboxy-terminal telopeptide of type I collagen cross-link; BSALP = bone-specific alkaline phosphatase.

baseline blood tests were taken and before any WBV was performed; their data have been excluded. The CONSORT diagram showing the flow of patients is provided in Fig. 1.

The baseline demographics are shown in Table 1. All participants but one were either prepubertal or in early puberty, and there was a slight preponderance of girls (7/12).



**Fig. 2.** (A) Change in adjusted P1NP (ng/mL) across the study period. Boxes represent second and third quartiles with the internal horizontal line showing the median; whiskers are 1.5 times the interquartile range. + shows arithmetic mean; ● shows outliers more than 3 times the interquartile range from the median. (B) Change in adjusted CTX (ng/mL) across the study period. Boxes represent second and third quartiles with the internal horizontal line showing the median; whiskers are 1.5 times the interquartile range. + shows arithmetic mean; ● shows outliers more than 3 times the interquartile range from the median. (C) Change in adjusted BSALP (ng/mL) across the study period. Boxes represent second and third quartiles with the internal horizontal line showing the median; whiskers are 1.5 times the interquartile range. + shows arithmetic mean; ● shows outliers more than 3 times the interquartile range from the median.

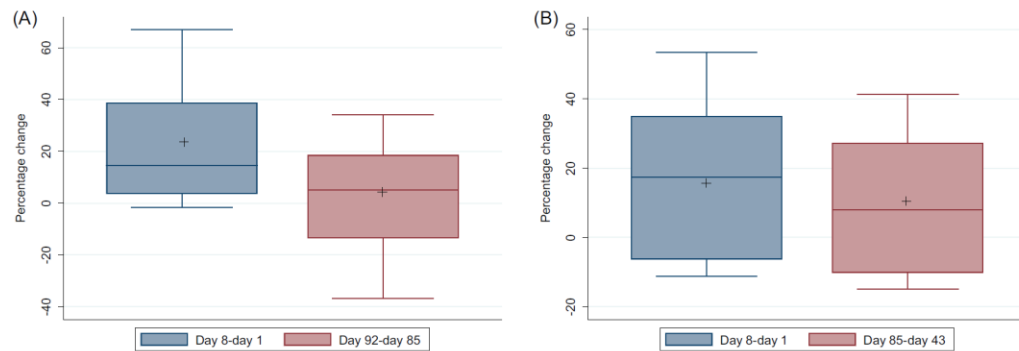


The summary unadjusted data for each biomarker at each time period are shown in Table 2. The baseline mean absolute values obtained for P1NP and CTX were around 65% of the mean values we obtained in healthy prepubertal boys<sup>(16)</sup> and 80% of those in our studies of younger children;<sup>(17)</sup> we did not measure BSALP in our previous studies.

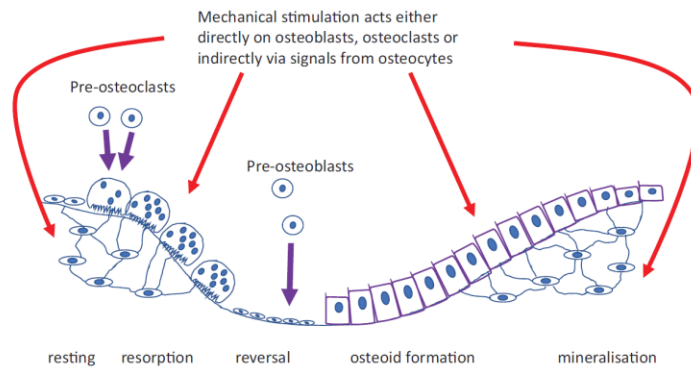
The percentage changes in the unadjusted means from D1–8 for P1NP and CTX, were thus an increase of 18.4% and 13.8%, respectively; both markers then fell slightly (7.3% and 2.0%, respectively) from D8 to D15 and were again increased with respect to baseline at D43, 19.8% and 19.2%, respectively. The change from D43–85 across the period of risedronate therapy

was effectively a return to previbration baseline levels. The percentage changes from D85–92 across the second period of vibration for P1NP and CTX, respectively, were an increase of 3.4% and 9.2%.

Fig. 2A–C shows the change in each of the bone biomarkers assessed at each time point in graphical form, with the means adjusted for age, sex, and weight. For P1NP and CTX, the pattern of change is similar. For P1NP, there was a significant initial increase from D1–8 ( $p = 0.010$ ), a plateau or slight decrement from D8–15, and then a second peak on D43 ( $p = 0.03$  versus D1) before the period during which risedronate, calcium, and vitamin D were administered. For CTX, there was a significant



**Fig. 3.** (A) Box plots showing comparison of P1NP percentage changes after whole body vibration period 1 (day 8–day 1) and period 2 (day 92–day 85). Boxes represent second and third quartiles with the internal horizontal line showing the median; whiskers are 1.5 times the interquartile range. + shows arithmetic mean. (B) Box plots showing comparison of CTX percentage changes after whole body vibration period 1 (day 8–day 1) and period 2 (day 92–day 85). Boxes represent second and third quartiles with the internal horizontal line showing the median; whiskers are 1.5 times the interquartile range. + shows arithmetic mean.



**Fig. 4.** Schematic showing the possible sites of action of mechanical stimulation.

initial increase from D1–8 ( $p = 0.014$ ), a plateau or slight decrement from D8–15, and then a second peak on D43 ( $p < 0.001$  versus D1).

After the administration of the medication between D43 and D85, P1NP fell significantly ( $p = 0.016$ ). The change in mean adjusted CTX did not reach statistical significance ( $p = 0.086$ ).

The subsequent percentage change from D85–92 in P1NP was less than it had been from D1–8 (3.7% versus 19.5%;  $p < 0.05$ ) but the difference in percentage change in CTX (9.8% versus 14.4%) did not reach significance; Fig. 3).

The pattern of change in BSALP was different from that of P1NP and CTX. There was little change after the first period of whole body vibration from D1–42; BSALP appeared to fall from D42–85 during the period of treatment with risedronate and Calcichew and rose back toward the previbration baseline at the end of the study. The absolute changes in BSALP did not, however, reach significance at any time point (D1 versus D8  $p = 0.822$ ; D1 versus D43  $p = 0.621$ ; D43 versus D85  $p = 0.451$ ).

One child suffered a fractured forearm (radius and ulna) 2 days into the period of bisphosphonate administration. This was the only radiologically confirmed fracture among participants during the study.

#### 4. Discussion

In 12 children with osteogenesis imperfecta, we found that the response of the bone biomarker P1NP to a standardized form of mechanical stimulation, whole body vibration, was reduced after the administration of an oral bisphosphonate, risedronate, and concomitant calcium and vitamin D supplementation. The implications of this finding are significant for the management of children with osteogenesis imperfecta, the majority of whom are likely to receive bisphosphonate treatment. Interestingly, the falls in P1NP and CTX after medication did not reduce the absolute values of either biomarker below the previbration baseline. However, the reduction in response of the serum marker of bone formation brings into question how bisphosphonates are used in children with osteogenesis imperfecta.

This is the first time that successive periods of WBV have been used to assess the effect of bisphosphonate treatment on the dynamic skeletal response to mechanical stimulation.

Based on our prior experience with apparently healthy children,<sup>(16)</sup> we had anticipated that allowing a period of 5 weeks for the bone biomarkers to return to baseline would be adequate. We were mistaken. In contrast to the expected fall from D15 onward, we saw an increase at D43 in both P1NP and CTX compared with baseline. When we first reported on our use of this form of assessment in apparently healthy prepubertal boys, we speculated that the greater increase in P1NP as opposed to CTX was evidence of increased modeling activity in response to mechanical stimulation. On the basis of this new data, we suggest that a more plausible explanation is that WBV increases the activity of osteoblasts that are already producing bone matrix, as well as stimulating the initiation of additional remodeling events; this then is reflected in the pretreatment double peak for both P1NP and CTX; see Fig. 4 for an illustration. This action could be direct or mediated through the osteocyte network—our data are uninformative in this regard. The reduction in both P1NP and CTX after risedronate administration further reinforces the concept that cells engaged in remodeling, rather than modeling—which should be less affected by risedronate administration—are the main effectors of the response to

vibration. This is, in our view, the most likely biological explanation for our findings, but we fully accept that we are only looking at circulating biomarkers of cellular activity, not at the cells themselves.

The smaller rise in P1NP in response to WBV after risedronate treatment suggests that the numbers or activity of osteoblasts forming matrix is diminished by such treatment. This would be consistent with the reports of lower osteoid surface/bone surface found during bisphosphonate treatment in children with OI.<sup>(6)</sup> The fact that both CTX and P1NP did increase immediately after the second period of WBV does suggest that the capacity to respond to mechanical stimulation is still present, albeit reduced. The fact that the initial increase in CTX after WBV was not different across the two periods of vibration (D1–8; D85–92) might imply that the initiation of new remodeling events in response to vibration was similar at both times; however, the period of follow-up after WBV2 was insufficient to demonstrate a subsequent rise in P1NP.

The pattern of change in P1NP and CTX after each period of WBV was quite different from that for BSALP. BSALP changed little in response to the first period of WBV, fell during the period of risedronate and calcium/vitamin D administration, and then rose toward the previbration baseline during and after the second period of vibration. Mineralization must necessarily come after osteoid formation; if more osteoid has been formed previously in response to WBV, it would seem logical that more mineralization would occur subsequently. It is interesting, however, to note that markers of all activities—bone resorption, osteoid formation, and mineralization—fell during the period of risedronate and calcium/vitamin D treatment. It would be interesting to speculate that this implies continuing cross-talk between osteoclasts and osteoblasts persisting across both the period of osteoid formation and of mineralization. The change in BSALP in response to the first period of WBV is not suggestive of an immediate increase in the rate of mineralization. It is important to note, however, that none of the changes in BSALP were statistically significant.

This work has a number of limitations. The study was undertaken on a small group of children with OI and ideally the results should be confirmed in a larger group with more older children included. One child suffered a fracture during the period of bisphosphonate administration; that event may have impacted on subsequent bone marker measurements, although the individual data for that patient post-fracture are similar to those of the other children. We did not measure factors likely to reflect osteocyte activity or osteoblast–osteoclast cross-talk; in our previous studies, we found no acute change in circulating sclerostin or osteoprotegerin in response to WBV.<sup>(16)</sup> We did not check serum vitamin D and PTH during the study; however, all children under our care with osteogenesis imperfecta are advised to take regular daily vitamin D supplements, and we also give calcium supplements to those who report low dairy product intake. The administration of calcium and vitamin D in combination with the risedronate (following the manufacturer's recommendation) may have also impacted on short-term bone turnover. In our previous studies of children receiving risedronate in a placebo-controlled trial,<sup>(7)</sup> however, we saw no change in urinary cross-linked N-telopeptide of type 1 collagen at 3 months for those receiving calcium and vitamin D as placebo, in contrast to a significant decline for those also receiving risedronate. We cannot exclude the possibility that exposure to two successive episodes of WBV at this interval would result in altered bone turnover marker responses in the absence of risedronate, calcium, and

## 7.2 Study protocol



**TRIAL FULL TITLE – DO BISPHOSPHONATES ALTER THE SKELETAL RESPONSE TO MECHANICAL STIMULATION IN CHILDREN WITH OSTEOGENESIS IMPERFECTA: A PILOT STUDY**

**TRIAL SHORT TITLE – BAMES**

**TRIAL NUMBER – (from R&D at registration)**

**DATE AND VERSION NUMBER – 24.10.16 v3.4**

**EUDRACT NUMBER – 2016-003606-14**

**ISRCTN NUMBER -**

### **LAY SUMMARY (max 300 words)**

Osteogenesis Imperfecta (OI) or Brittle Bone Disease is a genetic disorder characterised by bones that break easily because the bone material is brittle and the structure of the bones is defective. Current medicines cannot affect the material itself, instead increase bone strength by making bones wider and “filling in” the holes in the bone walls that weaken it.

These medicines are bisphosphonates, given either by a drip intravenously or taken by mouth. Their major action is to prevent bone breakdown by stopping the normal process of removing and then replacing old bone tissue, so in parts of the bone, new bone formation is reduced. Bisphosphonates studies in OI children have shown increased bone mineral density and improved exercise tolerance that could positively affect new bone formation; some have shown reduced fracture rate.

Bone is highly responsive to dynamic loading and adapts its architecture and mass to mechanical stimulation. Whole body vibration (WBV) is a standardized form of mechanical stimulation. Sitting or standing on a vibration platform (whole body vibration) has been shown to improve bone mineral density in individuals with narrow bones. We have previously used WBV to look at bone turnover markers (blood tests assessing the formation and breakdown of bone) in healthy children and found that 10 minutes WBV for 5 days increases bone formation by 25%.

Little is known whether bisphosphonates affect the response of the skeleton to mechanical stimulation. We will determine the response to mechanical stimulation in OI children by looking at bone turnover markers following WBV in those who are and are not treated with bisphosphonates. This study will help us to understand whether skeleton in children with OI is normally responsive to mechanical stimulation, and whether bisphosphonates alter that responsiveness in a way that is either beneficial or not for increasing bone strength

*Protocol number – EUDRACT 2016-003606-14  
Trial name/abbreviated title BAMES study  
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**GENERAL INFORMATION**

<b>Sponsor:</b> Sheffield Children's NHS Foundation Trust Western Bank Sheffield S10 2TH United Kingdom	<b>Sponsor's Representative/Contact:</b> Dr Paul Dimitri Director of Research & Development  Phone: 0114 2717354 Fax: 0114 2267844 Email: <a href="mailto:paul.dimitri@sch.nhs.uk">paul.dimitri@sch.nhs.uk</a>
<b>Chief Investigator:</b> Name: Professor Nick Bishop Address: Academic Unit of Child Health Oncology and Metabolism Dept Sheffield Children's Hospital Telephone: 01142717303 Fax 01142755364 Email: <a href="mailto:n.j.bishop@sheffield.ac.uk">n.j.bishop@sheffield.ac.uk</a>	<b>Principal Investigator</b> Name: Sivagamy Sithambaram Address: Academic Unit of Child Health Oncology and Metabolism Dept Sheffield Children's Hospital Telephone: 01142717561 Fax: Email: <a href="mailto:s.sithambaram@sheffield.ac.uk">s.sithambaram@sheffield.ac.uk</a>
<b>Co-Investigator:</b> Name Address Telephone Fax Email	<b>Co-Investigator:</b> Name Address Telephone Fax Email
<b>Statistician:</b> Name Address Telephone Fax Email	<b>Laboratory/other key service provider</b> Name Address Telephone Fax Email

## 1.0 **BACKGROUND**

Osteogenesis Imperfecta (OI) is a spectrum of genetic disorders with decreased bone density and increased bone fragility. It is characterised by fractures associated with minimal or absent trauma, dentinogenesis imperfecta, blue sclerae and progressive hearing loss. OI has a prevalence of approximately 6-7/100 000<sup>1</sup> and the fracture risk is greatest during childhood.

At a tissue level, the characteristic feature of bone in OI is its brittle nature. This is largely because of an excess in the amount of mineral present relative to the fibre content of the bone material, and also altered 3-dimensional structure of the fibrous component of the matrix. The altered fibrous component of the matrix comes from the combination of reduced amounts of type 1 collagen, and in more severe cases, abnormal type 1 collagen. At a microscopic level, this results in reduced amounts of bone tissue, with both the cortical and trabecular bone being affected. The cortices are narrower, with large intracortical pores and the trabecular connectivity is disrupted. The overall width of tubular long bones is reduced. This combination of material brittleness, disrupted micro architecture and reduced bone size all combine to reduce fracture resistance and increase bone fragility.

In children, bone is a dynamic tissue continually undergoing modelling and remodelling. During remodelling in adults, bone resorption occurs at a rate equal to that of bone formation, in a process referred to as coupling; in children, more bone is formed than is removed (approximately 4% more per remodelling cycle), allowing for bone mass accretion during growth. In modelling, osteoblasts and osteoclasts are active on opposite sides of the cortex and thus are not directly coupled (the osteoblasts' and osteoclasts' activity are not occurring at an equal rate). When bone resorption exceeds formation, loss of bone mass occurs, thus leading to bone fragility. Bone mass can be increased in two ways; one is by increasing osteoblast activity and the other is by decreasing osteoclast activity<sup>2</sup>. In children with OI, bone turnover is increased; the replacement of resorbed bone is defective compared to normal, and the number of osteocytes (terminally differentiated osteoblasts that are embedded within the bone substance and that form part of the pressure- sensing network in bone) is increased.

Bisphosphonates are potent antiresorptive agents that inhibit osteoclast function. Bisphosphonates are deposited onto naked bone surfaces and endocytosed during osteoclastic bone resorption. The nitrogen-containing bisphosphonates act on specific enzymes in the mevalonate pathway, reducing prenylation activity and effectively poisoning the osteoclasts, resulting in reduced function and apoptosis. Bisphosphonates have a variable residence time in bone, depending on the individual bisphosphonate's mineral binding avidity.

Bisphosphonates have been widely used in the treatment of children with OI over the last 20 years. At a tissue level, the changes seen in children with OI treated with bisphosphonates include an increase in trabecular number, thickness and connectivity, reduced intracortical porosity and increased cortical thickness. At a whole bone level, restoration of vertebral shape and size as well as increased bone width in long bones and reduced long bone bowing deformity have been reported. However, two recent reviews have highlighted that despite these observed changes, the effects of such therapy on fracture frequency are equivocal, though multiple studies (RCTs and observational cohorts) report this independently and no studies report an increased fracture rate with treatment<sup>3</sup>.

Whole body vibration (WBV) therapy is targeted at musculoskeletal strengthening and has been trialled in a variety of conditions. WBV has been shown to have therapeutic advantage in various osteopenic populations such as post-menopausal women (improved mobility, muscle strength, postural strength and bone density) and children with osteogenesis imperfecta (improved mobility)<sup>4-12</sup>. Our recent studies of WBV in healthy boys showed that even a short period of exposure could increase a bone formation marker in excess of one reflecting bone resorption<sup>13</sup>.

Previous studies evaluating the combined effect of vibration therapy and bisphosphonates in osteoporotic women have shown inconsistent results. Iwamoto et al (2005), showed that the increase in lumbar bone mineral density (BMD) and the decrease in urinary NTX and serum ALP levels were similar in both the bisphosphonate only group and the bisphosphonate with vibration exercise groups. However, the reduction in chronic back pain was greater in the bisphosphonate with vibration group than the bisphosphonate only group<sup>14</sup>. Hatori et al (2015), showed that combined effect of high frequency loading and bisphosphonates did not produce any additive effect on bone micro-architecture<sup>15</sup>. Chen et al (2014), showed that whole body vibration enhanced the effect of alendronate (bisphosphonate) in ovariectomized rats by inducing further improvements in trabecular architecture. However, there were no significant additive effect on bone turnover markers (osteocalcin and CTx)<sup>16</sup>.

The treatment of children with OI does not depend solely on administering bisphosphonates. Children need a variety of inputs to address their needs; many children complain of weakness, fatigue and significantly limited mobility. Physiotherapy is a major component of the management of many such children, but often the response to even carefully targeted interventions seem rather limited.

*Protocol number – EUDRACT 2016-003606-14*

*Trial name/abbreviated title BAMES study*

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It is unclear to what extent the skeleton in children with OI is responsive to mechanical stimulation; it may be that it is normally responsive, but the osteoblastic activity that occurs in response to mechanical stimulation is defective. It could be that the higher intrinsic levels of bone turnover in OI reduce the capacity of bone to further respond by increasing bone formation in excess of bone resorption. It might also be the case that the use of bisphosphonates abrogates the ability of bone tissue and cells to respond to mechanical stimulation. As bisphosphonates inhibit bone resorption, bone formation is also inhibited in remodelling as the osteoblasts and osteoclasts are coupled with resorption preceding formation. However, vibration therapy could still stimulate bone formation independently in remodelling as then the osteoblasts and osteoclasts are not coupled.

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## 2.0 TRIAL OBJECTIVES AND PURPOSE

We need to know more about the ability of OI bone to respond to mechanical stimulation, and what factors might influence that response. We therefore propose to undertake prospective studies to ascertain this information. It is important to understand the ability of OI bone to respond to mechanical stimulation, and if bisphosphonates might influence the response as, in an ideal world, we want to reduce bone loss but at the same time stimulate bone formation for optimal benefit. The results of the study will be relevant in determining the extent (if any) to which bisphosphonates reduce the response of bone to mechanical stimulation, and form the basis for future studies that assess whether bisphosphonate dose might need to be adjusted to allow adequate bone formation in response to mechanical stimulation.

This study is undertaken in part fulfillment of an MD.

Primary objective: To look at the change in bone formation and resorption markers following whole body vibration in children with osteogenesis imperfecta who are and are not treated with bisphosphonates.

## 3.0 TRIAL DESIGN

This is a pilot study. We will recruit 15 patients (assumed dropout rate of 20%, final n=12) with osteogenesis imperfecta not treated with bisphosphonates. Our choice of sample size is pragmatic, and not informed by power. A literature review of sample size for pilot and feasibility studies found a few papers with recommendations for two-arm trials but nothing of good quality for single arm interventions<sup>17</sup>. The smallest number suggested for a two-arm trial was 12 patients per group<sup>18</sup> while others have suggested larger numbers<sup>19</sup> (Hertzog 2008). Given this is a pilot study the data will not be over analysed but presented as a mainly descriptive account<sup>20</sup>.

Regular assessment of children with OI to be entered into the study is undertaken in the metabolic bone clinics at Sheffield Children's Hospital; where there is clinical evidence of underlying disorders such as renal failure or hypocalcemia that would be contraindications to the use of a bisphosphonate, appropriate investigations are undertaken. However, such problems are vanishingly rare in OI (i.e. not seen in our clinical experience of any such children in the last 20 years). Nevertheless, calcium and vitamin D supplementation will be given during the period of risedronate administration as is the recommended practice in adults receiving the drug. During clinical risedronate administration in children with OI, safety assessments are focused on ensuring that excessive suppression of bone turnover is not caused by drug administration, by measurement of bone formation and resorption markers at six monthly intervals. In this protocol, such measurements will be undertaken 7 times in 99 days.

A flowchart showing the processes for the study is attached in the Appendix at the end of the protocol.

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Essentially, subjects will have a baseline assessment (WBV1) of their bone turnover marker response to a week-long period of whole body vibration (10 minutes/day), followed by a "washout period" of 5 weeks during which bone turnover is expected to return to normal. Following this, there will be a period of 6 weeks of treatment with risedronate (1 mg/kg/week). Immediately following this will come a second assessment (WBV2) of the bone turnover marker response to a week-long period of whole body vibration (10 minutes/day) as previously.

The subjects stand on the vibration platform for 10 minutes for 7 days on 2 occasions. The vibration is delivered as 4 "blocks" of 2.5 minutes each, with 30 seconds rest in between each block. The initial Whole Body Vibration (WBV) on day 1 will be undertaken in the Sheffield Children's Hospital Clinical Research Facility (SCHCRF) under supervision. Subsequent WBVs D2-D7 and D85-91 will be done in the participants' homes. Participants will be asked to record the administration and timing of WBV in a diary.

Blood samples will be taken after an overnight fast according to the following schedule: Pre-WBV1 D1; D8 (postWBV); D15; D43 (immediate pre-risedronate); D85 (post-risedronate and pre-WBV2); D92 (post-WBV2) and D99 (final). 7 samples are taken altogether.

The blood tests are bone turnover markers (Alkaline phosphatase[ALP], Procollagen Type 1 N-Terminal Propeptide[P1NP] and C-Terminal Telopeptide of Type 1 Collagen[CTX]). The first blood test will be done by the researcher (Dr Sithambaram) in the SCHCRF and the subsequent 6 blood tests can be done by the research nurse/researcher at the participant's home. Blood samples taken will be allowed to clot for ½ an hour. Samples will be spun at 2500 rpm for 10 minutes at 4°C. The centrifuged sample will be stored in SCHCRF at -80°C. Blood tests will be analysed in the Mellanby Centre for Bone Research, University of Sheffield.

Participants will be taking risedronate (oral bisphosphonate, once weekly), rounded to the nearest 5 mg) together with Vitamin D and calcium for 6 weeks. Vitamin D and Calcium will be given as Calcichew 500mg/200 IU tablets, 1 tablet for participants weighing less than 30 kg and 2 tablets for participants weighing 30 kg or more. Risedronate Sodium belongs to Bisphosphonates group of medicine. As per BNF, it is not licensed for use in children. The trade name is Actonel® Warner Chilcott). This study will use 5mg and 35mg film-coated tablets.

Pubertal status of every patient will be assessed using a questionnaire (Appendix 1&2) as puberty affects both bone turnover and the rate of bone growth.

Pregnancy testing will be carried before risedronate is given to any female subject aged 10 years or more. Any child found to be pregnant will be withdrawn from the study immediately.

#### *Summary of drug characteristics and administration*

Risedronate sodium is a pyridinyl bisphosphonate that binds to bone hydroxyapatite and inhibits osteoclast-mediated bone resorption. The bone turnover is reduced while the osteoblast activity and bone mineralisation is preserved.

In preclinical studies risedronate sodium demonstrated potent anti-osteoclast and antiresorptive activity, and dose dependently increased bone mass and biomechanical skeletal strength. The activity of risedronate sodium was confirmed by measuring biochemical markers for bone turnover during pharmacodynamic and clinical studies. Decreases in biochemical markers of bone turnover were observed within 1 month and reached a maximum in 3-6 months ([www.medicines.org.uk](http://www.medicines.org.uk)).

Risedronate has been shown to increase bone mineral density and reduce fracture frequency in mild osteogenesis imperfecta; our paper in the Lancet showed a 47% decrease in fracture risk over the first year of treatment.

It is administered orally and the dose we will use is 1mg/kg/week (rounded to the nearest 5mg). We know from a previous dose-ranging study<sup>25</sup> that this dose is sufficient to maintain bone mass in relation to body size in moderately to severely affected children with OI. 1mg/kg/week was the average dose administered in the prospective RCT of risedronate in mild OI.<sup>26</sup> We found no adverse effects or change in efficacy if the tablet was crushed and dissolved in water before taking. Risedronate will be taken once weekly from week 7 to week 12 (6 weeks in total). Vitamin D & Calcium will be taken orally each evening during this period.

Risedronate has to be taken either whole with a glass of tap water, or crushed and dissolved in a glass of tap water, on an empty stomach, typically first thing in the morning. The patient must ingest nothing other than tap water for at least the following 30 minutes (to ensure maximal drug absorption), and remain upright to reduce the risk of mucosal damage in the lower oesophagus. In adults there are a variety of side effects reported including gastrointestinal upset and musculoskeletal aches and pains;

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our previous large scale RCT of risedronate in children with OI<sup>26</sup> showed no excess of such side effects in treated children as opposed to those receiving placebo.

We will provide these instructions to the patient both verbally and in written form and add that they should immediately stop taking any further study medication and notify us if there is any evidence of hypersensitivity or allergic reaction, or SAE (see safety assessments section).

Vitamin D and Calcium will be taken in evenings. Vitamin D and Calcium combination tablets are available as Calcichew -D<sub>3</sub> (chewable). This contains calcium carbonate 1.25 g (calcium 500 mg or Ca<sup>2+</sup> 12.5 mmol), colecalciferol 5 micrograms (200 units). In this study, participants will be asked to take 1-2 tablets per day as recommended by BNF (<30kg one tablet; ≥30kg 2 tablets). The combination tablet is regarded as a non-IMP for the purposes of this study as its role is simply to ensure adequate calcium intake and absorption in the face of reduced calcium release from bone, consequent on the actions of the bisphosphonate medication.

The medication can be taken by the participant themselves or given by parents of participants. Participants will be taking these medication under the supervision of parents and will be asked to record the timings of drug administration in a diary.

These medication will be stored and dispensed by the sponsor's Pharmacy Department in accordance with Good Clinical Practice. The IMP(s) will be dispensed and labelled in accordance with Annex 13 of Good Manufacturing Practice.

#### 4.0 **SELECTION OF SUBJECTS**

Participants will be selected from children attending OI service at Sheffield Children's Hospital. The service was established in 1998 and is now the largest of its kind in Western Europe; Sheffield is the lead centre for the 4 designated centres providing the Highly Specialised Severe Complex and Atypical Osteogenesis Imperfecta Service for England, so access to patients is excellent.

Inclusion criteria:

- Age 4-16 years
- Able to speak fluent English
- Diagnosed with osteogenesis imperfecta
- Able to stand
- Not treated with bisphosphonates

Exclusion criteria:

- Presence of other chronic illnesses including renal failure
- Balance problems
- Recent fracture (in the last 6 months)
- Recent (last 12 months) or current treatment likely to affect bone – this does not include inhaled or intermittent oral therapy with steroids for asthma
- Involvement in another interventional research project
- Hypocalcemia
- Pregnancy or lactation
- Known hypersensitivity to risedronate or any of the excipients

#### 5.0 **SUBJECT RECRUITMENT**

Prof Bishop's team will identify potential participants who meet the eligibility criteria for this study by searching the database of children attending hospital with osteogenesis imperfecta.

Only members of the clinical team involved in the care of the patients will access their records for the purposes of identification. The researcher will not contact the families directly. Any approach to discuss potential participation will be undertaken by Professor Bishop or another member of the clinical team in the first instance. This approach will take place in clinic or by letter if the clinic appointment is more than six months beyond the commencement date of the project.

Informed consent will be obtained by the researcher Dr. S.Sithambaram. Researcher will first talk through the study to the potential participant together with their parents. Researcher will explain the purpose of the study and the likely benefits of the study. The role of the participants and the procedures involved will be explained in detail. A written information sheet with contact details of the researcher and the research nurse will be provided. Researcher and the research nurse can be contacted for queries related to the research study.

At least a day will be given for the potential participant to make the decision. It will be made very clear

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to the potential participants that they do not have to participate in the research if they don't wish to and that it would not affect their clinical care one way or the other if they participate. Researcher will ensure that participants understand that they are free to withdraw from the study at any time.

Assent will be obtained from children by using age-appropriate assent forms after providing age-appropriate information.

Every participant will be given £50 in total (£25 at the beginning of the study and £25 at the end of the study). This is felt to be appropriate remuneration as a compensation for the number of blood tests and time involved in this study.

Detailed timeline of recruitment of patients and data collection form is attached in appendix (EXCEL sheet). Recruitment will take at least 15 months to complete. Every participant will be involved in this study for 99 days.

## **6.0 DATA HANDLING AND RECORD KEEPING**

The researcher is responsible for data collection, recording and quality. Data will be entered in Sheffield Children's Hospital computers and stored in password protected files (electronic) or a locked filing cabinet (paper). Participants will be identified by id codes only, with personal details stored separately. Data will be collected and retained in accordance with the Data Protection Act 1998. Trial documents (paper and electronic) will be retained in a secure location during and after the trial has finished. All source documents will be retained for a period of 5 years following the end of the trial. Where trial related information is documented in the medical records – those records will be retained for 5 years after the last patient last visit.

## **7.0 ACCESS TO SOURCE DATA**

The researcher, Prof Bishop, statistician and the research nurse will have access to participants' personal data after obtaining consent.

The Sponsor will permit monitoring and audits by the relevant authorities, including the Medicines and Healthcare products Regulatory Agency (MHRA). The investigator will also allow monitoring and audits by the sponsor, and will provide direct access to source data and documents upon request.

## **8.0 STATISTICAL ANALYSIS**

This is a pilot study. We will recruit 15 patients (assumed dropout rate of 20%, final n=12) with osteogenesis imperfecta not treated with bisphosphonates. Our choice of sample size is pragmatic, and not informed by power. A literature review of sample size for pilot and feasibility studies found a few papers with recommendations for two-arm trials but nothing of good quality for single arm interventions<sup>17</sup>. The smallest number suggested for a two-arms trial was 12 patients per group<sup>18</sup> while others have suggested larger numbers<sup>19</sup> (Hertzog 2008). Given this is a pilot study the data will not be over analysed but presented as a mainly descriptive account<sup>20</sup>. Continuously distributed data will be summarized by the median (25th/75th centiles); categorical data by n(%). Missing data will be recorded but will not be considered otherwise in the analyses. The Stata statistical computer package will be used to analyse the data. This data will be used to inform another study.

## **9.0 SAFETY ASSESSMENTS**

As previously described, risedronate is not shown to cause any side effects/allergic reactions in clinical studies in the paediatric population. In the event of a serious allergic reaction, there is a 24-hour emergency paediatric team (including an anaesthetist) available and intensive care facilities. (SOP attached).

In adults there are a variety of side effects reported including gastrointestinal upset and musculoskeletal aches and pains; our previous large scale RCT of risedronate in children with OI (Lancet 2013) showed no excess of such side effects (or indeed any side effects) in treated children as opposed to those receiving placebo.

As the study is unblinded there will be no need to have in place plans for breaking randomisation codes.

All adverse events will be managed in accordance with the Pharmacovigilance Standard Operating Procedures of the Clinical Research Facility at the Sheffield Children's NHS Foundation Trust. These are taken from the requirements for safety reporting in part 5 of the Medicines for Human Use (Clinical Trials) Regulations 2004: SI 2004/1031 (as specified in ICH GCP EU Directive 2005/28/EC) (see pharmacovigilance section below).

*Pharmacovigilance: recording, managing and reporting adverse events*

- **Definitions as they apply to BAMES Study**
  - **Adverse event (AE)** – any untoward medical occurrence in a subject to whom a medicinal product (risedronate) has been administered, including occurrences which are not necessarily caused by or related to that product.
  - **Adverse drug reaction (ADR)** – any untoward and unintended response in a subject to risedronate which is related to any dose administered to that subject.
  - **Unexpected adverse reaction** – An adverse reaction the nature and severity of which is not consistent with the information about risedronate set out in the Summary of Product Characteristics.
  - **Serious adverse event (SAE) or serious adverse drug reaction (SAR) or suspected unexpected serious adverse reaction (SUSAR)** – any adverse event, adverse reaction or unexpected adverse reaction that
    - results in death
    - was life-threatening
    - required hospitalisation
    - resulted in persistent or significant disability or incapacity or
    - consisted of a congenital anomaly or birth defect.

Important medical events that are not immediately life-threatening or result in death or hospitalisation but may jeopardise the patient or may require intervention to prevent one of the other outcomes listed above will also be considered serious.

In children with osteogenesis imperfecta (OI) it is expected that mildly affected children will suffer one fracture every 2 years, on average. It is thus possible that one or more children will fracture during the course of the study and might need hospitalization. This would be regarded as an AE for the purposes of the study rather than an SAE, because of the likelihood of such an event occurring under normal circumstances. Similarly, children with OI suffer variable degrees of musculoskeletal pain; whilst it is unusual for this to result in hospitalization, it can do and again this would be regarded as an AE for the purposes of the study.

- **Investigator responsibilities**
  - The investigators will assess each event and assign it to one of the categories of adverse event described above. The CI and academic supervisor will take overall responsibility in deciding how to categorise the event.
  - The investigators will take responsibility for assessing each event for causality. If the SAE is judged as being likely to be a reaction to risedronate, it will be classified as a SAR.
  - Each event will be recorded on an adverse event form, a copy of which will go in the volunteers' study notes and Investigator Site File.
  - The investigators will report any SAE occurring in a subject **immediately** to the Sponsor.
  - The immediate report will be made orally or in writing and will be followed by a detailed written report on the event.
  - Where the event reported consists of, or results in, the death of a subject, the investigator will supply the Sponsor with any additional information requested. Where the death has been reported to the relevant Ethics Committee, the investigator will supply any additional information requested by the Committee.
- **Sponsor responsibilities**
  - The Sponsor will keep detailed records of all adverse events that are reported to them by the investigators.

- The Licensing Authority may require the Sponsor to send those records, or copies of such records, to the authority.
- The Sponsor will ensure all relevant information about a SUSAR, which occurs during the course of the clinical trial and is fatal or life-threatening is reported as soon as possible to the MHRA and the relevant Ethics Committee. This will be done not later than seven days after the Sponsor was first aware of the reaction. Any additional relevant information will be sent within eight days of the report.
- The Sponsor and investigator will take appropriate urgent safety measures to protect clinical trial subjects from any immediate hazard to their health and safety. The measures will be taken immediately and the MHRA informed in writing within 3 days.
- If the Sponsor halts the BAMES Study they will notify the MHRA and Ethics Committee immediately and at least 15 days from when the trial is temporarily halted.
- If the Sponsor terminates the trial before the date specified for its conclusion the Sponsor will notify the Ethics Committee within 15 days of the date of termination.

#### *Stopping/discontinuation rules*

Treatment will be discontinued if a patient experiences a serious adverse event (SAE) that it is considered at least possibly related to the study drug. Any child found to be pregnant will be withdrawn from the study immediately.

The vibrating platform has been used extensively both in clinical practice and sold 'over the counter' in countries within the EU including the UK. Minimal adverse events have been reported in the use of vibrating platforms. In our previous vibration study the boys tolerated the intervention well with only mild effects being reported. A few of the participants experienced an itching type response in the feet and legs during the vibration and anxiety during cannulation. It is possible that someone could fall off the platforms (which are about the size and shape of typical bathroom scales). Anyone who has a problem with balance or is concerned that they may fall off will be asked to hold the back of a chair to steady them self. In our previous studies reported in JMNI (2015), we found that one subject felt dizzy whilst standing on the platform, and one felt faint after having blood taken - both issues resolved after the subjects sat for a while.

The study will be monitored and audited in accordance with the Monitoring Standard Operating Procedures of the Research and Innovation Directorate at Sheffield Children's NHS Trust. All study related documents will be made available on request for monitoring and audits by the Sponsor, the relevant Research Ethics Committee and for inspection by the MHRA or other regulatory bodies.

#### Trial management group (TMG)

A TMG will be set up to review the progression of the study. The TMG will include Professor Bishop (Chief Investigator), Dr Sithambaram (Principal Investigator) and a representative from R&I monitoring team and will meet at monthly intervals.

#### Trial steering committee (TSC)

The members of the TSC will be independent of the study with the exception of the study CI (Professor Bishop). There will be no formal statistical input to the TSC, reflecting the "pilot" nature of the study, and the simplicity of the statistical testing required (paired T-test of change in P1NP consequent upon WBV, before and after receiving the risedronate/calcium/vitamin D). TSC will meet at 6 monthly intervals.

## **10.0 ETHICAL CONSIDERATIONS**

This study involves 7 episodes of blood sampling. The discomfort caused by the blood sampling will be minimised as it is done by the researcher/research nurse who are both trained in blood sampling. Topical anaesthetics will be used to numb the skin and reduce pain before the blood tests. Blood samples will be taken after an overnight fast. Fasting overnight is not very uncomfortable as children are asleep. The first blood test is done in the hospital and the subsequent 6 blood tests are done at the participant's home to minimise travelling time and inconvenience.

This study involves standing on the vibration platform for 10 minutes a day for 7 days on 2 occasions during the study. On the first occasion, participants will stand on the vibration platform for 7 days

without any prior bisphosphonate treatment. On the second occasion, participants will receive 6 weeks of risedronate treatment prior to standing on the vibration platform for 7 days (10 minutes a day). Participants will be taking risedronate (oral bisphosphonate) together with Vitamin D and calcium for 6 weeks. Risedronate has been shown to increase bone mineral density and be beneficial in mild osteogenesis imperfecta.

#### *Ethics and R&D approval*

The trial will be conducted subject to a Research Ethics Committee favourable opinion, HRA Approval, a Clinical Trials Authorisation from the MHRA /notification to MHRA if it is Type A trial and local Research and Development Capacity & Capability.

This clinical trial will be conducted in accordance with The Medicines for Human Use (Clinical Trials) Regulations 2004 SI: 1031 plus subsequent amendments; and Good Manufacturing Practice Annex 13.

#### **11.0 FINANCE AND INDEMNITY**

This study is funded by SPARKS charity and Professor Bishop's research account.

#### **13.0 JUSTIFICATION OF FINANCE AND RESOURCES**

The consumables essentially reflect the costs of undertaking the biomarker assays (15 patients, 7 tests each, 105 tests in all; cost per sample P1NP £25; CTx£20; ALP £20, i.e. £65 x 105 = £6825 plus the costs of consumables for sampling - needles, syringes, tubes; storing samples in the CRF); the time for a research nurse to visit; travel to and from patients homes; costs of supplying risedronate, calcium and vitamin D; clinical trials management costs (pharmacy); costs of dissemination and compensation for time spent by children and parents in participating in the study; statistics advice and support.

#### **14.0 REPORTING AND DISSEMINATION (max 300 words)**

The results of the study will be reported in medical journals, and presented at conferences both in the UK and abroad. Results will also be disseminated on the R&D website, Brittle Bone Society website and in the Trust newsletter. It is the intention that this work should form the basis of an MD. The results will also be presented at the Brittle Bone Society annual meeting.

#### **15.0 IMPACT (max 400 words)**

Direct benefit to the patient will depend on the outcome of the study - no effect of bisphosphonates on response to mechanical stimulation means that patients can be reassured that bisphosphonate treatment is still allowing the skeleton to "behave normally"; contrariwise, showing an effect may mean that different advice needs to be given, or monitoring procedures changed.

#### **16.0 OUTCOMES (max 400 words)**

If the study indicates that bisphosphonates alter the response to mechanical loading, then additional studies will be needed on a larger population to confirm this and also determine if there are "dose" effects of different types of bisphosphonate (there are a variety of oral and intravenous forms in current clinical use).

At a more general level, understanding how bisphosphonates actually alter bone's functioning may enable more rapid evaluation of novel treatments as they go through phase II studies, and also open up the possibility of using this form of testing in other drug-related studies eg effects of steroids on bone.

## 7.3 Study documents

### Information Sheets



#### PARENT/LEGAL GUARDIAN INFORMATION SHEET

##### **Study title**

BAMES study

We would like to invite you and your child to take part in our research study. Before you decide we would like you to understand why the research is being done and what it would involve. **One of our team will go through the information sheet with you and answer any questions you have.** Please talk to others about the study if you wish.

Part 1 tells you the purpose of this study and what will happen to you and your child if you take part.

Part 2 gives you more detailed information about the conduct of the study.

Please ask us if there is anything that is not clear.

##### **Part 1 – to give you first thoughts about the project**

##### **1. What is the purpose of the study?**

We want to try and find out if bisphosphonate (a medicine taken by mouth) affects bone response to vibration (gentle buzzing) in children with brittle bone disease (OI). Risedronate is a type of bisphosphonate that will be used in this study. We will be doing blood tests (bone turnover markers) to review the bone response to vibration. Vibration in this study is delivered by standing on the vibration platform for 10 minutes.

Bisphosphonates are the only medicine that we currently use to treat children with brittle bone disease. Most studies of bisphosphonates in children with OI have shown increased bone density and some have shown reduced fracture rate.

Activity to improve muscle and bone strength is a major part in caring for children with OI. Standing on a vibration platform has been shown in some studies to improve bone strength in some individuals with weak bones; it isn't clear if vibration strengthens bones in OI though.

In this study we are not using vibration as a treatment. We are using it to stimulate bone activity. We know from work we have done in children with healthy bones that standing on a vibrating platform for 10 minutes each day for only 5 days makes the bones more active (in terms of forming bone) by about 25%. We want to see if the bones respond the same way if you have OI, and also whether a bisphosphonate medicine like risedronate affects this response.

This study is undertaken as part of a research training program (MD).

##### **2. Why have we been invited?**

Your child has been chosen because he/she has brittle bone disease. We hope to have 15 children in this study.

### 3. Do we have to take part?

It is up to you and your child whether or not to take part in the study. We will explain the study and go through this information sheet. If you agree to take part, we will then ask you to sign a consent form. If your child is able to understand the research and is happy to take part and can write their name, they will be asked to sign an assent form with you, if they want to.

You will be given a copy of the information sheets and the signed consent and assent forms to keep for your records. You are free to withdraw at any time, without giving a reason. This would not affect the standard of care your child receives.

### 4. What will happen to my child if we agree take part?

Your child will be asked to choose and tick an image within the pubertal questionnaire to document where they think they are at in terms of their development.

Your child will stand on a vibrating platform (that is about the size and shape of typical bathroom scales) for 10 minutes for 7 days in a row. We will take blood samples on the first day before the vibration and then again on 3 more days (days 8, 15 and 43). Having breakfast may affect the blood tests we do, so we will ask your child not to have anything to eat or drink except water, before we take the blood samples. Your child can have something to eat straight after the blood samples have been taken. The amount of blood taken for each visit is 5ml (1 teaspoon). This is likely to be between 07.30-08.15, before school. This is the time for all 7 blood tests (during the initial visit and the subsequent visits at home).

For the next 6 weeks, we will ask your child to take the medicine by mouth (risedronate) once a week. This medicine has to be taken first thing in the morning before breakfast. Your child will also be taking another tablet (Vitamin D together with calcium) in the evenings everyday for the same 6 weeks' period. Risedronate and vitamin D are taken separately; risedronate is taken first thing in the morning and Vitamin D together with Calcium is taken in the evening.

After the 6 weeks of having the medicine, your child will have another blood test and then stand on the vibrating platform again for another 7 days. After this vibration, your child will have another 2 blood tests, the same as before.

The first vibration and blood test will take place in the hospital, the rest can happen in your own home.

Parents/carers will be encouraged to stay with their child and support them throughout all research procedures.

Day 1 - blood tests before vibration

Day 1 to day 7 – standing on the vibrating platform (10 minutes)

Day 8, Day 15 and Day 43 – blood tests

Day 43 to day 84 – medicine taken by mouth  
(Risedronate and vitamin D with calcium)

Day 85 – blood tests before vibration

Day 85 to day 92 – standing on the vibrating platform (10 minutes)

Day 92 and day 99 – blood tests

## 5. Expenses and payments

Parent information sheet  
Version 1  
Date 15.02.2016



A remuneration of £50 (£25 at the beginning and £25 at the end of the study) will be given for all the effort and time spent in this study.

#### **6. What will we have to do?**

It will be good if you could be present while your child is having the blood tests for support. It will be really important that you ensure that your child is standing on the vibrating platform correctly and having the medicines as directed.

#### **7. What are the possible disadvantages and risks of taking part?**

There may be discomfort caused by blood tests. Every effort will be made to reduce the anxiety felt by some children for example during a blood test. Local anaesthetic cream will be used to help numb any pain and your child will appreciate having you there for support. If at any time you or your child feels that the actual or perceived distress is too great, please don't hesitate to tell the research doctor/nurse.

The vibrating platform has been used extensively both in clinical practice and sold 'over the counter' in countries within the EU including the UK. Minimal adverse events have been reported in the use of vibrating platforms. In our previous vibration study the boys tolerated the intervention well with only mild effects being reported. A few of the participants experienced an itching type response in the feet and legs during the vibration. It is possible that someone could fall off the platforms (which are about the size and shape of typical bathroom scales). Anyone who is concerned that they may fall off will be asked to hold the back of a chair to steady them self. The manufacturer does list the following as possible side effects:

- a) skin lesions/blisters on contact surface
- b) itching in trained body parts
- c) nausea and dizziness

#### **8. What are the side effects of any treatment received when taking part?**

Our prior experience with both Risedronate in children with brittle bone disease and vibration in healthy prepubertal boys suggests that there are unlikely to be any side effects. We undertook a big study (147 children) of risedronate in OI and compared risedronate with a placebo (a blank pill with no activity) for the first 12 months; no side effects were found more often in children taking the risedronate. All the children took calcium and vitamin D each day as well without any obvious problems. This information was published in the Lancet in 2013.

If during the study you think your child has any problems, please contact:

Name: Sivagamy Sithambaram

Title: Dr

Hospital/Department: Sheffield Children's Hospital/Academic Unit of Child Health

Tel: 01142717561

#### **9. What are the possible benefits of taking part?**

We cannot promise the study will help your child but the information we get from this study will help to understand and improve the treatment of people with brittle bone disease.

**10. What happens when the research study stops?**

The vibration device is used a standardised test of mechanical stimulation and will not be continued following on from the study. We will collect all the information together and we will use it to help us design other bigger studies in children with brittle bone disease.

**11. What if there is a problem?**

Any complaint about the way you or your child has been dealt with during the study or any possible harm you or your child might suffer will be addressed. The detailed information on this is given in Part 2.

**12. Will my child's taking part in the study be kept confidential?**

Yes. We will follow ethical and legal practice and all information about your child will be handled in confidence. The details are included in Part 2.

**This completes Part 1.**

**If the information in Part 1 has interested you and you are considering your child's participation, please read the additional information in Part 2 before making any decision.**

**Part 2 of the information sheet****13. What if relevant new information becomes available?**

If new information becomes available that means that the study needs to be stopped for any reason, we will tell you and arrange your continuing care.

**14. What will happen if we don't want to carry on with the study?**

If you withdraw from the study, we will destroy all your child's identifiable samples if you wish, but we would like to use the data collected up to the point of their withdrawal.

**15. What if there is a problem?****Complaints**

If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions.

Name: Sivagamy Sithambaram

Title: Dr

Hospital/Department: Sheffield Children's Hospital/Academic Unit of Child Health

Tel: 01142717561

If you remain unhappy and wish to complain formally, you can do this by contacting:

Mrs Linda Towers

Patient Advice & Liaison Co-ordinator

Sheffield Children's NHS Foundation Trust

Tel: 0114 271 7594

**Harm**

In the event that something does go wrong and your child is harmed during the research and this is due to someone's negligence then you may have grounds for a legal action for compensation, but you may have to pay your legal costs. The normal NHS complaints mechanisms will still be available to you.

**16. Will my taking part in this study be kept confidential?**

All information, which is collected, about your child during the course of the research will be kept strictly confidential. Any information about your child, which leaves the hospital, will have their name and address removed so that they cannot be recognised from it. Once the study is complete all information will be kept for 5 years in their confidential notes.

Our procedures for handling, processing, storage and destruction of data are compliant with the Data Protection Act 1998.

Collected data and blood samples will be kept for up to 5 years after the study has finished. The research team will have access to view identifiable data.

We will also inform your family GP that your child will be taking part in the study.

Your child's medical notes may also be looked at by other people within the hospital involved in the running and supervision of the study to check that it is being carried out correctly.

**17. What will happen to any samples my child gives?**

Blood samples obtained will be collected, used and stored following hospital procedures. If in the future we decide to make any further tests on the stored blood samples, your consent would be sought. The research team and anyone needing to carry out checks on what we do will have access to the blood samples. The blood samples will only be identified by ID numbers.

**18. What will happen to the results of the research study?**

When the study has finished we will present our findings to other researchers, and we will put the results in medical magazines and websites that researchers read. We would also like to put a brief summary on the hospital research website so that you will be able to read about our results too. This will be available at the end of the study, hopefully October 2017, on [www.sheffieldchildrens.nhs.uk/research-and-innovation.htm](http://www.sheffieldchildrens.nhs.uk/research-and-innovation.htm). The results will also be included as part of the investigator's educational qualification. They will be anonymous, which means that your child will not be able to be identified from them.

**19. Who is organising and funding the research?**

The research is being organised by Sheffield Children's NHS Foundation Trust and paid for by SPARKS charity.

**20. Who has reviewed the study?**

All research in the NHS is looked at by an independent group of people, called a Research Ethics Committee, to protect your interests. This study has been reviewed and given a favourable opinion by Sheffield Research Ethics Committee. It has also been given approval by the Research Department to run at this hospital.

**21. How can I find out more?**

If you would like to find know more about research in general, the Clinical Research Facility at this hospital has an **Information for families** section on its website [www.sheffieldchildrens.nhs.uk/research-and-innovation.htm](http://www.sheffieldchildrens.nhs.uk/research-and-innovation.htm) or you could contact the hospital Clinical Research Facility:

Ms Wendy Swann  
R&D Manager  
Sheffield Children's NHS Foundation Trust  
Tel: 0114 3053478

If you would like to know more specific information about this research project, please contact the project co-ordinator:

Name: Sivagamy Sithambaram

Title: Dr

Hospital/Department: Sheffield Children's Hospital/Academic Unit of Child Health

Tel: 01142717561

If you would like advice as to whether your child should participate you could contact the project team, or one of your child's health care professionals.

If you have any concerns during the study, you should contact the project team.

**If you and your child decide to take part in this study, you will be given this information sheet and signed consent and assent forms to keep.**

**Thank you for taking the time to read this information sheet.**

**PARTICIPANT INFORMATION SHEET**  
**FOR YOUNG CHILDREN**

**Study title**

The "BAMES" study

**This information is to be shown and read by a  
parent/carer**



I am a doctor who is studying about weak bones in children



I would like to find out if medicine taken by mouth helps  
bones grow stronger with exercise in children with weak  
bones



To help us find out more, I would like you to stand on the vibrating platform and I would like to do some blood tests.



I would also like to ask you and your mummy and daddy some questions



If you don't want to do this - just say no thank you !



Thanks for reading this



**PARTICIPANT INFORMATION SHEET**  
**FOR CHILDREN AGED 6-12 YEARS**

To be shown and read by parent/carer if required

**Study title**

BAMES study

**1. What is research?**

Research is done to find out the answers to important questions.

**2. Why is this project being done?**

We want to try and find out if a special medicine affects bone's response to vibration (gentle buzzing) in children with brittle bone disease. This special medicine is called Risedronate.



**3. Why have I been asked to take part?**

You have been chosen because you have brittle bone disease and you can help us find answers that will help us in caring for children with this condition.

We are asking 15 children all together.



#### 4. Do I have to take part?

No you do not! It is up to you. We would like you to read this information sheet. If you agree to take part, we would like you to write your name, if you can, on two forms. We will also ask your mum, dad or carer to write their name on the forms and give one back to us. You can still change your mind later. If you don't want to take part, just say no!



#### 5. What will happen to me if I take part in the research?

On day 1, we will show you pictures that show you what happens during the growth spurt and ask you to tell us which one looks like you.

You will stand on a vibrating platform (the platform is about the size and shape of a set of bathroom scales) for 10 minutes for 7 days in a row. We will take some blood samples on the first day before the vibration and then again on 3 more days (days 8, 15 and 43). Having breakfast may affect the blood tests we do, so we will ask you not to have anything to eat or drink except water before we take the blood samples. You can have something to eat straight after the blood samples have been taken.

For the next 6 weeks, we will ask you to take the medicine risedronate once a week. This medicine has to be taken first thing in the morning before breakfast. We will explain exactly how you take it. You will also be taking another tablet (Vitamin D together with calcium) in the evenings every day for the same 6 weeks' period.

After the 6 weeks of having the medicine, you will have another blood test and then stand on the vibrating platform again for another 7 days. After this vibration, you will have another 2 blood tests, the same as before.

The first vibration and blood test will take place in the hospital, the rest can happen in your own home.

Day 1 - blood tests before vibration

Day 1 to day 7 – standing on the vibrating platform (10 minutes)

Day 8, Day 15 and Day 43 – blood tests

Day 43 to day 84 – medicine taken by mouth  
(Risedronate and Vitamin D with calcium)

Day 85 – blood tests before vibration

Day 85 to day 92 – standing on the vibrating platform (10 minutes)

Day 92 and day 99 – blood tests

**6. Will the medicine upset me?**

There are unlikely to be any side effects. We use the same medicine in other children with brittle bones.

**7. Will any of these hurt?**

Standing on the vibration platform will not hurt.

Before we take the blood samples, we will put a special cream, or use a cold spray on your hand or inside of your elbow so that the needle doesn't hurt as much when we take the blood sample.

**8. Will joining in help me?**

We cannot promise the study will help you but the information we get might help treat children and young people with brittle bone disease in the future.



**9. What if something goes wrong during the project?**

Your mum, dad or carer will be able to talk to someone who will be able to tell them what they need to do about it.

**10. Will anyone else know I'm doing this?**

The people in our research team will know you are taking part. The doctor looking after you while you are in hospital will know. We will also tell your GP. No one else will know because we will not use your name or address.

**11. What if I don't want to do the research anymore?**

If at any time you don't want to do the research any more, just tell your parents, doctor or nurse. They will not be cross with you. Your doctor will help you decide which medicine is best to use afterwards.



**Thank you for taking the time to read this - please ask any questions if you need to.**

**PARTICIPANT INFORMATION SHEET**  
**FOR CHILDREN/YOUNG PEOPLE AGED 13 TO 15****Study title**

BAMES study

We are asking if you would join in a research project to find out whether a bisphosphonate (a type of medicine taken by mouth) affects the bone response to vibration (gentle buzzing) in children and young people with brittle bone disease. Before you decide if you want to join in, it's important to understand why the research is being done and what it will involve for you. So please consider this leaflet carefully. Talk to your family, friends, doctor or nurse if you want to.

**Part 1 – to give you first thoughts about the project****1. Why are we doing this research?**

We want to try and find out if bisphosphonate (medicine taken by mouth) affects bone response to vibration (gentle buzzing) in children and young people with brittle bone disease. We will be doing blood tests (bone turnover markers) to review the bone response to vibration. Vibration in this study is delivered by standing on a vibrating platform, that is about the size and shape of a set of bathroom scales.

Answers from this study will help us understand about brittle bone disease better. A better understanding will help in the treatment of brittle bone disease.

This research is being done by Dr Sivagamy Sithambaram as part of research training program (MD) and is being supervised by Professor Bishop.

**2. Why have I been invited to take part?**

You have been chosen because you have brittle bone disease. We hope to have 15 children in this study.

**3. Do I have to take part?**

No! It is up to you. We will ask you for your agreement and then ask if you would sign a form called an "Assent form". We will give you a copy of this information sheet and your signed form to keep. You are free to stop taking part at any time during the research without giving a reason. If you decide to stop, this will not affect the care you receive.

**4. What will happen to me if I take part?**

You will stand on a vibrating platform (that is about the size and shape of typical bathroom scales) for 10 minutes for 7 days in a row. We will take some blood samples on the first day before the vibration and then again on 3 more days (days 8, 15 and 43).

Having breakfast may affect the blood tests we do, so we will ask you not to have anything to eat or drink except water, before we take the blood samples. You can have something to eat straight after the blood samples have been taken.

For the next 6 weeks, we will ask you to take the medicine risedronate once a week. This medicine has to be taken first thing in the morning before breakfast. We will explain exactly how you take it. You will also be taking another tablet (Vitamin D together with calcium) in the evenings every day for the same 6 weeks' period.

After the 6 weeks of having the medicine, you will have another blood test and then stand on the vibrating platform again for another 7 days. After this vibration, you will have another 2 blood tests, the same as before.

The first vibration and blood test will take place in the hospital, the rest can happen in your own home. On day 1, we will show you pictures that show you what happens during puberty/the growth spurt and ask you to tell us which one looks like you.

This study will take about 3 months in total.





Day 1 - blood tests before vibration

Day 1 to day 7 – standing on the vibrating platform (10 minutes)

Day 8, Day 15 and Day 43 – blood tests

Day 43 to day 84 – medicine taken by mouth  
(risedronate and vitamin D with calcium)

Day 85 – blood tests before vibration

Day 85 to day 92 – standing on the vibrating platform (10 minutes)

Day 92 and day 99 – blood tests

**5. What are the possible side effects of the medicine and vibration platform?**

There are unlikely to be any side effects. Some may have some itching while standing on the platform which will disappear on its own.

**6. What are the possible benefits of taking part?**

We cannot promise the study will help you but the information we get might help treat children and young people with brittle bone disease in the future.

**7. Contact for further information**

If you would like any further information about this study you could contact:

Name: Sivagamy Sithambaram  
Designation: Clinical Research Fellow  
Hospital/Department: Sheffield Children's  
Hospital/Academic Unit of Child Health  
Tel: 01142717561



**Thank you for reading so far - if you are still interested, please go to Part 2:**

## Part 2 - more detail – information you need to know if you want to take part.

### 1. What happens when the research project stops?

We will collect all the information together and we will use it to help us design other bigger studies in children with brittle bone disease.

### 2. What happens if new information about the research comes along?

Sometimes during research, new things are found out about brittle bone disease. Your doctor will tell you about this if it happens. If the study is stopped for any reason, we will tell you and arrange your continuing care.

### 3. What if there is a problem or something goes wrong?

Tell us if there is a problem and we will try and sort it out straight away. You and your mum, dad or carer can either contact the project co-ordinator:

Name: Sivagamy Sithambaram  
 Designation: Clinical Research Fellow  
 Hospital/Department: Sheffield Children's  
 Hospital/Academic Unit of Child Health  
 Tel: 01142717561



or the hospital complaints co-ordinator:

Mrs Linda Towers  
 Patient Advice & Liaison Co-ordinator  
 Sheffield Children's NHS Foundation Trust  
 Tel: 0114 271 7594

### 4. Will anyone else know I'm doing this?

We will keep your information confidential. This means we will only tell those who have a need or right to know. Wherever possible, we will only send out information that has your name and address removed.

We will also tell your family doctor (GP) that you are doing the study.

### 5. What will happen to any samples I give?

Blood samples obtained will be collected, used and stored following hospital procedures. In case, blood samples need to be used for future research, we would ask your permission. The researcher and research nurse will have access to the blood samples. The blood samples will only be identified by ID numbers.

### 6. Who is organising and funding the research?

The research is being organised by Sheffield Children's NHS Foundation Trust and paid for by SPARKS charity.

**7. Who has reviewed the study?**

Before any research goes ahead it has to be checked by a Research Ethics Committee. They make sure that the research is fair. This study has been checked by the Yorkshire & The Humber - Sheffield Research Ethics Committee.

It has also been checked by the Research Department at this hospital.

**Thank you for reading this – please ask any questions if you need to.**





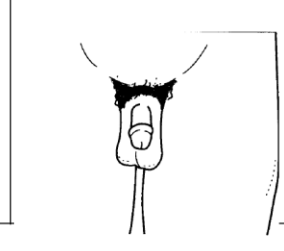
Pubertal Self Assessment questionnaire (boys)

Pubertal Self Assessment questionnaire (boys)

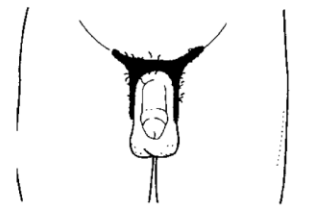
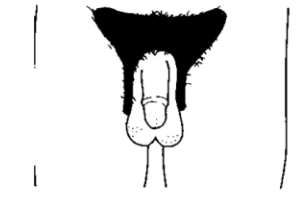
Subject number:-

FIRST look at the penis and scrotum (ONLY) in the picture. Please put a tick in the box that looks most like you now:-

SECOND look at the pubic hair (ONLY) in the picture. Please put a tick in the box that looks most like you now:-

1. Scrotum and penis same size as when you were younger	1. The scrotum has lowered a bit and the penis is a little larger	1. The penis is longer and the scrotum is larger
2. No hairs	2. Very little hair	2. Quite a lot of hair
		

1. The penis is longer and wider and the scrotum darker and bigger than before	1. The penis and scrotum are the same size and shape as an adult.
2. The hair has not spread over the thighs	2. The hair has spread on to the thighs

	
<input type="checkbox"/>	<input type="checkbox"/>

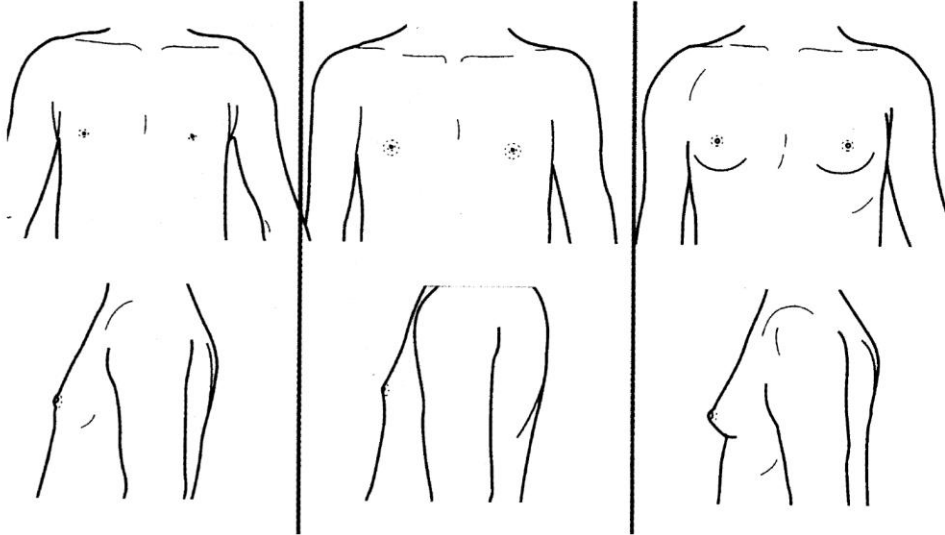
Pubertal Self Assessment questionnaire (girls)

Pubertal Self Assessment questionnaire (girls)

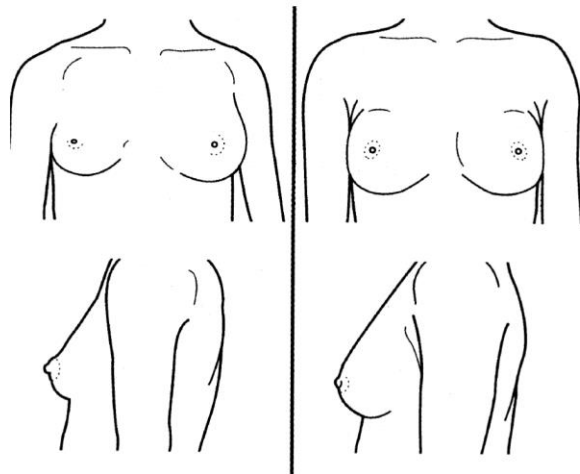
Girls (Breasts)

Subject number

Look at the breasts and put a tick in the box that looks most like you now:-



Your breasts are flat.	The breasts form small mounds.	The breasts form larger mounds than in 2.
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The nipple and the surrounding part (the areola) make up a mound that sticks up above the breast.	Only the nipple sticks out beyond the breast.
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Participant study number:

## PARENT/LEGAL GUARDIAN CONSENT FORM

**Title of project:** *BAMES Study*

Name of researcher:

Please initial box

1. I confirm that I have read and understand the information sheet dated 01.02.2017 (version 2.2) for the above study. I have had the opportunity to consider the information ask questions and have had these answered satisfactorily.
2. I understand that my child's participation is voluntary and that I am free to withdraw my child at any time, without giving any reason, without my child's medical care or legal rights being affected.
3. I understand that relevant sections of any of my child's medical notes and data collected during the study, may be looked at by researchers and those involved in the running and supervision of the study from Sheffield Children's NHS Foundation Trust or from regulatory authorities, where it is relevant to my child taking part in research. I give permission for these individuals to have access to my child's records.
4. I agree to my child's GP being informed of participation in this study.
5. I agree to my child taking part in the above study.
6. I agree to my child's blood samples being taken. If at any time, your child withdraws from the study, blood sample already collected with consent would be retained and used in the study. No further data or blood sample would be collected or any other research procedures carried out on or in relation to the participant.

\_\_\_\_\_  
Name of Parent/Guardian      Date      Signature

\_\_\_\_\_  
Name of Person taking consent      Date      Signature

When completed: 1 for parent; 1 for researcher site file; 1 (original) to be kept with hospital notes

Assent form

**ASSENT FORM FOR CHILDREN & YOUNG PEOPLE**  
 (To be completed by the child/young person and their parent/carer)

**Title of project:** BAMES Study

Participant study number:

Child (or if unable, parent on their behalf)/young person to circle all they agree with:

Has somebody else explained this project to you? Yes / No

Do you understand what this project is about? Yes / No

Have you asked all the questions you want? Yes / No

Have you had your questions answered in a way you understand? Yes / No

Do you understand it's OK to stop taking part at any time? Yes / No

Are you happy to take part? Yes / No

If any answers are 'no' or you don't want to take part, don't sign your name!

If you do want to take part, you can write your name below

Your name \_\_\_\_\_ Date \_\_\_\_\_

The person who explained this project to you needs to sign too:

\_\_\_\_\_  
 Name of Researcher                      Date                      Signature

Thank you for your help.

1 for participant; 1 for researcher site file; 1 to be kept with hospital notes