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Acute response of bone to whole body vibration in pre-pubertal boys with and without a history of fracture

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Summary

Research into risk factors for fracture have found that children who have narrower bones and lower bone mass are more likely to fracture than those with larger bones. Increased bone mass following whole body vibration has been demonstrated in postmenopausal women, young women with low bone mass and children with disabling conditions such as cerebral palsy. However little is known of the acute bone response to WBV in healthy children.

This thesis will determine the range and rate of the acute bone response to WBV in apparently healthy boys and in boys with a history of fracture. Boys were randomised to 10 minutes of WBV on 1, 3, or 5 consecutive days delivered by the Juvent 1000 (low magnitude, high frequency), Galileo Med M (high magnitude, high frequency) platforms or control. Fasted blood samples were collected pre- and post-vibration, on day 8 (and day 12 in the fracture cohort only) for markers of bone turnover, OPG and sclerostin.

P1NP and CTX increased from baseline to day 8 in the boys with no prior fracture by 25.1% and 10.9% respectively, but not in those who have a history of fracture. At day 12 the boys with a history of fracture demonstrated a non-significant decrease in CTX of 5%. No change was observed in either group in sclerostin, with a trend towards an increase in OPG in the boys with no prior fracture only at day 8.

This is a novel finding showing that apparently healthy pre-pubertal boys with a history of fracture do not respond to loading in the same way as those who have not fractured. If reduced responsiveness is present prior to fracture and is related to reduced bone accrual, this could to some extent explain increased fracture susceptibility in some children.

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Abbreviations

aBMD	Areal bone mineral density
BA	Bone area
BMC	Bone mineral content
BMD	Bone mineral density
BMP	Bone morphogenetic protein
BFR/BS	Bone formation rate/bone surface
BFR/BV	Bone formation rate/bone volume
BSALP	Bone specific alkaline phosphatase
BTM	Bone turnover marker
BV/TV	Bone volume/tissue volume
CT	Computed tomography
1CTP	C-telopeptide-1
CTX	C-terminal telopeptide of type 1 collagen
DKK1	Dickkopf 1
DPD	Deoxypyridinoline
DXA	Dual-energy x-ray absorptiometry
HMHF	High magnitude, high frequency
HRpQCT	High resolution peripheral quantitative computed tomography
Hyl	Hydroxylysine
Hyp	Hydroxyproline
Hz	Hertz
IGF-1	Insulin like growth factor 1
LMHF	Low magnitude, high frequency
LRP5/6	Lipoprotein receptor-related protein 5/6
LS	Lumbar spine
MAR	Mineral apposition rate
MESm	Minimum effective strain modelling
MESr	Minimum effective strain remodelling
MPa	Megapascals
MS/BS	Mineralising surface/bone surface
NTX	N-terminal telopeptide of type 1 collagen
OC	Osteocalcin
OPG	Osteoprotegerin
P1CP	Pro-collagen type 1 C-terminal propeptide
P1NP	Pro-collagen type 1 N-terminal propeptide
Pa	Pascals
pQCT	Peripheral quantitative computed tomography
PTH	Parathyroid hormone
PTHR1	Parathyroid hormone receptor 1
PYD	Pyridinoline
QCT	Quantitative computed tomography
RANK	Receptor activator of nuclear factor-κB
RANKL	Receptor activator of nuclear factor-κB ligand
RUNx2	Runt-related transcription factor 2
SMI	Structure model index
Tb.N	Trabecular number
Tb.Sp	Trabecular separation
Tb.Th	Trabecular thickness

TRAP	Tartrate-resistant acid phosphatase
vBMD	Volumetric bone mineral density
vTBMD	Volumetric trabecular bone mineral density
WBV	Whole body vibration
WHO	World Health Organisation
µCT	Micro computed tomography
µε	Micro strain

1. Hypothesis and aims of thesis

Research into risk factors for fracture have found that children who have narrower bones and lower bone mass are more likely to fracture than those with larger bones. Increased bone mass following whole body vibration has been demonstrated in postmenopausal women, young women with low bone mass and children with disabling conditions such as cerebral palsy. However skeletal benefits of WBV are not conclusive across all vibration studies, either in terms of the size of the effect or in its site specificity, and a number of studies have failed to show any skeletal benefits at all. This will be discussed in detail in Chapter 3, following an overview of bone in Chapter 2.

In healthy adult populations little or no effect of WBV on bone outcomes has been demonstrated, either in the immediate or longer term periods. Exercise studies show that the response from loading is greater in children than in adults suggesting that the response of bone to WBV may also be greater in the growing skeleton. However there are no published data on the acute effect of WBV in apparently healthy paediatric populations. It is not known if the bone response to vibration in such children would be similar to that seen in children with reduced bone mass.

Whilst the role of WBV as a therapeutic intervention to improve bone health is not clear, an alternative use for vibration would be as part of a stimulatory test to assess the skeleton's responsiveness to mechanical loading, similar to cardiac stress training using a treadmill. Use of such a form of testing could be advantageous if acute changes in bone formation or resorption were predictive of longer term response to pharmacological or other therapeutic interventions. The response to a standardised mechanical load could be assessed before, during and after an intervention in a variety of disease states both within and between groups of individuals. Such a test could also be used during clinical trials as a means to ensure that response to mechanical loading has not been abrogated by the new bone targeted compounds that are being developed. Knowledge of the acute paediatric bone response to WBV could be used to support the development of use of WBV in this manner.

Over the course of 2 studies (Chapters 6 and 7) this thesis will explore the acute response of bone to WBV to test the hypothesis that short term mechanical stimulation in the form of WBV will result in a change in bone turnover markers in an apparently healthy paediatric population.

The aims of these studies are to characterise the response of bone to whole body vibration, more specifically to identify the rate and range of response in a paediatric population. To further understand the acute bone response to WBV, the response of bone turnover markers in healthy boys with no prior fracture will be compared to those who have a history of having sustained at least one fracture. An exploratory aim is to compare different magnitudes of vibration (low magnitude <1g and high magnitude >1g) delivered on 2 different types of vibrating platforms to identify if any response is dependent on the size and method of delivery of the vibration signal. By characterising the acute bone response to WBV in a paediatric population, this thesis will generate new knowledge further contributing to what is already known about this type of intervention and will provide further scope on how it can be most effectively used in a clinical and/or paediatric setting.

2. Bone

2.1. Function

Bone has a number of functions. It provides support for soft tissues and point of attachments for muscles and tendons to give the body shape and form. When muscles contract, bones serve as levers to enable movement of parts of, or of the whole body. The rigid structure provides protection of the internal organs, for example the ribs protect the heart and lungs and the cranial bones of the brain. Additionally bone provides storage of several minerals such as calcium and phosphorus, and houses the marrow that has the function of producing cells. To serve these purposes bones vary significantly in shape and size, and need to be lightweight yet strong.

Classification of bones is based on shape with the human skeleton having five types; long bones which are longer than they are wide and include the femur and smaller bones such as the carpals; short bones are more cuboidal in shape such as those in the feet and hands; flat bones which are thin and curved such as in the skull; irregular bones are those that do not fit other shape categories such as the vertebra; and sesamoid bones which are found in tendons. This report will focus on long bones (femur, tibia and radius) and irregular bones (vertebrae).

2.2. Composition

Bone is a composite material made up mostly of a fibrous protein densely surrounded by mineral, along with water, living cells and blood vessels. The mineral, hydroxyapatite, is a crystalline form of calcium phosphate and accounts for 50-70% of bone, with the fibrous protein collagen (type 1) accounting for 20-40%. Collagen chains are twisted into triple helices which are linked together with covalent bonds into fibrils. The fibrils are arranged in layers and the mineral is deposited in between the layers (1). The mineral gives bone

stiffness, but is very brittle and on its own can be easily crushed. The collagen provides strength.

Bone is composed of a hard outer layer of densely packed compact bone, the cortical bone and an inner core of spongy trabecular bone. Covering the outside of the cortical bone is a membrane called the periosteum, whilst the inside is covered with a lining surface called the endosteum.

Cortical bone is organised into cylindrical shapes called osteons. Osteons are made up of concentric layers of lamellar bone surrounding a central (Haversian) canal that houses blood vessels and nerves. Distributed between the lamellar bone are small spaces (lacunae) containing osteocytes (mechanosensing cells). The lacunae are linked to each other by canals called canaliculi to provide a network throughout the bone. Processes radiate from the osteocyte into the canaliculi which also contain extracellular fluid allowing the osteocytes to communicate with other cells within the bone tissues (2).

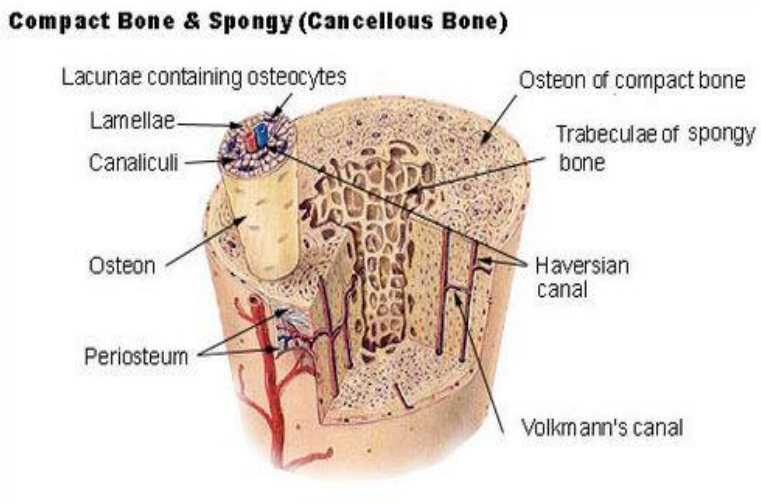


Figure 1 Cortical and trabecular bone. Image in the Public Domain taken from http://en.wikipedia.org/wiki/File:Illu_compact_spongy_bone.jpg

The inner core is made up of spongy, trabecular bone, which is formed in plate and rod-like struts with spaces in between. Like cortical bone the trabecular are also made up of lamellar bone containing lacunae and osteocytes. The spaces in between the struts are filled with marrow which provides a blood supply and nutrients to the bone. This organisation of trabecular bone allows for a material that is both strong and light.

Irregular bones are made up mainly of trabecular bone, with a thin cortical layer that provides support and protection. In long bones the shaft, diaphysis, is hollow and narrower than at the ends. The diaphysis widens out to the metaphysis and above this, separated by the growth plate, the epiphysis rounds off the ends of the long bone. This region is filled with trabecular bone covered by a thin layer of cortical bone. Along the diaphysis the cortical bone is thicker than it is at the ends to help resist the stress of loads placed upon it (3).

Three types of bone cells are contained within or along the bone surfaces and are responsible for growth, maintenance, and repair of bone tissue; osteoblasts, osteoclasts, and osteocytes.

Osteoblasts are the bone cells involved in bone formation. They originate from mesenchymal stem cells, and through a process of proliferation and differentiation, precursor cells mature into osteoblasts (4). The mature osteoblasts are responsible for the synthesis of the bone matrix and subsequent mineralization. During formation of the matrix some of the osteoblasts become trapped and differentiate into osteocytes (see below). Others at the end of the formation period will remain on the surface as bone lining cells (5, 6).

Osteoclasts are large multinucleated cells responsible for bone resorption. They are derived from the same haematopoietic lineage as macrophages (5). Osteoblasts have an indirect role in bone resorption through their production of receptor activator of nuclear factor-κB ligand (RANKL) and osteoprotegerin (OPG). RANKL binds to the RANK receptors on the osteoclast precursor cells allowing osteoclast formation and activation. OPG is the decoy receptor for RANKL, by blocking the interaction of RANK – RANKL, OPG interferes with the formation and survival of osteoclasts (7). Mature osteoclasts settle onto the bone surface and form a ruffled border where the cell is in contact with the bone (8). Through this, acid and proteolytic enzymes are secreted to mediate bone resorption. At the end of the resorption osteoclasts undergo apoptosis (5, 6).

The third type of bone cells, osteocytes, are terminally differentiated osteoblasts. They are located in the lacunae throughout the bone matrix and are able to communicate with other cells within the bone via processes that radiate from the cell body into the canaliculi (as stated above). Osteocytes are thought to be important in detecting, and co-ordinating responses of the bone to mechanical loading, sending out biochemical signals that influence bone formation and resorption (2, 7). Located within fluid filled spaces osteocytes sense the fluid flow and sheer stresses that are the result of bone deformation due to mechanical loading. Mechanically sensitive ion channels, G protein-coupled-receptors or focal adhesion complexes are thought to have a mechanosensing role within the bone cells (9, 10). Tension and stretch on the cell membrane instigates calcium signalling and the activation of a number of complex pathways that regulate the bone response to loading. Gap junctions and hemichannels, specifically connexin 34, enable intercellular communication between the bone cells and the passage of small signalling molecules (11, 12). Reported immediate and short term signalling responses to loading are an increase in cellular calcium concentrations and release of adenosine triphosphate, closely followed by Prostaglandin E2 and nitric oxide release, and then activation of extracellular signal-related kinase 1/2 (ERK 1/2). Later, down regulation of SOST/DKK1 occurs. Disruption of these events has been shown to inhibit bone formation (10, 13, 14). Sclerostin, a protein encoded by the SOST gene and secreted by the osteocytes, inhibits bone formation through the Wnt signalling pathway, which plays a key role in the regulation of bone formation. Secretion of sclerostin by the osteocytes is inhibited by mechanical loading and therefore sclerostin is considered to be a key factor in the anabolic response of bone to loading (12, 14, 15).

2.3. Bone Modelling and Remodelling

Bone, even after growth is completed is active. As stated above bone cells are constantly working to maintain, repair and adapt bone to the loads placed upon it. This is managed through processes of modelling and remodelling.

Bone remodelling, briefly, is a process whereby damaged (fatigued) or old bone is replaced with new. This occurs in a closely coupled cycle of activation, resorption, reversal, formation

and quiescence, see figure 2 (16). During the activation phase recruitment of osteoclast precursor cells and infiltration of the bone lining cells occurs. This leads to the attachment of the mature osteoclasts to the bone surface and resorption of the bone matrix creating resorption pits. The resorption phase is completed with osteoclast apoptosis. During reversal, signals are sent out to recruit osteoblasts to these resorption pits. Recruitment of the osteoblasts leads to the formation phase whereby new bone replaces that which has been taken away during resorption. On completion of this remodelling cycle the bone reverts back to a resting or quiescent phase (16). Resorption takes only a few weeks, whereas the formation occurs over several months (6).

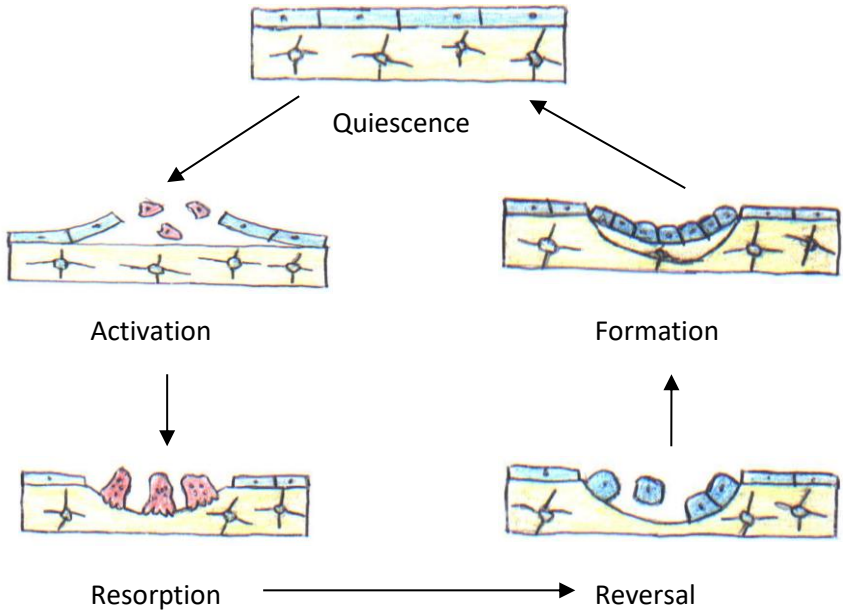


Figure 2 Bone remodelling cycle

Bone modelling is a process whereby formation and resorption is carried out independently to shape or reshape bone as is seen during growth or in response to mechanical loading (16, 17). Increased bone formation occurs in response to loading at skeletal sites experiencing the greatest loads (stresses) (18), and is repeatedly demonstrated by the changes in bone geometry and size as seen in the playing and non-playing arms in competitive tennis players (10). Expansion of the periosteal circumference occurs to increase bone size and therefore strength, whilst endosteal resorption occurs to expand the cavity of the bone ensuring

structural efficiency (10). Even within the same bone, formation is targeted at regions under the greatest load, showing that modelling is targeted to where it is needed the most (18).

Bone modelling due to growth is stimulated by a number of essential hormones. Growth hormone and insulin like growth factor 1 (IGF-1) are required for longitudinal bone growth, having crucial functions in the proliferation and stimulation of cartilage cells, growth at the epiphyseal plate and formation of collagen (19). Following longitudinal bone growth reshaping of the bone occurs to maintain its shape. During puberty, sex steroids influence further bone modelling. Periosteal expansion and changes within the trabecular microarchitecture are driven by testosterone in boys, with increases in bone size driven by IGF-1 in girls. Oestrogen also drives bone length through the accrual of bone mineral (20). Exercise itself has an influence directly on bone modelling through the process of increased loading, but additionally contributes to increased growth hormone secretion (20).

2.4. Biomechanics of Bone

2.4.1. Stress, Strain

It has been suggested that the most important property of bone is stiffness (21). Bone needs to be stiff enough so as not to bend too much under the various loads it is subjected to. Additionally it needs to be strong, a measure of the load it can bear before breaking. A load (stress) placed upon bone will result in a deformation (strain) even if the load is small. The stress, which is the force per unit area measured in pascals or megapascals (Pa or MPa), can be in compression, tension or shear, depending on how the load is applied. The resultant strain is reported as a percentage change in length or relative deformation (μstrain). A deformation of 1% = 0.01 strain = 10,000 μstrain (22, 23).

The relationship between stress and strain is shown in a load-deformation (stress-strain) curve. The load-deformation curve is divided at the yield point into an elastic region and plastic region. The yield point is the point below which bone can withstand a strain (deformation) and return to its normal size once the load has been removed, the elastic region. Above this point, the plastic region, bone is unable to recover from the deformation and permanent damage to the bone occurs.

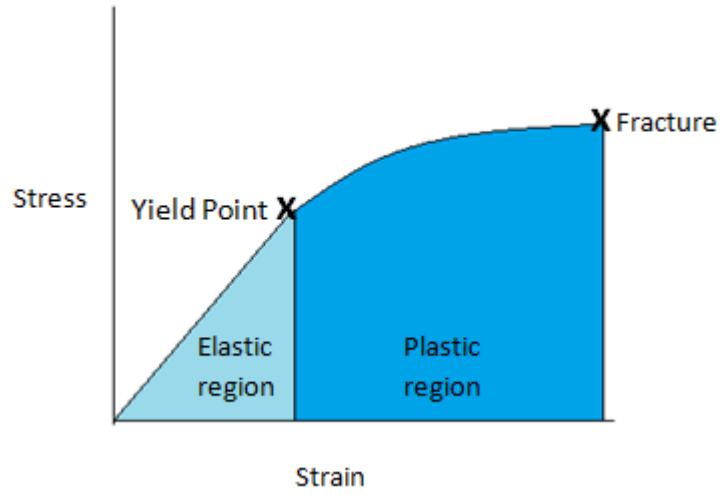


Figure 3 Stress strain curve

An increased yield load therefore demonstrates that the bone can withstand a greater load before damage occurs and thus is an indicator of increased toughness. Young's modulus of elasticity is a measure of the linear part of the stress-strain curve. The steeper this section the stiffer the material. Therefore an increase in the Young's modulus demonstrates an increased stiffness, which results in an increased resistance to deformation (23, 24).

2.4.2. Mechanostat Theory

Wolffs law first described the strong influence of function on the skeleton, acknowledging that bone responds and adapts to the loads placed upon it. Other than trauma and impulsive loads such as jumping from a height, the greatest loads on bone usually come from muscle contracting (25, 26).

Frost proposed a theory known as the Mechanostat, whereby strain thresholds control modelling and remodelling of bone in such a way as to ensure the minimum amount of bone tissue provides an optimum level of bone strength (25, 27). More mass than required to meet habitual daily loads would incur additional energy expenditure, create unnecessary bulk and weight and be inefficient (21, 28).

The Mechanostat theory states that thresholds exist that make the strains on a bone determine the bone strength (size, mineralisation and architecture). These strains switch on

and off the biological mechanisms that increase or decrease its strength. He referred to these thresholds as minimum effective strain for modelling (MESm) and remodelling (MESr). This theory proposes that strains experienced above the MESm will switch on modelling to strengthen the bone. Strains below the MESr will switch on disuse-mode remodelling whereby removal of bone occurs to reduce bone strength. The amount of strain required to reach the MESm is greater than for the MESr, where strains fall between these two thresholds a conservation mode of remodelling occurs and existing bone mass and strength is preserved (25, 27, 29). This is a dynamic process whereby changes in habitual daily loading may result in strains that move above or below the MES thresholds.

When strains exceed the MESm and the bone strengthens, under the same load the strain is subsequently reduced in the stronger bone. This reduced strain will then fall below the MESm threshold and modelling will switch off as further bone accrual is not required to meet the loading demand. Bone will revert back to the conservation mode of remodelling to maintain the adapted size and mass.

The same is the case when strains fall below the MESr, but in reverse. The disuse-mode of remodelling will result in a smaller weaker bone that under the same load will experience increased strains. Once these strains exceed the MESr disuse-mode remodelling will switch off as the bone will have reached an optimum strength and mass for the load it is placed under and further bone loss is not necessary. Therefore keeping habitual daily loads constant, that is between these two thresholds, will maintain bone mass and size.

It is thought that the low magnitude high frequency loads caused by muscles contracting during activities such as standing are just as important to bone adaptation as the more sporadic high magnitude loads caused by for example weight lifting (30).

2.4.3. Bone Adaptation to Unloading and Loading

There is much in the literature supporting the theory of bone adaptation to loading and unloading. This will not be discussed in detail here as this is not the focus of this report, however it is relevant in understanding the importance of loading (and ultimately vibration as a means of loading) on bone.

Following periods of bed rest in healthy male adults, loss of bone mass (bone mineral content – see section 2.5.2) has been observed in the leg (31), more specifically at the tibia, with the epiphyseal regions showing the greatest loss (32, 33). This has occurred following as little as 35 days of bed rest. Loss in bone density at the trochanter and total hip measured by dual-energy x-ray absorptiometry (DXA) has occurred after as little as 21 days of bed rest (34, 35). Although a small amount of bone loss in the forearm has been reported in one study (33) this has not been shown elsewhere (34-36). Bone loss during space flight in excess of that seen due to bed rest, has also been well documented with losses shown to occur at the spine, femur, legs and pelvis (36).

The effects of unloading by bed rest and space flight has also been seen in bone formation and resorption measured by serum and urinary markers of bone turnover. Formation has decreased (37, 38) and resorption increased (38-40) demonstrating the disuse-mode of remodelling as suggested by Frost above. This has occurred from as early as two days (resorption) from commencing bed rest (40) though the time of change in markers has varied according to the markers measured and due to the time points of sample collection. In girls with anorexia nervosa bed rest has resulted in a decrease in bone formation as early as three days (41). Others have reported no change or only minimal decreases in bone formation but acknowledge that there is still an uncoupling of the remodelling cycle and bone loss will occur as resorption exceeds formation (36, 40, 42, 43).

On return to weight bearing bone losses and changes in resorption markers are shown to return to baseline levels. However the time to recovery of bone loss takes longer than time taken for the loss due to disuse to occur, with suggestions of recovery occurring after 90 days of re-ambulation (31, 44). Resorption markers tend to return to pre bed rest levels by about 7 days (31, 38, 44), however there then appears to be a secondary transient increase after 2-3 weeks of re-ambulation. The formation markers have been shown to increase after return to weight bearing (31, 34, 36, 42) taking as long as 180 days to then return to baseline levels (44).

However this report is concerned with effects of loading on bone. Whilst the unloading literature supports the argument of bone as a dynamic organ that responds to increases and decreases in loading, only loading activities will be considered from here on. A number of exercise studies have indicated the impact of increased loading on bone and these will be discussed in Chapter 4. Chapter 3 will discuss the effects of WBV loading and report outcomes measured in bone.

2.5. Measuring Bone Properties and Activity

When evaluating the effect of an intervention on bone health a number of factors must be considered. The effect on size, shape, architecture, organisation, quality and quantity of bone are important features. A variety of methods has been used to investigate bone properties and activity, some of these will be outlined below.

2.5.1. Size

A simple measure of a whole bone is its size. This can easily be performed using plain x-ray to assess the length and width of the bone. Size is an important property as a bigger bone is a stronger bone. Doubling the size of the diameter of a bone will increase the (bending) strength by approximately eight times (29). Cortical thickness is also important and can be measured with more accuracy and detail using computed tomography (CT). This method of imaging can clearly differentiate between trabecular and cortical bone and can be used to calculate geometrical parameters such as the cross sectional area which is associated with bone strength.

2.5.2. Mass and Density

The terms bone mass and bone density are often used interchangeably and this can lead to confusion when assessing bone. Bone mass, referred to as bone mineral content (BMC) is concerned with the amount of bone present. Bone mineral density (BMD), however, is the amount of bone in a given area or volume (g/cm^2 or g/cm^3). Both mass and density can be assessed by dual-energy x-ray absorptiometry (DXA) or CT.

DXA, the method most used in clinical practice to measure BMD, is a measure of high and low energy x-rays transmitted through the body that discriminate between bone and soft tissue to calculate an areal bone mineral density (aBMD) and bone area; BMC is calculated aBMD x bone area (45). Its advantages include short scan times, low radiation doses, precision and wide availability of trained technicians and equipment (46). The World Health Organisation (WHO) classification of osteoporosis is based on bone densitometry measured using DXA. The level for diagnosis in adults is a T-score of ≤-2.5 SD below the young adult mean at the femoral neck (47), though other sites such as the lumbar spine and total hip can be used. In paediatric populations a diagnosis of osteoporosis should only be made when low bone mass adjusted for age and body size is present (a Z-score ≤-2.0) and there is a clinically significant fracture history (48).

CT can also be used to measure mass and density. Clinically it is used less than DXA due to higher radiation exposure and the need for repeated assessment, though is often used in research as less examinations are likely to be required. Although the radiation exposure is greater, Quantitative CT (QCT) has a number of advantages over DXA. DXA which measures a two dimensional areal BMD is affected by bone size, this can be problematic in short individuals or growing children. QCT is not size dependent as it measures a volumetric BMD (vBMD) (49). Additionally it can provide structural and geometrical parameters of bone, and unlike DXA is able to measure BMD at the trabecular and cortical compartment level rather than BMD of bone as a single unit. As trabecular bone is more metabolically active than cortical bone this enables QCT to be more sensitive to changes than DXA (50).

2.5.3. Architecture

Advances in QCT techniques have made it possible for parameters beyond density and geometry to be assessed. Detailed quantification of trabecula scale, topology and orientation can occur. These include amongst others, parameters such as trabecular number (Tb.N), thickness (Tb.Th) and separation (Tb.Sp), shape, connectivity, and orientation. The resolution to obtain these images is high, needing to be equal to or finer than the trabecular (51). At central skeletal sites the doses of ionising radiation required to obtain these images can be high, though similar to other types of x-ray imaging such as spinal radiographs (50).

However high-resolution peripheral QCT (HRpQCT) has been developed to measure architecture at the distal tibia or radius in vivo.

Historically histomorphometry techniques (microscopic analysis of bone tissue) have been used to measure bone architecture. However samples are analysed ex vivo, either biopsy samples from humans or whole bone samples from animals. The samples have to be processed and prepared, slices cut and placed onto slides. Micro-CT (µCT) which is at the highest end of the resolution spectrum of CT imaging can obtain similar static parameters of bone architecture to histomorphometry. Samples can be analysed whole so remain intact, though these still need to be ex vivo and only biopsy or small whole bone samples can be imaged at present. Both histomorphometry techniques and µCT are currently used in research.

2.5.4. Material Properties

In addition to the size and structure mentioned above, bone material needs to be of sufficient quality to serve its purpose. For this reason investigators may look to measure biomechanical parameters of bone.

Biomechanical tests such as loading a bone or piece of bone in compression or via three point bending are used to measure the strength and stiffness of bone. These methods correspond to the loads that are most likely to be placed on bone in daily activities, such as normal weight bearing, movement, falls and trauma.

Finite element modelling, which is analysis of the structural or mechanical performance of an object using a computer programme, can be used to simulate loading conditions and monitor the response of bone.

Mineral density backscattered electron microscopy and toughness atomic force microscopy can also be utilised to assess material properties of bone but will not be discussed further here.

2.5.5. Cellular Activity

Histomorphometry can be used to measure dynamic indices and therefore cellular activity as well as structural parameters of bone tissue. The most commonly reported dynamic indices in animal vibration studies are mineral apposition rate (MAR), mineralising surface/bone surface (MS/BS) and bone formation rate. MAR and MS/BS look at the speed and extent of mineralisation activity on the bone surface (52). A labelling agent is ingested or injected at two time points to deposit fluorescent labels at sites where bone is actively mineralising (53). MS/BS is a percentage of the surface that is labelled, whereas MAR is a measure of the distance between the surfaces that are doubly labelled (53). Bone formation rate is a measure of the volume or surface of bone formed per unit of time (54), calculated from the MS/BS.

Bone turnover markers (BTM) reflect the extent of bone formation and resorption and are also used to measure cellular activity. The breakdown products of formation activity can be detected in serum and of resorption in serum and urine. The markers that can be measured are listed in table 1. Recent research using BTMs as outcome measures have focused primarily on pro-collagen type 1 N-terminal propeptide (P1NP), bone specific alkaline phosphatase (BSALP), osteocalcin (OC), the C and N-terminal telopeptides of type 1 collagen (CTx, and NTx), and the pyridinolines (PYD and DPD). Analysis or imaging of samples and whole bones will give a picture of the skeletal activity at those sites only. BTMs are not site specific so give a picture of whole skeletal activity. This may be of relevance in certain metabolic bone conditions where treatment and monitoring is required throughout the skeleton.

Table 1 Bone turnover markers used in research and clinical practice

Formation Marker	**Resorption Marker (Serum)**	**Resorption Marker (Urine)**
Pro-collagen type 1 N-terminal propeptide (P1NP)	C-terminal telopeptide of type 1 collagen (CTX)	C-terminal telopeptide of type 1 collagen (CTX)
Osteocalcin (OC)	C-telopeptide-1 (1CTP)	N-terminal telopeptide of type 1 collagen (NTX)
Bone specific alkaline phosphatase (BSALP)	Pyridinoline (PYD)	Pyridinoline (PYD)
Pro-collagen type 1 C-terminal propeptide (P1CP)	Deoxypyridinoline (DPD)	Deoxypyridinoline (DPD)
	Tartrate-resistant acid phosphatase (TRAP)	Hydroxyproline (Hyp)
	Receptor activator of nuclear factor-κB ligand (RANKL)	Hydroxylysine (Hyl)
	Osteoprotegerin (OPG)	

3. Vibration Therapy

3.1. Types of Platforms

A variety of platforms to provide WBV therapy are now commercially available (this thesis will focus mainly on two). The main difference between these platforms is the method of delivery of the vibration stimulation. Vibration can be applied in a synchronous vertical or side-alternating direction. The Galileo vibration platforms (Novotec Medical GmbH, Pforzheim, Germany) are side alternating, working like a seesaw so that when standing on the platform as one end tilts downwards the other end tilts upwards (Figure 4). This motion is designed to mimic gait experienced during walking. Synchronous platforms such as the Juvent 1000 Dynamic motion therapy platform (Marodyne, Lakeland, Florida, USA) deliver a direct synchronous vertical vibration.

Figure 4 Top panel shows the Galileo platform, images from http://www.galileo-training.com/de-english/products/galileo-training-devices/vibration-training.html. Bottom panel shows Juvent 1000 platform

Juvent platforms, as stated by the manufacturer, deliver low magnitude high frequency (LMHF) WBV at a frequency of 32-37Hz, acceleration of 0.3g (where 1g = earth's gravitational field, or 9.8 m/s^2) and a displacement (amplitude) of 0.085mm. The Galileo Med M enables the user to set the frequency on the control panel from a range of 12-27Hz and the amplitude ranging from 0-4.5mm peak to peak by adjusting foot position on the platform to deliver high magnitude high frequency (HMHF) WBV. The acceleration will therefore be dependent on these two factors, though it has been reported to be as much as 15g (55). As the Galileo platform delivers side alternating WBV the amount of force transmitted through the body will be dependent on site and posture. Standing in a very erect position with legs straight (not recommended by the manufacturer) will mean that some acceleration will be felt in the upper body and head, with the knees bent this is diminished.

Strain within the human adult tibia (measured from an implanted strain gauge) following WBV delivered by the Juvent platform has been reported as tensile strain of 100 με to 150με and compressive strain of -70με, and in the Galileo 900 platform at 15Hz and an amplitude of 5mm this has been reported as 300με to 600με and -250με to -400με (tensile and compressive strain respectively). The strains recorded in the Juvent platform are comparable to walking, whereas in the Galileo these are comparable to jumping (56).

Listed side effects of the vibration platforms by the manufacturers are; skin lesions/blisters on contact parts, itching, nausea and dizziness, quick temporary drop in blood pressure, and drop in blood sugar in diabetics for the Galileo with none listed for the Juvent (discussed further in section 3.6.2). It is advised that WBV is contraindicated or should be used with caution in the following conditions:

Pregnancy

Congestive heart failure

History of deep vein thrombosis and/or pulmonary embolism

History of thrombophlebitis within 5 years

Sensitivity to motion sickness

Known retinal conditions

Joint implants

Pacemakers and implantable cardioverter defibrillators

Treatment/surgery for spinal conditions

Acute inflammation in the musculoskeletal system or recent fracture

Acute migraine

Directly post-surgery

Rheumatoid arthritis

Gall, bladder and kidney stones

Epilepsy

3.2. How the Platforms Work

There are two theories as to how WBV has an anabolic effect on bone. One suggestion is that the vibration causes low level strains to occur directly on the bone. Though the bone deformation (strain) is small the high frequency magnifies the signal, elevating intramedullary pressure and increasing fluid flow out of the bone (57). The shear stresses arising from fluid flow through the extracellular spaces in the canaliculi and lacunae (58) are thought to be detected by the osteocytes (the mechanosensing cells) which send out signals that cause osteoblasts and osteoclasts to be activated (2, 7).

The second school of thought is that WBV is osteogenic through the musculoskeletal forces acting on the body (59). As stated previously the greatest loads on bone come from the muscles (25). When standing on the platform the vibrations are transmitted into the muscles. Muscle spindles are stimulated by this vibration and activate the motoneurons which results in involuntary muscular contractions, known as the ‘tonic vibration reflex’. This reflex activity (short rapid changes in muscle length) detected as a degree of muscle stiffness, is an attempt to dampen the vibration occurring within the tissues (60-63).

3.3. Animal Models

Development of WBV interventions has been led by work from animal models, most of which have delivered LMHF synchronous WBV vibration. Studies have focused on adult and growing animal models and in some cases animals have additionally been exposed to ovariectomy or unloading. The reduced oestrogen levels caused by ovariectomy are associated with bone loss. As discussed in Chapter 2 disuse, which in the animal studies reviewed here is a result of hind-limb unloading, is also associated with bone loss. These models therefore consider not only the potential of vibration to increase bone, but also to prevent bone loss. Variations in the protocols used makes direct comparison between studies complicated, this will be discussed later in section 3.5, and different outcomes have been reported as presented below.

3.3.1. Biomechanics

In compression testing (Table 2) femoral bone from sheep exposed to LMHF WBV demonstrated increases in stiffness and failure strength of 12.1% and 26.7% respectively (64). This was confirmed by finite element modelling using the same data, which also showed increased stiffness in the anterior-posterior and medio-lateral directions of cubes of bone sampled from the femoral condyle (65). Work in rats also showed an increase in maximum load before fracture however it was dependent on the frequency of WBV (66) with only animals exposed to a higher frequency of 45Hz showing a significant difference. In the fourth lumbar vertebra of rats an increased Young's Modulus (measure of stiffness) and increased yield load (measure of toughness) has also been demonstrated (67).

Unlike compression testing, three point bending tests have generated contradictory results with a number of studies showing no effects of WBV on strength and stiffness in the tibia and femur (Table 2) (68-70). These studies exposed rats to WBV at frequencies of 50-90Hz. In other experiments when exposed to lower frequencies at 17Hz and 45Hz bending strength was increased by 12% and 8% respectively in the tibia (66), though not at 30Hz, suggesting that there may be an optimum frequency to improve bone strength. Young's modulus was shown to be higher in adult rats exposed to WBV compared to those not by as much as

Table 2 Biomechanical testing

Author, year	Sample studied, age	Groups studied	WBV duration	WBV parameters	Mechanical test	Change in experimental group	Sites studied
Brouwers et al 2010	23 Wistar rats, female, 6 months old	1. Sham OVX 2. OVX 3. OVX+WBV	20 mins x2, x5 weekly for 6/52	0.3g, 90Hz	3 point-bending	NS	tibia
de Oliveira et al 2010	30 Wistar rats, male, 3 months old	1. Control 2. Glucocorticoids 3.Glucocorticoids+WBV	30 mins x5 weekly for 9/52	1g, 60Hz	3 point-bending	NS	tibia
Flieger et al 1998	32 Wistar rats, female, 12 weeks old	1. Sham OVX, 2. Sham WBV 3. OVX 4. OVX+WBV	30 mins x5 weekly for 12/52	2g, 50Hz	3 point-bending	NS	tibia, femur
Jing et al 2016	24 diabetic mice, male, 12 weeks old	1. diabetic *db/db*control 2. diabetic *db/db*+WBV 3. WT control	60 mins daily for 12 weeks	0.5g, 45Hz	3 point-bending	+20.5% max load, +24.6% yield load, +42.1% stiffness in *db/db*+WBV v *db/db*	femur
Judex et al 2003	18 sheep, female, 6-8yrs old	1. Control 2. WBV	20 mins x5 weekly for 12 months	0.3g, 30Hz	Compression testing-Finite element modelling	+17% Stiffnes in longitudinal direction, +29% in anterior-posterior, +37% in medial-lateral direction	femur
Oxlund et al 2003	81 Wistar rats, female, 12 months old	1. control, 2. sham OVX 3. OVX 4-6. OVX+WBV	30 mins daily for 90 days	0.5g 17Hz, 1.5g 30 Hz, or3.0g 45Hz	3 point-bending, compression testing	Bending (tibia) @ 17 and 45Hz max bending strength +12% and +8%, Compression (femur) @45Hz max load +19%, @17, 30, 45Hz max stress +15%, +17%, and +17%	tibia, femur
Rubin et al 2002	18 sheep, female, 6-8yrs old	1. Control 2. WBV	20 mins x5 weekly for 12 months	0.3g, 30Hz	Compression testing	+12.1% Stiffness in longitudinal direction, +26.7% failure strength in longitudinal direction	femur

Author, year	Sample studied, age	Groups studied	WBV duration	WBV parameters	Mechanical test	Change in experimental group	Sites studied
Sehmisch et al 2009	60 Sprague Dawley rats, female, 6 months old	1. Sham OVX, 2. Sham WBV, 3. OVX, 4. OVX+WBV	15 mins daily for 5/52	0.5g, 90Hz	Compression testing	Yield load, Youngs modulus increased	LS
Vanleene et al 2013	24 B6C3Fe wild type, 24 B6C3Fe *oim* mice, female, 2 weeks old	1. Wild type WBV 2. Wild type Sham 3. OIM WBV 4. OIM Sham	15mins x5 weekly for 5weeks	0.3g, 45Hz	3 point-bending	+11.2% stiffness, +10.8% yield load in wild type WBV v wild type (did not reach significance in oim WBV v oim sham)	femur
Yang et al 2009	49 Sprague Dawley rats, male, adult	1. Control for 28 days, 2. HLU for 28 days, 3. WBV 28days, 4. HLU+WBV for 28days, 5. Control 49days, 6. HLU 28days then load bearing 21days, 7. HLU 28days then wbv 21days	15 mins daily for 28 days or 21 days post HLU	0.1-1.0g, 10-60Hz	3 point-bending	After 28 days +27.7% elastic modulus, (+43.3% HLU+WBV v HLU), HLU inhibited stiffness and ultimate force 35.3% and 19.6%, Days 29-49 (recovery) stiffness and ultimate stress +19.9% and +9.8% in HLU then WBV v HLU then load bearing	femur

Brouwers WBV commenced 8 weeks post OVX, Sehmisch WBV commenced 3 months post OVX, Rubin 1cm cubes harvested from medial condyle of left femur

HLU – hindlimb unloading, NS – not significant, OVX – ovariectomy, oim - osteogenesis imperfecta WBV – whole body vibration

27.7% (71). In leptin receptor deficient (*db/db*) mice and skeletally immature mice stiffness has been increased by 42.1% and 11.2% following 12 and 5 weeks of WBV respectively at 45Hz (72, 73), increases in yield loads of 24.6% and 10.8% were also observed in these models. When comparing two groups that had undergone hind limb unloading the group exposed to 15 minutes of WBV in addition to unloading had a 43.3% higher Young's modulus. Rats returned to weight bearing after 28 days of hind limb unloading had greater stiffness and strength when exposed to WBV also, though this did not reach control levels (71).

The lack of accord in the results from the biomechanical tests may be reflective of the type of bone being studied; trabecular bone is assessed by compression testing and cortical bone by three point bending. Therefore the consensus of results in compression testing illustrates that trabecular bone may be more responsive to vibration than cortical bone.

3.3.2. Bone Mineral Density

Most often interventions concerned with bone health report changes in BMD, the animal studies reflect this. A significant mean increase of 0.017g/cm^2 in tibial aBMD has been demonstrated in adult sheep exposed to 29 weeks of WBV compared to a decrease of 0.030g/cm^2 in controls (Table 3). A trend that was apparent though not significant after 54 weeks (57). Positive effects of WBV on aBMD have also been observed in bone loss models. In the early post-ovariectomy period (five weeks) WBV has been shown to diminish bone loss in the femur and tibia in rats by as much as 17% (69). Likewise the effect of unloading on aBMD has been suppressed in rats concurrently exposed to WBV by 10.1% and 7.1% in the femur and tibia respectively compared to rats exposed to unloading only (71).

However these findings are not conclusive, other studies have shown no change in aBMD as a result of WBV in the whole body or tibia of young adult and aged mice (74). Comparison of rats exposed to WBV with and without glucocorticoid steroids and controls also showed no difference in aBMD in the tibia (70). Although it has been demonstrated that WBV is able to suppress the effects of unloading, this intervention has been unable to restore aBMD to control levels following a period of unloading (71).

Table 3 Characteristics of animal studies measuring DXA

Author, year	Sample studied, age	Groups studied	WBV duration	WBV parameters	Change in aBMD	Sites studied
de Oliveira et al 10	30 Wistar rats, male, 3 months old	1. Control 2. Glucocorticoid 3. Glucocorticoid+WBV	30 mins x5 weekly for 9/52	1g, 60Hz	NS	Tibia
Flieger et al 1998	32 Wistar rats, female, 12 weeks old	1. Sham OVX, 2. Sham WBV, 3. OVX, 4. OVX+WBV	30 mins x5 weekly for 12/52	2g, 50Hz	NS Sham-V v Sham-C Loss of BMD in OVX v sham At 5/52 OVX 13.8-17% < OVX+WBV @ femur and tibial metaphysis	femur, tibia
Lynch et al 2009	82 BALB/c mice, male, 7/12 or 22/12	1. 7/12 sham, 2. 7/12 0.3g, 3. 7/12 1.0g, 4. 22/12 sham, 5. 22/12 0.3g, 6. 22/12 1.0g	15 mins x5 weekly for 5/52	0.3 or 1.0g, 90Hz	NS[a]	tibia, whole body
Rubin et al 2002	18 sheep, female, 6-8yrs old	1. Control 2. WBV	20 mins x5 weekly for 12 months	0.3g, 30Hz	At 29/52 WBV 0.044% > control @ tibia	Femur (ex vivo), tibia
Yang et al 2009	49 Sprague Dawley rats, male, adult	1. Control for 28 days, 2. HLU for 28 days, 3. WBV 28days, 4. HLU+WBV for 28days, 5. Control 49days, 6. HLU 28days then load bearing 21days, 7. HLU 28days then WBV 21days	15 mins daily for 28 days or 21 days post HLU	0.1-1.0g, 10-60Hz	NS - control v WBV HLU @ femur -18.8%, tibia -16.7%, LS -29.1% v control HLU+WBV @ femur +10.1% and tibia +7.1% v HLU HLU+WBV < control 28 days HLU then WBV or load bearing 21 days < control	femur, tibia, LS

[a]Significant 5% increase in BMC of tibia of 7 month old mice exposed to WBV

Total volumetric bone mineral density (vBMD) measured by pQCT, of the proximal femur of sheep was not improved by WBV (57). However, when this was separated into cortical and trabecular compartments, trabecular density was 34.2% greater in the experimental animals. Volumetric bone density loss in rat tibial trabecular and cortical bone caused by ovariectomy was not prevented by WBV (75). This model did though show a greater cortical area in the ovariectomised rats exposed to WBV at 30Hz, 0.3g compared to those not, indicative of an attempt to strengthen bone by size if not by density.

The findings from these studies are mixed. Of the five studies reported above only one (57) demonstrated a change in aBMD or vBMD in healthy animals. It could be suggested that WBV in animal models may be most beneficial in preventing bone loss as opposed to increasing bone density, as measured by DXA or QCT.

3.3.3. Bone Morphology

Areal and volumetric BMD does not give a complete picture of the response of bone tissue to WBV. pQCT offers more detail than DXA by differentiating between trabecular and cortical bone, though µCT as stated before can provide even further detail of the structure and configuration of bone tissue. Evidence of improvements in the amount of trabecular bone following WBV has been shown measured by µCT . Sheep exposed to one year of WBV have shown an increase in trabecular bone volume/tissue volume (BV/TV) of 10.2% at the femoral condyles (64). A similar though not significant trend was shown in the distal femora in mice, with a significant increase of 43% BV/TV in the proximal tibia of the same animals (76). In disease models, diabetes and osteogenesis imperfecta, increases of BV/TV of up to 72.3% in the femur and tibia have been shown (72, 73). Other papers have failed to confirm these findings (Table 4).

However bone strength is not only concerned with the amount of bone present. Bone distribution, orientation, and shape may be important in terms of strength and stiffness. Alterations in trabecular number (Tb.N), trabecular thickness (Tb.Th), trabecular separation (Tb.Sp), connectivity and trabecular shape have been demonstrated. In the femur increases in Tb.N of 8.3-54.4% (64, 72) and of Tb.Th by +11% (77) have been shown. At the tibia a

Table 4 Characteristics of animal studies using µCT

			Trabecular						Cortical			
Author	**Site**	**Model**	**BV/TV**	**Tb.Th**	**Tb.N**	**Tb.Sp**	**Conn.D**	**SMI (TBPf)**	**Ct.Ar**	**Ec.Ar**	**Ps.Ar**	**Ct.Th**
Brouwers et al 2009	Tibia	OVX	NS	NS	NS	NS	NS	NS	na	na	na	NS
	Femur		NS	**4%**	NS	NS	NS	**-43%**	na	na	na	na
Chritiansen et al 2006[a]	Tibia	adult	**+43%**	**+12.1%**	**-11.1%**	**+5.9%**	na		na	na	na	na
	Femoral Condoyle		NS	**-8.4%**	**-4.6%**	NS	na		na	na	na	na
	Distal femur		NS	NS	**-9.3%**	**+9.7%**	na		na	na	na	na
	Proximal femur		NS	NS	NS	NS	na	NS	na	na	na	na
	Vertebra - L5		NS	NS	NS	NS	na	NS	na	na	na	na
de Oliveira et al 2010	Tibia	Glucocorticoid steroids	NS	NS	**+29.8%**	**-26.5%**	na	na	**na**	na	na	**na**
Garman et al 2006[b]	Tibia	local vibration	NS	NS	NS	NS	NS	NS	**+8.4%**	NS	NS	**+8.2%**
Gnyubkin et al 2016	Femur	adolescent	NS	NS	NS	NS	NS	NS	**+6%**	na	na	**+5%**
	Vertebra - L2		NS	NS	NS	NS	NS	NS	na	na	na	na
Jing et al 2016	Femur	diabetic mice	**+72.3%**	NS	**+54.4%**	**-37.3%**	na	na	**+28.7%**	na	na	**+26.6%**
Judex et al 2007	Femur (epiphysis)	OVX	NS	**+11%**	NS	na	**-35%**	na	na	na	na	na
Lynch et al 2010	Tibia	adult v aged	NS	NS	NS	NS	NS	NS	NS	NS	na	NS
Rubin et al 02	Femoral condyle	adult	**+10.2%**	NS	**+8.3%**	**-11.3%**	na	**-24.2%**	na	na	na	na
Vanleene et al 2013	Femur	Osteogenesis	NS	NS	na	NS	na	na	**Inc**	na	na	**Inc**
	Tibia	imperfecta mice	**Inc**	NS	na	NS	na	na	**Inc**	na	na	**Inc**
Xie et al 2006	Tibia	adolescent	NS	NS	NS	NS	NS	NS	NS	NS	NS	na
Xie et al 2008	Tibia	adolescent	NS	NS	NS	NS	NS	NS	**+11%**	**+12%**	**+12%**	NS

[a]Control versus 0.1g WBV group only, [b]Vibration applied to tibia only - not WBV

Inc - increased (values not reported), na - not applicable/data not reported, NS - not significant

similar increase in Tb.Th was found although a decrease in Tb.N was shown (76). When comparing rats treated with glucocorticoid steroids those exposed to WBV did demonstrate greater tibial Tb.N of +30%, though this was still 24% less than for the rats in the control group (70). There is some contradiction in the findings presented here and also from other studies (see Table 4), with different methodologies being used. Though these findings suggest that WBV has an effect on bone, it is not clear whether more, thinner trabecular is more important than less, thicker structures (21).

Indices of connectivity (connectivity density and trabecular pattern bone factor), which is seen as important in bone strength (78), have shown contradictory results. Structure model index (SMI) is a measure of the shape of the trabecular struts. This increased in rats following ovariectomy (68) indicative of the more rod-like trabecular that is associated with osteopenia. In this study the rats exposed to WBV had a significantly lower SMI than those not suggesting the prevention of deterioration in bone trabecular structure.

Less data is available on cortical bone changes due to WBV, although significant increases in cortical area of up to 11% have been demonstrated in mice tibiae and 28.7% in femora (72, 79, 80), similar to findings from pQCT assessment (75), and cortical thickness (72, 80) which will enhance the strength of the bone. In these instances the mice were adolescent (including adolescent diabetic) or the vibration stimulus was applied directly to the limb and therefore may have been more receptive to the vibration signal.

The μCT findings are however potentially indicative of modelling of bone tissue into a structurally stronger organ in response to WBV.

3.3.4. Histomorphometry

Further, microscopic analysis of slices of bone tissue to assess for dynamic changes in bone tissue has also been reported, with the main focus on MAR, MS/BS, and bone formation rate (Table 5).

MAR & MS/BS

In the papers reviewed here two demonstrated a significant effect on MAR at the femur versus controls; an increase of 60% in cortical endosteum in growing mice following 3 weeks of WBV (81) and of 171.6% in trabecular at the epiphysis in adolescent diabetic mice (72). Both groups represent a time of rapid bone turnover due to longitudinal growth and therefore may have been more receptive to the vibration signal.

MS/BS in trabecular bone of the tibia and femur was as much as 2.4 fold greater as a result of a vibration intervention (30, 57, 72, 79-81). In cortical tibial bone an increase of 20% was seen as a result of vibration applied directly to the leg in mice (80) with a similar increase in an osteogenesis imperfecta mouse model (73). This is in contrast to the lack of a vibration effect seen in ovariectomy models.

Disuse suppressed MAR and MS/BS. These values were however normalised to that of weight bearing controls in the group of rats exposed to disuse with ten minutes a day of WBV (30).

BFR

Animal models (adolescent, adult and ovariectomy) have demonstrated an increase in the bone formation rate with bone surface as referent (BFR/BS) at the tibial metaphysis (77, 82), tibial mid diaphysis (66), femur (though referent not consistently stated) (57, 72, 81) and vertebra (81) as a result of WBV. An increase in BFR/BS of 88% has also been shown in the tibia when non-weight bearing vibration was applied directly to the legs of mice (80), suggesting that the increase is not reliant on body weight to enhance the vibratory signal.

Rats exposed to ten minutes a day of WBV showed an increase in tibial BFR/BV of 97% over controls (30). This study also looked at unloading; all groups exposed to disuse had suppressed BFR/BV compared to normal weight bearing. However for the disuse and ten minutes a day WBV group BFR/BV was only 7% below the normal weight bearing (control)

Table 5 Histomorphometry analysis of animal studies

				Trabecular			Cortical		
	Animal	**Model**	**Site**	**BFR/BS**	**MS/BS**	**MAR**	**BFR/BS**	**MS/BS**	**MAR**
Garman et al 06	C57BL/6J mice	Adult- local vibration	Tibia metaphysis for Tb. diaphysis for Ct.	**+88%**	**+64%**	NS	NS	**+20%**	NS
Gnyubkin et al 2016	C57BL/6J mice	7 weeks old	Femur	**Inc**	**Inc**	**Inc**	na	na	**+60%**
			Vertebra L2	**Inc**	na	**Inc**	na	na	na
Judex et al 02	BALB/cByJ mice	Adult	Tibia metaphysis	**+32.1%**	na	NS	na	na	na
Judex et al 07	Sprague-Dawley rats	OVX	Tibia metaphysis	**+159%**	NS	NS	**+31%**	NS	NS
Jing et al 2016	Diabetic *db/db*mice	12 weeks old	Femur	**+238.6%**	**+171.6%**	**+21.7%**	na	na	na
Lynch et al 10	BALB/c mice	Adult, Aged	Tibia metaphysis for Tb. diaphysis for Ct.	NS	NS	NS	na	NS	na
Oxlund et al 03	Wistar rats	OVX	Tibia diaphysis	na	na	na	**+84%**	NS	NS
Rubin et al 02	Warhill Sheep	Adult	Femur (proximal)	**+2.1 fold**	**+2.4 fold**	na	na	na	na
Rubin et al 01	Sprague-Dawley rats	Disuse	Tibia metaphysis	**+97%**	**+76%**	NS	na	na	na
Rubinacci et al 08	Sprague-Dawley rats	OVX	Tibia diaphysis	na	na	na	NS	na	na
Vanleene et al 2013	Osteogenesis imperfecta mice	3 weeks old	Tibia	NS	NS	NS	NS	**+17.2%**	NS
Xie et al 06	BALB/cByJ mice	Adolescent	Tibia metaphysis	NS	na	NS	**+30%**	na	NS
Xie et al 08	BALB/cByJ mice	Adolescent	Tibia metaphysis	NS	**+75%**	NS	na	na	na

Inc - increased (values not reported), na - not applicable/data not reported, NS - not significant

Garman- vibration to tibia only and ?low bone density strain of mice

group, demonstrating normalisation of the bone formation rate due to ten minutes a day of WBV.

In ovariectomy models increases in BFR/BS of 31% and 84% were detected in tibial cortical bone (66, 82). Loss of oestrogen is associated with an increase in cortical width and these animals may therefore have been more receptive to the vibration signal. In an adolescent mouse model bone formation rate declined over the duration of the study in the age-matched versus baseline controls by 70% (82). Bone formation measured by bone turnover markers decreases in the later stages of puberty (83-85), therefore this was an expected finding. However this decline was attenuated by WBV with mice exposed to vibration demonstrating a BFR/BS 30% greater than age-matched controls

Increased bone formation due to WBV has not been conclusively proven. A number of studies have identified no significant differences between groups. Judex et al showed that significant differences in bone formation rates was dependent on the referent used and the mouse model investigated, in their study low, mid, or high bone mineral density genotypes. Additionally the vibration parameters used may determine significance of the results. Bone formation rate was only significantly increased at 90Hz and not 45Hz compared to controls (86).

Bone Resorption

Whilst the effects of WBV on bone resorption have not been extensively considered, when investigating effects in adolescent mice, an age related decrease in osteoclastic activity of 25% in the tibial epiphysis was found, with a further decline of 31% in the mice exposed to WBV (82). A decline of 33% was detected in the tibial metaphysis though this region did not demonstrate an age related effect. These findings have though been contradicted by a non-significant effect of WBV on osteoclastic activity in this region (74, 79).

3.3.5. Biochemistry

Many of the procedures reported above that investigate bone responses to WBV require collection of partial or whole bone samples. They are therefore very invasive and cannot be

easily measured in humans. For this reason biochemical markers of bone turnover are used extensively for monitoring in clinical practice and to assess bone response to therapy. As changes can be observed directly within site specific bone tissue in animal models, less attention has been paid to serum biochemical markers. However it useful where they have been measured to acknowledge findings as this will help with translation of data from the bench to bedside. Also it may help to make sense of the serum changes observed in humans which are a measure of global skeletal change rather than site specific change.

No difference between animals exposed to WBV and controls were observed in the bone formation marker alkaline phosphatase (ALP) (67, 71, 87). However when comparing rats that underwent hind-limb unloading, those exposed to 15 minutes of daily WBV in addition to the unloading had a greater ALP than those not (71), suggesting that WBV may have a protective effect against bone loss. Changes in osteocalcin (OCN) were mixed with no evidence of a response in adult rats exposed to 6 weeks or less WBV (67, 87, 88), but an increase in leptin-receptor deficient *db/db* mice and ovariectomised rats versus controls after 8-12 weeks of intervention (72, 89, 90). P1NP was shown to be increased following 7 days of WBV in rats (91). Additional groups in this study were immobilised for 2 weeks in hind-limb pelvipedal casts; no difference was detected in P1NP between the groups exposed to WBV or sham WBV following casting, suggesting that a short period of WBV does not have a superior remobilisation effect.

Bone resorption measured by serum pyridinoline (PYD) was not different between groups exposed to WBV and those not (71). Normal weight bearing compared to WBV following a period of hind-limb unloading resulted in a PYD level 48.7% less in the WBV group, indicating less bone resorption. This was similar to the group who were normal weight bearing throughout the study. TRACP 5b and CTX were reduced in *db/db* mice and were not different to wild type controls, suggesting that 12 weeks of WBV can normalise bone resorption in this group (72). Inconclusively CTX has been shown to be increased following 7 days of WBV (91), decreased (up to 50% lower than controls) following 12 weeks and 6 months of WBV (72, 88), and not changed following 8 weeks of WBV versus controls (90). Potentially indicating a transient increase in bone resorption that tapers out and results in a

decrease over longer periods of WBV. No changes were observed in serum sclerostin (81) or RANKL (88).

3.4. Human studies

3.4.1. Bone mineral density

A number of skeletal sites that reflect those measured in clinical practice have been investigated for changes in BMD. Participants exposed to WBV have shown increased aBMD measured by DXA in response to WBV at the lumbar spine, femoral neck and total hip (Appendix 1).

Changes in spine BMD from baseline in groups exposed to WBV have been reported to be as high as +10.2% (92), with a net benefit of up to 3.35% in the treatment groups versus control groups (93). However findings have been variable and contradictory between studies, also significant differences between intervention and control groups have not consistently been shown (92, 94-103). Factors in addition to WBV may have influenced outcomes, for example one study using HMHF WBV (92) included concurrent treatment with Bisphosphonate therapy which is known to increase spine BMD. Two studies (LMHF WBV) recruited adolescents and young women with low bone mass (94, 104); bone mass accrual in the spine continues into early adulthood (105-107). A third study demonstrated an effect of HMHF WBV on spine BMD only in the group exposed to both WBV and resistance training (97). Other studies showing increases in BMD have included concurrent treatment with calcium and Vitamin D or physiotherapy; all but 1 of these studies were unable to demonstrate a difference between the WBV and control groups (94, 100, 102, 108-111). Compliance to the intervention and weight have also been shown to be important factors when evaluating changes in lumbar spine BMD following WBV (93, 110).

When considering other skeletal sites, BMD response at the total hip and femoral neck appears to be as variable between studies, though increases at the femoral neck and hip of up to 4% have been observed in groups exposed to both HMHF and LMHF WBV (97, 100, 108, 111-117). However difference in response between intervention and control groups

have been demonstrated in only a small number of studies (reported in 3 studies) with no difference between groups reported considerably more often (17 studies). At the radius no change in BMD (93, 101, 115) or loss of 3.5% compared to controls (108) has been reported, supporting the view that WBV has a beneficial effect on bone mass at weight bearing skeletal sites rather than a global systemic effect. Whilst there is some evidence to support an increase in BMD in response to WBV across weight-bearing skeletal sites this is clearly not consistent. A number of studies have failed to demonstrate within or between group BMD responses to WBV across varied skeletal sites (96, 101, 118-120).

To date a treatment effect of WBV on aBMD has not been shown in young healthy populations. Firstly this population has not been studied in detail despite the importance given in the literature to understanding and promoting bone health at an early age to protect bone health in old age. Secondly it has been suggested that in populations where bone mass is within normal limits an anabolic effect of WBV may not be seen. The lack of consistency in the findings of BMD measured by DXA could be due to the use of various vibration platforms, differing study protocols and intervention strategies, or the inability of DXA to detect small changes in BMD. Where trabecular rather than cortical changes have occurred (trabecular bone is more metabolically active) these may not be detected by DXA.

QCT

Though more sensitive to change a smaller number of studies have measured bone outcomes using QCT, most likely due to it being utilized less than DXA in the clinical setting for assessment of bone metabolism and monitoring of treatment. In research settings the effect of WBV on lumbar spine vBMD has been contradictory (Table 6). An increase of 3.8% over controls was detected in young women with low bone mass who complied most with the intervention (94), but not in older adults (121) or children with disabling conditions including cerebral palsy (122, 123). The lack of effect in the paediatric populations may reflect shorter intervention durations and fewer episodes of WBV. Additionally the children with disabling conditions, although able to stand independently, had limited mobility. Altered standing stance and posture may have limited the transmissibility of the vibration signal to the spine. As bone mass accrual in the spine continues into early adulthood, this

Table 6 Characteristics of studies measuring QCT

Author, year	Sample studied, age	N=	Concomitant therapy	WBV duration	WBV parameters	Control group	Change versus controls	Sites studied
Gilsanz et al 2006	Females with low bone mass, 15-20yrs	48	Calcium carbonate 500mg	10mins daily for 12/12	Vertical, 30Hz, 0.3g	Yes	+3.9% net benefit LS[a] +2.9% net benefit femoral cortical area	spine, femur
Gomez-Cabello et al 2014	Elderly	49		7.5mins x3 weekly for 11 weeks	Power Plate, 40Hz, 2mm	Yes	-0.89% net loss vBMD tibia	Tibia, radius
Hogler et al 2017	Osteogenesis Imperfecta, 5-16yrs	24	Bisphosphonate naïve, >2yrs, or 6/12 post	3x3mins x2 daily for 5/12	Galileo 20-25Hz amplitude 1-3	Age matched controls	NS	tibia
Kiel et al 2015	Older adults	174	Calcium 1000mg, Vitamin D 800iu	10 mins daily for 24-36/12	Vertical, 37Hz, 0.3g	Yes	NS	lumbar spine, hip
Lam et al 2012	Females with osteopenia and idiopathic scoliosis, 15-25yrs	149		20mins x5weekly for 12/12	Vertical,30Hz, 0.3g	Yes	0.084mm greater increase in tibial cortical perimeter	Tibia, radius
Liphardt 2015	Postmenopausal, osteopenic, 50-65yrs	42		10mins x2-3 weekly for 12/12	Galileo, 20Hz, 3-4mm	Yes	NS	Radius, tibia
Pitukcheewanont et al 2006	Girls with low bone density, 9.7 +/-1.5yrs	8		30mins x3weekly for 8/52	Vertical, 30Hz, 0.3g	No	+6.2% LS , +2.1% femur (not versus controls)	LS, femur

Author, year	Sample studied, age	N=	Concomitant therapy	WBV duration	WBV parameters	Control group	Change versus controls	Sites studied
Russo et al 2003	Postmenopausal,	29	Calcium carbonate 1g, Vitamin D 0.25µg	3-6mins x2 weekly for 6/12	Galileo, 12-28Hz, 0.1-10g	Yes	NS	tibia
Soderpalm et al 2013	Duchenes Muscular dystrophy 5-12yrs	6	Not stated	2mins x2-3 weekly for 2 weeks, then 6mins for 12 weeks	Galileo 16-24 Hz, 2.1-4.6g, 4mm	No	NS	tibia
Slatkovska et al 2011	Postmenopausal, 44-79yrs	202	Calcium 1200mg, Vitamin D 100iu	20mins daily for 12/12	30Hz 0.3g, 90Hz 0.3g or control	Yes	NS, but greater decrease in tibial tbth, tbsp, and increase in tbno in >60yrs or>10 yrs since menopause	tibia, radius
Torvinen et al 2003	Healthy non-athletic, 19-38yrs	56	No	4mins x3-5 weekly for 8/12	Vertical, 25-45Hz, 2-8g, 2mm	Yes	NS	tibia
Ward et al 2004	Disabling conditions, 4-19yrs	20	No	10mins x5 weekly for 6/12	Vertical, 90Hz, 0.3g	Yes	+17.7% net benefit tibia	spine, tibia
Wren et al 2010	Cerebral Palsy, 6-12 yrs	31	Not stated	10mins daily for 6/12 then 6/12 off platform	Juvent, 30Hz, 0.3g	Yes - crossover	tibial CtBA increased more during WBV (8.5%) than standing (4.9%)	spine, tibia

LS - lumbar spine, NS - not significant, tbth - trabecular thickness, tbsp - trabecular separation, tbno - trabecular number

[a]Per protocol analysis, lowest compliers pooled with controls

may partly explain the discrepancy in the response between the young women and older adults.

A beneficial effect of WBV on tibial volumetric trabecular bone mineral density (vTBMD) was found, with a +17.7% greater change in the intervention than control group in the children with disabling conditions (122), whereas in the elderly a net loss of 0.9% was observed (95). Increases in femoral and tibial cortical area of 2.9% and 3.6% have been shown in the young women and children with cerebral palsy respectively (94, 123) and in tibial cortical perimeter of 0.14mm in adolescents with osteopenia and idiopathic scoliosis (104). However, other studies have failed to detect any differences in change between the intervention and control groups at the spine, tibia or hip (96, 99-101, 120, 121, 124). The 4 studies that measured the radius were unable to detect any change compared to controls (95, 99, 100, 104) again supporting the view that non-weight bearing sites may not respond to WBV. The difference in findings may reflect the varied populations observed; children with disabling conditions, young women with low bone mass, healthy adults, postmenopausal women and older adults. Length of duration of WBV either in months, or minutes of the intervention, does not seem to effect the significance of the findings. Of interest the 3 studies in the younger populations that observed differences in response between the intervention groups and controls were conducted on low magnitude platforms, no differences were observed in those exposed to high magnitude platforms.

Whilst a significant increase in vBMD was found in the spine of young women with low bone mass compared to controls this was not detected when measured by DXA, highlighting the increased sensitivity of QCT in detecting change. The lack of consistency in findings from both DXA and QCT studies strongly support the need for further investigation of this intervention. Other outcomes should be considered to support or refute current findings.

3.4.2. Bone Turnover Markers

Clinically biochemical markers of bone turnover are used in addition to DXA to assess bone metabolism and patient response to therapy. Bone formation or resorption can be measured in serum and urine to provide a picture of remodelling or modelling activity (125)

and therefore response of bone to unloading and loading conditions. The rate of bone turnover measured using biochemical markers is associated with BMD and fracture risk, particularly in postmenopausal women (126-128). As changes in bone turnover markers can be detected more rapidly than changes in BMD measured by DXA or QCT, they are ideally suited to evaluating short term effects of interventions on bone metabolism.

A number of different bone turnover markers have been measured in response to WBV in human studies. Similar to outcomes measured by DXA and QCT findings are diverse. A large number of studies have reported no change in bone turnover markers in response to WBV and/or no difference in the response between intervention and control groups (101, 108, 112, 115, 120, 121, 124, 129-131), whereas others have shown evidence of a significant response to WBV (92, 93, 97, 117, 132-137). However findings are not consistent between these studies with some showing changes in specific markers that others do not. Most commonly the bone formation markers measured have included osteocalcin, bone specific alkaline phosphatase (BSALP), and P1NP. The variety of markers should capture different periods of the bone formation process and therefore should detect both acute and longer term formation activity. Five studies that detected changes in bone formation included an increase in osteocalcin of approximately 10% following 1 episode of WBV (137) that was not detected in other studies at time points ranging from 3-12 months; an increase in P1NP of up to 35% at 12 weeks (132) that was not detected following 1 exposure and up to 2 years of intervention; an increase in BSALP of 16.6% at 8 weeks (138) and in alkaline phosphatase (not bone specific) of 24% at 3 months (117); and a decrease in alkaline phosphatase of 28% in postmenopausal women on concomitant bisphosphonate therapy (92). Bisphosphonate therapy is associated with a decrease in bone turnover markers (139), and was the most likely cause of the decrease in bone formation in this last study as there was no difference in response between the WBV and control groups. Evidence of increased bone formation following WBV measured by biochemical markers is therefore very limited. The variance in sampling time points may partially explain why some studies have been able to observe changes whereas others have not. It is likely that any response to WBV in bone turnover markers is transient and not sustained over long periods of time.

The resorption markers have more consistently but still not conclusively shown a reduction in bone resorption following WBV. CTX, NTX and hydroxyproline have decreased by as much as 12.6-30.9%, 34.6-56.7% and 3% respectively following a single episode and up to 12 months of WBV (92, 93, 131, 133, 135, 136). TRAP5b has been shown to increase immediately post vibration then decrease by 3.8-9.9% in line with other resorption markers (131, 135). Changes that have been detected in bone turnover markers have occurred in girls with low bone density, younger healthy adults and postmenopausal women. Populations that have not demonstrated a response in bone resorption markers are mixed and include healthy younger and older adults, postmenopausal and oestrogen deficient women, adults with osteoporosis and chronic stroke, and younger adults with low bone mass. Two studies investigating paediatric populations, Duchene's Muscular Dystrophy and severe motor disability, failed to demonstrate a response in either bone formation or resorption markers. A third study in overweight pre-pubertal boys observed an increase in CTX in the control group with no change in the intervention group, suggesting an abrogation of resorption following WBV.

In all cases where change following WBV has been seen it has indicated an uncoupling of bone turnover in favour of bone gain either by increased formation or a reduction in bone resorption. Beneficial effects on bone turnover markers have generally been observed in healthy populations rather than those with conditions known to impact bone metabolism, but not to date in a healthy paediatric population.

Other biochemical markers associated with bone remodelling have also been investigated to identify the likely pathways involved in response to WBV loading. Osteopontin, which initiates the ruffled border in osteoclastic bone resorption and has a role in osteoclastogenesis, was shown to be decreased after 16 weeks of twice weekly WBV plus resistive exercise in healthy adult females (97), indicating a negative effect on bone resorption and possible explanation for decreased CTX observed in other studies. Sclerostin, an important inhibitor of bone formation expressed by the mechanosensing osteocytes, has also been measured. In healthy younger men and postmenopausal women following 8 weeks and 1 episode of WBV respectively no change in sclerostin was observed. However in

younger healthy women sclerostin was shown to increase by 91% at 10 minutes post vibration on day 1 and decrease at the same time point on day 5 by 31% with an increase over the 5 days in the pre WBV level (140). Whilst this indicates an immediate response to WBV it does not explain the increase in bone formation that has been observed in the limited number of studies discussed above.

BTMs in Non WBV exercise

WBV has been proposed as an alternative method of exercise to load bone. Where the effect of WBV on bone turnover markers has been inconclusive, studies investigating traditional methods of exercise may offer some clarification as results have been more consistent. A transient rise in the formation marker osteocalcin of 11% and in the resorption marker CTX of 16% has been demonstrated in male adult cyclists after fifty minutes of using a cycle ergometer (141). In this same group BSALP increased by 12% as early as thirty minutes after commencing the exercise. All of the bone markers returned to pre exercise levels within 15 minutes of recovery. This demonstrates an immediate effect on bone formation and resorption that is limited to the period of exercise and immediate recovery only. Though not significant a similar trend was detected in P1NP in pre and late pubertal boys immediately post cycling and in early to mid-pubertal boys sixty minutes post cycling (142).

In contrast another study showed no change or a decrease in osteocalcin and P1NP, and no change in CTx and TRAP after 60 minutes of varying intensities on a cycle ergometer in adult athletes and controls (143). This contradiction in findings may possibly be due to the fact that as demonstrated by Maimoun et al levels could return to baseline within 15 mins of recovery; these samples were taken at 3 and 24 hours post exercise and may have missed the period during which a change in biomarkers could be demonstrated. In a polymetric jumping study boys and young men however did show changes 24 hours post-exercise in BSALP of +24.4% and +9.9%, in NTX of +23.5% and -5%, and in OPG of 5.1% and 16.1%. No change was detected in RANKL (144). Although the differences between the boys and men was not statistically significant the boys do appear to have a more pronounced response. The difference in the response at 24 hours between the 2 studies may be due to the

different types of exercise being assessed, high impact loading versus non-weight bearing activity. Treadmill running for 60 minutes in adult males at varying intensities demonstrated increases in P1NP of up to 31% during exercise but not in recovery, decreases in OCN of up to 5%, and increases in BSALP of 1-7% 3 and 4 days post exercise (145). OPG increased during exercise and recovery and was maintained 3 days post. CTX decreased during exercise and up to 3 hours of recovery though this may reflect the circadian rhythm rather than an exercise effect.

In assessing ongoing levels of activity on bone turnover markers, boys aged 12-18years old who regularly participate in a variety of sports have demonstrated a higher rate of bone formation (BSALP) than non-active boys (146). Interestingly boys participating in swimming sports had the highest BSALP levels, though it was the boys who played weight bearing sports that had a greater aBMD. In adult females both bone formation and aBMD at selected sites were greater in those undertaking high and medium impact sports as opposed to non-impact sports, as would be expected (147).

In a cross-sectional study of habitual physical activity in preadolescent girls, P1NP and BSALP was found to be higher and CTX lower in those who undertook higher levels of physical activity compared to lower levels of physical activity (18,695 +/-1244 and 7633 +/-1099 steps a day respectively measured by a pedometer over 7 days), with no difference in OCN (148). A higher level of unstructured physical activity (measured by questionnaire) is also positively associated with OCN and BSALP in adolescence (149).

Intense military training has been shown to increase the formation markers BSALP and P1NP from baseline at 2 months in both men and women (P1NP by 5.2% in men and 20.6% in women, BSALP by approximately 11-16%). The resorption markers CTx and tartrate-resistant acid phosphate (TRAP5b) also increased between 0 and 2 months. CTx returned to baseline levels at 4 months, whilst the formation markers remained elevated (150).

Increased BSALP, osteocalcin and CTx has been shown elsewhere (151, 152) but these programmes included an element of weight loss which is associated with an increase in

bone resorption and formation, and may have hidden any true effect of exercise. Though it is believed that the degree of resorption seen in one of these studies was less than would have been expected through weight loss alone and therefore exercise may have had a protective effect on bone by limiting the amount of resorption.

Bone remodelling is conducted in closely controlled bone modelling units, with the degree of resorption and formation finely coupled. What is not clear from these WBV and exercise studies, though has been suggested (147), is whether there is an uncoupling of this process resulting in greater formation than resorption. Changes in formation and resorption markers may not be significant per se but when considered alongside each other may have a significant effect on bone accrual during remodelling and modelling activity.

3.5. Variation in Vibration Regimes

It is clearly evident from all the studies that have been reviewed that there is inconsistency in reported outcomes and methodologies. To fully elucidate the impact of WBV loading on bone the type of platform used, the parameters (amplitude, frequency, acceleration) utilised and magnitude of the signal, the duration of interventions, exposure time to WBV, and populations studied should be considered.

3.5.1. Synchronous or Side-Alternating Parameters

A variety of platforms have been used in these studies delivering either synchronous, side-alternating, or combination vibration. The Juvent platform delivers a low magnitude signal below 1g. Other synchronous platforms and the Galileo side-alternating platform deliver forces that are much greater than this, reported as being up to 15g (55). Four studies have directly compared magnitude and method of WBV delivery as a means to assess superiority of one device over another or as a means to prove that WBV is anabolic to bone regardless of magnitude or method of delivery. In healthy young men a decrease in bone resorption (CTX) was observed in the Galileo (at 3.8g) but not Juvent (0.3g) group, however the change from baseline was not different between the platform groups (133). Similarly Corrie et al were unable in older adults to demonstrate a difference in bone formation response (P1NP)

between the Power Plate (synchronous) at 1.5g and Galileo at 3.6g (132). Likewise no differences between platform groups in aBMD change were observed in postmenopausal women when comparing Vibrafit (vertical) and Qionic (rotational) platforms both at 8g (110), and Juvent and Galileo (at ~1g) platforms (116). Only 1 of the studies was powered to detect between group differences but due to participant withdrawals did not have sufficient numbers in each group to achieve this. The two theories of how the platforms work in terms of strain (deformation) directly on bone creating shear stresses arising from fluid flow within the bone tissue, or indirectly via musculoskeletal forces attempting to dampen the vibration signal have not been clearly addressed. Currently evidence suggests that method of vibration delivery or magnitude of the signal in adults does not alter bone response to WBV.

3.5.2. Platform Parameters

Between the studies there is also great variability in the platform parameters; frequency, amplitude, and acceleration of the WBV (Table 7). Low magnitude platforms have generally delivered WBV (as stated) at frequencies of 30Hz but from 12-90Hz, amplitude (where reported) <0.1-0.5mm, and acceleration of 0.2-0.9g. High magnitude synchronous platforms have delivered WBV at 20-50Hz, 1-6mm, and 1.5-8g, and high magnitude side-alternating platforms have used 12.5-30Hz, 0.7-4.2mm, and 1-10g. It is difficult to make a definitive conclusion on the effectiveness of WBV when different parameters are used and the different studies present conflicting results. Improvements in bone stiffness and strength and in aBMD in the animal studies have been seen at frequencies of 50Hz or less only suggesting that there is a dose limiting effect. To determine if there is a similar limiting effect across all the parameters requires further investigation.

3.5.1. Duration of Intervention

The duration of the intervention has also varied between the studies. This is in terms of the length of time that the studies have been conducted over and the periods of exposure to the intervention. WBV has been applied for as little as two to thirteen weeks in animal models with the exception of one study lasting twelve months. In human studies the intervention has mostly occurred from three to twelve months, however a small number of studies have investigated only a single episode of WBV. The overall shorter duration of

animal studies is most likely due to the fact that the use of far more invasive techniques than in the human studies means outcomes can be detected earlier. The life span of the animals used is shorter than that of humans and any age related changes in bone health will be detected over a shorter time period. Human studies of less than 11 weeks duration have not assessed bone outcomes using DXA or QCT. To detect significant changes using DXA or QCT the measurements need to be taken at separated time points that allow for changes to be greater than the least significant change.

Table 7 WBV study parameters

	High magnitude synchronous platforms	**High magnitude side-alternating platforms**	**Low magnitude platforms**
Number of studies including type of platform*	16	15	14
Frequency (Hz)	20-50	12.5-30	12, 37, 32-37, 90
Amplitude (mm)	1-6	0.7-4.2	0.085, 0.5
Acceleration (g)	1.5-8	1-10	0.2-0.9
Minutes	1.5-30	3-13.5	10-30
Episodes of WBV (day or week)	1-5	1-5	twice weekly - twice daily
Duration (weeks/months)	once only - 12 months	once only - 12 months	8 weeks-36 months
Study populations	Overwieght pre-pubertal boys	Children with Duchene's Muscular Dystrophy	Children with disabling conditions
	Postmenopausal osteoporosis	Children with Cerebral Palsy	Children with Cerebral Palsy
	Postmenopausal eostrogen deficient	Children with Osteogenesis Imperfecta	Postmenopausal
	Postmenopausal	Postmenopausal osteoporosis	Older adults
	Obese postmenopausal	Postmenopausal osteopenic	Females with low bone mass
	Chronic stroke	Postmenopausal	Healthy young men
	Elderly	Elderly	
	Healthy 19-38yrs	Healthy young women	
	Healthy young women	Healthy young men	

* 4 studies investigated 2 different types of platforms

3.5.2. Frequency of WBV exposure

The frequency of exposure to WBV has varied from as little as once only and once a week to daily, and the period of exposures from 1.5 to 30 minutes at a time. This makes comparisons

of the studies very difficult as the actual total time that the animals or humans have been exposed to WBV is vastly different. Additionally reporting and accounting for participant compliance to the intervention is variable.

3.5.3. Cycles/rest periods

Some loading studies have looked at insertion of rest periods and the most effective number of cycles required to elicit bone responses to loading. Insertion of rest periods appears to increase the anabolic response of bone at the periosteal and endocortical surfaces (153). Strain magnitudes of 1000-1600 με delivered as 10-250 loading cycles were investigated showing that at the lower end of the ranges a four point bending load in mice was barely stimulatory, though resulted in a greater response when 10 second rest intervals were inserted. Robling et al (154) also found short rest intervals of 14 seconds to be effective when delivering 36 loading cycles, resulting in endocortical MS and BFR/BS that were 66-109% higher than in rats exposed to no, 3.5, or 7 second rest periods between each load. Insertion of longer rest periods when delivering 360 load cycles divided into four bouts of 90 cycles showed an 8 hour rest period to increase BFR/BS by 125% compared to no rest and by 102% with 0.5 hour recovery. It has been suggested that the bone response to loading saturates quickly and that rest periods allow the cells to recover their sensitivity to the mechanical signal (155). Therefore to obtain an osteogenic effect a lower number of cycles and lower strain magnitudes can be used when rest periods are inserted (156, 157).

The vibration studies reviewed here used a variety of regimes for time on and off the vibration platforms. Overall the animal models applied vibration over one cycle at each time, for example 10 minutes once a day, though two studies did provide WBV twice a day (67, 68) and one delivered the vibration over nine cycles (further detail was not given) (71). The studies concerning humans varied. Some followed the same pattern as with the animal studies, two separated the vibration into two cycles delivered twice a day, one specifying at least 10 hours between cycles. A number of protocols inserted short rest periods between cycles of 30 seconds to 2 minutes (or length unspecified) that stayed the same or reduced over time. It is likely that rest periods have been used in some instances as training to the platforms or for comfort. Other protocols either did not include rest periods or did not

report them. It is possible that insertion of rest periods may increase bone response to WBV to a level of significance that is not detected when rest periods have not been used. However, as suggested above this makes evaluation of the outcomes of these studies difficult. In addition to frequency, amplitude, acceleration and duration of WBV, cycles and rest periods should also be considered when comparing the reported findings.

3.5.4. Variability in study populations

The models or populations investigated have ranged in age, health, and physical abilities, often with small sample sizes used. In the animal models different species have been investigated. Though data collected from animal models can be used to inform human research, it may not be appropriate to directly apply findings especially in regard of growth and development (158-160).

In regard of the human studies, when evaluating BMD, which is the most used clinical assessment of bone health, increases as a result of WBV have been demonstrated in postmenopausal women, low bone mass cohorts, children with severe motor and disabling conditions, older adults, elderly and in combination with resistance training. Positive effects have not been reported in healthy populations, but WBV has not been assessed in healthy children. Gains in bone health, mass and strength, due to exercise and mechanical usage are greater in childhood than in adulthood (25, 161), suggesting that bone in children is more responsive to loading. It is possible therefore that a WBV intervention delivered over a period of time prior to the attainment of peak bone mass could be more effective also.

Having considered the variation in the vibration regimens and parameters used it is apparent that further work is required to make sense of the data collected to date. There is clearly some evidence to support the beneficial effects of WBV on bone, however no strong conclusions can be made, a consensus held by a small number of systematic reviews of WBV in older adults and children with disabilities (162-166). Further studies are required to clarify the optimal therapeutic regimens to ensure the safest and most effective outcomes of WBV interventions.

3.6. Safety

3.6.1. ISO guidelines

Before any medical equipment can be introduced to the clinical environment its effectiveness, appropriateness and safety must be established. Many studies in the realm of occupational medicine have looked at the dangerous and undesirable effects of WBV on the human body. Negative effects have been reported in a number of physiological systems including the spine, peripheral nervous system, digestive system, visual and vestibular system, female reproductive system, and also to the foetus in pregnancy (167-170). International standards have been devised as to the acceptable levels of vibration that people can be exposed to, ISO 2631-1. Investigation into the amount of exposure as experienced during typical WBV training regimes has shown that the estimated vibration dose value on both side-alternating and synchronous (high magnitude) platforms may exceed the recommended daily exposure as per the ISO guidelines (167). This view has been supported by others, however they argue that the high transmissibility of the signal can be minimised (171). Maintaining a squat or semi-squat posture whilst standing on the platform is preferable and sitting or lying on vibrating platforms should be avoided. It should also be noted that the ISO guidelines were based on data collected regarding vibration in seated occupational environments and is likely that the limits cannot be directly transferred to WBV in therapy or rehabilitation (59). WBV should however be used with caution.

3.6.2. Side effects of WBV therapy

Minimal side effects have been reported in the use of vibration platforms to deliver WBV. Where side effects have occurred they have resolved quickly once exposure to the intervention has been removed. Faintness, a seasick like reaction, headache, itchiness of the feet, and localised pain at the end of a medullary rod have been reported (93, 167, 169, 172).

In a cystic fibrosis study evaluating the effects of WBV a number of adverse events were reported (173). These included a discomfort in the head during vibration, a manifestation of diabetes mellitus, joint effusions of the knee, and in a patient with an intravenous port, a

new thrombosis of the superior vena cava. These effects could be explained however as resulting from the participants' underlying diseases and therefore it is unclear if they can be attributed to the vibration intervention. This does though make it clear that the health or disease state of the persons exposed to WBV must be considered prior to embarking on such an intervention.

Generally in all the studies reported the vibration intervention has been well tolerated. At the end of one placebo controlled trial, participants were asked to guess whether they had been exposed to the intervention (synchronous WBV) or placebo (93). Approximately 30% of the control group wrongly thought the intervention, and even 20% of those exposed to WBV could not tell. Although WBV regimes have been well tolerated compliance has been variable (94, 122, 174) with reports of compliance as low as 1%.

4. Relevance in childhood

4.1. Variation of WBV studies in childhood

The effect of WBV on bone in paediatric populations has only been investigated in a small number of studies to date with conflicting results. Only 3 out of 11 were able to detect any beneficial effect of WBV measured by DXA or QCT compared to controls (104, 122, 123); 3 were unable to demonstrate any response to WBV at all (96, 118, 120); the remaining 5 demonstrated improvements that were not significantly different to controls or studies did not include a control group (94, 109, 129, 134, 138). Similarly conflicting results were found in bone turnover markers. Therefore understanding of the effects of WBV on the growing skeleton is limited. If WBV is considered to be an alternative method of physical activity to load bones, then the literature regarding more traditional types of exercise can be used to guide understanding of the importance of exercise on bone health in paediatric and adolescent populations. For a number of years leaders in the field of bone health have been postulating the importance of optimal bone health in childhood as a means to protect bone health in later life, especially in regard of the expected bone loss and potential for fractures. This is working from the premise that the more bone that is accrued during childhood the longer it will take for bone loss to become clinically significant in adulthood.

4.2. Effects of exercise on bone during childhood

Positive effects of exercise on bone have repeatedly been demonstrated in childhood and adolescence. Elite pre pubertal gymnasts have demonstrated significantly greater aBMD at total body, spine, legs and arms compared to controls and have continued to increase aBMD 30-85% more rapidly over a twelve month period (except at the arm) (175). Comparison of the playing and non-playing arms in forty-seven competitive tennis players showed an increase in BMC of 12% at the humerus in pre-pubertal players, with a similar difference seen in the peri- and post-pubertal players (176). In the pre-pubertal group cortical area was 8-11% greater in the playing arm. Similar findings have been demonstrated in male tennis

players with increases in BMC at the playing humerus of 17%, 27.5%, and 18.1% in the pre-, peri-, and post-pubertal groups (177). Increases in cortical area of 12-32.7% were detected across the three groups and as with BMC the greatest difference was seen in the peri-pubertal players.

Studies looking at less intense exercise regimes (the above athletes participated in tennis for 6-14 hours a week or up to 36 hours a week of gymnastics), have also demonstrated differences in bone parameters as a result of physical activity. Increases in aBMD at the femoral neck (178), and in BMC at the proximal femur, femoral neck, lumbar spine, total body, legs and distal forearm have been reported (179-184) as a result of interventions such as jumping, aerobics, and other weight bearing exercise. Non-interventional studies, whereby participants have self-selected and self-reported physical activity levels, have also demonstrated positive associations with bone mineral accrual across different skeletal sites (TB, LS, femoral neck and hip BMC). In the first of these studies weight bearing physical activity levels were greater than the 3 hours a week recommended by the US Health and Human Services department (185). The second study demonstrated that the greatest effects of physical activity were in those reporting vigorous physical activity compared to those reporting low physical activity (finding sports outside of school quite hard to extremely hard versus not hard at all to a bit hard) (186); highlighting that although some exercise is good more is better. Physical activity in childhood has also been positively associated with indices of bone strength (187, 188).

However the findings from these studies have varied by their extent, skeletal sites, and gender of participants. Additionally differences between intervention and control groups have not been demonstrated across all pubertal stages. Some studies have failed to detect an effect of reported physical activity or exercise intervention strategies on bone size, mass or biomechanical changes in pre-pubertal girls (189-191) or early pubertal girls (191). In a study where peri-pubertal female dancers demonstrated greater BMC versus controls at the total body, femoral neck, lumbar spine and lower limbs of 0.6% to 1.3%, the pre-pubertal dancers only showed an increase (of 4%) in the femoral neck (192). Moreover pre- but not post-menarcheal girls have been found to be responsive to a nine month step aerobic

programme (181). Pubertal stage of boys has also been shown to influence bone response to activity. A daily jumping intervention was effective at increasing the bone strength index in pre- but not early pubertal boys (187). As mentioned before the humerus in peri-pubertal boys appeared more responsive to playing tennis than the pre- and post-pubertal boys (177).

Additionally outcomes have also differed between boys and girls with some intervention strategies resulting in benefits to bone health in girls that were not seen in boys (forearm) (179) and vice versa (weight bearing sites) (187, 191).

This inconsistency in detecting a positive association of exercise and bone outcomes in the different gender and pubertal groups suggests that there may be a time during growth when bone is the most responsive to loading. It has been suggested that the early pubertal period rather than pre- or post- results in greater increases in bone parameters (193). This view has been supported by others who suggest that physical activity is associated with greater bone mineral accrual around the period of peak height velocity and peak BMC velocity, which occurs during early to mid-puberty (183, 194). With girls reaching maturity before boys it is important to note that the window of opportunity to increase bone mass or size beyond that expected by growth alone may be shorter in girls.

4.3. Maintenance of bone into adulthood

Much debate has occurred over the maintenance of the gains seen in bone mass during childhood and adolescence into adulthood, and also following the cessation of exercise or study interventions. Bass et al (98) in their study of elite gymnasts found that the greater aBMD versus controls observed with active gymnasts was maintained 8 years from retirement by 6-16% at the total body, spine, legs and arms. Boys who started a sport activity at a mean of 8.7 years of age and played for an average of 5.4 years, showed improved bone outcomes 3.4 years after ceasing to be active versus boys who were always inactive (195). At the tibia and radius periosteal circumference was 3.2% and 2.1% greater, and trabecular vBMD 3.5% and 5.3% greater respectively. One year after cessation of a 9

month jumping intervention girls had a percentage increase in LS BMC that was 6% greater than controls (196). In contrast the 2% gain in LS BMD in male and female children who participated in a 7 month jumping intervention disappeared as early as 7 months from cessation of jumping (197). This group did maintain an increase in hip BMC 7 years post intervention though it had dropped from 3.6% to 1.4% (198).

Other studies have reported a smaller change in LS BMC and aBMD in young females who discontinued physical activity for at least a year during the 3 to 7 year period of a 7 year follow up (199). More so this group lost BMC and aBMD at the femoral neck supporting other evidence that continuing activity is required to maintain the gains in bone mass due to exercise. However it is not reported whether this loss resulted in bone mass being the same as, less or greater than controls.

As with the vibration studies comparison of the reported bone outcomes from exercise studies is complex. Intervention strategies have varied in duration and intensity, methods of assessment and reporting have differed, study populations have differed and study designs have not been consistent. However the data presented here suggest that quick, easy and cheap physical activity interventions can have a positive effect on bone health in pre- and early pubertal children (180). It is still not clear if the beneficial effects of loading exercise on bone are maintained into adulthood or indeed what exercise regime is best in terms of duration, intensity, and frequency (200). Despite this it has been suggested that recommending exercise during growth may help to develop a habitual active lifestyle and promote bone health in adulthood (194). Further work in this area of bone health is required, and additionally the role that WBV can play in this arena should be considered.

5. Fractures in Children

Much of the drive for improving bone health in adulthood is to reduce the burden and economic impact of fracture particularly in older age. As previously stated leaders in the field of bone research now recognise that fracture prevention should occur over the life course and establishing good bone health in childhood is of paramount importance. Nonetheless interventions to better understand and improve fracture incidence in later life are also important for bone health during childhood. This chapter will highlight a number of factors that are associated with fractures in childhood demonstrating the complexity of the issues surrounding bone health in paediatric populations.

5.1. Incidence and type of fractures in childhood

Fractures in children are common. Approximately 50% of children will sustain a fracture during growth (201-203), corresponding to a fracture incidence of 103-197/10,000 person years for girls and 162-257/10,000 person years for boys (204-208). The consequences of fractures during childhood include missed days at school, activity restriction (5-26 days depending on site of fracture), hospital appointments, and clinical complications such as compartment syndrome, growth disturbance, and impaired peak bone mass accrual (209-212).

Fracture incidence has increased over time. Landin et al (208) reported a two-fold increase in the risk of fracture in boys and girls over the thirty year period from 1950 to 1979. The same increase was reported in Japanese school children comparing the period of 1979-1987 and 1999-2007 (213). Specifically an increase in forearm fracture incidence in boys of 32% from 1999-2001 compared to 1969-1971 and 56% over the same time periods in girls has been reported in the US (214) and an increase of a 31% in girls in Sweden comparing data from 1993-1994 to the 1975-1979 data reported by Landin et al (206). However despite the increase in forearm fractures an overall decrease in fracture incidence in children was reported in this later study. The observed increase in fracture incidence is thought to be

associated with an increase in sports participation, with the greater increase in girls reflecting a greater number of girls participating in organised sports.

As highlighted above, boys are more likely to fracture than girls, with approximately 60% of fractures in children 0-16 years old occurring in males. The average age at fracture is 11-14 years for boys and 8-11 years for girls (202, 204, 205, 214) and at all ages boys have a higher rate of fracture than girls (215). The age at increased risk of fracture for boys and girls corresponds to early-mid stages of puberty (207) and the period of peak height velocity. This period of relative skeletal fragility is thought to be due to a period of high linear growth with a lag in bone mineralisation and cross sectional changes in bone area that leave bone less resistant to bending and fracture (216-218). That peak BMC velocity has been documented to occur approximately 0.7 years after peak height velocity supports this argument (183). Additionally in early adolescence changes in behaviour such as increased risk taking and uptake of vigorous physical activity are thought to impact fracture risk, especially in males.

Across the studies reporting fracture incidence, upper limb fractures are the most frequently reported fractures (nearly two thirds of all fractures) during childhood with forearm fractures, in particular distal radius/ulna, being the most common followed by the hand/fingers and humerus. The least reported fractures occur at the pelvis/hip and spine (202, 204, 205, 215, 219, 220).

5.2. Associations and risk factors for fracture in childhood

5.2.1. Bone mass and bone size

Fractures are more common in children with low bone mass. Case-control studies have repeatedly but not conclusively shown bone mass to be associated with risk of fracture in childhood (221, 222). In prospective studies where children have undergone bone mass assessment and subsequently been observed for episodes of fracture BMD, BMC, bone area and width have been shown to be lower in the children who have gone on to fracture than in those who have not. Observing children for future fracture episodes increases the likelihood that lower bone mass increases the risk of fracture rather than fracture resulting

in a decrease in bone mass (223). Measured by DXA, BMD at the radial diaphysis and metaphysis, femoral neck, femoral diaphysis, total hip, and lumbar spine in boys (202) and TB in boys and girls (224) has been shown to be lower in those who went on to fracture. Lower BMC has been detected in girls at the radial diaphysis and metaphysis, LS and femoral trochanter but not femoral neck or diaphysis (225). Also in girls bone area and width at the radial diaphysis (in pre-pubertal girls) and LS (at pubertal maturity) has been shown to be reduced in those who subsequently fractured (225).

pQCT measurements from prospective studies support the data obtained by DXA. vBMD has been shown to be lower at the distal radius in girls during pubertal growth and into early adulthood who experienced an upper limb fracture (223). In boys tibial HR-pQCT recorded at 15.2 years showed a lower distal trabecular vBMD and trabecular number, with increased trabecular spacing (202) and reduced bone strength (stiffness and failure load). Stiffness, failure load and apparent modulus were also lower (-9.2%, -8.6% and -11.2% respectively) in females at 20.4 years who had experienced a fracture during observation over the previous 12 years (226).

Each SD decrease in BMD at the radius, femoral neck, femoral diaphysis, total hip and LS increases the risk of fracture expressed as an odds ratio by 1.46-1.64 at age 7.4 years and by 1.62-1.90 at 15.2 years (not at the radius) (202). This inverse association was also detected in humeral aBMD and adjusted vBMD. Each SD decrease in TBLH BMC increases the risk of fracture in the subsequent 2 year period by 89% (220), with lower TB and spine BMC and BMD at 8 years of age being a predictor for upper limb fracture for up to 8 years (224). More specifically reduction in BMD at the hip, spine and TB in girls and at the radius in boys is associated with increased risk of upper limb fracture but not lower limb fractures (227). Greater bone length of the radius has also been identified as an indicator for upper limb fracture (223), supported by findings that boys with a history of forearm fracture were taller than boys with a history of other fractures or no fracture (186).

The difference in BMD and BMC between those who have fractured and those who have not is also affected by puberty with some of the differences only present at a certain stage(s) of

development. In pre-pubertal girls (Tanner stage 1) BMC at the radial diaphysis was lower, at early puberty (Tanner stage 2) BMC was also lower at the LS, and at pubertal maturity (Tanner stage 5) BMC was decreased in the ultradistal radius, LS and femoral trochanter in the girls who fractured over the course of the 8.5 year observation period. Additionally BMC accrual at the radial diaphysis during pubertal growth was reduced, and calculated BMC over height gain at the ultradistal radius and LS was lower in the girls who fractured (225). Later menarcheal age has also been observed as a risk factor for fracture, with an inverse association between later menarche and lower aBMD at the radius in childhood and adolescence (226). In boys the differences in bone detected at 15.2 years (lower tibial vBMD, trabecular number and reduced bone strength) (202) was not present at 22.6 years of age (228) suggesting that bone deficits in males who fracture during childhood are resolved by the end of adolescence. In contrast females from this study at age 20.4 years, who had fractured during growth, had lower vBMD, trabecular bone density and trabecular thickness at the distal radius (-7.2 to -9.6%) than in those who had not. Measures of bone strength, (stiffness, failure load and apparent modulus -9.2%, -8.6% and -11.2% respectively) were also lower in this group (226). This difference between genders in resolution of bone deficits is important when considering bone health and fracture risk in later life.

Clarke et al (229) have further considered bone mass and level of trauma. They found that in children experiencing fracture due to low level trauma TB BMD, BMC and BA, and humeral estimated vBMD was lower than in children who had not fractured. Even in children who fractured as a result of moderate/severe trauma parameters of bone fragility (humeral estimated vBMD and TB bone mass for bone size) were lower than controls.

Farr et al (230) in their review paper have reported a 11-13% reduction in bone strength estimated by micro finite element analysis in 8-15 years old who had a mild trauma distal forearm fracture compared to controls and reductions in CtTh of 13-14% and CtA of 23-26%. Those who fractured due to moderate trauma had ‘similar values’ to controls.

5.2.2. Physical activity

Physical activity has been shown to increase BMC and BMD across various skeletal sites in childhood (discussed in Chapter 4); increased BMC and BMD has been shown to reduce risk of fracture as discussed above. Therefore there is an assumption that physical activity will have a protective effect on risk of fracture. However the association between physical activity and fracture risk is not so clear.

A school based physical activity intervention programme in a Swedish population has shown a decrease in fracture risk following 8 years of the intervention (40 minutes a day of teacher led physical activity compared to the standard 60 minutes a week) (231). However in the first year following the intervention fracture risk increased. Other studies have shown no difference in physical activity levels between those who have fractured and those who have not (186, 202) or indeed have shown that increased physical activity is in fact a risk factor for fracture (232). Physical activity intensity and frequency in particular are related to fracture risk with participation in competitive sports being higher in pre-pubertal children who had fractured compared to those who had not (233). The risk of fracture is doubled in children undertaking vigorous physical activity (including running, dance, gymnastics, swimming) at least once daily compared to less than four times a week (234). This increased risk is seen in children regardless of vBMD and bone size relative to body size therefore suggesting that it is not an effect of weaker or smaller bones that causes fractures to occur in this group. Girls who participate in more than 8 hours a week of physical activity compared to those taking part in less than 4 hours are also at double the risk of fracture. Again it is the intensity of physical activity that is driving the increase risk of fracture, in this study each hour of high-impact activity (including running, football, cheerleading/gymnastics, basketball) increased the risk of stress fracture by 8% (HR = 1.08; 95% CI, 1.05-1.12) (235). However the results from an Australian population based case-control study (children aged 9-16 years) only showed an increased risk of forearm fracture in boys, with a risk reduction in girls who participated in sports (high-risk, competitive and contact sports, boys; high-risk and non-contact sports girls) (236). Differences in type of sports activity, contact and competitive, may account for some of the difference observed in fracture risk between males and females. It has been suggested that vigorous physical

activity predisposes children to falls and trauma therefore increasing their risk of fracture and this overrides the beneficial osteogenic effects of physical activity in reducing fracture risk in children (234).

Less surprisingly sedentary behaviour is also associated with increased fracture risk. The same Australian study discussed above looked at hours spent television, computer or video viewing and found a dose-dependent association between this and forearm fractures. Additionally they found that light physical activity was associated with a reduction in forearm fractures (independent of bone strength assessed by DXA)(236). Weight-bearing exercise calculated as an activity score has been shown to be protective of recurrent fractures, with children aged 4-16 years who sustained recurrent fractures having a lower score (less weight bearing exercise) than those having only 1 or no fracture (237). Also in this study general activity levels (measured by METS metabolic equivalents) were lower in the group who recurrently fractured compared to those who had sustained only 1 fracture.

Confusingly physical activity therefore has been shown to both increase and decrease the risk of fracture. Most studies looking at the osteogenic value of physical activity have not had fracture risk as an outcome but rather the effects on bone mass and size as mentioned previously. Certainly there are few studies that have implemented a physical activity intervention and recorded fracture as the main outcome. The Swedish school study discussed here has produced many publications reporting over time though it was only the more recent paper that demonstrated a positive effect of a non-specific physical activity intervention on fracture risk.

5.2.3. Environmental and societal

Many other factors thought to be associated with risk of fracture have been investigated. The role of calcium and Vitamin D in skeletal homeostasis has been reported in detail. Dietary calcium and Vitamin D has been clearly shown to be important in paediatric bone health and therefore its effect on fracture risk has been considered. Across a number of studies it has been shown that children with a history of fracture or recurrent fractures have reduced milk consumption (smaller volumes and less frequently), adverse symptoms to

cow's milk, and lower calcium intake from dairy products than age-matched controls (237-239) particularly in children aged 6-15 years. Even where no difference in calcium consumption between fracture and control groups was found in a New Zealand population, more boys in the fracture group had a calcium intake below recommended levels than controls (240).

There is some evidence to suggest an association between low serum Vitamin D levels and increased risk of fracture (241-243), though this has not been shown conclusively across cross-sectional and case-control studies (244-246). Vitamin D supplementation however, has been shown to be associated with a decreased risk of fracture in childhood (244). In regard of recent interest in maternal and neo-natal Vitamin D status on fracture risk, studies have failed to show an association between childhood fractures and early life Vitamin D levels (247), despite evidence of maternal non-supplementation and deficiency/insufficiency leading to reduced BMC, whole body area and BMD at birth (248) lasting up to 9 years of age (249). The most reliable means to test Vitamin D status and fracture incidence would be through intervention studies, however the duration of observation and number of participants required for such studies make such trials unfeasible (246).

Carbonated beverage intake as a dietary risk factor has also been considered, with increased intake associated with an increased risk of fracture (237), and more specifically regarding cola beverages and forearm fractures (250). This has been hypothesised though not proven to be due to carbonated drinks replacing milk consumption, but regardless of milk consumption carbonated drink intake is a risk factor for fracture.

Obesity has also been clearly associated with risk of fracture. Children who fracture are more likely to have a higher BMI, fat mass, percentage body fat, are likely to be heavier and/or overweight than age matched controls (237-240). With a 1.5 fold increased risk of fracture at any site and of 1.7 at the forearm (251). However in pre-pubertal children fat mass is positively associated with gains in TB BMC with overweight children having greater measure of bone strength. By late adolescence, particularly in girls, there is however a negative association between increased fat mass and bone size and reduced strength. It is

thought that fat accumulation during childhood may have a detrimental effect on bone strength. The findings are not consistent across all studies with studies assessing bone microstructural parameters and strength using micro finite element analysis showing no difference between lean and obese children (252). Despite the discussion regarding bone mass, bone strength and obesity, consistently being over-weight or obese has been shown to increase the risk of fracture in childhood. It has been suggested that this may be caused by an increased number of falls in this group or by the increased body weight resulting in an increased load to bone mass ratio (251, 252). In obese children TB BMD may be age-appropriate but insufficient for their weight.

Children from lower socioeconomic backgrounds or with parents of lower levels of educational attainment appear to be over represented in groups of those who have fractured versus those who have not. However there is insufficient data to be clear on an association between this and risk of fracture (215).

6. Methodology

6.1. Study 1 - Acute Response of Bone to whole Body Vibration in Healthy Pre-Pubertal Boys

Study 1 was published in the journal of Musculoskeletal and Neuronal Interactions (Appendix 2) (253).

6.1.1. Research Aims and Purpose

The purpose of this three phase randomised comparative pilot study was to determine the range and rate of the acute bone responses in apparently healthy boys to standing on a vibrating platform. Previous studies in paediatric populations have focused on the longer term use of vibrating platforms with primary outcomes concerning change in bone mass and/or muscle function. However little is known of how soon bone responds to a single exposure to vibration or consecutive daily short term exposure to vibration.

Additionally there is a paucity of data regarding the effects of vibration in healthy children. Bone outcomes have been investigated in children with disabling conditions such as cerebral palsy and Duchene's Muscular Dystrophy, osteogenesis imperfecta, low bone mass conditions, and in overweight boys over periods of a eight weeks to twelve months. To date no data has been collected on the effect of vibration in healthy pre-pubertal boys. There is also limited data available on bone turnover markers in children exposed to WBV.

A number of devices are now readily available to deliver WBV. Only a small number of studies have directly compared different vibration devices, none in a paediatric population. Vibration regimes have varied significantly between the published studies making it difficult to make direct comparisons of the effectiveness of the platforms and any differences that may result from the different magnitude of vibration. This study compared two of the commercially available platforms, the Juvent MDT 1000 (Marodyne, Lakeland, Florida, USA) and the Galileo Med M (Novotec Medical GmbH, Pforzheim, Germany). The first delivers low magnitude synchronous vibration, the second provides a high magnitude side-alternating stimulus.

To gain a broader understanding of how the two devices work, either directly on bone tissue or via musculoskeletal forces, this study also measured skin surface temperature in the lower legs using an infra-red thermography camera pre- and post-vibration, as a surrogate measure for blood flow and muscle activity.

6.1.2. Hypotheses

1. Ten minutes of WBV in pre-pubertal boys will result in a change in serum bone turnover markers P1NP and CTx.
2. An increase in skin surface temperature pre and post intervention will occur, which will be dependent on the type of vibration platform used (high or low magnitude vibration).

6.1.3. Ethical Approval and funding

Ethical approval for this study was obtained from South Humber Research Ethics Committee. Funding was awarded by Sheffield Children's Hospital Charity to a total of £22,420.

6.1.4. Study Design

The final version of the study protocol, patient and parent/guardian information sheets, and parent letters can be found in Appendices 3 and 4.

Phase 1

Twelve boys stood on either the Juvent or Galileo platform for ten minutes on one occasion (Figure 5). Blood samples were taken prior to the intervention, ten minutes and sixty minutes post

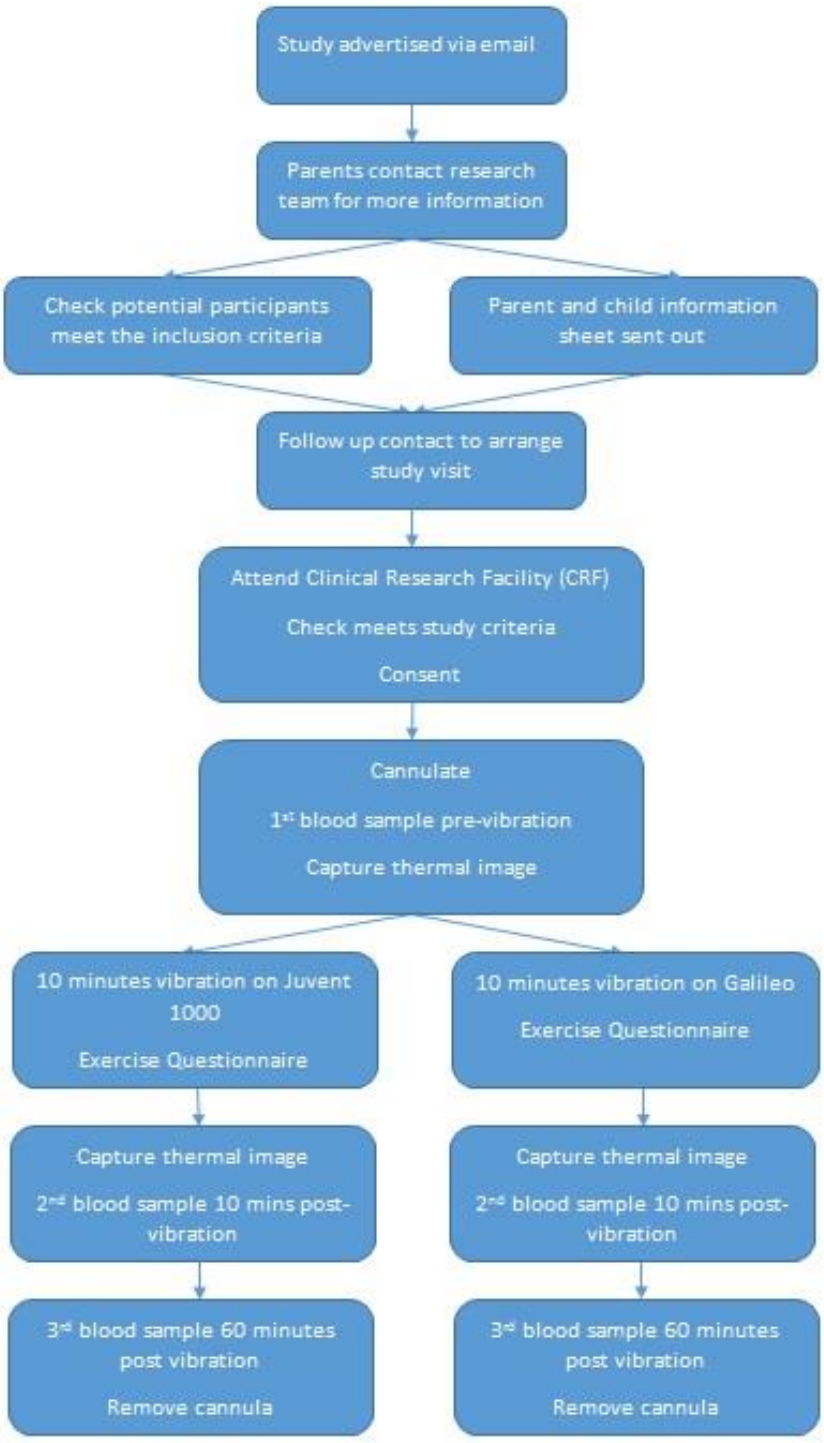


Figure 5 Participant involvement - phase 1

intervention. A validated questionnaire (254) regarding exercise and sport activity over the seven days previous to the study visit was administered during the intervention. Thermal images were taken immediately pre- and post-intervention to measure skin surface

temperature. All visits occurred in the mornings at the Clinical Research Facility at Sheffield Children's Hospital and were completed by the researcher.

Phase 2

In phase 2 24 boys were exposed to 3 or 5 days of vibration on either the Juvent or Galileo platform (Figures 6 and 7). There were 4 groups in phase 2; Group a) 3 days Juvent vibration (n=6), Group b) 5 days Juvent vibration (n=6), group c) 3 days Galileo vibration (n=6), and group d) 5 days Galileo vibration (n=6). Blood samples were taken immediately pre- and 10 minutes post- vibration on days 1 and 3 in all groups and on day 5 from the boys in the 5 day group. A blood sample was also collected on days 5 and 8 in the 3 day groups and 5 day groups respectively at times corresponding to the pre-vibration blood samples. The variation in the timing of the last samples in each group (either 2 or 3 days post-vibration) reflected the need to fit in with the boys' school and family commitments (sample timing was planned to allow normal school attendance and avoid weekend attendance). Study visits took place in the child's home before school or at school before lessons began which ever was the most convenient for the study participants. The researcher carried out all study visits and was present for the full duration of study visits to ensure compliance to the study protocol.

Phase 3

A control group was recruited to account for potential change in bone turnover markers and skin surface temperature over time. 18 boys were recruited to phase 3. Thermal images were captured before and after standing on a non-vibrating platform in the same cycles as boys standing on the platforms in phase 1 (as described below in section6.1.13). Blood samples were collected at 30 minute intervals (0 to 120 minutes) to cover the duration of the intervention in the boys in phase 1. The samples collected at 0, 30 and 90 minutes corresponded to the pre-, 10 minutes post-, and 60 minutes post-vibration samples respectively and were used for comparison between the control and intervention groups. As with phase 1, all visits occurred in the mornings at the Clinical Research Facility at Sheffield Children's Hospital and were completed by the researcher.

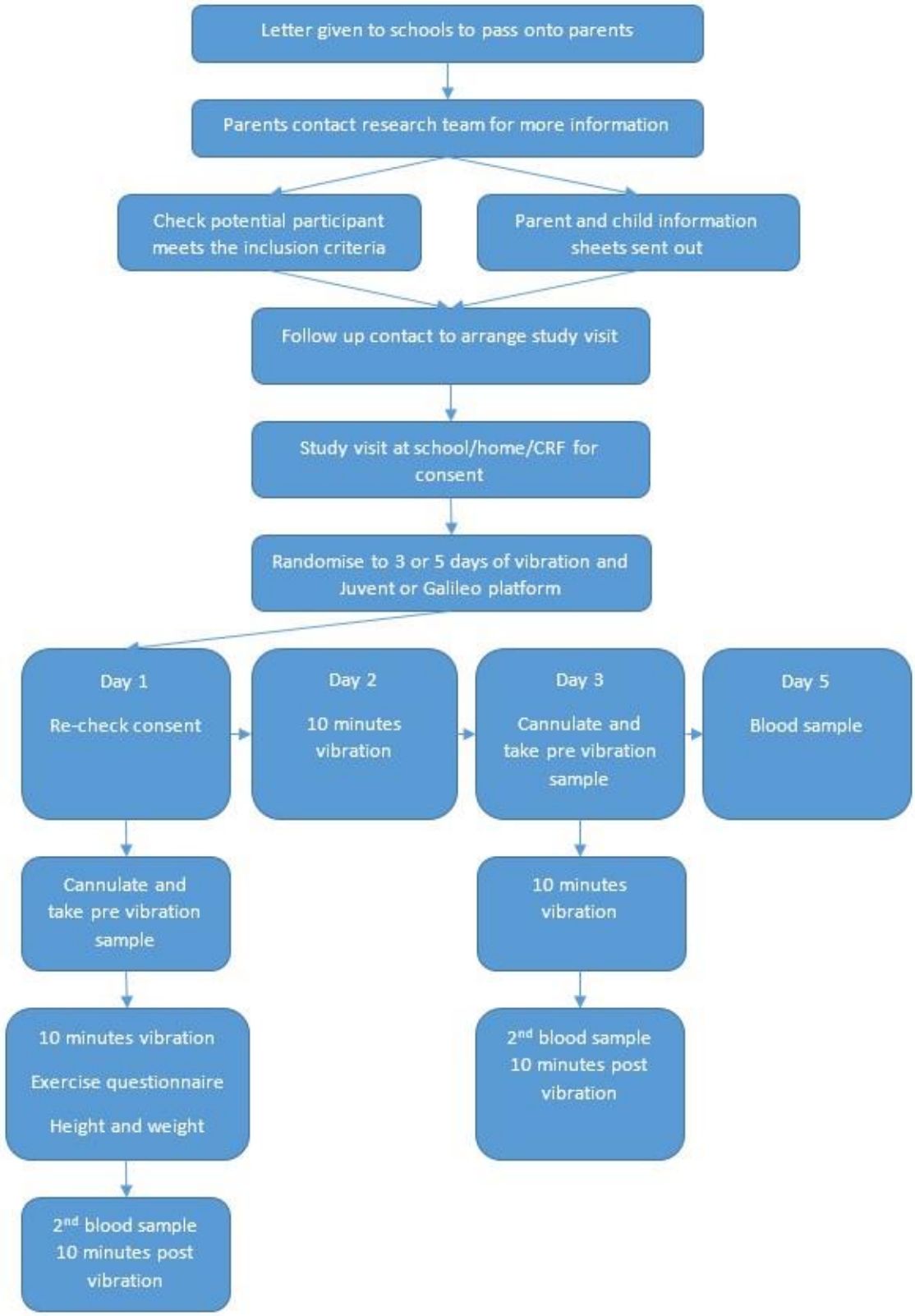


Figure 6 Participant involvement - phase 2, 3 days WBV (groups a and c)

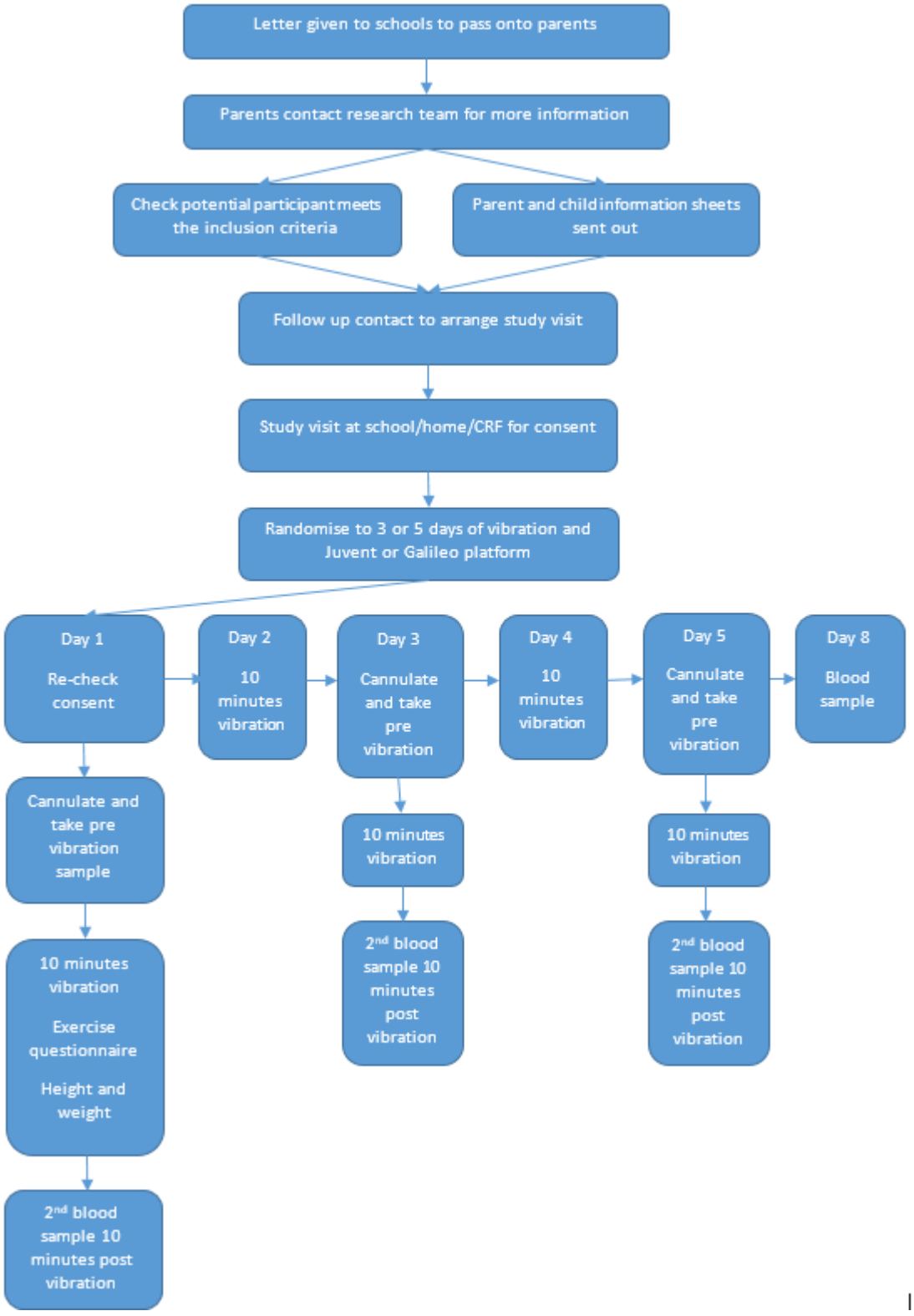


Figure 7 Participant involvement - phase 2, 5 days WBV (groups b and d)

6.1.5. Participant Recruitment

In total 64 pre-pubertal boys aged 9-12 years were recruited. Participants were recruited from local schools. The Head teacher was approached for permission for letters to be given to pupils and parents inviting them to participate in the study. Contact details for the study team were included in the letters so that parents could contact the team if they wished for their children to participate. An invitation for participation in the study was also sent via email to staff working at Sheffield Children's NHS Foundation Trust, the University of Sheffield, and Sheffield Teaching Hospitals NHS Foundation Trust asking for interested persons to contact the research team for further information.

Information sheets detailing the study procedures and contact details for the study team were posted out to potential participants and their parents who expressed an interest in the study prior to their attendance. Age appropriate information sheets were included for the participants. A minimum of 24 hours was given for participants to consider their involvement. Parents and participants were given the opportunity to discuss the study further with the research team prior to giving consent. Screening for the inclusion/exclusion criteria was done by interview. Written informed consent for the study was obtained from a parent/legal guardian and assent from the child prior to any study procedures.

6.1.6. Inclusion/Exclusion Criteria

Inclusion criteria:

White Caucasian

Aged 9-12yrs (pre-pubertal)

First language English

Exclusion criteria:

Pre-existing chronic illness

Known bone disease

History of one or more fractures

Recent (last 12 months) or current treatment likely to affect bone – not including inhaled or intermittent oral therapy with steroids for asthma

Balance problems

Continuing involvement in more than one other research study

As a small pilot study funding for this study was limited and a decision was made to restrict recruitment to pre-pubertal children only. This would eliminate any potential effect of the different pubertal stages on bone turnover markers and bone response to loading (as discussed in chapter 4), therefore reducing the required sample size and study costs. Initial planned recruitment was from local secondary schools of participants aged 11-12 years. As the reported age of onset of puberty in girls is from 10 years, and later in boys from 11.5 years (255, 256), most girls would not be eligible for study inclusion; it was decided to focus the study on boys only. Once recruitment was extended to include primary schools and therefore a lower age group, the study was still restricted to boys only to eliminate any gender effect.

6.1.7. Randomisation

In phase 1 participants were randomly allocated to either the Juvent (low magnitude) or Galileo (high magnitude) platforms. In phase 2 they were also randomised to 3 or 5 consecutive days of ten minutes of WBV. Randomisation in phase 1 was by the participants selecting and opening an opaque envelope containing the randomisation code. In Phase 2 a similar system applied of successive opening of envelopes, however a block randomisation system was used. This was to ensure that equal numbers of boys were allocated to the 4 intervention groups; Juvent for 3 consecutive days, Juvent for 5 consecutive days, Galileo for 3 consecutive days, and Galileo for 5 consecutive days.

Neither the researchers nor participants were blinded to the intervention groups as the platforms look different and provide the vibration stimulus in different ways. The Juvent platform provides vertical vibration that is of such low magnitude it is hardly detectable. The Galileo platform delivers side-alternating vibration of a higher magnitude.

The participants were supervised during the vibration at each visit so compliance to the intervention was be monitored.

6.1.8. Pubertal Status Assessment

Pubertal status was determined during interview by self-assessment using a gender appropriate validated pictorial scale depicting the different stages of puberty (Appendix 5) (257). Although it is recognised that assessment of pubertal status by an experienced clinician is the most accurate way to assess the stage of pubertal development, self-assessment of puberty is frequently used in research. Self-assessment by the child is often the most convenient method and is much less embarrassing for the child. Self-assessment through the use of line drawings has been shown to be reliable with good to substantial agreement of stage of puberty being established between self and clinician assessment (258, 259). Within some age groups reliability has been questioned with the suggestion that there is a tendency for children to overestimate pubertal stage in early puberty and underestimate in mid to late puberty (259-261). As the study required participants to be pre-pubertal, it was felt that if the boys did over estimate their stage of puberty they would have excluded themselves from the study. The researcher was aware that this could have made recruitment more difficult although it added confidence that only suitable boys were included in the study. However only 8 boys were excluded from the study on the basis of their Tanner stage.

6.1.9. Vibration Regime

On each occasion WBV occurred for 10 minutes divided into 4 cycles of two minutes thirty seconds separated by a 30 second rest period. The boys stepped off the platforms for the 30 second rest period and stepped back on again for each vibration cycle. It is recognised that the response of bone to loading can be enhanced by the insertion of rest periods as discussed in Chapter 3.

Often researchers have allowed a run in period whereby participants gradually increase the duration of the WBV to familiarise themselves with the devices and for comfort. As this study investigated the immediate response of bone, WBV could not be gradually increased. Therefore it was felt inserting brief rest periods would also serve a purpose of participant comfort.

The Juvent platform delivers a set frequency, acceleration and amplitude of vibration (listed in table 8) with only the duration of vibration being controlled by the user. In contrast parameters for the Galileo can be altered by the user; WBV on the Galileo platform was delivered at the settings below for participant comfort.

Table 8 Platform parameters for phase 1 and 2

	Frequency	Acceleration	Amplitude
Juvent 1000	32-37Hz	0.3g (low magnitude)	0.085mm (displacement)
Galileo Med M	20Hz	6.4g (high magnitude)	4mm

WBV was delivered on 1, 3, or 5 consecutive days to ascertain the rate of bone response to vibration. Prior to this study it was not known how soon bone in this population would respond to this mechanical stimulation. Studies in children and adults exposed to other exercise interventions have detected changes in bone turnover markers after only one episode. Unloading studies have demonstrated changes following 3 days of bedrest. On this basis it was decided to have the 3 groups. It was considered that the low magnitude group may take longer to respond than the high magnitude group, it was felt that 5 days would be sufficient time to detect any changes from baseline.

6.1.10. Exercise Questionnaire

A validated questionnaire (254) regarding exercise and sport activity over the 7 days previous to the study visit was completed during the intervention (Appendix 6). The questions asked in the Godin-Shepard Leisure –Time questionnaire are considered to successfully discriminate between active and sedentary people (254). Reliability and validity of this tool to assess physical activity in children has been demonstrated (262). The boys were asked two questions regarding the frequency of strenuous, moderate, or mild exercise undertaken for at least fifteen minutes during their free time. Activity undertaken during physical education lessons was not included. Strenuous exercise is defined as exercise that causes the heart to beat rapidly, moderate exercise as not exhausting, and mild exercise as minimal effort. The frequencies of the types of exercise given in answer to these questions were then multiplied by the anticipated metabolic equivalents (METs) of nine, five, and

three for strenuous, moderate, and mild exercise respectively, to provide a score for comparison. The participants were asked to continue with their usual levels of activity for the duration of the study.

6.1.11. Weight and Height

Weight and height were recorded at day 1 of the study and from this body mass index (BMI) was calculated for each boy. Weight was measured to the nearest 0.1kg with the participant wearing light clothing using electronic balance scales (Seca GmbH & Co, Hamburg, Germany). Participants were asked to remove additional layers of clothing beyond trousers/shorts or t-shirts and to empty their pockets of any items such as keys, phones etc. Height was measured to the nearest 0.1cm without shoes using a portable stadiometer (Leicester height measure, Invicta, Leicester, UK).

6.1.12. Blood Sampling

The bone turnover markers Pro-collagen type 1 N-terminal propeptide (P1NP) and C-terminal cross-linked telopeptide of type 1 collagen (CTx) were measured for bone formation and bone resorption at each sampling time point as stated previously. P1NP is a measure of the formation of type 1 collagen. As the collagen molecules are formed, prior to assembly into fibrils, the ends are cleaved off by enzymes and released into the blood stream (139)(ASBMR). As bone is resorbed the crosslinks that form bonds between the collagen fibrils are released. Some have fragments of collagen attached; these are telopeptides which can be measured in urine or serum, NTx and CTx (125, 263); only CTx was measured in this study. An additional bone formation marker Osteocalcin (OCN), a non-collagenous protein expressed by mature osteoblasts (264), was measured at baseline and on day 8 in the boys who received 5 days of vibration. This was decided after the study had been completed using left over serum. There was insufficient sample to measure at the intermediate time points. The majority of osteocalcin which is found in the bone matrix, though some will enter the circulation, is an important factor in matrix mineralisation (264) and would therefore be expected to be detected in serum later in the course of bone formation than P1NP.

In addition factors known to affect bone turnover, sclerostin (bone formation) and osteoprotegerin (bone resorption) were also measured. As discussed in Chapter 2, sclerostin, expressed by osteocytes in response to mechanical loading, is a key factor in the anabolic response of bone to loading. OPG is the decoy receptor for RANKL, by blocking the interaction of RANK – RANKL, OPG interferes with the formation and survival of osteoclasts (7) and therefore has an important impact on bone resorption activity.

Samples were collected between 7.30am and 10.30am in a fasted state as is recommended to reduce variability in samples due to food intake and circadian rhythm (265, 266). Sample timing occurred so that all the samples for each individual patient were collected at approximately the same time.

Bone formation markers need to be measured from serum. P1NP is a stable marker as it can tolerate prolonged frozen storage and transport without degradation of the propeptides (263). CTx was chosen as the resorption marker due to its stability and reliability as long as the above criteria for collection are met. Resorption markers can be measured in urine also but their intra-individual variability is large. Since the time points between sampling in this study were small the likelihood of collecting or detecting a change in urine markers was very small and likely to be confounded by factors such as incomplete prior voiding. Additionally as the boys underwent venepuncture for the formation marker, only a small extra amount of blood was needed to be collected and this reduced the number of procedures that the boys were subjected to.

2mls of blood was collected from the participants and transferred into a blood bottle containing a serum separating gel. This was left for at least 30 minutes to allow the sample to clot and then placed in a centrifuge and spun at approximately 3500rpm for 15 minutes. Serum was then be aliquoted into 1.5ml Sarstedt tubes and frozen at -80^{o}C until analysis. To ensure stability of the samples they were frozen within two hours of collection. No freeze thaw cycles occurred other than for sample assays. All tubes were labelled with the patient study identification number, date of collection and time point.

P1NP, OCN and CTx was measured using electrochemiluminescence Immunoassay (Elecsys total P1NP kit; intraassay %CV <1.7% and Elecsys β-CrossLaps/serum kit; intraassay % CV 2.8-8.4%, Cobas E411, Roche Diagnostics, UK, and OCN; intrasaay %CV 1.4-3.3% Cobas E411, Roche Diagnostics, Germany). Sclerostin and OPG was measured using manual enzyme-linked immunosorbent assays (Sclerostin Enzyme Immunoassay; intraassay %CV <7%, Biomedica Gruppe, Germany and OPG ELISA; intraassay %CV 2.5-4.9%, BioVendor, Czech Republic; intraassay %CV 2.5-4.9%). Assays were performed by Dr Fatma Gossiel at the Mellanby Bone Centre, University of Sheffield as per the manufacturer's guidelines.

6.1.13. Thermal Imaging

A secondary outcome of skin surface temperature of the lower legs was measured using a Land Guide M4 hand held thermal imaging camera (temperature range -20^{o}C-250^{o}C and a sensitivity of 0.12^{o}C, CV 2%, Asten Instruments Limited, England). Lower limb temperature change following WBV has been demonstrated in a small number of studies using skin probes (267), via a thermometer inserted directly in to the active muscle (vastus lateralis) (268), and also using thermal imaging cameras (269, 270). Varying exercise studies have also demonstrated the utility of thermal imaging to measure temperature change over the region of the active muscles in healthy adults (271-274). To date there are no published data in a paediatric population. Thermal images were taken immediately pre- and post-intervention during phase 1 and phase 3 of the study only, whilst the boys were standing on the platform. Boys in the control group (phase 3) were asked to stand on and off the Juvent platform, which was not turned on, in the same cycle as the boys exposed to vibration, that is 2.5 minutes on 30 seconds off. The camera was aimed at the back of their legs to include behind the posterior knee down to the bottom of their feet as the area of interest was the gastrocnemius and soleus muscles. They were asked to wear shorts so that the lower legs were fully exposed. The same room was used for the thermal imaging (and WBV), with windows closed to exclude draughts and the blinds drawn to reduce heat from the sun, room temperature was recorded. The vibrating platforms were left in position for use and the thermal image captured by the researcher from the wall behind the platforms.

Images were analysed using the manufacturer's software. A within subject temperature difference pre- and post-vibration was reported with type of vibration platform as covariant.

6.1.14. Statistical Analysis

No sample size calculation was performed for this study as it was pilot study to determine the range of responses to the period of vibration. Therefore it was not possible to estimate the number of participants needed. The decision to recruit thirty-six participants, twelve to phase 1 and twelve to each of the three and five consecutive days of vibration in phase 2, was based on recommendations in current literature regarding sample sizes for pilot studies (275, 276). Twelve participants were recruited per group (1, 3 or 5 days of WBV) to assess the primary endpoint of bone turnover marker change in the intervention versus control group, comparison between platforms was exploratory (6 boys per platform per days of WBV). Eighteen participants were recruited for phase 3 to match the number of boys exposed to each platform for at least one day.

SPSS version 19 (IBM, New York) was used for the statistical analysis. Baseline characteristics of each group were considered but formal statistical testing was not performed. Both within group change (pre- to post-vibration) and between group differences in bone biomarkers (P1NP, CTx, OPG and sclerostin) were analysed. A repeated measures ANOVA was used to assess within group changes in the phase 1 up to 60 minutes post-vibration. Day 1 data from phase 1 and phase 2 groups were combined to assess the immediate bone biomarker response to vibration (sampling time points pre- and 10 minutes post-vibration). Paired t-tests were used to test for change within a group from pre- to immediately post-vibration on each day (days 1, 3, and 5).

Changes between the high and low magnitude vibration and control groups on day 1 pre- to immediately post-vibration were compared using ANOVA and ANCOVA, adjusting for baseline bone turnover markers, number of days of vibration, age and activity score. Imbalances in the baseline activity score were observed between the groups, therefore this was included as a covariate. Adjustment for days of vibration was included to account for

the recruitment of groups of subjects in successive phases. This adjust for the potential differences between the boys recruited in the different phases.

Day 1 data was also analysed for changes pre- to 60 minutes post-vibration in the bone biomarkers between groups and on days 3 and 5 pre- to immediately post-vibration using ANOVA and ANCOVA (adjusting for baseline bone turnover marker). No other covariates were included due to a lack of power. The comparison for day 3 and 5 data did not include phase 1 or phase 3 participants as data was only collected from these boys on 1 day.

Change in bone markers (P1NP, CTX and OCN) and bone cell derived factors (OPG, sclerostin) after 5 days of vibration were combined across the WBV groups due to limited data. Paired t-tests were used to test for change from baseline using day 1 pre-vibration and day 8 measurements.

To account for camera temperature drift between the recorded images, the post-image temperature was adjusted and within participant temperature change pre to post WBV reported as the outcome. For each participants' pre- and post-image a reference area not expected to change in temperature over the short time lapse between the images (i.e. where the camera was pointed at the wall) was determined with the region temperature recording used to adjust for any camera drift.

6.2. Study 2 – Acute Response of Bone to whole Body vibration in Pre-Pubertal Boys with a History of Fracture

Study 2 was designed on the completion of Study 1 to further investigate the acute response of bone to WBV.

6.2.1. Research Aims and Purpose

The primary aim of this study was to determine the acute skeletal response to high or low magnitude whole body vibration in boys who have a history of having sustained at least one fracture. A second purpose was to determine if bone in this group of children responds to vibration in the short term in the same way as it does in boys who have not previously fractured. Data collected from this fracture cohort was compared to data collected in study 1 investigating the bone response to WBV in apparently healthy pre-pubertal boys.

6.2.2. Hypotheses

1. Ten minutes of WBV on five consecutive days in pre-pubertal boys with a history of fracture will result in a change in the serum bone turnover markers P1NP and CTx at day 8.
2. The response of the serum bone turnover markers in boys with a history of fracture will be different to that of the healthy boys in Study 1.

6.2.3. Ethical Approval and Funding

Ethical approval for this study was obtained from Yorkshire and the Humber - Leeds West Research Ethics Committee. Funding for this study was awarded by Orthopaedic Research UK to a total of £73,040.22. The study was adopted and listed on the NIHR Clinical Research Network Portfolio as 'Vibration in boys with a history of fracture' number 91811.

6.2.4. Study Design

The results of study 1 informed the design of study 2. Appendices 7 and 8 contain the final version of the study protocol, patient and parent/guardian information sheets, and parent letters. The increase in P1NP and CTx detected at day 8 only, determined that in study 2 all the boys were exposed to 5 consecutive days of WBV (Figure 8). Blood samples were collected at the same time points as boys exposed to 5 days of WBV in study 1. However an additional sample was collected at day 12 to see if the response detected at day 8 was still present a week after 5 consecutive days of 10 minutes WBV. Thermal imaging was not measured in study 2.

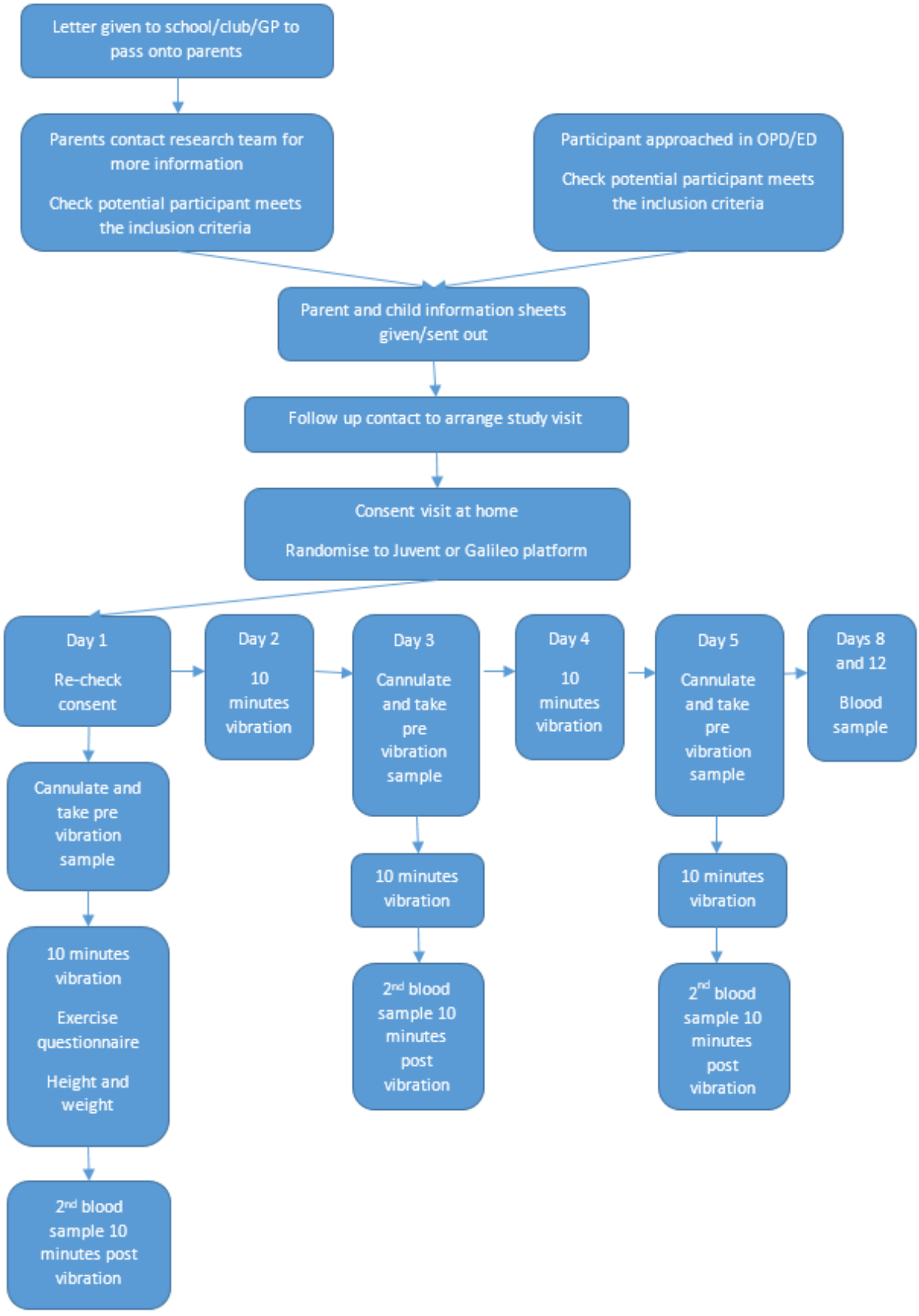


Figure 8 Participant involvement in study 2

6.2.5. Participant Recruitment

Participants were recruited from local schools and youth/activity clubs. As for Study 1 the Head teacher/club leader was approached for permission to display an advertisement for the study and for letters to be given to pupils and parents inviting them to participate in the study. Contact details for the study team were included so that parents could contact the team if they wished for their child/children to participate. An invitation for participation in the study was also sent via email to staff working at Sheffield Children's NHS Foundation Trust, the University of Sheffield, Sheffield Hallam University, and Sheffield Teaching Hospitals NHS Foundation Trust asking for interested persons to contact the research team for further information. In addition to the above methods in Study 2 boys were also recruited from Out-patient (OPD) and Emergency (ED) departments at Sheffield Children's Hospital. Boys aged 7-13 years who attended fracture clinic or ED were approached to see if they were interested. As participants had to have had no recent fractures (within 6 months) details of those that were attending due to a fracture were kept on a future participants list and contacted again as they approached 6 months post fracture to see if they still wished to take part. Boys who had attended fracture clinics more than 6 months previously were also identified from the orthopaedic lists; their GP was asked to forward on a letter to them inviting them to participate in the study. An advertisement for the study was displayed in the Hospital and through the media, including social media and on Sheffield Children's NHS Foundation Trust website.

6.2.6. Inclusion/Exclusion Criteria

The inclusion/exclusion criteria for study 2 were similar to the criteria for study 1. Where as in study 1 boys with a history of one or more fracture were excluded from the study, this became a requirement for inclusion in study 2. However participants were excluded if the fracture was current or a healing fracture. This was to ensure that the WBV intervention did not impact on fracture healing and also to ensure that the bone marker measurements were not affected by bone remodelling following the fracture. We required the boys to be at least 6 months post their last fracture. Additionally the fracture, or at least one of the fractures if the boys had sustained 2 or more, had to be the result of mild or moderate trauma as

categorised by Landin (229) (Appendix 9). Fracture(s) history was as reported by the parent or participant and was not radiologically verified. The age range for study 2 was extended to include pre-pubertal boys aged 7-13 years. Recruitment to study 1 was slow, extending the age range allowed recruitment from a larger pool of potential participants.

6.2.7. Randomisation

Participants were randomly allocated to either the Galileo or Juvent platform. Randomisation occurred by the participants selecting and opening an opaque envelope containing the name of the platform they have been allocated to. As with study 1 neither the researchers nor participants were blinded to the intervention groups as the platforms look and deliver the vibration stimulus differently (synchronous or side-alternating).

6.2.8. Pubertal Status Assessment

Pubertal stage was self-assessed to ensure that participants were at pubertal Tanner stage 1 as discussed previously.

6.2.9. Vibration Regime

As in Study 1 WBV occurred for 10 minutes divided into 4 cycles of two minutes thirty seconds separated by a 30 second rest period. The boys stepped off the platforms for the 30 second rest period and stepped back on again for each vibration cycle. The settings used for the platforms are listed in table 8 above.

6.2.10. Exercise Questionnaire

The Godin-Shepard Leisure –Time questionnaire was again used to ascertain physical activity levels in the 7 days prior to standing on the platforms.

6.2.11. Weight and Height

Weight and height were recorded as described in section 6.1.11.

6.2.12. Blood Sampling

As discussed in section 6.1.12 P1NP, CTx, OPG, and Sclerostin were collected in a fasted state pre- and 10 minutes post-vibration and on days 8 and 12, coinciding with the timing of the pre-vibration samples.

6.2.13. Statistical Analysis

No sample size calculation was performed for this study as it was pilot study to determine the range of responses to the period of vibration in boys with a history of fracture. Therefore it was not possible to estimate the number of participants needed. It was decided to recruit 12 boys to each of the platform groups (Juvent and Galileo) based on recommendations in current literature regarding sample sizes for pilot studies (275, 276). SPSS version 24 (IBM, New York) was used for the statistical analysis. Baseline characteristics of the boys with a history of fracture were considered but no formal testing was performed. Both within group change (pre- to post- vibration) and between group differences in bone biomarkers (P1NP, CTX, OPG and sclerostin) were analysed. Paired t-tests were used to test for within group change from pre- to 10 minutes post-vibration on each day (days 1, 3, and 5) and at days 8 and 12 from baseline. Independent samples t-test was used to compare between group differences at the same time points.

Data from boys exposed to 5 days of WBV in study 1 was used to compare any difference in the response of bone to WBV in boys with a history of fracture and those without (healthy boys). Differences in baseline characteristics between the fracture and non-fracture groups were explored using independent samples t-tests.

Changes (unadjusted) in bone markers (P1NP, CTX and OCN) and bone cell derived factors (OPG, sclerostin) after 5 days of vibration were compared between the fracture and non-fracture groups using independent samples t-tests. ANCOVA was used to adjust for age, activity score and baseline bone turnover markers as in study 1. Imbalances in the baseline activity score between fractures and non-fractures was observed and the age range for study 2 was expanded over the course of recruitment, therefore these were included as covariates.

7. Results

7.1. Study 1 - Acute Response of Bone to whole Body Vibration in Healthy Pre-Pubertal Boys

7.1.1. Baseline Characteristics

In total 64 boys consented to study participation. Of these 8 were excluded on the basis of pubertal stage, 2 withdrew consent prior to data collection and commencement of the intervention, and 3 withdrew due to difficulties in obtaining blood samples on day 1. Data were collected and analysed on 51 boys in total; 12 boys in phase 1 (1 day only of WBV), 24 boys in phase 2 (3 or 5 days of WBV), and 15 boys in the control group (no WBV). No participants were siblings. The baseline characteristics of each group are shown in Table 9. Age, height, body mass index (BMI), and weight were similar between the groups. The activity scores appear to be different across the intervention groups with the Galileo group scoring higher; it should be noted that these scores have a large standard deviation (SD). Baseline P1NP, osteocalcin, CTX, OPG, and sclerostin values were also similar between the groups. The time taken between the pre and post vibration samples on day 1 was slightly longer in the Juvent group. One boy in this group felt faint after cannulation and rested prior to standing on the vibration platform, accounting for the greater time lag and larger SD in this group. None of the boys reported current or recent use of oral steroids for asthma.

7.1.2. Bone turnover markers – changes across individual cycles of vibration

Values of P1NP, osteocalcin and CTX for each participant are listed in Appendix 10.

Within control group (n=14)

P1NP decreased by 7.8% (CI -13.4 to -2.2; p=0.008, paired t-test) at 10 minutes, and by 12.0% (CI -19.3 to -4.7; p=0.04) at 60 minutes compared to baseline. Osteocalcin was not measured. CTX decreased by 12.0% (CI -19.3 to -4.7; p=0.04) at 10 minutes and by 7.0% (CI -13.7 to-0.4; p=0.03) at 60 minutes (Table 10, actual sampling time points were 0, 30 and 90 minutes to correspond to pre-, 10 minutes and 60 minutes post-vibration samples respectively in the low and high magnitude intervention groups).

Table 9 Baseline characteristics of participants by intervention group

	Juvent (low magnitude) platform n=18			**Galileo (high magnitude) platform n=18**			**Control n=15***		
	Mean	SD	n	Mean	SD	n	Mean	SD	n
Age (years)	10.4	0.8	18	10.4	0.9	18	10.8	0.6	15
Height (cm)	141.8	6.5	18	145.4	8.5	18	143.2	7	15
Weight (kg)	34.3	4	18	37.1	6.8	18	38.6	10.9	15
Body Mass Index (kg m^2)	17.1	1.7	18	17.4	1.8	18	18.6	4.1	15
Activity score (METS units)	79	25	18	94	33	18	67	26	15
P1NP Day 1 pre (ng/ml)	671.5	281.1	18	794.5	204.6	17	679.5	186.5	14
OCN Day 1 pre (ng/ml)	86.4	15.5	5	88.3	32.2	5	-	-	-
CTX Day 1 pre (ng/ml)	1.85	0.55	18	2	0.48	17	1.91	0.4	14
OPG Day 1Pre (pmol/L)	3.67	0.53	16	3.71	0.97	17	3.07	0.46	14
Sclerostin Day 1 Pre (pmol/L)	25.54	6.25	16	29.13	6.22	17	24.25	7.11	14
Time between samples day 1 (minutes)	33.06	17.01	17	29.76	5.21	17	31.07	2.27	14

SD= standard deviation, n= number of participants, *sample collected on 14 participants

METS: metabolic equivalents

P1NP: pro-collagen type 1 N-terminal propeptide

OCN: osteocalcin

CTX: C-terminal cross-linked telopeptide of type 1 collagen

OPG: osteoprotegerin

SCL: sclerostin

Table 10 Change in serum P1NP and CTX values from baseline at 10 and 60 minutes post WBV by platform group

		Day 1 Mean change (unadjusted)						
		Control[a]		**Juvent (low magnitude) platform**		**Galileo (high magnitude) platform**		p value
		mean (95% CI)	n	mean (95% CI)	n	mean (95% CI)	n	ANOVA
P1NP	10min post	-61.3 (-103.9 to -18.8)	14	-67.6 (-108.7 to -26.5)	17	-71.3 (-164.5 to 21.8)	16	0.97
ng/ml	60min post	-83.7 (-135.1 to -32.2)	14	-58.5 (-160.1 to 43.0)	6	-16.5 (-172.4 to 139.4)	5	0.43
CTX	10min post	-0.11 (-0.21 to 0.05)	14	-0.17 (-0.25 to -0.09)	17	-0.08 (-0.21 to 0.05)	16	0.36
ng/ml	60min post	-0.14 (-0.27 to -0.02)	14	-0.17 (-0.37 to 0.03)	6	-0.07 (-0.15 to 0.01)	5	0.64
		Day 1 Adjusted mean change (adjusted*)						
								ANCOVA
P1NP	10min post	-71.6 (-160.4 to 17.3)	14	-70.7 (-122.6 to -18.9)	17	-64.8 (-121.6 to -8.0)	16	0.91
ng/ml	60min post	-76.6 (-131.4 to -21.7)	14	-75.6 (-155.4 to 4.1)	6	-15.8 (-123.4 to 91.7)	5	0.17
CTX	10min post	-0.11 (-0.27 to 0.05)	14	-0.17 (-0.26 to -0.07)	17	-0.09 (-0.19 to 0.01)	16	0.14
ng/ml	60min post	-0.15 (-0.26 to -0.03)	14	-0.20 (-0.37 to -0.03)	6	-0.02 (-0.25 to -0.21)	5	0.59

*Adjusted for baseline CTX/P1NP, length of treatment, age, activity score, time between samples
CI= confidence interval
[a]Samples in the control group were collected at 0, 30 and 90 minutes to correspond to pre-, 10 and 60 minutes post-vibration respectively

Within low magnitude group, first cycle of vibration (n=17)

P1NP decreased by 7.9% (CI -14.0 to -1.9; p=0.003, paired t-test) at 10 minutes, and by 0.18% (CI -20.6 to 21.0; p=0.20) at 60 minutes (n=5). CTX decreased by 6.2% (CI -12.2 to -0.2; p=0.04) at 10 minutes post WBV and by 9.4% (CI -21.1 to 2.2; p=0.08, not statistically significant) at 60 minutes post vibration (actual values Table 10).

Within high magnitude group, first cycle of vibration (n=16)

No change was seen in P1NP (decreased by 6.8%, CI -18.4 to 4.9; p=0.1) at 10 minutes, or at 60 minutes (+6.04%, CI -9.2 to 21.3; p=0.78; n=6). Neither was there a change in CTX at 10 minutes (n=16; 3.6% decrease observed, CI -9.9 to 2.8; p=0.2) or 60 minutes (2.6% decrease; CI -5.4 to 0.1; p=0.08; actual values Table 10).

Changes across individual vibration cycles; Day 3 and Day 5

On day 3 P1NP decreased pre to post vibration in the low magnitude group (n=11) by 17.5% (CI -22.6 to -12.5; p<0.001) and in the high magnitude group (n=10) by 13.3% (CI -20.2 to -6.3; p=0.004). CTX decreased following WBV in the low magnitude group by 6.2% (CI -10.4 to -2.1; p=0.007; n=11), but did not change in the high magnitude group (day 3: 3.4% decrease observed, CI -8.1 to 1.3 p=0.2; n=10; actual values Table 11).

Day 5 showed a decrease following WBV in P1NP in the low magnitude group (n=5) of 15.9% (CI -20.2 to -6.3; p=0.008) and in the high magnitude group (n=4) of 10.6% (CI -20.2 to -0.9; p=0.05). CTX decreased following WBV in the low magnitude group by 8.1% (CI -10.3 to -5.7; p=0.004; n=5) and was unchanged in the high magnitude group (0.5% increase observed, CI -16.5 to 17.4; p=0.9; n=4; actual values Table 11).

7.1.3. Differences in bone marker responses between platforms

There were no differences between the control and platform groups in the day 1 P1NP and CTX response to vibration (ANOVA and adjusted ANCOVA; table 11). There was also no difference between the platform groups in response to WBV on days 3 and 5 from immediately pre-vibration to 10 minutes after vibration (no control data collected) though as on day 1 within group changes were detected, as shown above.

Table 11 Mean bone turnover marker (P1NP, CTX) values pre and post WBV on days 1, 3, 5

		Juvent (low magnitude) platform				**Galileo (high magnitude) platform**				**Control[#]**			
		n	Mean	SD	p value	n	Mean	SD	p value	n	Mean	SD	p value
P1NP ng/ml	Day 1 pre	17	669.8	289.7	**0.003**	16	786.8	208.8	0.12	14	697.5	186.5	**0.008**
	Day 1 10 mins post		602.2	245.5			715.4	191.6			618.2	141.7	
	Day 1 pre*	6	600.6	409.7	0.2	5	830.1	287.3	0.78	14	697.5	186.5	**0.04**
	Day 1 60 mins post		542.1	332.7			813.6	249.4			595.8	194.5	
	Day 3 pre	11	703.2	184.4	**<0.001**	10	749.8	122	**0.004**		-	-	
	Day 3 post		575.6	139.3			646.8	103.8			-	-	
	Day 5 pre	5	712.6	212.7	**0.008**	4	763.7	169.4	**0.05**		-	-	
	Day 5 post		608.9	225			681	145			-	-	
CTx ng/ml	Day 1 pre	17	1.83	0.56	**<0.001**	16	2	0.5	0.2	14	1.91	0.4	**0.04**
	Day 1 10 mins post		1.67	0.49			1.93	0.5			1.8	0.45	
	Day 1 pre*	6	1.71	0.73	0.08	5	2.4	0.59	0.08	14	1.91	0.4	**0.03**
	Day 1 60 mins post		1.54	0.65			2.34	0.56			1.76	0.39	
	Day 3 pre	11	1.89	0.51	**0.007**	10	1.93	0.3	0.2		-	-	
	Day 3 post		1.76	0.47			1.86	0.28			-	-	
	Day 5 pre	5	1.91	0.66	**0.004**	5	1.99	0.16	0.9		-	-	
	Day 5 post		1.76	0.62			2.01	0.33			-	-	

Mean pre and post WBV P1NP and CTx per intervention group for samples included in the paired t-test analysis of pre and post values on each day of sample collection

SD - standard deviation, N - number of participant samples included in the analysis

*Only participants allocated to 1 day of WBV or control group had blood collected at 60 minutes post WBV (or equivalent time in the control group); these 6 boys are a subgroup of the 17 measured on day 1, #Samples collected at 0, 30 and 90 minutes to correspond to pre, 10 and 60 minutes post

7.1.4. Changes in bone turnover markers from baseline after 5 days of vibration

In contrast to the decrease shown in the immediate pre to post WBV time period, boys exposed to 5 consecutive days of WBV (platform groups combined, n=11, measurements on day 8 vs baseline measurements) had a significant increase in P1NP of 25.1% (CI 12.3 to 38.0; paired t-test p=0.005; Figure 9). No significant change was detected in the formation marker OCN (measured at day 1 and day 8 in the 5 day subjects only n=11; change +11.5% CI -8.3 to 31.2; p=0.2; Figure 9). At day 8, CTX was greater in the boys exposed to 5 days of WBV on both of the platforms than at baseline with an increase of 10.9% (CI 3.6 to 18.2; paired t-test p=0.009; Figure 9).

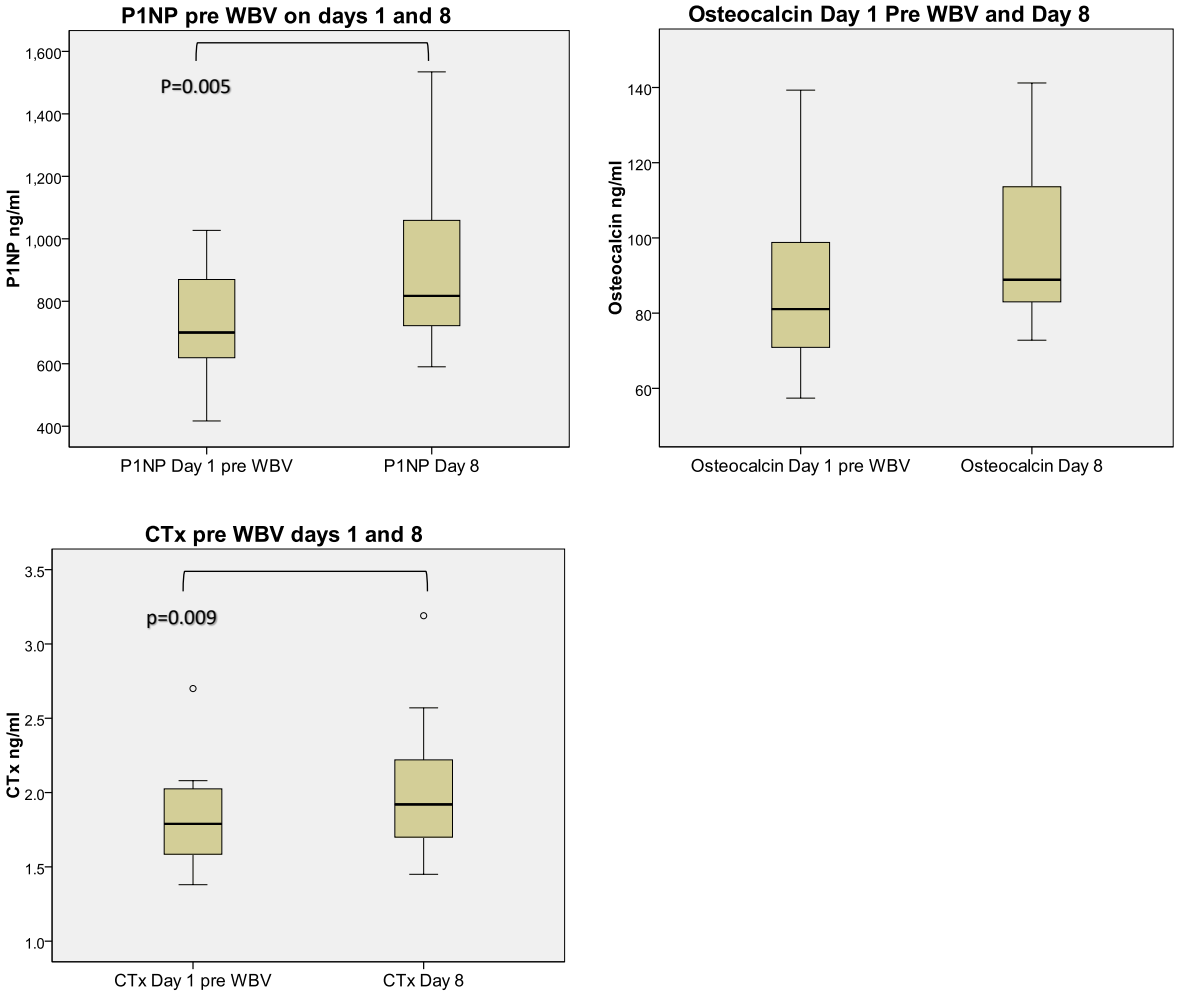


Figure 9 Box and whisker plots illustrating the absolute values of P1NP (top left), osteocalcin (top right) and CTX (bottom left) at baseline and day 8 for boys exposed to 5 consecutive days of WBV

7.1.5. OPG and Sclerostin

OPG and sclerostin values for each participant are listed in Appendix 10.

No changes were observed in OPG or sclerostin within or between groups at 10 or 60 minutes post-vibration on day 1. Combining the platform groups and comparing to the control group did not alter the significance of the results.

Within low magnitude group (n=6)

No change was observed in OPG at 10 minutes post-vibration (decreased by 2.3%, CI -10.7 to 6.1; p=0.5) or 60 minutes post-vibration (-0.8%, CI -8.0 to 6.5; p=0.8). Sclerostin similarly did not change (-5.7%, CI -15.8 to 4.4; p=0.2 and 1.1%, CI -8.4 to 10.6; p=0.6 at 10 and 60 minutes respectively).

Within high magnitude group (n=5)

No response to WBV was observed in OPG or sclerostin within the high magnitude group. OPG change of -0.9% (CI -17.1 to 15.3; p=0.8) at 10 minutes and -6.6% (CI -21.1 to 7.8; p=0.2) at 60 minutes; sclerostin increased 3.9% (CI -15.5 to 23.3; p=0.5) at 10 minutes and +8.8% (CI -16.9 to 34.5; p=0.5) at 60 minutes.

Within control group (n=14)

The control group also did not show a change in OPG or sclerostin over the equivalent time period to the vibration groups. OPG change at 10 minutes of -5.3% (CI -12.6 to 1.9; p=0.1) and -3.5% (CI -11.6 to 4.6; p=0.3) at 60 minutes. Sclerostin was +3.3% (CI -8.8 to 15.4; p=0.9) and -2.8% (CI -11.1 to 5.4; p=0.2) at 10 and 60 minutes respectively.

Change from baseline: Day 3 and 5 (platform groups both n=10)

OPG and sclerostin were also measured pre-vibration on days 3 and 5. As in the immediate pre- to post vibration period, on day 3 and day 5 no change was detected in either biochemical marker. At day 3 OPG response was +3.2% (CI -8.0 to 14.4; p=0.5) in the low magnitude group and -4.8% (CI -12.4 to 2.7; p=0.7) in the high magnitude group, sclerostin response was +3.6% (CI -6.4 to 13.5; p=0.3) and +3.6% (CI -4.1 to 11.3; p=0.5) respectively.

On day 5 OPG change was +0.7% (CI -8.3 to 9.7; p=0.6) and -2.9% (CI -10.8 to4.9; p=0.9) and sclerostin +11.1% (CI -3.4 to 25.6; p=0.3) and -1.9% (CI -8.1 to 4.4; p=0.1) in the low and high magnitude groups.

Change from baseline: Day 8 (platform groups combined n=11)

OPG showed a trend for an increase of 7.2% (CI -1.4 to 15.8; p=0.08) on day 8 compared to baseline (Figure 10). No change was detected in sclerostin (+8.2%, CI -5.65 to 21.88; p=0.3).

Together with no within group difference in OPG and sclerostin, no difference was detected at any time point between groups.

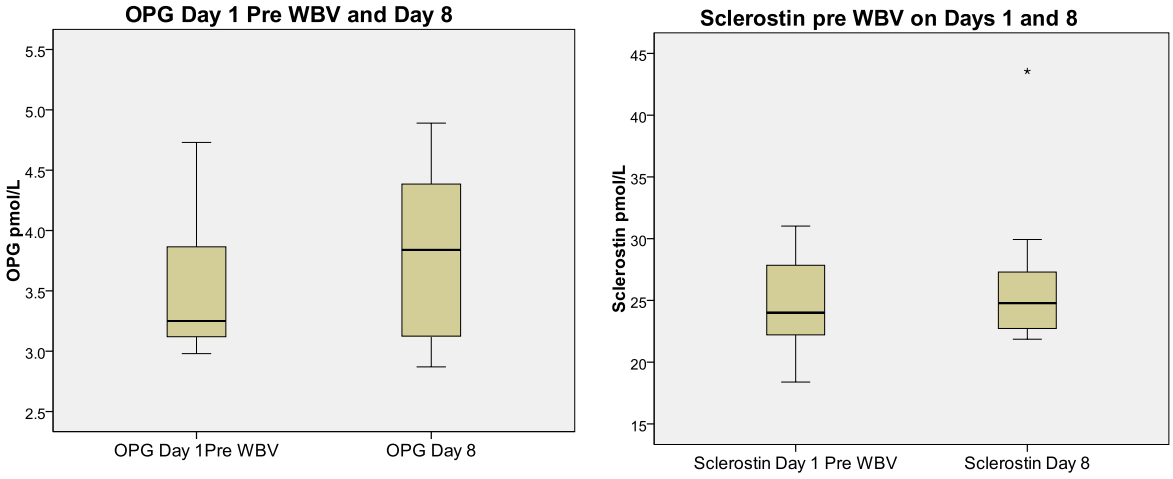


Figure 10 Boxplots illustrating the absolute values OPG (left) and Sclerostin (right) at baseline and day 8 for boys exposed to 5 consecutive days of WBV

7.1.6. Thermal Imaging

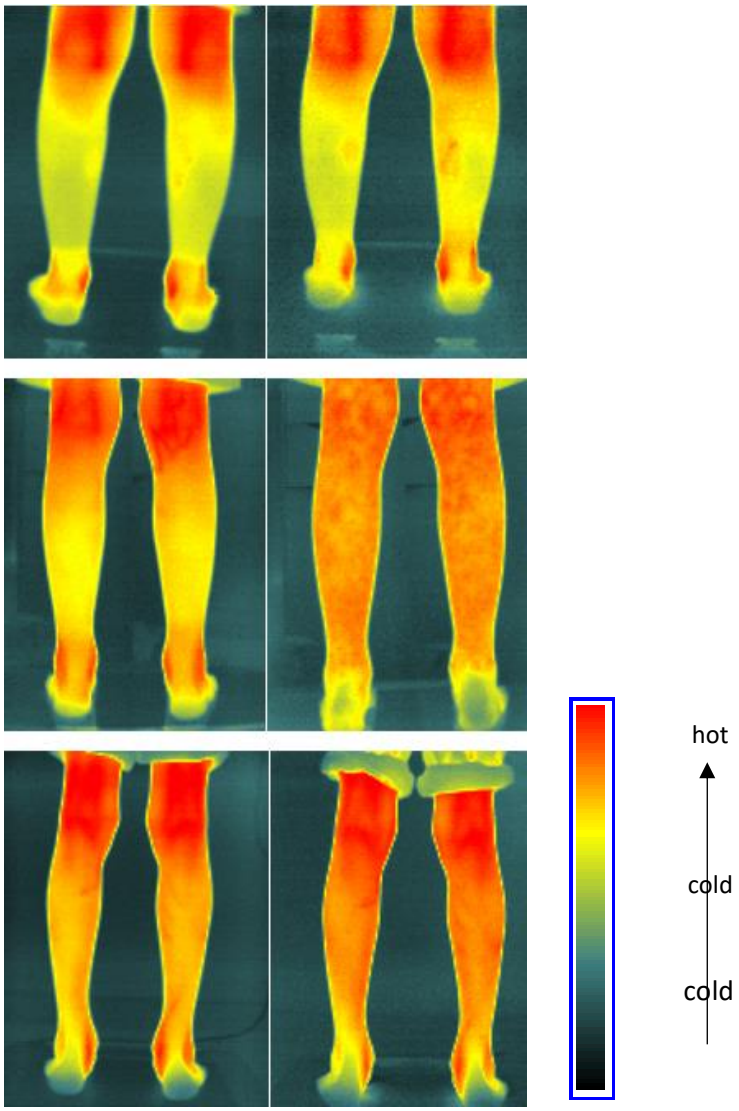
Thermal images were captured on 7 boys (randomly selected) in the control group and on all boys exposed to 1 day only of vibration (n=12). However it was not possible to compare images in 2 of the boys due to difficulties in marking the outlines of the regions of interest, and these were excluded from the analysis. Images therefore were analysed on 5 boys exposed to each of the vibrating platforms and 7 boys from the control group. The change in

skin surface temperature pre- to post vibration ranged from -1°C to 1.6°C (mean 0.3°C, CI -0.4 to 1.1) in the control group, 1.4°C to 4.2°C (mean 2.9°C, CI 1.5 to 4.4) in the high magnitude group, and 0.2°C to 2.8°C (mean 0.9°C, CI -0.4 to 2.3) in the low magnitude group. There was a significant difference in the response of the boys in the high magnitude group compared to the control (p=0.002) and low magnitude groups (p=0.02; ANOVA, bonferroni post hoc test). In addition, when visually assessing the pre and post images, a difference in the temperature distribution was seen in the boys exposed to the high magnitude platform that was not seen in the control or low magnitude groups (Figure 11).

7.1.7. Adverse Events

WBV was well tolerated by the study participants. Minimal side effects were reported; itching or "weird feeling" in the calves or legs (high magnitude), tickling sensation in the feet (low magnitude) and anxiety in relation to cannulation. In all cases these resolved on or shortly after completion of the WBV. At this time it is not clear the exact number of participants who experienced these side effects as the study documentation where this was recorded has been destroyed in line with the study sponsor requirements (5 years from the end of study completion). However from recall less than half of the participants reported any side effects.

a)



b)

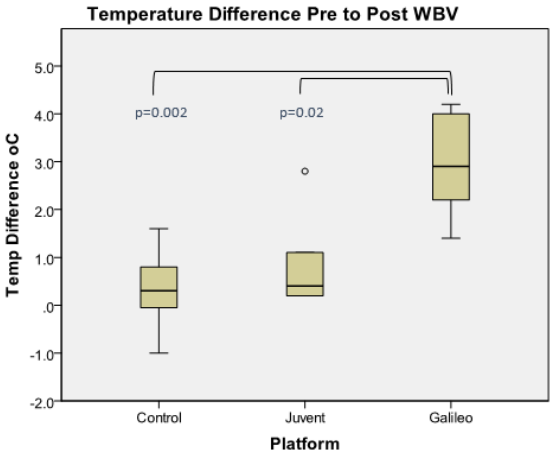


Figure 11 a) Thermal images taken immediately before (left panels) and after (right panels) vibration in the Juvent (top), Galileo (middle), and control (bottom) groups. b) Boxplots showing comparison of the pre- to post-vibration temperature change between the groups

7.2. Study 2 – Acute Response of Bone to whole Body vibration in Pre-Pubertal Boys with a History of Fracture

7.2.1. Baseline Characteristics

Twenty boys consented to participate in study 2. As with study 1 no participants were siblings and none of the boys reported current or recent use of oral steroids for asthma. Two withdrew, 1 due to refusing to have blood samples taken after consent had been obtained; 1 due to difficulties cannulating on day 1. Data was not collected on these 2 participants. Due to difficulties in recruitment (discussed in Chapter 9) the target of 24 was not reached. A decision was made following statistician advice that sufficient data was collected from 18 boys to answer the hypothesis and to end the study prior to reaching the target. A statistically significant result was seen at day 8 in 11 data sets in study 1; complete data at day 8 was collected in 15 boys in study 2 (due to difficulties in cannulation samples were not collected from 3 participants).

The baseline characteristics between the Juvent and Galileo groups appear to be similar (Table 12) although the Juvent group were slightly older. The activity scores were almost identical but in both groups the standard deviation is large. Similar P1NP and CTX scores were seen in the groups however the standard deviation in the Juvent group was larger than that of the Galileo group.

Table 12 Baseline characteristics by platform

	Low magnitude platform n=10			High magnitude platform n=8		
	Mean	SD	n	Mean	SD	n
Age (years)	11.1	1.41	10	10.3	1.20	9
Height (cm)	146.3	10.46	10	144.0	5.93	7
Weight (kg)	39.8	11.12	10	39.5	7.59	7
Body Mass Index (kg m^2)	18.3	3.65	10	19.0	3.18	7
Activity score (METS units)	64.7	25.90	10	64.8	27.68	8
P1NP Day 1 pre (ng/ml)	595.4	203.71	10	577.9	72.70	8
CTX Day 1 pre (ng/ml)	1.9	0.45	10	2.02	0.13	8
OPG Day 1Pre (pmol/L)	3.79	0.69	9	4.47	0.57	8
Sclerostin Day 1 Pre (pmol/L)	33.01	7.55	9	41.33	15.78	8
	Median	Range	n	Median	Range	n
Months since last fracture	17.5	6-39	8	9	6-44	8
History of>1 fracture	-	-	5	-	-	3

SD= standard deviation, n= number of participants

METS: metabolic equivalents
P1NP: pro-collagen type 1 N-terminal propeptide
OCN: osteocalcin
CTX: C-terminal cross-linked telopeptide of type 1 collagen
OPG: osteoprotegerin
SCL: sclerostin

Type and severity of fractures

Thirty-two episodes of trauma resulted in 41 fractures for the 18 boys (at least 6 months prior to study inclusion). Of the 18 boys, 10 boys had 1 episode of trauma resulting in a fracture or fractures, 3 had 2 episodes of trauma, 4 had 3, and 1 had 4. More fractures occurred in the wrist (20 out of 41) than at any other site. In total 32 fractures occurred in the upper limbs including the hand and fingers, 8 occurred in lower limbs including the feet and toes with 1 other (nose). The severity of trauma was classified as mild for 19 of the episodes, moderate for 11 and not recorded for 2. The 19 mild episodes of trauma accounted for 22 of the fractures and the 11 moderate episodes accounted for 17.

Table 13 Number of trauma episode and fractures by platform

	Low magnitude platform	High magnitude platform
Mild trauma episodes	10	9
Moderate trauma episodes	8	3
Number of fractures	26	15

Trauma severity not recorded for 2 episodes

Baseline characteristics comparing study 1 to study 2

Only data from the boys in study 1 who were exposed to 5 days of WBV has been used as the comparison for the data collected in study 2 due to comparable vibration exposure. Table 14 shows the baseline characteristics for the fracture and non-fracture groups split by platform. Within the fracture and non-fracture groups the baseline characteristics were similar across the low and high magnitude platforms. The exceptions to this were sclerostin and OPG. The baseline sclerostin in the non-fracture group was lower in the boys exposed to the low magnitude platform compared to the high magnitude platform (mean 22.16 [SD 2.46] versus 27.01 [SD 3.94] p=0.04). In the fracture groups OPG was lower in the boys exposed to low magnitude platform (mean 3.79 [SD 0.67] versus 4.47 [0.57] p=0.04). Due to the similarities between the platforms within both the fracture and non-fracture groups, the platform data were combined so that analysis was made between the fracture and non-fracture groups.

Table 14 Baseline characteristics by platform, fracture and non-fracture groups exposed to 5 days of WBV

	Non fracture						Fracture					
	Low magnitude platform n=6			High magnitude platform n=6			Low magnitude platform n=10			High magnitude platform n=10		
	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n
Age (years)	9.9	0.36	6	10.1	0.33	6	11.1	1.41	10	10.3	1.20	9
Height (cm)	139.4	4.91	6	143.2	4.74	6	146.3	10.46	10	144.0	5.93	7
Weight (kg)	35.1	3.83	6	36.7	4.44	6	39.8	11.12	10	39.5	7.59	7
Body Mass Index (kg m^2)	18.1	2.1	6	17.8	1.8	6	18.3	3.65	10	19.0	3.18	7
Activity score (METS units)	87.7	27.18	6	81.5	19.29	6	64.7	25.90	10	64.8	27.68	8
P1NP Day 1 pre (ng/ml)	691.2	238.91	6	742.68	121.42	6	595.4	203.71	10	577.9	72.70	8
CTX Day 1 pre (ng/ml)	1.9	0.46	6	1.8	0.24	6	1.9	0.45	10	2.02	0.13	8
OPG Day 1Pre (pmol/L)	3.4	0.45	5	3.66	0.65	6	3.79	0.69	9	4.47	0.57	8
Scl Day 1 Pre (pmol/L)	22.2	2.46	5	27	3.94	6	33.01	7.55	9	41.33	15.78	8

SD= standard deviation, n= number of participants

METS: metabolic equivalents

P1NP: pro-collagen type 1 N-terminal propeptide

CTX: C-terminal cross-linked telopeptide of type 1 collagen

OPG: osteoprotegerin

SCL: sclerostin

When comparing the boys with a history of fracture and the non-fracture groups, the non-fracture boys had a higher activity score (84.6 [SD 22.7] versus 64.7 [SD 25.9] p=0.04), higher P1NP (716.9 [SD 182.7] versus 587.6 [SD 155.7] p=0.05), lower OPG (3.55 [SD 0.55] versus 4.11 [SD 0.67] p=0.03, and lower sclerostin (24.01 [SD 4.07] versus 36.92 [SD 12.48] p=0.001) at baseline (table 15 and figure 12). The difference in age between the fracture and non-fracture groups did not reach significance. No difference in CTX was observed between the groups.

Table 15 Baseline characteristics by fracture and non-fracture groups exposed to 5 days of WBV

	Non Fracture			**Fracture**						
	Mean	SD	n	Mean	SD	n	Mean Difference	95% CI lower	95% CI upper	p value
Age (years)	10.0	0.35	12	10.7	1.34	19	0.66	-1.34	0.08	**0.053**
Height (cm)	141.3	5.02	12	145.4	8.72	17	4.06	-9.81	1.69	0.16
Weight (kg)	35.9	4.05	12	39.7	9.58	17	3.81	-9.19	1.57	0.16
Body Mass Index (kg m^2)	18	1.85	12	18.6	3.38	17	0.63	-2.65	1.38	0.52
Activity score (METS units)	84.6	22.70	12	64.7	25.90	18	19.86	1.01	38.71	**0.04**
P1NP Day 1 pre (ng/ml)	716.93	182.67	12	587.63	155.65	18	129.3	1.97	256.62	**0.047**
CTX Day 1 pre (ng/ml)	1.86	0.36	12	1.95	0.34	18	0.09	-0.36	0.18	0.49
OPG Day 1Pre (pmol/L)	3.55	0.55	11	4.11	0.67	17	0.56	-1.08	-0.05	**0.033**
SCL Day 1 Pre (pmol/L)	24.01	4.07	11	36.92	12.48	17	12.12	-18.92	-5.32	**0.001**

SD= standard deviation, n= number of participants

METS: metabolic equivalents

P1NP: pro-collagen type 1 N-terminal propeptide

CTX: C-terminal cross-linked telopeptide of type 1 collagen

OPG: osteoprotegerin

SCL: sclerostin

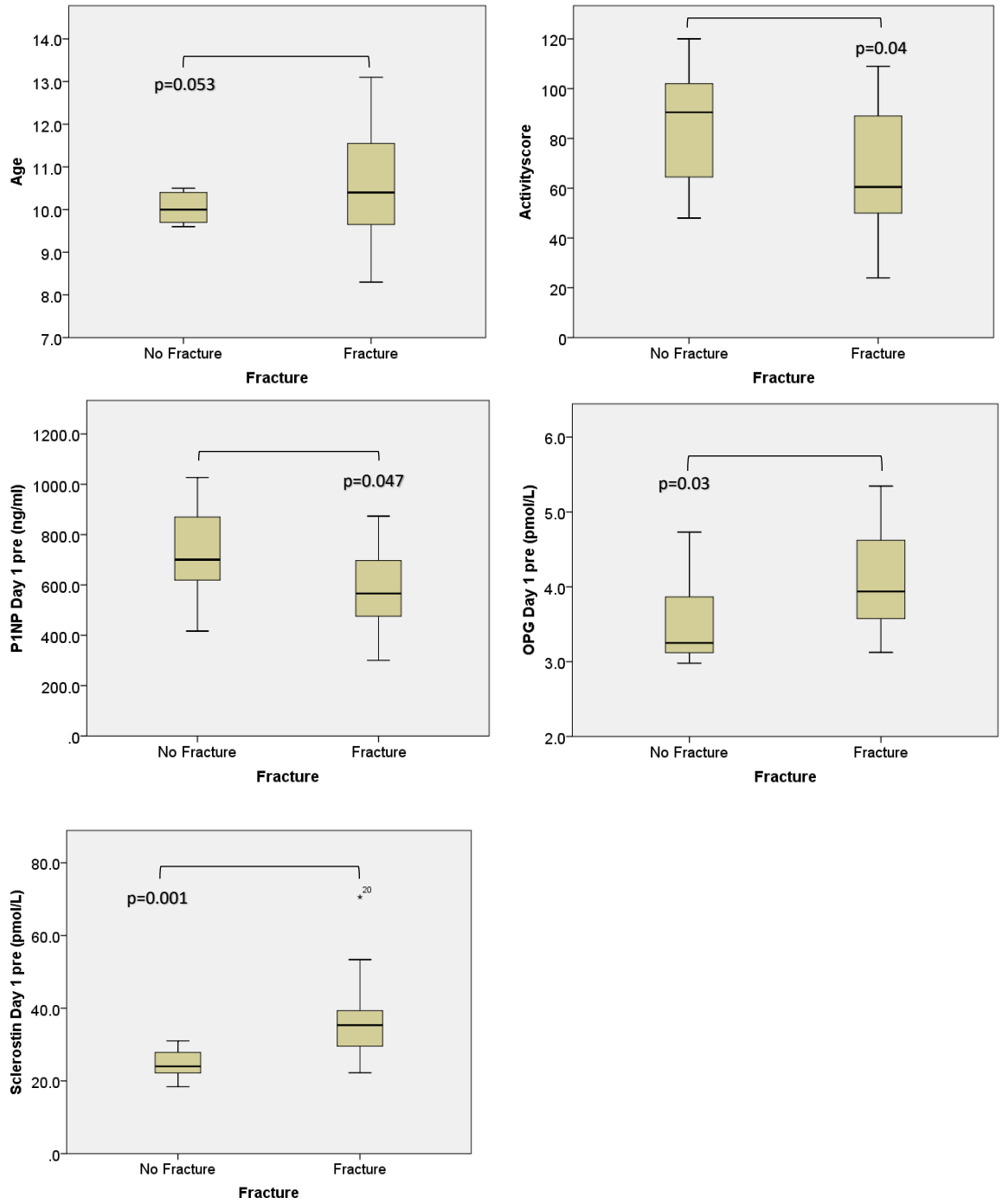


Figure 12 Boxplots illustrating baseline characteristics by fracture and non-fracture groups

Correlation of baseline characteristics

No correlation was found between age, height, weight, or activity score and P1NP. Though there was positive correlation between P1NP and CTX (r=0.598; p=0.03) at baseline. A positive correlation was also found between CTX and age (r=0.519; p=0.03) but no other baseline characteristics. A negative correlation between P1NP and weight (r=-0.33; p=0.08)

and a positive correlation between sclerostin and height (r=0.339; p=0.08) did not reach statistical significance.

7.2.2. Changes from baseline after 5 days of vibration

Values of P1NP, CTX, OPG and sclerostin for each participant are listed in Appendix 11.

There was no change in P1NP at day 8 (following 5 days of WBV) from baseline in boys with a history of fracture according to mode of vibration: low magnitude -0.3% (CI -7.0 to 6.4; paired t-test p=0.9; n=9), high magnitude 3.7% (CI -16.1 to 23.5; paired t-test p=0.7; n=6). However there was a difference in the response of P1NP between the fracture and non-fracture groups (figure 13). The boys without prior fracture (n=11) had a significant increase in P1NP of 25% (CI 12.3 to 38.0; paired t-test p=0.005) compared to 1.3% (CI -6.0 to 8.6; paired t-test p=0.8; n=15) in the boys with a history of fracture (mean difference 23.8; CI 10.8 to 36.9; independent samples t-test p=0.001).

CTX showed a similar trend with no change at day 8 from baseline in the low magnitude fracture group (-1.7%; CI -6.2 to 2.7; paired t-test p=0.5; n=9) or high magnitude fracture group (0.5%; -21.3 to 22.2; paired t-test p=0.9; n=6). As with the formation marker a difference in the CTX response of healthy boys (+10.9% 3.6 to 18.2; paired t-test p=0.009, n=11) compared to the boys with a history of fracture (-0.9%; CI -8.2 to 6.5; paired t-test p=0.9; n=15) was observed (mean difference 11.8%, CI 1.7 to 21.8; independent samples t-test p=0.02). The difference between the fracture and non-fracture groups in both P1NP and CTX remained after adjusting for baseline P1NP/CTX, age, and activity score.

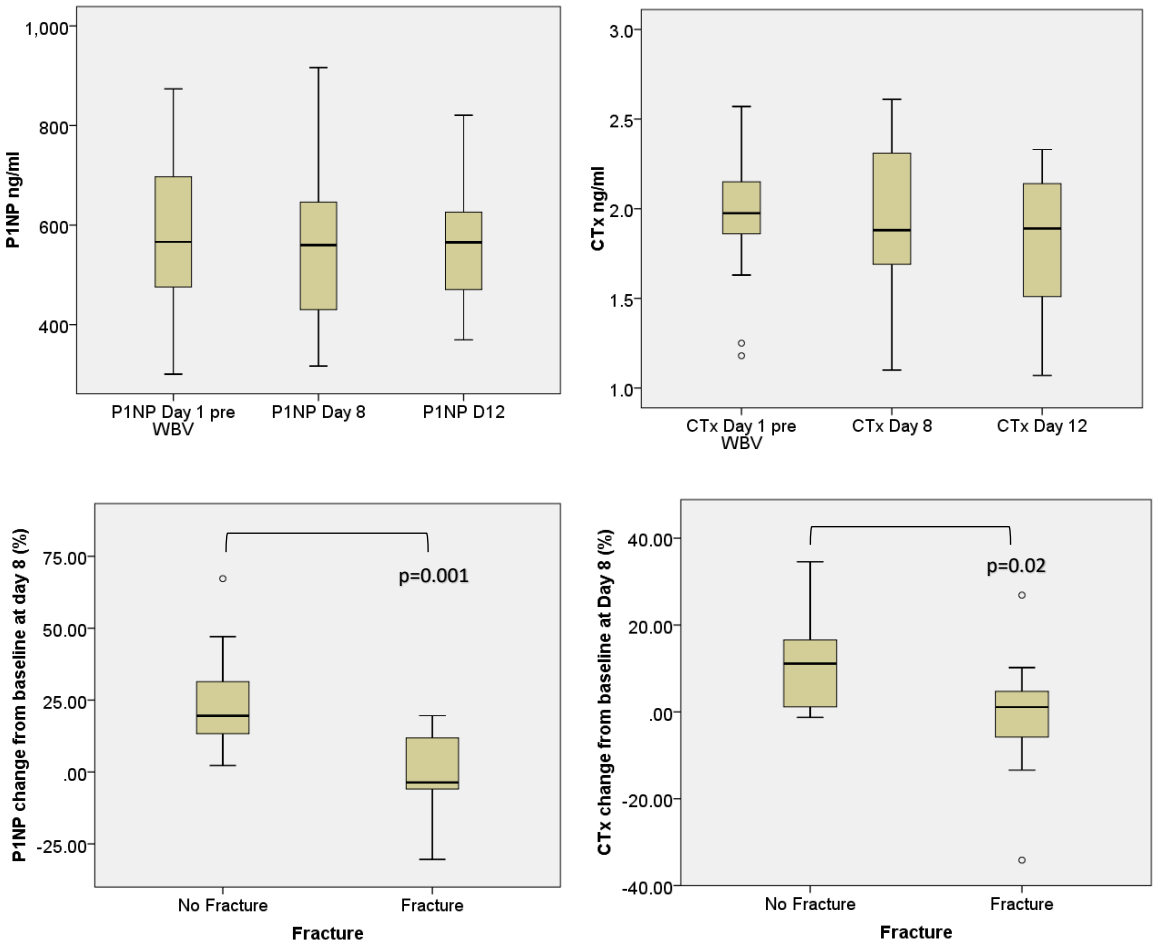


Figure 13 Boxplots illustrating the absolute values of P1NP (top left) and CTX (top right) on days 1, 8, and 12 of WBV in boys with a history of fracture (study 2), and percentage change in P1NP (bottom left) and CTX (bottom right) from baseline at day 8 by fracture and non-fracture group

7.2.3. Change from baseline at day 12

At day 12 there was no change from baseline in P1NP in the boys with a history of fracture (0.33%; CI-7.82 to 8.49; p=0.7; n=17). According to mode of vibration the change observed in P1NP was -0.8% (CI -13.0 to 11.4; paired t-test p=0.5; n=10) in the low magnitude group and 2.0% (CI -12.0 to 16.1; paired t-test p=0.8; n=7) in the high magnitude group. The response was not different between the two groups. P1NP was not measured at day 12 in the non-fracture group so no comparison can be made.

As with P1NP there was also no change in CTX at day 12 (-5.04%; CI -11.49 to 1.41; p=0.07) However when split by platform group CTX decreased by 7.4% (CI -15.4 to 0.6; paired t-test p=0.02; n=10) in the low magnitude group but was not changed in the high magnitude group (-1.6%; CI 14.9 to 11.8; paired t-test p=0.8; n=7). No difference in response between

the two platforms groups was observed. CTX was not measured in the non-fracture group at day 12.

7.2.4. Bone turnover markers – changes across individual cycles of vibration

Within fracture group

Day 1

Looking at the data from boys with a history of fracture only, on day 1 P1NP decreased pre- to 10 minutes post WBV in the low magnitude group (n=9) by 11.5% (CI -20.65 to -2.32; paired t-test p=0.04) with no change in the high magnitude group (n=8; 6.8% decrease observed, CI -15.59 to 1.95; p=0.1). CTX also decreased following WBV by 10.0% (CI -14.76 to -5.30; p=0.001) in the low magnitude group and by -6.4% (CI -12.01 to -0.78; p=0.03) in the high magnitude group. There was no difference in the response of P1NP or CTX to WBV between the platform groups.

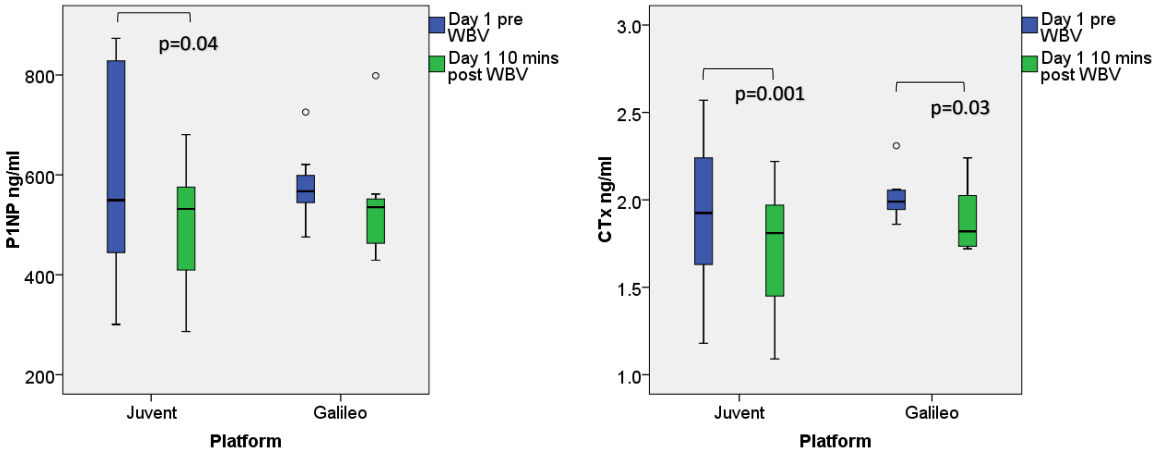


Figure 14 Boxplots illustrating P1NP and CTX pre- to 10 minutes post-WBV on day 1 by platform

Day 3

On day 3 pre-to post WBV P1NP decreased by 19.4% (CI -27.78 to -11.11; p=0.02) in the low magnitude group (n=10) and by 10.5% (CI -18.44 to -2.54; p=0.02) in the high magnitude group (n=6). CTX decreased by 12.7% (CI -16.29 to -9.13; p<0.001) in the low magnitude group and by 7.6% (CI -11.07 to -4.04; p=0.002) in the high magnitude group. Whilst there was no difference in the response of P1NP to WBV between the platform groups, there was

a significant difference in the response of CTX between the groups (mean difference -5.2%, CI -10.13 to -0.19; p=0.04).

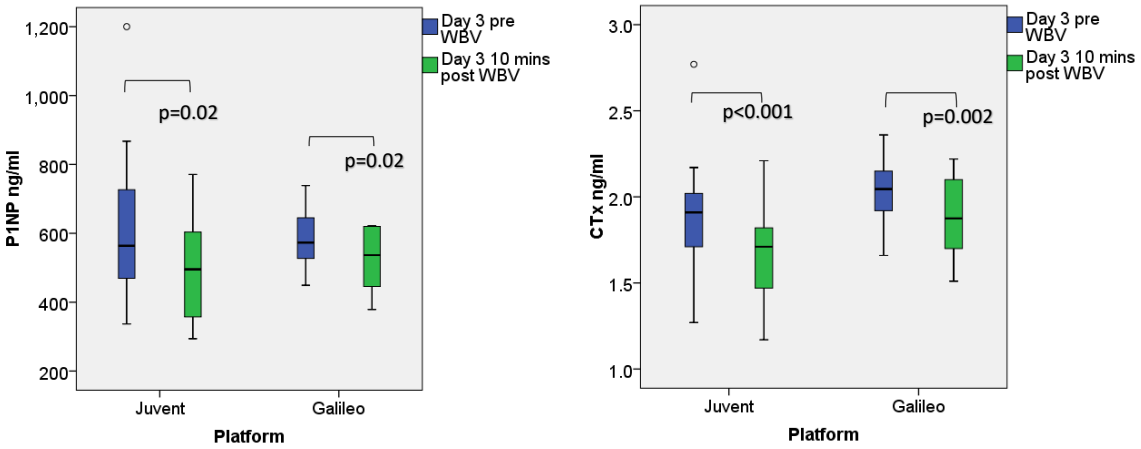


Figure 15 Boxplots illustrating P1NP and CTX pre- to 10 minutes post-WBV on day 3 by platform

Day 5

On day 5 there was no change in P1NP in the low magnitude group (observed decrease of 8.0%, CI -31.83 to 15.92; p=0.2; n=6), there was an observed decrease of 9.5% in the high magnitude that approached significance (CI -19.93 to 0.99; p=0.07; n=6). CTX decreased by 8.1% (CI -15.51 to -0.71; p=0.05) in the low magnitude group and by 9.0% (CI -11.90 to -6.07; p=0.002) in the high magnitude group. As with day 1 there was no difference in the response of P1NP and CTX to WBV between the platform groups.

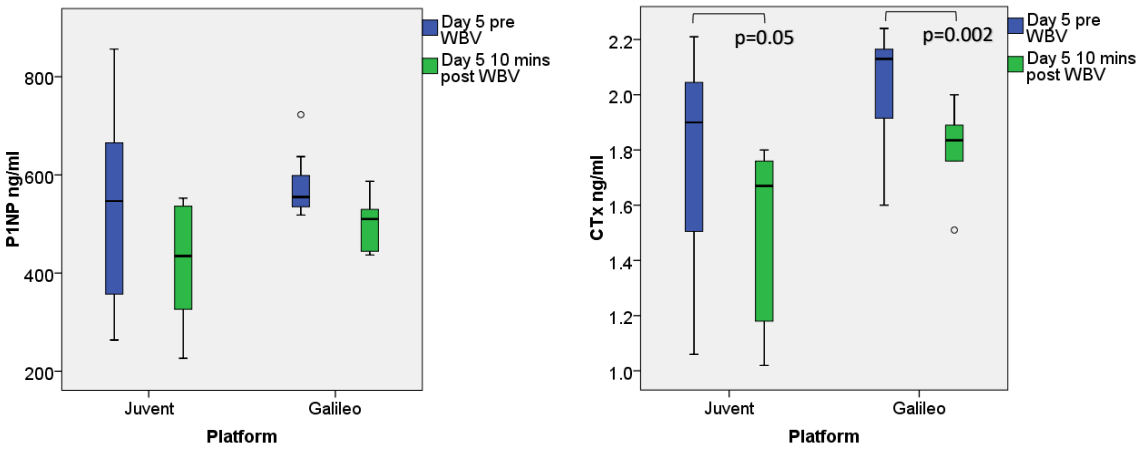


Figure 16 Boxplots illustrating P1NP and CTX pre- to 10 minutes post-WBV on day 5 by platform

Between groups fracture versus non-fracture

Day 1

In the non-fracture group there was no change in P1NP (observed decrease of 3.6%, CI -14.01 to 6.71; p=0.3; n=10) on day 1, and a decrease of 9.3% (CI -15.05 to -3.54; p=0.01) in the fracture group (n=17). There was also no change in CTX in the non-fracture group (observed decrease of 3.0%, CI -9.82 to 3.89; p=0.2) with a decrease of 8.3% (CI -11.66 to -4.98; p<0.001) in the fracture group. There was no difference in the response between the fracture groups in either P1NP or CTX on Day 1 (Table 16).

Day 3

On Day 3 P1NP in the non-fractures (n=10) decreased by 17.8% (CI -24.74 to -10.88; p=0.001) and in the fractures (n=16) by 16.1% (CI -21.94 to -10.24; p=0.005). There was also a decrease in CTX in both groups, the non-fracture group decreased by 6.4% (CI -10.82 to -1.94; p=0.01) and fracture group decreased by 10.8% (CI -13.47 to -8.09; p<0.001). Whilst there was no difference between the groups in the response of P1NP to WBV, CTX showed a near significant between group difference (mean difference 4.4%, CI -0.19 to 8.98; p=0.06).

Day 5

Both groups had a decrease in P1NP pre to post WBV on day 5, the non-fracture group (n=9) decreased by 13.6% (CI 19.81 to -7.29; p<0.001) and fracture group (n=12) decreased by 8.7% (CI -19.36 to 1.94; p=0.03). There was no change in CTX in the non-fracture group (observed decrease of 4.3% (CI -10.41 to 1.92; p=0.2) and a decrease in the fracture group of 8.5% (CI -11.81 to -5.29; p<0.001). As on day 1 there was no difference between the groups in the response of P1NP or CTX.

Pre WBV bone turnover marker values

Pre WBV P1NP and CTX was not different on days 1, 3, and 5 within either the non-fracture or fracture groups or the 2 groups combined, any pre- to post-vibration changes observed had returned to baseline by the next time point. In the fracture boys the mean (SD) P1NP on day 1 was 567.4ng/ml (136.9), 574.7ng/ml (146.3) on day 3 and 549.0ng/ml (166.0) on day 5 (ANOVA, n=13; p=0.5). For the non-fracture boys this was 707.5ngml (198.7) on day 1,

729.9ng/ml (189.0) on day 3 and 735.3ng/ml (184.7) on day 5 (ANOVA, n=9; p=0.8). CTX in the fracture boys was 1.86ng/ml (0.34) on day 1, 1.94ng/ml (0.28) on day 3 and 1.87ng/ml (0.39) on day 5 (ANOVA, n=13; p=0.5). In the non-fracture group CTX was 1.87ng/ml (0.39) on day 1, 1.95ng/ml (0.34) on day 3 and 1.95ng/l (0.48) on day 5 (ANOVA, n=9; p=0.7). No difference in pre-vibration values was observed over the three time points when compared by platform or fracture groups.

Table 16 Changes pre- to post-vibration on days 1, 3, and 5

		Non Fracture				Fracture			
		n	Mean	SD	p value	n	Mean	SD	p value
P1NP ng/ml	Day 1 pre	10	698.5	189.5	0.3	17	572.4	146.0	0.01
	Day 1 10 mins post	10	658.0	144.5		17	513.5	122.5	
	Day 3 pre	10	728.8	178.8	0.001	16	611.6	204.5	0.005
	Day 3 10 mins post	10	596.0	142.1		16	502.2	129.8	
	Day 5 pre	9	735.3	184.7	<0.001	12	516.3	161.2	0.03
	Day 5 10 mins post	9	640.9	186.1		12	460.8	108.1	
CTX ng/ml	Day 1 pre	10	1.83	0.39	0.2	17	1.96	0.35	<0.001
	Day 1 10 mins post	10	1.76	0.29		17	1.79	0.31	
	Day 3 pre	10	1.93	0.34	0.01	16	1.96	0.34	<0.001
	Day 3 10 mins post	10	1.8	0.32		16	1.75	0.3	
	Day 5 pre	9	1.95	0.48	0.2	12	1.83	0.38	<0.001
	Day 5 post	9	1.87	0.5		12	1.66	0.29	

7.2.5. OPG and Sclerostin

There was no change in OPG at day 8 (+5.9%; CI -3.4 to 15.2; paired t-test p=0.2, n=14) or day 12 (-1.2%; CI -8.4 to 6.0; p=0.6, n=16) from baseline in the boys with a history of fracture (Figure 17). Similarly there was no change in sclerostin from baseline at day 8 or day 12 (+4.3%; CI -8.1 to 16.6; paired t-test p=0.7, n=14 and +5.4%; CI -6.7 to 17.4; p=0.7, n=16 respectively). No change was observed either within or between platform groups. This was similar to the lack of response seen in the healthy boys at day 8 from study 1.

7.2.6. Adverse Events

As with Study 1, WBV was well tolerated by the study participants. Nine boys reported a total of 18 adverse events during WBV. Itching in the legs, feet and ankles (3 boys in the high and 1 boy in the low magnitude group), feet tingling (1 boy high magnitude), “pins and

needles" in the legs (1 boy high magnitude), feeling faint and/or dizzy (4 boys low magnitude), and feeling sick (3 boys low magnitude) were reported. Itching and tingling was reported on more than one occasion for 4 of the 5 boys. The 3 boys who reported feeling sick also reported feeling faint and/or dizzy, this was once only for each boy. One boy reported feeling dizzy much later in the day so it is not clear if this was related to the WBV. In all cases any adverse events resolved on completion of, or shortly after the completion of, the WBV session. All participants were happy to continue with the study.

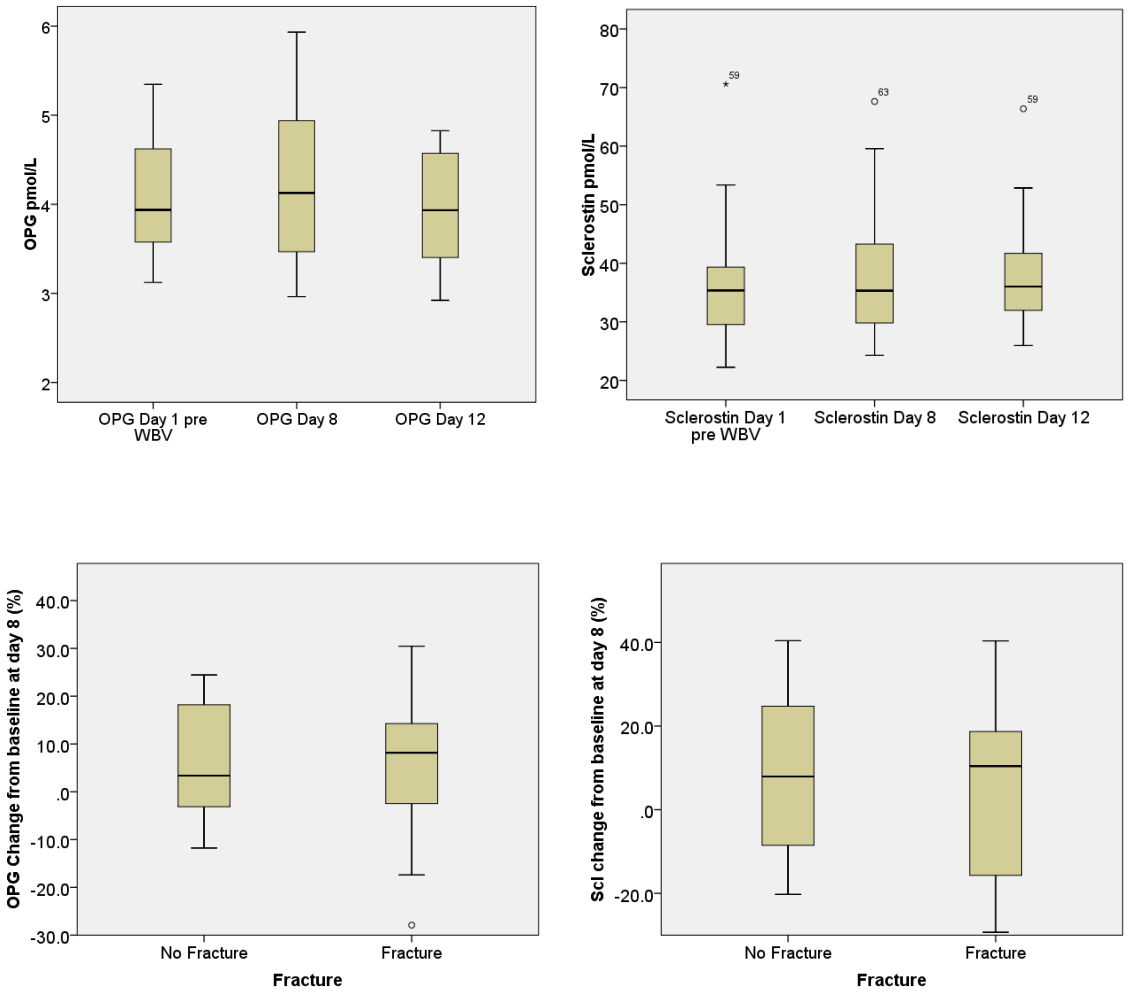


Figure 17 Boxplots illustrating the absolute values of OPG (top left) and Sclerostin (top right) at baseline, day 8 and day 12 in boys with a history of fracture (study 2), and percentage change in OPG (bottom left) and sclerostin (bottom right) from baseline at day 8 by fracture and non-fracture groups

7.3. Summary of findings

In the boys with no prior fracture P1NP and CTX decreased over time in the control group, at 10 minutes post WBV only in the low magnitude group but not in the high magnitude group. On days 3 and 5 P1NP decreased pre- to post WBV in both intervention groups whilst CTX decreased in the low magnitude group only. No difference between group responses was detected. In contrast to the immediate pre- to post WBV periods, by day 8 boys exposed to 5 consecutive days of WBV (low and high magnitude groups combined) showed a significant increase in both P1NP and CTX. No within or between groups difference was detected in OPG or sclerostin at any time point, though there was a trend for an increase in OPG on day 8 (low and high magnitude groups combined).

In boys with a history of fracture P1NP decreased pre to post WBV on day 1 in the low magnitude group but not the high magnitude group, on day 3 it decreased in both groups, whereas no change occurred in either group on day 5. No difference was observed between the group responses. CTX decreased in both groups on days 1, 3 and 5, with a decrease that was greater in the low magnitude group on day 3. No change was observed in P1NP at days 8 or 12, or in CTX at day 8, however there was a trend for a decrease in CTX at day 12. In accord with the boys with no prior fracture, no change was detected in OPG or sclerostin.

When comparing groups by fracture history (platform groups combined) P1NP decreased in the fracture group on days 1, 3 and 5, and on days 3 and 5 in the non-fracture boys; the response was not different between the groups. CTX also decreased on each of the 3 days in the fracture group, but only on day 3 in the boys with no prior fracture. There was a trend for a greater decrease in the fracture group on day 3, but no difference between groups on days 1 or 5. On day 8 the response of both P1NP and CTX was markedly different between the 2 groups with an increase in both bone turnover markers in boys with no prior fracture that was not seen in those with a history of fracture.

8. Discussion

Five consecutive days of WBV in the boys without a history of fracture (low and high magnitude groups combined) increased the bone formation marker P1NP by 25.1% and bone resorption marker CTX by 10.9% between baseline and day 8. The second formation marker osteocalcin was unchanged. OPG showed a trend towards an increase over the same time period and should be associated with reduced bone resorption which was not seen in our results. Sclerostin, a key inhibitor of bone formation, was unchanged at day 8 and is likewise unable to explain our observation of increased P1NP. This study demonstrates that WBV provides a relatively quick and easy means to measure and assess the response of bone to loading in a paediatric population.

In our second study, boys with a history of fracture demonstrated no response in bone turnover markers to mechanical stimulation at day 8. At day 12 however there was a trend for a decrease of 5% in the bone resorption marker CTX. No change was observed in P1NP or the associated bone factors OPG and sclerostin. This difference in response of bone to WBV in boys with and without a history of fracture is a novel finding showing that apparently healthy pre-pubertal boys with a history of fracture do not respond to loading in the same way as those who have not fractured.

Differences between the 2 groups were also observed in the biochemical markers at baseline. P1NP was 20% lower, whilst OPG and sclerostin were 15% and 50% higher respectively in the boys with a history of fracture. CTX was not different between the 2 groups. The higher level of sclerostin may have made the boys less receptive to the anabolic effect of loading on bone which was shown by change in P1NP at day 8 and may partly explain why boys with a history of fracture do not respond to loading in the same way as those who have not. However sclerostin does not appear to be the regulator of the bone formation response here following WBV. The same level of bone resorption (CTX), with a reduced level of bone formation (P1NP) detected at baseline in the boys with a history of fracture, could be indicative of reduced bone accrual in this population. This in combination

with the reduced response to loading could to some extent explain increased fracture susceptibility in some children.

The response to loading observed in the biochemical markers was not altered by magnitude of WBV. However change in skin surface temperature following WBV was different between groups; the high magnitude side-alternating platform group had a greater increase in skin surface temperature than the low magnitude synchronous platform and control groups (increase of 10%, 2.8% and 1.1% respectively). It is not clear if bone responds to vibration directly within the bone tissue or indirectly via musculoskeletal forces. An increase in skin surface temperature, a surrogate measure of muscle activation, would suggest the latter. Nevertheless the increase in bone turnover markers seen in both platform groups would suggest muscle activation is not a prerequisite for bone to respond to mechanical stimulation.

8.1. Difference in response of boys with and without a history of fracture

Whilst the difference in response of bone turnover markers in boys with and without a history of fracture has been clearly shown by our data, the reason for the difference is not so easily explained. In our cohort the boys were exposed to vibration as recently as 6 months and up to 3.5 years post fracture and we cannot be certain that the reduced response to loading would have been the same prior to their fractures. We cannot discount that the reduced loading response is as a result of sustaining a fracture(s) rather than a contributing factor to fracture. Participants were required to be at least 6 months post fracture at the time of vibration to limit any possibility of ongoing fracture healing altering the bone response to loading or obscuring changes in bone turnover marker levels. P1NP and CTX are known to be elevated post fracture peaking at 4 and 12 weeks (CTX and P1NP respectively) before decreasing, levels may nonetheless remain elevated for up to a year (277, 278). At baseline CTX was not different between our fracture and non-fracture groups and P1NP was in fact lower in the fracture group. It is therefore unlikely that fracture healing could be the cause of the difference in bone turnover marker response between the 2 groups.

Physical activity has an anabolic effect on bone shown repeatedly in studies measuring bone size, mass and strength. In our cohort, the boys who had experienced fracture(s) were less physically active in the week prior to WBV. It is possible that this is the cause of lower bone formation (P1NP) in this group at baseline. In a cross-sectional study of habitual physical activity in preadolescent girls P1NP was found to be higher in those who undertook higher levels of physical activity compared to lower levels of physical activity (18,695 +/-1244 and 7633 +/-1099 steps a day respectively) (148). The boys were asked to maintain their normal level of physical activity during their exposure to WBV. If the boys without a history of fracture continued to be more active over the vibration period (concurrent physical activity was not measured), this in combination with the 5 days of vibration could have resulted in the increase seen in P1NP at day 8. However Humphries et al (97) in their study of healthy young women found no difference after 16 weeks in either bone outcomes measured by DXA or serum bone markers between WBV only and WBV plus resistive exercise groups, suggesting that additional exercise may not explain the difference in response seen in our study.

The trend towards a decrease in bone resorption of 5% at day 12 but lack of response at day 8 might suggest that the response of bone to WBV takes longer to reach the level of significance in boys with a history of fracture. Additionally the increase in CTX of 10.9% seen at day 8 (it was not measured at day 12) in boys with no prior fracture is a response of greater magnitude, suggesting that these boys may not only respond more quickly but also be more receptive to the vibration signal than those with a history of fracture. The direction of response also differed with the fracture group response indicating a decrease in resorption activity. The reduction in CTX with absence of change in P1NP could be indicative that in boys with a history of fracture bone responds to WBV by slowing down remodelling activity. In the boys with no prior fractures increases in both CTX and P1NP were observed, due to the short period of time it is unlikely formation change is a result of remodelling activity, but would reflect instigation of independent pockets of bone modelling in response to WBV. Either way bone turnover response indicates a positive effect of WBV on bone. As no change was observed in bone formation in the boys with a history of fracture it could be suggested that WBV serves the purpose of preventing bone loss in this population as

opposed to prompting bone formation. However, as the response of CTX in the boys with a history of fracture was not conclusive, the result did not reach significance, this is only speculation and needs further investigation. Therefore it is also possible that 5 days of WBV was not sufficient time to elicit a response in this group.

Alternatively the magnitude of the load may not have been sufficient to produce a response in boys with a history of fracture. We compared two different magnitudes of vibration, 0.3g in the Juvent groups (low) and 6.4g in the Galileo groups (high), a difference in response between the low and high magnitude vibration groups both in and between boys with and without a history of fracture was not observed. Other studies comparing different magnitudes of vibration have similarly been unable to establish a difference in response of bone turnover markers to magnitude of vibration. Data from older adults exposed to different levels of high magnitude vibration (Galileo platform at 3.6g or Powerplate platform at 1.5g) 3 times a week for 12 weeks, demonstrated increases in P1NP of 35% and 26% respectively that was not different between platform groups (132). Similarly Elmantaser et al 2012 (133) did not report a significant difference in response of CTX in a high magnitude group (Galileo, 3.8g) compared to a low magnitude group (Juvent, 0.3g) following 8 weeks of WBV in healthy adult males. This is despite a decrease in CTX in the Galileo group but not in the Juvent group. We also observed in a decrease in CTX of 7.4% in the Galileo group that was not present in the Juvent group (day 12 in boys with a history of fracture only) and likewise the response between the 2 groups was not different. Whilst our data also did not show a significant difference between the platform groups at day 8 (or any time point other than in CTX pre- to 10 minutes post- WBV on day 3), the data at this time point does suggest that the boys exposed to the high magnitude platform (both fracture and non-fracture groups) may have had a greater percent increase in P1NP than those exposed to the low magnitude platform (4% and 5.2% greater increase in boys with and without history of fracture respectively). A similar effect at 12 weeks (9% greater effect in the high magnitude group) was observed by Corrie et al (132). With larger sample sizes, the above 2 adult studies only had 60 and 10 participants respectively, a difference between platform groups and therefore vibration magnitude might become apparent.

Chapter 5 highlights a number of factors associated with fractures and fracture risk that may be pertinent to the different response to vibration seen here in boys with and without a history of fracture. All the boys in our studies were pre-pubertal, Tanner stage 1, so differences in stage of puberty and/or growth cannot explain the difference in response between our 2 groups; height and weight were similar between the groups. Also discussed in Chapter 4 there is much evidence to support an association of pubertal-stage and bone accrual following exercise, it is possible that a similar effect could occur on response to WBV loading. With a larger sample size it would be appropriate to consider level of trauma resulting in fracture and the response to WBV. Farr et al (230) have suggested that children who fracture following mild trauma have stronger evidence of bone fragility than those who have not fractured. It could be in this group of children specifically that the response to mechanical stimulation is attenuated. Other factors known to affect fracture risk such as dietary, environmental and genetic factors were not measured in our cohort and therefore cannot be ruled out as possible influences on group differences.

The suggested association of low serum Vitamin D and fracture risk discussed in Chapter 5, is further supported by Borg et al (279) who demonstrated in a murine model that vitamin D deficiency in early life leads to decreased bone strength. Additionally they found evidence of a reduced response of growing bone to mechanical loading with effects continuing into adult life. Work by a colleague within the Academic Unit of Child Health, Sujatha Gopal, looking at the effect of WBV in 4-5year children whose mothers were given Vitamin D supplementation or placebo is just completed and due to be presented at the International Conference on Children's Bone Health in June 2019. This cohort of patients were recruited from the MAVIDOS study which has demonstrated that children born in winter months (December to February) to mothers who received Vitamin D supplementation during pregnancy had greater BMC, whole body area and BMD (mean difference 5.5g, 11.5cm^2, and 0.01g/cm^2 respectively) at birth than those born to mothers who received placebo (248). Even at 9 years of age maternal serum Vitamin D deficiency/insufficiency, reduced ultraviolet-B exposure and lack of Vitamin D supplementation in late pregnancy are associated with reduced whole body BMC, BA and BMD, and LS BMC and BMD (249). Taken with the observations by Borg et al early life Vitamin D deficiency if present may in part

explain the reduced response to loading seen in our fracture cohort. Of interest more boys in the fracture group were exposed to WBV during the months of December to May when there is a greater risk of serum Vitamin D insufficiency in British children (280, 281) than in the non-fracture group (10 out of 17 boys, and 5 out of 11 respectively). However neither maternal Vitamin D status at birth nor participant Vitamin D were measured in our cohort and therefore cannot be confirmed or disputed as the cause of reduced response to loading in boys with a history of fracture.

8.2. Day 8 change in boys without prior fracture

The greater increase over the eight day period in the formation marker as opposed to the resorption marker (in the boys with a history of fracture only) suggests that there is an uncoupling of bone turnover in favour of formation in response to vibration. These changes are consistent with the reported effects of increased activation of the canonical wnt-signalling pathway through LRP5/6 where bone formation is increased and there is increased osteoblastic expression of osteoprotegerin. Our data indicated this with a trend towards an increase in OPG in the boys without a history of fracture. However increased expression of OPG should also be associated with reduced bone resorption which is not confirmed by our CTX results.

Only a small number of studies have investigated the response of bone turnover markers to WBV in a paediatric population. In girls with low bone density an increase in BSALP of 16.6% was detected following 8 weeks of WBV (138), similar to the increase in our healthy boys. Two paediatric studies, one in children with Duchene's Muscular Dystrophy and another in children with severe motor disability where WBV occurred over 3-6 months, were unable to detect any bone turnover response (120, 129). A third study in overweight boys also didn't find a response after 10 weeks of high magnitude vibration (134), however this was different to the control group who saw an increase in CTX of 11%, suggesting abrogation of bone resorption following WBV. This is in contrast to our healthy boys exposed to vibration where resorption measured by CTX was increased. Increased bone resorption with no change in formation in the overweight control group indicates uncoupling of bone turnover

in favour of bone loss. Attenuation of resorption with no change in formation in the overweight boys exposed to WBV would have a protective effect via an uncoupling of bone turnover in favour of formation as we found in our cohort.

A greater number of studies regarding the effect of WBV on bone turnover markers have been conducted in a variety of adult populations. In accord with our results, Corrie et al (132) found an increase in P1NP following 12 weeks of WBV that was 25-36% greater than controls (dependent on platform as discussed above in section 8.1), but no change in CTX. In contrast to the increase we saw in resorption, Turner et al (136) showed a 34% decrease in urinary NTx following 6 weeks of WBV in postmenopausal women, a third of whom had osteoporosis. In a similar population Iwamoto et al (92) also found a reduction in NTx, however participants were concomitantly receiving Alendronate, a bisphosphonate whose function is to reduce bone turnover, this may have obscured or diminished any vibration effect. Following one episode of vibration and resistive exercise Sherk et al (135) also found a decrease in CTX in healthy young women exposed to one episode of high magnitude synchronous WBV. The decrease was greater at 30 minutes post exercise when the women were exposed to WBV immediately prior to resistance exercise than following resistance exercise only (-12.5% vs -1.3%). In a similar study healthy young men showed a decrease in CTX immediately post WBV, but unlike in the women CTX was not different between the groups post exercise (WBV immediately prior to resistive exercise versus resistive exercise only) (131). In both of these studies TRAP5b, another marker of bone resorption, appeared to increase immediately post WBV, however when corrected for plasma volume there was no change in the women and a decrease of 9.9% in the men as there was with CTX. Neither study showed a change in bone formation markers in response to WBV. Whether in accord or contrast to our findings these studies nonetheless demonstrate a change in bone turnover markers that suggests an uncoupling of bone turnover in favour of formation as we found in our healthy paediatric population. In contrast Rubin et al (282) found only a slight decrease of 3% in the resorption marker hydroxyproline in their active vibration group with a much greater decrease in the placebo group of 16%. The difference between the 2 groups (postmenopausal women) in the resorption marker would suggest greater bone loss in the

WBV group, this however contradicts their DXA outcomes whereby the intervention group had less bone loss then the placebo group.

The second bone formation marker we measured in the boys without a history of fracture, osteocalcin, did not change over the period of observation. This may be because osteocalcin is produced later in the process of endochondral ossification, when mineralisation is taking place. By contrast, P1NP is produced early in the bone formation process, when the osteoid matrix is being formed and deposited. Other studies have also been unable to detect a change in osteocalcin following 10 weeks to 12 months of WBV (101, 115, 117, 133, 134, 282, 283). It is possible that a change was not detected in these studies as the formation marker was measured too late and the level may have returned to a pre WBV value. Bowtell et al (137) detected a change in osteocalcin at 48 hours post vibration in healthy adult females exposed to a single episode of WBV. Osteocalcin was measured 72 hours post-vibration in our cohort and only in the boys exposed to 5 days of vibration. WBV may therefore only elicit a transient increase in this formation marker.

Our focus on healthy children and the response of bone in the growing skeleton rather than on a fully developed skeleton may explain some differences seen between our results and those of others. The increase in resorption and greater increase in formation in our group, not typically seen in the adult populations, may reflect the enhanced response to loading that has been well reported in the growing as opposed to adult skeleton. Eight separate papers (101, 108, 120, 121, 124, 129, 130, 283) found no change in bone markers following 3 months to 3 years WBV in healthy young adults, older adults, adults with history of chronic stroke, children with motor disabilities and postmenopausal women. However, this may reflect the adaptation of bone structure and bone remodelling to the continuing stimulus over a prolonged period. Bone response to loading is more apparent in the initial stages of loading with no further benefit of continuous loading; over time bone formation levels will return to normal (284) and this could be reflected by the studies above. In considering Frost's Mechanostat Theory once bone has responded to the increased load, and size, mass or microarchitectural adaptions have occurred reducing strain to normal levels, a new equilibrium will be set and bone modelling would cease.

8.2.1. Pathway for change in bone formation and resorption

Sclerostin is widely recognised to be a key inhibitor of bone formation by the canonical wnt-signalling pathway through LRP5/6 (285) and is an important factor in bone response to mechanical loading (286). However no significant change in serum sclerostin was detected either over time or between our fracture and platform groups (or within the fracture or non-fracture groups by platform) and could not therefore explain our observations of increased P1NP in the boys without a history of fracture. Similarly no change in sclerostin was seen in healthy adult males following 8 weeks of WBV (133) or in postmenopausal women following 1 episode of WBV (287). In contrast Cidem et al (140) demonstrated in healthy women an increase in sclerostin pre- to 10 minutes post-vibration on day 1 and a decrease over the same period on day 5. As we did not measure sclerostin immediately pre- to post-vibration on days 3 or 5 it is not apparent if a similar response occurred in our cohort. Furthermore a gradual increase in pre-vibration levels over the 5 days of WBV was observed by Cidem et al that we did not; bone formation markers were not measured in the healthy women therefore a corresponding change in bone formation cannot be ascertained. In contrast in vitro mechanical vibration of primary rabbit osteoblasts has shown decreased sclerostin gene expression (288) and animal studies have shown down-regulation of sclerostin production at sites of new bone formation following even short periods of mechanical loading (18, 286). Sclerostin response has been shown to be dose dependent with sections of greater strain along the ulnar diaphysis (proximal, mid-shaft and distal regions) and cross section (medial, lateral and central cortex) showing a greater reduction in sclerostin positive osteocytes with a corresponding strain dependent increase in bone formation across the same sites (18). Circulating serum sclerostin was however unchanged following loading despite the observed decrease in SOST and sclerostin expression. Therefore the lack of response in sclerostin observed in our cohort of pre-pubertal boys may be due to changes in serum sclerostin not reflecting accurately or quickly on changes occurring at a tissue level.

Alternatively activity in other signalling pathways or regulators of the wnt-signalling pathway may explain the acute response of bone formation and resorption observed in our cohort. Like sclerostin, DKK-1 and WISE are highly expressed in osteocytes, and bind to

LRP5/6 preventing binding of Wnt and the subsequent activation of the Wnt β-catenin signalling pathway (12, 289). Loss of function studies of the LRP5/6 complex have repeatedly demonstrated low bone mass phenotypes and likewise high bone mass in gain of function mutations (290). Other compounds such as nitric oxide and prostaglandin E2 also produced by the osteocytes in response to mechanical stimulation have a positive role in bone formation regulation (14) via crosstalk with the wnt-signalling pathway (12). Noncanonical Wnt signalling pathways such as the planar cell polarity, Rho/Rac GTPase, and Gprotein-coupled receptor signalling pathways are also thought to have a role in skeletal development but are less understood than the canonical Wnt βcatenin signalling pathway. Other pathways considered to control bone formation include the bone morphogenetic protein (BMP) signalling pathway via expression of Runt-related transcription factor 2 (Runx2) which is crucial for osteoblast differentiation, and the sympathetic nervous system which negatively regulates bone formation and increases bone resorption through stimulation of the β_2 adrenergic receptor (14). In addition to these pathways hormonal interactions with the bone cells occur resulting in regulation of bone formation and resorption. PTH prevents osteoblast and osteocyte apoptosis increasing osteoblast number and bone formation, and by interaction via the PTH-PTHR1 complex with LRP6, initiates signalling in the absence of Wnt-ligands (289). Additionally PTH has a resorptive effect through enhancing the expression of RANKL. Insulin-like growth factor 1 (IGF-1) has a role in osteoclastogenesis and therefore resorption directly through the IGF receptor and upregulation of RANKL. Expression of RANKL is also stimulated in the presence of the active form of Vitamin D, 1,25 $VitD_3$. The role of Vitamin D via the Vitamin D receptor as both an anabolic and catabolic hormone in bone homeostasis has been described with its importance in the role of RANKL expression thought to be dependent on the maturation of the osteoblast (291). The number of pathways, compounds and bone cell interactions involved in bone modelling and remodelling activity highlight a complicated physiological process involved in bone response to mechanical loading.

In addition to measuring sclerostin, we measured OPG at baseline, day 5 and day 8, and additionally at day 12 in the boys with a history of fracture. No change was detected in OPG at any time point in the boys with a history of fracture. OPG did however show a trend

towards an increase from baseline at day 8 in boys without a history of fracture. OPG expressed by osteoblasts is the decoy receptor for RANKL which is sited on the surface of osteoblasts and osteoblast precursors. RANKL/RANK signaling is essential for osteoclast proliferation and activation, by interacting with the RANK receptor on osteoclast precursors OPG plays a pivotal role in osteoclast differentiation and its resorptive activity (292). OPG should be associated with reduced bone resorption which was not seen in our results where bone resorption measured by CTX in fact increased in the boys without a history of fracture. The ratio of OPG/RANKL is considered to be important in determining bone mass (293), however RANKL was not measured in our cohort as at the time of designing the study the assays for measuring this in serum were not seen to be very reliable.

8.3. Daily pre- to 10 minutes post- vibration change

Bone turnover markers were also measured pre- and 10 minutes post-vibration on days 1, 3 and 5 of the intervention to ascertain the rate and range of bone response to WBV. An immediate decrease in P1NP and CTX pre- to post vibration on days 1, 3, and 5 was observed in most of the groups (control group measured on day 1 only, and vibration groups split by platform and fracture). On day 1 P1NP and CTX decreased in the control group at 30 mins and 90 minutes (corresponding to 10 and 60 minutes post WBV), at 10 mins post WBV in the low magnitude groups, but not in the high magnitude groups, this is regardless of fracture history. Samples were collected in a fasted state between 07:30 and 10:00 am to reduce biological variability, as discussed in Chapter 6. Decreases in P1NP and CTX of up to 12% were observed in the control group; the size of change is similar to that reported due to the circadian variation (294, 295) and was therefore not unexpected. Other controllable factors thought to effect bone turnover markers, such as lifestyle factors (specifically smoking and alcohol) and intense physical activity the day before sampling, (277) were not accounted for, but were unlikely to be relevant in this cohort and were not different between groups for the duration of the participants' study involvement. Dietary factors, as discussed in section 8.1, were also not measured, however the baseline values (including height, weight, and BMI) between the control and platform groups were similar.

Stress hormone effects of venepuncture and cannulation have been reported, showing elevated salivary cortisol levels before and 20-30 minutes after cannulation in children (296). Many of the boys in our study were anxious about the blood sampling process (observed by or as voiced to the researcher) and it is likely that a similar response would occur in our cohort; cortisol was not however measured. Serum cortisone and cortisol levels are negatively correlated with the bone formation marker osteocalcin, with a positive or no correlation with CTX and urinary NTx (297-299). The inhibitive effect of corticosteroids on bone formation has been widely reported. However there is very little data to support an acute cortisol effect on bone turnover markers. The impact on osteocalcin is the most widely reported but we did not measure this in the control group. It is not possible to state whether or not the stress effects of cannulation could have influenced the bone turnover markers in our control or platform groups (including any effect on bone response to loading) over the time period measured.

As with time of sampling and fasting, any stress hormone affect would be expected to be similar between the control and platform groups. The change in bone turnover markers in the platform groups did point towards a diminished reduction in P1NP and CTX at 60 minutes post WBV (90 minute sample in the controls), with an increase in P1NP in the high magnitude group, however this was not significant within the groups and was not significantly different to the response over time in the controls; the bone turnover marker change in the controls was therefore similar to that of the boys exposed to WBV.

Pre- to post vibration on day 3 there was no decrease in CTX in the high magnitude no fracture group, whilst the decrease observed in the high magnitude fracture group was less than that observed in the low magnitude fracture group. Possibly suggesting that there is a trend for high magnitude WBV to limit bone resorption over the time period measured; the post-vibration sample was collected approximately 30 minutes after the pre-vibration sample. As on day 3, again on day 5 CTX did not decrease in the high magnitude no fracture group, but did in all others. P1NP did not decrease pre- to post-vibration in the low magnitude fracture group. Other than the day 3 CTX pre- to post vibration change there was

no difference in the response to vibration between groups. P1NP and CTX returned to the baseline (day 1 pre vibration) values prior to vibration on days 3 and 5.

As stated above participants were fasted on each day that blood sampling occurred as is the correct approach to reduce variability in bone turnover markers, specifically CTX (265, 266). However any possible effect of fasting on bone response to WBV was not considered. Fasting occurred on days 1, 3 and 5 but was not required on days 2 and 4 despite exposure to WBV on each of these days. An increase in CTX following endurance cycling has been shown in non-fasted elite male athletes (300), which was not shown in our fasted cohort in the immediate pre- to post vibration period. However this study specifically measured an oral calcium dose effect on bone resorption (demonstrating suppression of the rise in CTX) and did not compare a fed/fasted state. In support of the design of our studies, response of bone turnover markers to acute weight-bearing exercise regimes in adults (running, resistance training and plyometric exercise) does not appear to be affected by a fed or fasting state (301, 302). Three consecutive days of 60 minutes treadmill running at restricted energy intake compared to a balanced energy intake in healthy adult males showed a decrease in P1NP but no difference in NTx from pre-exercise to 1 day post-exercise (303). We saw a similar response in P1NP in the immediate pre- to post vibration period, but an opposite effect 3 days post vibration in our study. However the boys in our study did not have their energy intake restricted over the period of the 5 day WBV intervention. Other than an overnight fast prior to blood sampling there were no other dietary restrictions; all the boys ate breakfast following blood sampling and were instructed to eat as normal up until bedtime the night prior to sampling. There is little reported evidence to suggest that the response of bone turnover markers to WBV would have been altered in a non-fasted state in our cohort.

Whilst the daily pre- to post vibration results in our cohort are interesting they are not conclusive of what is happening to bone turnover immediately following WBV. One reason for this could be the small number of participants; bone turnover data was collected from as few as 4 in the no fracture high magnitude group on day 5 with a maximum group size of 10 in the low magnitude fracture group on day 3. Due to the small numbers data were

compared by platform or fracture groups but not both. On day 1 the comparison with the control group included study 1 data only. Where changes from the boys with a history of fracture is reported compared to boys without, only data from boys exposed to the full 5 days of vibration is included in the analysis. Within the groups the SD were quite large, the variance in the data highlights that more participants would be needed in each group to sufficiently power a study to detect any statistically significant differences either between the platform groups or between boys with and without a history of fracture. As pilot studies neither study 1 nor 2 were powered to detect a significant change, the purpose of the studies was to assess the range and rate of the response of bone to WBV in the pre-pubertal boys. Prior to this work it was not known how soon a response to WBV could be detected in healthy boys or what size of response could be expected.

8.4. Differences in baseline characteristics

Baseline differences between the boys who had previously fractured and those who had not were observed in P1NP, OPG and sclerostin, but not CTX. P1NP was 20% lower whilst OPG and sclerostin were 15% and 50% higher respectively in the boys with a history of fracture. The 20% reduction in bone formation (P1NP) with a similar level of bone resorption (CTX) at baseline indicates uncoupling of bone turnover that could result in reduced bone accrual and potentially reduced bone mass in boys with a history of fracture. As discussed previously (in chapter 5) these are associated with increased risk of fractures (202, 220, 223-225, 227) and therefore may partly explain the fracture susceptibility in this group. This is however stated with caution as we did not measure bone size and mass in our cohort. It is possible that an inherent abrogated response to loading is the cause of the discrepancy between the 2 groups in the biochemical markers at baseline.

The higher level of sclerostin at baseline may have made the boys less receptive to or blocked the anabolic effect of the additional WBV load on bone which was shown by change in P1NP at day 8 in the boys with no prior fracture. This may partly explain why boys with a history of fracture do not respond to loading in the same way as those without. The effect of increased sclerostin expression on reduced bone formation has been widely reported (14, 15) and P1NP and sclerostin levels at baseline in our fracture cohort would correspond with

this. The difference in baseline sclerostin levels between the fracture and non-fracture groups may highlight a role of sclerostin in adolescent radial fractures as suggested by Kirmani et al (304) who observed in boys and young men a positive correlation between serum sclerostin and apparent cortical porosity of the radius, which peaks at the time of greatest incidence of radial fractures in childhood. However the lack of change observed in sclerostin at day 8 in both groups and the absence of a correlation between changes in sclerostin and P1NP would suggest that sclerostin is not the regulator of the bone formation response seen in P1NP in our study.

In addition to the differences in sclerostin and P1NP at baseline between the 2 groups, OPG was greater in the boys with a history of fracture. This increase cannot be explained by bone formation (increased osteoblast activity) as you would expect to see an increase in both OPG and P1NP if this was the case; P1NP was lower. Additionally with a greater level of OPG a decrease in bone resorption would be expected which was not shown in our results; CTX was similar between the 2 groups. The lack of effect of OPG on CTX at baseline is similar to lack of effect that change in OPG following WBV had on change in CTX (both increased, though increase in OPG was not significant).

Interpretation of markers of bone metabolism and their association with bone mass is complicated in children by the fact that closely coupled remodelling activity is occurring alongside independent modelling activity whereby bone size and mass is increased due to childhood growth (305). Biochemical markers are unable to differentiate between local tissue activity and whole skeletal changes, therefore site specific pockets of reduced or increased bone activity due to loading may also be obscured by activity due to growth. Evidence of association of bone turnover markers and BMD, BMC and bone accrual velocity in children is mixed, with studies demonstrating evidence of bone turnover markers being positively (178, 306-308) negatively (83, 308-312) or not associated (307, 313, 314) with BMD, BMC and bone mineral accrual at different skeletal sites and stages of puberty. Negative associations between bone turnover markers and bone mass during early to mid-puberty may be partly explained by the reported 0.7 year delay in peak BMC velocity that follows peak height velocity (183). Bone turnover markers reflect rapid alterations to bone

metabolism whereas it takes longer to detect any change in bone measured by DXA or HRpQCT. By recruiting only pre-pubertal boys we would expect to control for this, nonetheless the self-reporting of pubertal stage may not have been as accurate a predictor of pubertal stage as expected. The boys ranged in age from 9.6-10.5 years in the non-fracture group and 8.3-13.1 years in the fracture group, hormonal effects of puberty on bone may have begun even though the boys assessed themselves as pre-pubertal. Baseline P1NP and CTX in both the fracture and non-fracture groups was greater than some other reported values pre-puberty (307, 315, 316) suggesting that in fact our cohort of boys were likely to be pubertal. The age range of the boys in the fracture group was larger, if in fact this group included boys in the early- to mid-stages of puberty we would expect to see increases in both P1NP and CTX in this group as demonstrated in reference curves for pubertal stage (84, 317). CTX was not however elevated in this group suggesting that pubertal effects are not the reason for difference in bone formation in this group. It is possible that the boys with a history of fracture were having a period of higher growth velocity which is seen in children who are heavier and taller for age. The boys with a history of fracture were heavier (3.8kg) and taller (4.1cm), but also slightly older (0.7 years); differences between the 2 groups in height and weight were not significant and age only approached significance ($p=0.053$). Greater growth velocity would be reflected in increased bone turnover markers (305) which was not shown in CTX in our groups.

Any effect of the difference in the physical activity levels in the week prior to baseline between those who had fractured and those who had not (discussed in section 8.1) should also not be overlooked. As discussed in Chapter 3, higher levels of habitual physical activity are associated with an increase in bone formation markers including P1NP (146-149), and may therefore explain the difference in baseline P1NP in the 2 groups. Although the boys were recalling their physical activity over only the 7 days prior to the WBV intervention, anecdotally this was generally consistent with their usual activity levels. Exercise effects on bone resorption markers are less well-defined with observation of both increased and decreased CTX, though more intense exercise regimes are associated with higher levels of CTX (148, 150, 318-320); not seen in our cohort. OPG, which was higher in the boys with a history of fracture, has been shown to be higher in postmenopausal women who have

undertaken a 1 year exercise programme compared to controls (319), this is in contrast to our less active group, but not in adolescent gymnasts (321). Likewise the difference in sclerostin is not easily explained by the difference in activity levels between our 2 groups of boys. When comparing less intense physical activity participation, lower levels of sclerostin have been observed in more active groups than in sedentary groups (322), as seen in our cohort. However sclerostin has also been shown to be higher in adult and adolescent weight bearing athletes than non-athletic controls (318, 323). Again the intensity of the physical activity (professional football players and more than 4 hours a week of weight-bearing exercise compared to less than 2) may have impacted on the sclerostin concentrations. In our cohort for most of the boys time spent participating in physical activity during leisure time was less than 4 hours (as voiced to the researcher but not recorded). Unlike with P1NP, differences in baseline sclerostin and OPG between our fracture and non-fracture groups may not therefore be explained by their physical activity levels. As no significant correlations were found between the biochemical markers and physical activity score either at baseline or in change from baseline this is not unexpected. Lack of correlation could be due to the small sample size in our studies being unable to detect any association between the measures or a true absence of any associations.

8.5. Thermal imaging

In addition to bone biomarkers, in study 1 we measured the effect of WBV on skin surface temperature over the lower legs in a sub-group of the healthy boys pre and post vibration on day 1, as a surrogate measure of muscle activation. The mechanism of the bone response to WBV is thought to be due to a direct response within the bone tissue to loading or via muscle; either by contractions loading the bone or due to increased muscle mass and/ or force increasing load to bone (324). Increased muscle activity and blood flow as a result of WBV have been reported in a number of studies (62, 325-330). Our aim was to determine if muscle activation following WBV was different between the low and high magnitude groups. Although we found no difference between groups in bone turnover marker response in the immediate pre to post vibration period, thermal imaging detected a 10%

increase in skin surface temperature in boys exposed to the high magnitude platform that was significantly different to the 2.8% increase in the low magnitude platform and 1.1% increase in the controls. Skin surface temperature has been shown to be increased in passive vibration (331) as well as weight bearing vibration (267) suggesting that some degree of muscle activation occurs regardless of the magnitude or method of the stimulation. Nevertheless, our results suggest muscle activation is not a prerequisite for bone to respond to mechanical stimulation.

8.6. Discussion conclusions

The aim of these studies was to identify the rate and range of response of bone to WBV in apparently healthy pre-pubertal boys. We have shown that in apparently healthy boys 10 minutes of WBV on 5 consecutive days is sufficient to increase the bone formation marker P1NP by 25.1% and the bone resorption marker CTX by 10.9%. Demonstrating that WBV provides a quick and easy means to assess bone response to loading in a paediatric population. As the response observed cannot be explained here by sclerostin or OPG, other pathways could be involved in the bone response to vibration in this group. However the lack of any response may be due to these pilot studies not being powered to detect this change, a larger sample size may be required.

A novel finding of this work is that boys with a history of one or more fracture do not respond to loading in the same way as boys with no prior fracture. Five consecutive days of WBV was not sufficient to elicit a bone response in boys who have fractured. If reduced responsiveness is present prior to fracture and is related to reduced bone accrual, this could to some extent explain increased fracture susceptibility in some children. Baseline differences observed in P1NP, sclerostin and OPG between the 2 groups suggest that there could be an inherent difference in bone response to habitual daily loads, though this is not clear as difference in P1NP at baseline may partly be explained by the increased activity score recorded in the boys with no prior fracture.

Whereas history of fracture did have an effect on bone response, the magnitude or mode of vibration did not. No difference was detected in bone turnover markers or bone factors between groups when compared by platform type only. In the immediate pre- to post vibration periods within group changes were seen in the control and low magnitude platform groups but not the high magnitude group in the boys with no prior fracture. A difference in skin surface temperature change following WBV was recorded on day 1 in a sub group of boys with no prior fracture, suggesting a difference in muscle activation between platform groups, however as stated this did not translate into a difference in response of the bone turnover markers. Due to high individual intra-variability in bone turnover markers larger sample sizes may be required for differences between platform effects, if they exist, to become apparent.

9. Lessons learnt and future work

9.1. Recruitment

Recruitment to both the healthy boys study and the boys with a history of fracture study proved more difficult than anticipated. The initial plan for study 1 was that the study visits would take place in secondary schools, as it was thought that they would be more amenable to blood sampling occurring within school than in primary schools. For that reason the initial age for recruitment was 11-12 years old. As the target population was pre-pubertal children (to prevent the confounder of pubertal stage) boys and not girls were targeted as the onset of puberty in girls is reported to occur from age 10 and girls would therefore not be eligible for recruitment; onset of puberty is later in boys from 11.5 years (255, 256). Permission to conduct study visits in school needed to be obtained from the Head teachers. This proved to be very difficult; where the researcher was able to speak to them most were reluctant to get involved. It quickly became apparent that having a contact in a school provided easier access to the Head teacher. In one case within 24 hours of a parent approaching their child's school on behalf of the study team permission was granted to distribute the invitation letters. The researcher had been trying to seek permission from this same school for over two weeks. Due to this barrier the researcher went back to the REC and requested that study visits could take place in school or at the participants own home. Additionally the age range was extended to include 9-12 year olds to increase the number of schools that could be approached, as primary schools could then also be targeted. This proved fruitful with 22 out of 40 primary schools that we contacted agreeing to hand out a total of 1095 letters on our behalf to pupils. This was compared to only 1 out of 8 secondary schools agreeing to help. Additionally, the researcher learnt to approach all suitable schools at the same time rather than waiting for a response from each individual school before approaching the next.

Recruitment was still slow so once we had permission to conduct study visits in participants homes we went back to the REC to request that we could approach other organisations such as sports clubs and youth groups to disseminate invitation letters to the study on our behalf.

Study 1 was expected to take 9 months to complete, difficulties in recruitment almost doubled the time to completion.

It was anticipated that recruitment to study 2 would be more straightforward and in keeping with the study plan, as we had learnt numerous lessons from study 1. Despite the lessons learnt, recruitment remained a challenge throughout study 2. The initial recruitment strategies were incorporated into the study design. However as only a small number of boys were ineligible for study 1 due to a history of fracture, it was felt that these strategies were not the most effective use of time. Therefore in addition children who attended the Emergency Department (ED) and Out-patient Department (OPD) at Sheffield Children's Hospital (SCH) were approached for Study 2. Fracture clinic lists were screened for potential participants and any attenders in ED, regardless of reason for attendance, were approached if appropriate. Recruiting in this manner was time consuming and demotivating. Often patients were not available in clinic/ED, could not be found in the department, had lengthy consultations with their clinician whilst the researcher was waiting to discuss the study with them, did not attend for appointments, or had appointments at times when the research staff were not available to approach them. When potential participants were approached many were not interested in taking part in research. Over a 2 year period (August 2015 to September 2017) 666 children were identified from clinic/ED bookings. Some of these did not meet the inclusion criteria, or did not attend their appointments. 180 of the boys declined to take part when first approached, 33 took away information sheets for further consideration and only 7 of these took part in the study. Again the researcher went back to REC to extend the age range to 7-13 years olds in an attempt to increase recruitment. We had found when approaching potential participants that younger siblings were sometimes keen to take part but were in these circumstances excluded on the basis of fracture history and age. Originally we had thought that blood sampling would be too big a burden for the younger children. The top of the age range was increased but the criteria of pre-pubertal status was still required for study inclusion.

Blood sampling had the biggest impact on refusal to participate in the study. Many were discouraged due to the nature and timing of the study procedures. Once we completed the data analysis from study 1 we considered reducing the number of sampling time points in

study 2 to correspond to significant time points in study 1; day1, day 8 and the additional day 12 samples. However we had a flurry of interest in the study and it was thought the recruitment target would be met. Study visits needed to occur in the mornings with participants fasted for the blood sampling, for many families study visits before work and school were too difficult to complete. We had anticipated that this would be difficult and obtained approval for study visits to occur at school or in the participants home rather than at SCH, however this was still not possible for some families. Ultimately the target of 24 participants was not met but as this was a pilot study it was decided that the data collected on 20 participants would be sufficient to meet the requirements of study 2. For future work careful consideration should be given to the timing and number of samples required, keeping this to the absolute minimum required to answer the research question. Experience would suggest that this may help to improve participant recruitment. However for some of the children approached for these studies it was clearly not the number of blood samples to be collected that was the issue, but that blood samples were collected at all.

In addition to difficulties in recruitment due to lack of interest, the researcher had 2 periods of maternity leave during study 2. Staff within the Clinical Research Facility at SCH were allocated to work on the study but due to conflicting workloads and the time consuming nature of approaching potential participants, recruitment stalled. Over this 29 month period only 2 boys were recruited.

9.2. Study design

9.2.1. Blood sampling

1, 3 or 5 days WBV on one of two platforms or control was a complicated study design. The number of days were chosen as it was not known how soon a response of bone to the vibration could be detected, timings were guided by other studies looking at exercise or unloading. Sample size for the studies was low, whilst interesting results were observed on the daily pre- to post-vibration values a clear effect of WBV on bone markers response between fracture and/or platform groups was not identified. Future work would require sample size calculations to sufficiently power the study.

Samples were not collected from all the participants at all time points. Reasons for this were due to difficulties cannulating and therefore being unable to obtain pre- and post-WBV samples or the cannula not working for post-WBV sampling. This resulted in complete data sets on only 8 out of 12 boys exposed to 5 days of WBV in study 1 and only 10 out of 18 in study 2. Reducing the number of sampling time points would limit the number of incomplete data sets but it may not be possible to exclude missing samples entirely. Missing data was not replaced or accounted for and therefore only complete data sets were used in the statistical testing limiting the sample size. For example in a small number of participants difficulties occurred in obtaining samples on day 5. These participants were excluded from the day 5 pre- to post-vibration analysis but were included at other time points such as change from baseline at day 8. Whilst missing data can lead to bias in studies, in this case the missing data was completely random (failure of the cannula/cannulation) and not as a result of participant non-compliance, adverse events, or factors likely to influence the response of bone to WBV. However, due to the large variance in the bone turnover marker values and the small sample sizes it is possible that even 1 or 2 missing values may influence the significance of the results. As these 2 studies were exploratory pilot studies to identify the rate and range of bone response to WBV they were not powered to detect changes. The recommendation for pilot studies to have sample sizes of 12 was followed, in study 1 samples were obtained from only 11 participants on day 8 however in study 2 this was 17 participants. Despite this a significant effect of WBV on P1NP and CTX was still observed in study 1. The sample sizes for the platform groups were too small to draw definitive conclusions on any difference in bone response due to method or magnitude of WBV.

The final sample on the boys with no prior fracture was obtained at day 8. Based on the increase in the bone turnover markers at day 8, it was decided to collect a later sample in study 2 to observe if any changes at day 8 were maintained at day 12 or had returned to baseline values. As no change was observed at day 8 (though there was a trend towards a decrease in CTX) this question was not answered. However it is unlikely that the increases seen in P1NP and CTX would have been observed for long after cessation of the WBV intervention. In future studies observing for the duration of increase in bone turnover markers could help to guide clinicians in determining appropriate duration of WBV

interventions. It is possible that WBV could be used as an intermittent tool for increasing bone mass rather than as a continuous long term therapy. If intermittent bursts of WBV are more beneficial this may increase patient compliance, reduce costs as less platforms would be required, and decrease the therapy burden on patients. Additionally WBV could be used as a short term intervention at times of greater need.

The study in the boys with no prior fracture commenced in 2009 and in the boys with a history of fracture in 2011. The initial study design included measuring just P1NP and CTX, osteocalcin, OPG, and sclerostin were included when it was recognised that there was sufficient serum left over for further testing. This restricted measurement to only when any leftover serum was available, which was not all time points for all participants. Measuring RANKL alongside OPG was considered, however advice at that time from the laboratory conducting the sample assays was that the assay for measuring RANKL in serum was not considered a reliable test. Improvements in the assays would now merit observation of RANKL alongside OPG to assess the RANKL/OPG ratio to help explain the increase in resorption that was measured in the boys with no prior fracture. Other factors associated with bone turnover such as DKK1, WISE, PTH, and Vitamin D could also be assessed to determine the pathways that regulate the response of bone to WBV in pre-pubertal boys.

9.2.2. Baseline assessments

Baseline differences in the biochemical markers between the boys with a history of fracture and those without were greater than expected and only provide a snap shot of bone turnover at that time. Assessment of bone volume and density would have given a clearer picture of differences in bone health between the 2 groups. By recruiting boys with a history of fracture an assumption was being made based on literature that these boys may have smaller bones and/or lower bone mass than the boys without. Assessment by DXA or QCT would have confirmed this, however there were insufficient funds to cover the cost of DXA or QCT. Additionally to include participants based on fracture history and bone mass would have meant recruiting a higher number of participants in the first instance anticipating that some would not meet the inclusion criteria based on bone mass. As discussed above

recruitment was already difficult and it is likely that this would have added to this. DXA or QCT assessment would give a more thorough picture of the participants and would be useful for future work. The purpose of these studies were however exploratory to inform the design and sample size and direction of future work.

Additional assessment of other factors known to influence fracture risk would have been useful for a more thorough comparison between the boys with a history of fracture and those without. A more detailed physical activity questionnaire to identify number of hours and intensity of physical activity would have been useful as would dietary evaluation and information on Vitamin D status. However given the small sample sizes in these studies it would have been difficult to come to strong conclusions about the effect of these on the difference in response between the 2 groups of boys.

9.2.3. Pubertal stage, gender and ethnicity

Studies 1 and 2 looked at the effect of WBV on pre-pubertal boys only. However as highlighted in Chapter 8 Baseline P1NP and CTX in both the fracture and non-fracture groups was greater than some other pre-pubertal reported values, suggesting that in fact our cohort of boys were likely to be pubertal. This reflects the limitation of self-assessment of tanner stage used in our studies. Self-assessment of pubertal stage is often used in research as it is perceived to be more acceptable to participants and more convenient than clinician assessment. However it is recognised that under and over estimation of pubertal stage occurs when self-assessment is utilized, and is acknowledged to only be reliable to within one tanner stage (332, 333). Clinician assessment is accepted as the gold standard though was not possible in our studies. Serum assessment of growth and sex hormones (luteinizing hormone, follicle stimulating hormone, and/or testosterone) could have occurred to further clarify pubertal status as blood samples were already being obtained at baseline. The plan to include only pre-pubertal boys in the studies was primarily to eliminate any effect of gender and puberty on bone response to vibration and to reduce the number of participants that would be required to control for this. Studies regarding exercise in childhood, as discussed in Chapter 4, highlight the differences observed in changes in bone mass and size following exercise or in physically active children at differing stages of puberty

and between genders. Whilst greater benefits have been seen at pre-puberty in boys but not girls, it has been suggested that early puberty around the time of peak height and peak BMC velocity may be the optimum time for greater increases in bone parameters. If the purpose of WBV interventions are to increase bone mass in childhood with an aim of improving bone health in adulthood and older age, then determining the optimum pubertal stage in boys and girls to deliver WBV could be of great importance. Future work should therefore look at response of bone to vibration across all stages of childhood and adolescence, and even into the period of continuing bone mass accrual in early adulthood.

Alongside this ethnicity for the studies was limited to white Caucasian boys. As with gender and pubertal stage more participants would be required to control for any potential differences in response due to ethnicity. It is possible that ethnicity may impact on the response of bone to WBV. However this has not been fully explored in current published studies of WBV and could be considered in future larger studies looking at the acute response of bone to WBV. The decision to limit recruitment to white Caucasian boys only was based on the fact that this is the main ethnicity of the population of Sheffield and therefore would provide a larger sample of boys to participate in the study.

9.3. Positioning on platforms

Boys were asked to stand on the vibrating platforms with their knees slightly bent as is recommended for the Galileo platform. It was decided that the same stance should be used for both devices, however this may have dampened the effect of the low magnitude vibration signal delivered by the Juvent platform. The advice for this platform is to stand in a relaxed upright posture with legs straight and feet positioned beneath the shoulders. Although no differences were detected between the Juvent and Galileo platforms at any time points responses occurred in the Galileo platform that were not detected in the Juvent platform. This may in part be due to incorrect stance on the platform.

9.4. Thermal imaging

Thermal imaging was recorded in a clinical room with a window. Though the blinds were closed to reduce the effect on the room temperature of draughts or sunlight this could not be completely reduced. Ideally thermal imaging should be recorded in an imaging suite to limit variations in environmental factors. Additionally the platforms and cameras were not fixed in the room as it was used for other purposes also, therefore the distance between the platform and child and the camera was not constant between participants and this may have resulted in a variation in the recordings. However the camera and platform positions did not move during each study visit, the distance between the camera and platform for the pre- and post-WBV images was constant for individual participants. The pre- and post-vibration readings on each participant were taken 12 minutes apart therefore there was unlikely to be a significant change in the room temperature. Room temperature was however, measured and accounted for in the analysis and we restricted entry/exit from the room during the whole process of recording the thermal images; that is from the first image immediately pre-vibration, during vibration, up until the immediately post-vibration image was taken. The number of people in the room was restricted to the participant, parent, researcher and camera technician.

9.5. Future work

Whilst our findings clearly highlight that bone responds to a short term WBV intervention, it is restricted to a small defined population, healthy pre-pubertal boys. This was proof of concept work. In order to generate normative data regarding the acute response of bone to WBV much larger studies across different ages, gender and ethnicities would need to be undertaken. This should also incorporate some of the earlier suggestions made in this chapter including increased physical activity and dietary assessment, inclusion of other biochemical markers to help identify the pathways involved in mechanosensing activity, longer duration of blood sampling to identify how long the response is maintained, and measurement of bone size and mass.

In further investigation of the acute response of bone to WBV in boys or other populations with a history of fracture, it would be appropriate to explore any correlation of response to WBV with level of trauma resulting in fracture. As discussed in Chapter 5 it has been suggested that children who fracture following mild trauma have stronger evidence of bone fragility. It would be interesting to see if in this group bone is also less responsive to loading.

10. Conclusions

At the start of this PhD only 3 studies had been reported on WBV in paediatric populations. There was belief that this intervention could be used as a therapeutic agent in the management of bone diseases in childhood either independently or concomitantly with pharmaceutical therapy. However over the last 10 years more studies have come to light that question the use of this intervention in paediatric populations to improve bone health. As discussed in this thesis, findings from studies are inconclusive and contradictory. In addition to longer term WBV interventions, short term effects of WBV have been investigated in healthy adult populations, but not in paediatric populations. The studies undertaken for this PhD are to our knowledge the first to consider the acute response of bone to WBV in healthy pre-pubertal boys and to compare the response between boys who have a history of fracture and those who do not.

We have shown increased bone turnover measured by P1NP (+25.1%) and CTX (+10.9%) following 5 consecutive days of WBV in healthy pre-pubertal boys; this response was regardless of the method (synchronous or side-alternating) or magnitude (less than or greater than 1g) of WBV. No response was observed in boys with a history of fracture. The difference in response of bone to WBV in boys with and without a history of fracture is a novel finding showing that apparently healthy pre-pubertal boys with a history of fracture do not respond to loading in the same way as those who have not fractured. As no significant change was detected in serum sclerostin we are unable here to explain our observation of increased P1NP in boys without prior fracture. Differences between the 2 groups of boys were also observed in biochemical markers at baseline (P1NP, OPG and sclerostin) suggesting that boys with a history of fracture have reduced bone formation. Reduced response to loading if associated with reduced bone accrual could to some extent explain fracture susceptibility in some children.

Our studies demonstrate that WBV can provide a relatively quick and easy means to measure and assess the response of bone to loading in a paediatric population. WBV could be used as a tool to assess skeletal health, rather than as a therapeutic intervention,

particularly in identifying those at risk of fragility fractures. It could be used to target bone health strategies such as dietary adjustments and supplementation, or falls prevention programmes in those most at need. Identifying those that are less responsive to loading early will enable clinicians to promote fracture prevention strategies for patients over the life course with an aim to reducing the burden and economic impact of fracture particularly in older age.

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12. Appendices

12.1. Appendix 1 Human WBV Studies – DXA

Table 17 Human studies measuring DXA outcomes

Author, year	Sample studied, age	Sample number	Concomitant therapy	WBV duration	WBV parameters	Control group	Change from baseline	Change versus controls	Sites studied
Beck et al 2006	Females with low bone mass, 18-45yrs	4	No	10mins x2 daily for 12/12	Optimass 1000, 30Hz, 0.2g	No	+2.03% femur	NA	TB, LS, proximal femur, distal radius
Beck et al 2010	Postmenopausal,	47		15mins x2 weekly (J), 3mins x2 x2weekly (G) for 8/12	Juvent 30Hz, 0.3g, Galileo 12.5Hz, 1g	Yes (3 groups, J, G & control	-6.0% trochanter BMC, -5.7% LS BMC, -6.6% LS area - controls -2.1% TB BMC, -1.4% forearm BMD, +2.3% femoral neck area - Juvent	NS	TB, femoral neck, trochanter, forearm
Bemben et al 2010	Postmenopausal, 55-75yrs	55	Calcium 1500mg	15secs then 60 secs x2 x3 weekly for 8/12	Power Plate 30Hz-40Hz, 2-4mm, 2.16-2.8g	Resistance training (RT), WBV+RT, or control	-1.48% radius -WBV+RT +2.07% radius-control, -0.72%, -0.33%, -0.29% right total hip and -1.36%, -0.35%,-0.24% right femoral neck - WBV+RT, RT, control	net 3.55% loss radius in WBV+RT	LS, femoral neck, trochanter, total hip, forearm
Gilsanz et al 2006	Females with low bone mass, 15-20yrs	48	Calcium carbonate 500mg	10mins daily for 12/12	Vertical, 30Hz, 0.3g	Yes	+0.02 g/cm^2 LS in both groups	NS	TB, LS
Gomez-Cabello et al 2014	Elderly, >65yrs	49		7.5mins x3 weekly for 11 weeks	Power Plate, 40Hz, 2mm	Yes	TB area +1.5% WBV, +1.1% control	NS	TB, LS, hip, Femoral neck

Author, year	Sample studied, age	Sample number	Concomitant therapy	WBV duration	WBV parameters	Control group	Change from baseline	Change versus controls	Sites studied
Gusi et al 2006	Postmenopausal, 66yrs (mean age)	28	No	6mins x3 weekly for 8/12	Galileo, 12.6Hz, 3mm	Walking control group	+0.02 $gr \cdot m^{-2}$ femoral neck	+4.3% femoral neck	LS, femur
Hogler et al 2017	Osteogenesis Imperfecta, 5-16yrs	24	Bisphosphonate naïve, >2yrs, or 6/12 post	3x3mins x2 daily for 5/12	Galileo 20-25Hz amplitude 1-3	Age matched controls	NS	NS	TB, LS, hip
Humphries et al 2009	Healthy females, 18-30yrs	27	1 group + resistance training (RT)[a]	1.5-3mins x2 weekly for 4/12	NEMES (vertical), 50Hz, 1-6mm	Yes	+2.7% femur-WBV +2.0% femur-WBV+RT +1.0% LS-WBV+RT	NS	LS, femoral neck
Iwamoto et al 2005	Postmenopausal ostoeporosis, 55-88yrs	50	Alendronate	4mins x1 weekly for 12/12	Galileo, 20Hz, 0.7-4.2mm	Yes	+10.2% LS-WBV +8.4% LS-control	NS	LS
Kilebrant et al 2015	Severe motor disability, 5.1-16.3yrs	19		x2 weekly for 6/12	Hoppolek, a self-controlled dynamic platform with vibration, jumps and rotatio 5-15 mins, 0.2mm, 40-42Hz	No	TB BMC & BMD inc @6/12, TB BMC inc @12/12, LS & calcaneous no change		TB, LS, calcaneous
Lam et al 2012	Females with osteopenia and idiopathic scoliosis, 15-25yrs	149		20mins x5weekly for 12/12	Vertical,30Hz, 0.3g	Yes	Dominant FN +0.015g/cm^2 BMD, +1.17g/cm^2 LS BMC		LS, femoral necks
Lai et al 2013	Postmenopausal, 46-69yrs	28	No	5mins x3 weekly, for 6/12	LV-1000, 30Hz, 3.2g	Yes	+2.03% WBV, -0.05% controls	net 2.08% gain	LS

Author, year	Sample studied, age	Sample number	Concomitant therapy	WBV duration	WBV parameters	Control group	Change from baseline	Change versus controls	Sites studied
Leung et al 2013	Postmenopausal, >60yrs	710		20mins, x5 weekly for 18 months	35Hz, 0.3g, <0.1mm	Yes	LS +0.08% WBV, -0.64% controls hip -1.86% WBV, -1.89% controls	NS	LS, hip
Liphardt 2015	postmenopausal, osteopenic, 50-65yrs	42		10mins x2-3 weekly for 12/12	Galileo, 20Hz, 3-4mm	Yes	aBMD femoral neck dec @ 16/12 &20/12	NS	LS, femoral neck
Ruan et al 2008	Postmenopausal osteoporsis, 50's-70'syrs	94	No	10mins x5 weekly for 6/12	ZD-10 (vertical) 30Hz, 5mm	Yes	+4.3% LS +3.2% femoral neck	NA	LS, femoral neck
Rubin et al 2004	Postmenopausal, 47-64yrs	56	No	10mins x2 daily for 12/12	Vertical, 30Hz, 0.2g	Yes	NA	+3.5% LS	LS, proximal femur, distal radius
Ruck et al 2010	Cerebral Palsy, 6-12yrs	20	Physiotherapy	9mins x5 weekly for 6/12	Galileo, up to 18Hz, up to 4mm, up to 2.6g	Yes	NS	difference of 0.06g/cm^2 in favour of control at femoral neck (diaphysis)	LS, distal femur
Santin_Medeiros et al 2015	71-93yrs	37	Not recorded	x2 weekly for 8/12	Fitvibe 20Hz, 2mm	Yes	NS	NS	total hip
Slatkovska et al 2011	Postmenopausal, 44-79yrs	202	Calcium 1200mg, Vitamin D 1000iu	20mins daily for 12/12	30Hz 0.3g, 90Hz 0.3g or control	Yes	-0.008g/cm^2 LS 30Hz -0.006g/cm^2 LS 90Hz -0.007g/cm^2 LS control -0.006g/cm^2 femoral neck 30Hz	NS	LS, femoral neck, total hip

Author, year	Sample studied, age	Sample number	Concomitant therapy	WBV duration	WBV parameters	Control group	Change from baseline	Change versus controls	Sites studied
Soderpalm et al 2013	Duchenne Muscular Dystrophy, 5.7-12.5yrs	6	No	2mins for 2 weeks then 6mins x2-3 weekly for 3/12	Galileo, 4mm, 20-24Hz, 3.2-4.6g	No	NS	NA	TB, LS, hip, left heel
Stark et al 2010	Cerebral Palsy, 9.7yrs	78	Physiotherapy	9mins x2 daily for 6/12	Galileo, Hz and mm variable	No	+2.3% TB BMD +5.7% TB BMC	NA	TB
Torvinen et al 2003	Healthy non-athletic, 19-38yrs	56	No	4mins x3-5 weekly for 8/12	Vertical, 25-45Hz, 2-8g, 2mm	Yes	NS	NS	LS, femur, calcaneus, distal radius
Verschueren et al 2004	Postmenopausal osteoporosis, 58-74yrs	70	No	max 30mins x3 weekly for 6/12	Power Plate, 35-40Hz, 1.7-2.5mm	Yes, 1 group resistance training only	+0.93% hip	+1.5% hip	TB, total hip
Verschueren et al 2011	Postmenopausal, >70yrs	113	Calcium 1000mg and Vit D 880 or 1600IU/day	up to 12 mins x3 weekly for 6/12	Power Plate, 30-40Hz, 1.6-2.2g,	Yes, 4 groups -WBV with high/low dose Vit D and high/low dose Vit D	+0.75-0.88% hip	NS	Hip
Von Stengel et al 2011	Postmenopausal, >65yrs	151	Calcium 1500mg, Vitamin D 44 IE	6mins +training x2 weekly for 6/12, and training x2 weekly	Vibrafit (vertical) 25Hz 1.7mm	Yes, Training group (TG), WBV+TG, controls	+1.5% in WBV +2.1% TG LS	NS	LS, femur
Von Stengel et al 2011	Postmenopausal, 65-70yrs	108	Calcium 1200mg, Vitamin D 800iu WBV+ strength training	15mins x3 weekly for 12/12, plus exercises on the platform	Vibrafit (vertical) 35Hz 1.7mm, Quionic (rotational) 12.5Hz 12mm, both ~8g	Yes	+0.7% LS rotational	LS rotational increase v control no change was significant femoral neck NS	LS, femoral neck

Author, year	Sample studied, age	Sample number	Concomitant therapy	WBV duration	WBV parameters	Control group	Change from baseline	Change versus controls	Sites studied
Zaki et al 2014	Obese postmenopausal, 50-68yrs	80		10mins +resistive exercise x3 weekly for 8/12	OMA-701A (side-alternating), 16Hz	Yes, resistance training	+1.03-1.16% in WBV +1.02-1.08 in controls at all sites except femoral neck	NS	LS, femoral neck, greater trochanter, ward's triangle
Zha et al 12	Adults 50-60yrs and seniors +65yrs, 51-93yrs	68, (53 female)	No	20mins x3 weekly for 6/12	custom vertical and tilting, 0.3g-0.8g, 45-55Hz	Adult and senior controls	+2.52% and +1.63% LS - seniors and adults WBV +3.22% and +2.06% femoral neck - seniors and adults WBV - 0.44% femoral neck - seniors control	Greater increase in WBV compared to controls Increase in seniors in LS greater than adults	LS, right femoral neck

LS - lumbar spine, NA - not available, NS - not significant, TB - total body, WBV - whole body vibration

[a]3 groups: WBV, WBV+RT, control

[b]When accounting for compliance and weight <65Kg

12.2. Appendix 2 Study 1 Paper

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Original Article

Hylonome

Acute bone response to whole body vibration in healthy pre-pubertal boys

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Abstract

The skeleton responds to mechanical stimulation. We wished to ascertain the magnitude and speed of the growing skeleton's response to a standardised form of mechanical stimulation, vibration. 36 prepubertal boys stood for 10 minutes in total on one of two vibrating platforms (high (>2 g) or low (<1 g) magnitude vibration) on either 1, 3 or 5 successive days (n=12 for each duration); 15 control subjects stood on an inactive platform. Blood samples were taken at intervals before and after vibration to measure bone formation (P1NP, osteocalcin) and resorption (CTx) markers as well as osteoprotegerin and sclerostin. There were no significant differences between platform and control groups in bone turnover markers immediately after vibration on days 1, 3 and 5. Combining platform groups, at day 8 P1NP increased by 25.1% (CI 12.3 to 38.0; paired t-test p=0.005) and bone resorption increased by 10.9% (CI 3.6 to 18.2; paired t-test p=0.009) compared to baseline. Osteocalcin, osteoprotogerin and sclerostin did not change significantly. The growing skeleton can respond quickly to vibration of either high or low magnitude. Further work is needed to determine the utility of such "stimulation-testing" in clinical practice.

Keywords: Child, Bone, Vibration, Bone Turnover Markers, Stimulation Test

Introduction

Children in the UK suffer 100,000 fractures annually accounting for 10% of attendances at accident and emergency departments[1]. The incidence of childhood fractures has increased in the last 30 years by approximately 40%[2] and 20-30% of children who fracture are likely to fracture again[3-5]. Evidence suggests that children with narrower, i.e. more slender bones and a low bone mass are more likely to fracture than children with larger, wider bones[6,7]. Studies have shown that mechanical loading in childhood can increase bone size and mass[8-10], and that these gains can persist into adult life[11-13], potentially improving skeletal health across all ages.

Whole body vibration (WBV) delivered via vibrating platforms has been investigated as a technique to load bone, with the desired outcome of increasing bone size and strength. The vibratory stimulus provided differs between platforms. One type is designed to mimic low level postural strains that form a dominant component of the skeleton's 24 hour strain history[14], low magnitude, high frequency synchronous (vertical) vibration. The second type is designed to fatigue muscle and stimulate increases in loading to bone via the muscle by applying high magnitude, high frequency vibration that can be delivered in a synchronous or side-alternating motion. The majority of animal investigations have employed low magnitude high frequency WBV, and demonstrated increased bone density, size, formation, and strength[14-22]. Clinical studies over 6-12 month periods of children with disabling conditions such as cerebral palsy, young women with low bone mass and women with post-menopausal osteoporosis have also shown increased bone mass at the tibia, femur, and spine using synchronous, low magnitude, high frequency WBV[23-25] and at the femoral neck and hip using asynchronous high magnitude, high frequency WBV[26,27]. However findings across the studies are not conclusive, either in terms of the size of the effect or

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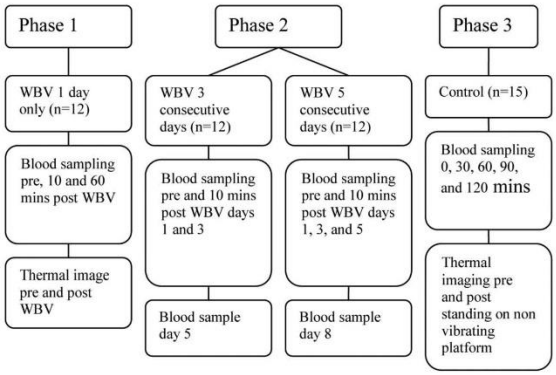


Figure 1. Study design showing number of days of whole body vibration, blood sampling regime, and thermal imaging by phase of the study.

its site specificity, and a number of studies have failed to show any skeletal benefits at all[28-31].

Adult studies suggest that WBV has little or no effect on bone outcomes in apparently healthy subjects when given 2-7 times a week for up to 12 months[29,30,32,33]. We know from exercise studies that the response to loading is greater in children than in adults however, suggesting that children may also have a greater capacity for a bone response to vibration. There are no published data on the acute effect of vibration on the apparently healthy growing skeleton. It is not known if the bone response to vibration in such children would be similar to that seen for children with reduced bone mass.

An alternative use for vibration would be as part of a stimulatory test to assess the skeleton's responsiveness to mechanical loading, similar to cardiac stress testing using a treadmill. There could be advantages to such a form of testing if acute changes in bone formation or resportion were predictive of longer term response to either pharmacological or mechanical interventions. The response to mechanical loading before, during and after intervention could be assessed in a variety of disease states both within individuals and between groups of individuals. Such a test could also be used in assessing outcomes for phase II studies of bone-targeted compounds to reassure investigators that their intervention had not abrogated responses to mechanical loading.

We wanted to determine the extent and timing of any changes in bone turnover markers following very short periods of WBV and whether this was influenced by the type of vibration stimulus, either high magnitude or low magnitude, used. This is a pragmatic approach, reflecting the fact that these two types of machine, providing either high magnitude, side-alternating (signal intensity >2.0 g) or low magnitude, vertical (signal intensity <1.0 g) vibration are widely available, but nothing is known as to their short term effects on bone metabolism. We undertook thermal imaging of calf muscles as a proxy for muscle activation[34] to determine if this was similar between the high and low magnitude vibration groups. The overall aim of this study therefore was to assess the acute response of bone to low and high magnitude WBV in the growing skeleton.

Materials and methods

The study was approved by the South Humber Local Research Ethics Committee and was carried out in accordance with the Declaration of Helsinki. Informed written consent was obtained from the parents, and written assent from the study participants.

Study participants

Healthy white Caucasian pre-pubertal boys (Tanner stage 1) aged 9-12 years were recruited to the study by invitation letters which were handed out in local schools and through staff at local university hospitals. Boys with a history of bone disease or other chronic illness, those currently taking medication known to affect bone and those who had previously fractured were excluded from the study. In total 1138 letters were sent out; the parents of 166 boys expressed an interest in the study (14%), and 64 (38%) signed a consent form.

Study design

The study was conducted in 3 phases as illustrated in Figure 1. Each boy participated in one phase only. In phase 1 we assessed the immediate response of bone biomarkers to vibration; in phase 2, we assessed the biomarker response to repeated episodes of vibration; and in phase 3 the variation in biomarkers immediately after standing on a non-vibrating platform. Gradually increasing overall periods of vibration were used in phase 2 because of uncertainty as to what duration of exposure (i.e. mechanical stimulation dose) would be

needed in order to generate a response in biomarkers.

In phase 1 (immediate response), boys were randomly allocated to platform type, either Juvent (low, <1.0 g magnitude) or Galileo (high, >2.0 g magnitude). Blood samples were collected immediately before and 10 and 60 minutes after vibration.

In phase 2 (response after repeated exposure), boys were randomly allocated to platform type, and duration of intervention. Half the boys received three cycles of vibration, the remainder five cycles.

In those receiving three cycles, blood samples were taken immediately before and 10 minutes after vibration on days 1 and 3, with a further sample being taken 2 days later.

In those receiving five cycles, blood samples were taken immediately before and 10 minutes after vibration on days 1, 3 and 5 with a further sample being taken 3 days later.

The variation in the later sample collection timings reflected both the need to fit with boys' school and family commitments (sample collection was timed to allow normal school attendance and avoid weekend attendance) and the uncertainty as to whether a biomarker response would occur either immediately or in a more delayed manner.

In phase 3, boys stood on a non-vibrating platform and samples were taken at 30 minute intervals over a 2 hour period, similar to the timing of samples in those exposed to vibration, in order for us to allow for any underlying time-related changes.

A thermal image was recorded pre and post vibration of the legs below the knee in the phase 1 boys exposed to a single 10 minute period of vibration and at 30 minute intervals (the same time points as for blood sampling) in the control group.

Recruitment to each phase of the study followed completion of the previous phase. Boys were randomly assigned to vibration platform (phase 1 and 2) and number of consecutive days of intervention (phase 2 only) by the successive opening of opaque envelopes containing the randomisation code; the randomisation to duration and platform was balanced. Neither the participants nor the researcher could be blinded to the intervention groups due to the difference in shape and size of platforms and the mode of vibration. Study visits occurred in the mornings; for the boys in phase 1 and the control group they took place in the Clinical Research Facility at Sheffield Children's Hospital. Phase 2 visits took place in the child's home or school for participant convenience. All visits were carried out by the same researcher (RH).

Loading regime

Participants stood either on the Galileo Advanced platform (Novotec Medical GmbH, Pforzheim, Germany) which delivers a high frequency high magnitude side-alternating signal, or the Juvent MDT 1000 platform (Marodyne, Lakeland, Florida, USA) which delivers a high frequency low magnitude synchronous (vertical) signal. The Galileo platform was set at a frequency of 20 Hz with the boy's feet positioned on the foot plate to give a peak to peak displacement of 4 mm, providing a peak to peak acceleration of approximately 6.4 g (earth's gravity; 1 g= 9.81 m/s^2), although the acceleration experience by the head is never >1 g. The Juvent delivers 32-37 Hz, displacement 0.085 mm, at an acceleration of approximately 0.3 g. All participants were asked to stand barefoot on the platforms facing forward with their knees slightly bent for 4 cycles of 2.5 minutes on the platform and 30 seconds off, totalling 10 minutes vibration. Insertion of a rest period between cycles of loading is thought to enhance the anabolic response of bone[35-38]. A training period to familiarise participants to the platforms was not possible as the intervention was conducted over such a short time scale. The rest periods therefore also helped participants to tolerate the full 10 minutes of vibration from day 1.

Outcome measures/blood sampling

Blood samples were collected to measure the bone formation markers pro-collagen type 1 N-terminal propeptide (P1NP; Elecsys, Cobas E411, Roche Diagnostics, UK; intraassay %CV <1.7%) and osteocalcin (OCN; Cobas E411, Roche Diagnostics, Germany; intraassay %CV 1.4-3.3%), and the bone resorption marker C-terminal cross-linked telopeptide of type 1 collagen (CTx; Elecsys β-CrossLaps/serum kit, Cobas E411, Roche Diagnostics, UK; intraassay %CV 2.8-8.4%). Factors affecting bone resorption - osteoprotegerin (OPG; ELISA, BioVendor, Czech Republic; intraassay %CV 2.5-4.9%) - and bone formation - sclerostin (SCL; Enzyme Immunoassay, Biomedica Gruppe, Germany; intraassay %CV <7%) - were also measured. Sampling was undertaken as per Figure 1. All samples were collected in the mornings starting at approximately 08:00 following an overnight fast. Samples were centrifuged, separated and stored at -80°C within 2 hours of collection. Serum samples were assayed in duplicate. P1NP, CTx OPG and SCL were assayed for each sample; OCN only for the baseline and day 8 samples in those receiving 5 days of vibration (insufficient sample for intermediate measures).

Thermal imaging

Thermal images of the legs below the knee were obtained pre- and post vibration on boys exposed to 1 day only of WBV (n=12) and on half of the boys (randomly selected) in the control group (n=7) to measure skin surface temperature. Images were recorded with the participants standing on the platform prior to commencement of WBV and immediately upon completion of the 4 cycles of vibration. Boys in the control group were asked to stand on and off the Juvent platform at the same timing as for the boys exposed to WBV, with the platform turned off. Images were captured using a hand held Land Guide M4 thermal imaging camera (Asten Instruments Ltd, Market Rasen, UK) with a temperature range of -20°C to 250°C and a sensitivity of 0.12°C, CV 2%, and were analysed using the manufacturer's software. All images were recorded by one of two of the researchers (CH or HR) in the same room with windows and doors kept closed and blinds drawn to block out sunlight and/or draughts. As the region of interest was the surface area over the gastrocnemius and soleus muscles, boys were asked to wear shorts or to roll their trouser legs up above their knees. Thermal images were recorded as a non-invasive surrogate measure of blood flow to the skin over the calf mus-

		Control mean (95% CI)	n	low magnitude platform mean (95% CI)	n	high magnitude platform mean (95% CI)	n	p value ANOVA
Day 1 Mean change (unadjusted)								
P1NP	10 min post	-61.3 (-103.9 to -18.8)	14	-67.6 (-108.7 to -26.5)	17	-71.3 (-164.5 to 21.8)	16	0.97
ng/ml	60 min post	-83.7 (-135.1 to -32.2)	14	-58.5 (-160.1 to 43.0)	6	-16.5 (-172.4 to 139.4)	5	0.43
CTx	10 min post	-0.11 (-0.21 to 0.05)	14	-0.17 (-0.25 to -0.09)	17	-0.08 (-0.21 to 0.05)	16	0.36
ng/ml	60 min post	-0.14 (-0.27 to -0.02)	14	-0.17 (-0.37 to 0.03)	6	-0.07 (-0.15 to 0.01)	5	0.64
Day 1 Adjusted mean change (adjusted*)								
								ANCOVA
P1NP	10 min post	-71.6 (-160.4 to 17.3)	14	-70.7 (-122.6 to -18.9)	17	-64.8 (-121.6 to -8.0)	16	0.91
ng/ml	60 min post	-76.6 (-131.4 to -21.7)	14	-75.6 (-155.4 to 4.1)	6	-15.8 (-123.4 to 91.7)	5	0.17
CTx	10 min post	-0.11 (-0.27 to 0.05)	14	-0.17 (-0.26 to -0.07)	17	-0.09 (-0.19 to 0.01)	16	0.14
ng/ml	60 min post	-0.15 (-0.26 to -0.03)	14	-0.20 (-0.37 to -0.03)	6	-0.02 (-0.25 to -0.21)	5	0.59

**Adjusted for baseline CTx/P1NP, length of treatment, age, activity score, time between samples*
CI= confidence interval

Table 2. Change in serum P1NP and CTx values from baseline at 10 and 60 minutes post WBV by platform group.

		Low magnitude platform				High magnitude platform				Control			
		N	Mean	SD	p value	N	Mean	SD	p value	N	Mean	SD	p value
P1NP ng/ml	Day 1 pre	17	669.8	289.7	**0.003**	16	786.8	208.8	0.12	14	697.5	186.5	**0.008**
	Day 1 10 mins post		602.2	245.5			715.4	191.6			618.2	141.7	
	Day 1 pre*	6	600.6	409.7	0.2	5	830.1	287.3	0.78	14	697.5	186.5	**0.04**
	Day 1 60 mins post		542.1	332.7			813.6	249.4			595.8	194.5	
	Day 3 pre	11	703.2	184.4	**<0.001**	10	749.8	122	**0.004**		-	-	
	Day 3 post		575.6	139.3			646.8	103.8			-	-	
	Day 5 pre	5	712.6	212.7	**0.008**	4	763.7	169.4	**0.05**		-	-	
	Day 5 post		608.9	225			681	145			-	-	
CTx ng/ml	Day 1 pre	17	1.83	0.56	**<0.001**	16	2.00	0.50	0.2	14	1.91	0.40	**0.04**
	Day 1 10 mins post		1.67	0.49			1.93	0.50			1.80	0.45	
	Day 1 pre	6	1.71	0.73	0.08	5	2.40	0.59	0.08	14	1.91	0.40	**0.03**
	Day 1 60 mins post		1.54	0.65			2.34	0.56			1.76	0.39	
	Day 3 pre	11	1.89	0.51	**0.007**	10	1.93	0.30	0.2		-	-	
	Day 3 post		1.76	0.47			1.86	0.28			-	-	
	Day 5 pre	5	1.91	0.66	**0.004**	5	1.99	0.16	0.9		-	-	
	Day 5 post		1.76	0.62			2.01	0.33			-	-	

Mean pre and post WBV P1NP and CTx per intervention group for samples included in the paired t-test analysis of pre and post values on each day of sample collection.
SD - standard deviation, N - number of participant samples included in the analysis.
**Only participants allocated to 1 day of WBV or control group had blood collected at 60 minutes post WBV (or equivalent time in the control group); these 6 boys are a subgroup of the 17 measured on day 1.*

Table 3. Mean (SD) bone turnover marker (P1NP, CTx) values Pre and post WBV on days 1, 3, 5.

day 1, and pre to immediately post-vibration on day 3 and day 5 were compared between groups using ANOVA and ANCOVA (adjusting for baseline bone turnover marker). No other covariates were included due to lack of power. After day 1 data was only collected in boys exposed to the vibration (high and low magnitude WBV groups, not the control group); the comparison for day 3 and day 5 are between WBV groups only.

Combined change at day 8

Change in bone markers (P1NP, CTx, OCN) and bone cell-derived factors (OPG, SCL) after 5 days of vibration were combined across the WBV groups due to limited data. Paired t-tests were used to test for change from baseline using day 1 (pre-test) and day 8 measurements.

To account for camera temperature drift between the recorded images, the post-image temperature was adjusted and within participant temperature change pre to post WBV reported as the outcome. For each participants' pre- and post image a reference area not expected to change in temperature over the short time lapse between the images (i.e. where the camera was pointed at the wall) was determined with the region temperature recording used to adjust for any camera drift.

Results

In total 64 boys consented to study participation. Of these 8 were excluded on the basis of pubertal stage greater than Tanner 1, 2 withdrew consent prior to data collection and commencement of the intervention, and 3 withdrew due to difficulties in obtaining blood samples on day 1. Data was collected and analysed on 51 boys in total; 12 boys in phase 1 (1 day/10 minutes only of WBV), 24 boys in phase 2 (3 or 5 days of WBV, 12 subjects in each group), and 15 boys in the control group (no WBV); see Figure 1. The baseline characteristics of each group are shown in Table 1. No formal statistical testing was undertaken. Age, height, body mass index (BMI), and weight were similar between the groups. The activity scores may appear to be different across the intervention groups with the high magnitude group scoring higher but it should be noted that these scores have a large standard deviation (SD). Baseline P1NP, OCN, CTx, OPG, and SCL values were similar between the groups. The time taken between the pre and post vibration samples on day 1 was slightly longer in the Juvent group. One boy in this group felt faint after cannulation and rested prior to standing on the vibration platform, accounting for the greater time lag and larger SD in this group.

Bone turnover markers – changes across individual cycles of vibration

Within control group (n=14)

P1NP decreased by 7.8% (CI -13.4 to -2.2; p=0.008, paired t-test) at 10 minutes, and by 12.0% (CI -19.3 to -4.7; p=0.04) at 60 minutes compared to baseline. Osteocalcin was not measured. CTx decreased by 12.0% (CI -19.3 to -4.7; p=0.04) at 10 minutes and by 7.0% (CI -13.7 to-0.4; p=0.03) at 60 minutes (actual values, Table 2).

Within low magnitude group, first cycle of vibration (n=17)

P1NP decreased by 7.9% (CI -14.0 to -1.9; p=0.003, paired t-test) at 10 minutes, and by 0.18% (CI -20.6 to 21.0; p=0.20) at 60 minutes (n=5). CTx decreased by 6.2% (CI -12.2 to -0.2; p=0.04) at 10 minutes post WBV and by 9.4% (CI -21.1 to 2.2; p=0.08, statistically not significant) at 60 minutes post vibration (actual values Table 2).

Within high magnitude group, first cycle of vibration (n=16)

No change was seen in P1NP (decreased by 6.8% CI -18.4 to 4.9; p=0.1) at 10 minutes, and by 6.04% (CI -9.2 to 21.3 p=0.78) at 60 minutes (n=6). Neither was there a change in CTx at 10 minutes (n=16; 3.6% decrease observed, CI -9.9 to 2.8; p=0.2) or 60 minutes (2.6% decrease; CI -5.4 to 0.1; p=0.08; actual values Table 2).

There were no changes in either osteoprotegerin or sclerostin over the period from baseline to 60 minutes post WBV for either low or high magnitude WBV.

Changes across individual vibration cycles; Day 3 and Day 5

On day 3 P1NP decreased pre to post vibration in the low magnitude group (n=11) by 17.5% (CI -22.6 to -12.5; p<0.001) and in the high magnitude group (n=10) by 13.3% (CI -20.2 to -6.3; p=0.004). CTx decreased following WBV in the low magnitude group by 6.2% (CI -10.4 to -2.1; p=0.007; n=11), but did not change in the high magnitude group (day 3: 3.4% decrease observed, CI -8.1 to 1.3 p=0.2; n=10; actual values Table 3).

Day 5 showed a decrease following WBV in P1NP in the low magnitude group (n=5) of 15.9% (CI -20.2 to -6.3; p=0.008) and in the high magnitude group (n=4) of 10.6% (CI -20.2 to -0.9; p=0.05; Table 3). CTx decreased following WBV in the low magnitude group by 8.1% (CI -10.3 to -5.7; p=0.004; n=5) and was unchanged in the high magnitude group (0.5% increase observed, CI -16.5 to 17.4; p=0.9; n=4; actual values Table 3).

Differences in bone marker responses between platforms

There were no differences between the control and platform groups in the day 1 P1NP and CTx response to vibration (ANOVA and adjusted ANCOVA; Table 2). There was also no difference between the platform groups in response to WBV on days 3 and 5 from immediately pre-vibration to 10 minutes after vibration (no control data collected) though as on day 1 within group changes were detected, as shown above.

Changes from baseline after 5 days of vibration

Bone turnover markers – P1NP, OCN, CTx

In contrast to the decrease shown in the immediate pre to post WBV time period, boys exposed to 5 consecutive days of WBV (platform groups combined, n=11, measurements on day 8 vs baseline measurements) had a significant increase in P1NP of 25.1% (CI 12.3 to 38.0; paired t-test p=0.005; Figure 2). No significant change was detected in the formation marker OCN (measured at day 1 and day 8 in the 5 day subjects only n=11; change +11.5% CI -8.3 to 31.2; p=0.2; Figure 2). At day 8, CTx was greater in the boys exposed to 5 days of WBV on both of the platforms than at baseline with an increase of 10.9% (CI 3.6 to 18.2; paired t-test p=0.009; Figure 2).

OPG and sclerostin

OPG and sclerostin were analysed at each time point. OPG showed a trend for an increase of 7.2% (CI -1.4 to 15.8; p=0.08) on day 8 compared to baseline (n=11; Figure 3). No difference was detected at any other time point within or between groups. Additionally no change within or between

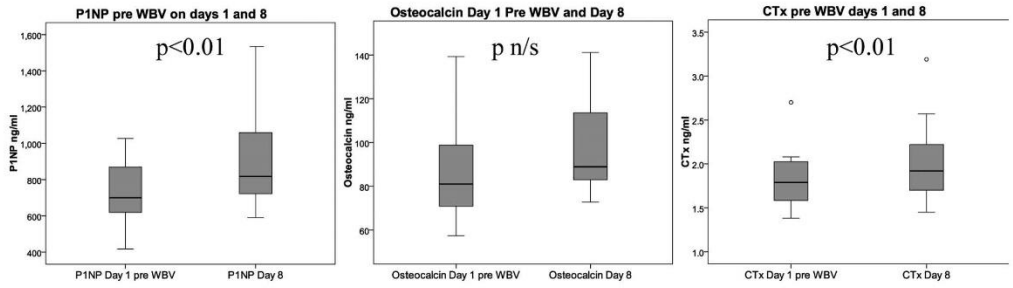


Figure 2. Boxplots illustrating the absolute values of a) P1NP, b) Osteocalcin and c) CTx at baseline and day 8 for boys exposed to 5 consecutive days of WBV.

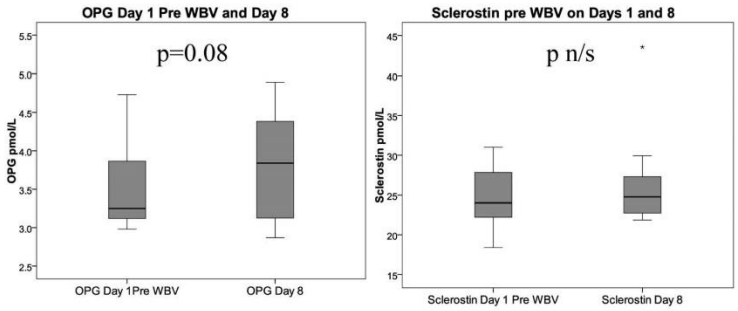


Figure 3. Boxplots illustrating the absolute values of a) OPG and b) Sclerostin at baseline and day 8 for boys exposed to 5 consecutive days of WBV.

groups was detected at any time point for sclerostin (change of +7.2% CI -7.45 to 21.73; p=0.3).

Thermal imaging

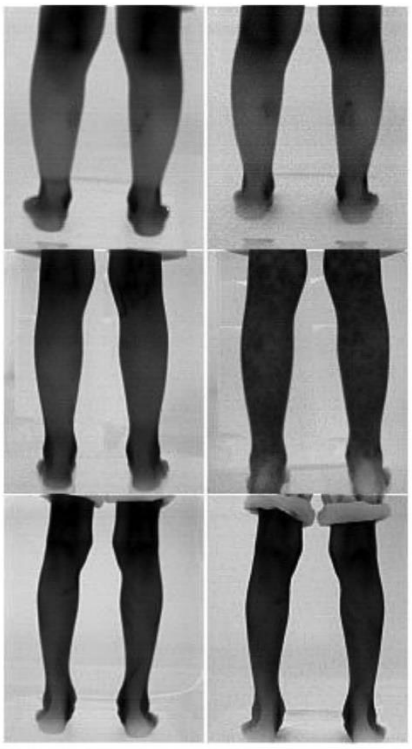
Thermal images were captured on 7 boys in the control group and on all boys exposed to 1 day only of vibration (n=7). However it was not possible to compare images in 2 of the boys and these were excluded from the analysis. Images therefore were analysed on 5 boys exposed to each of the vibrating platforms and 7 boys from the control group. The change in skin surface temperature pre- to post vibration ranged from -1°C to 1.6°C (mean 0.3°C, CI -0.4 to 1.1) in the control group, 1.4°C to 4.2°C (mean 2.9°C, CI 1.5 to 4.4) in the high magnitude group, and 0.2°C to 2.8°C (mean 0.9°C, CI -0.4 to 2.3) in the low magnitude group. There was a significant difference in the response of the boys in the high magnitude group compared to the control (p=0.002) and low magnitude groups (p=0.02; ANOVA, bonferroni post hoc test). In addition, when visually assessing the pre and post images, a difference in the temperature distribution was seen in the boys exposed to the high magnitude platform that was not seen in the control or low magnitude groups (Figure 4).

Adverse events

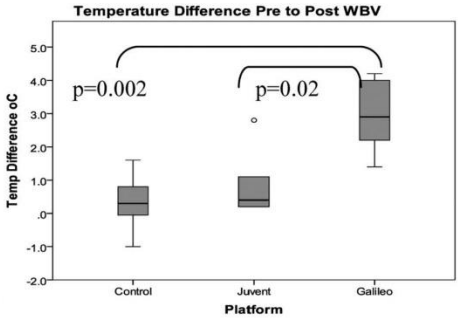
WBV was well tolerated by the study participants. Minimal side effects were reported; itching or "weird feeling" in the calves or legs (high magnitude), tickling sensation in the feet (low magnitude) and anxiety in relation to cannulation. In all cases these resolved on or shortly after completion of the WBV.

Discussion

We observed a fall in both P1NP and CTx ten minutes after vibration using the low magnitude platform on days 1, 3 and 5 and on days 3 and 5 following vibration using the high magnitude platform. Similar reductions were seen, however, in boys who stood on a platform that did not vibrate over the same time period. There were no differences between the groups in the changes in P1NP or CTx across the individual cycles of vibration exposure.



a)
Top left: Control pre WBV, top right: Control post WBV
Middle left: Galileo pre WBV, middle right: Galileo post WBV
Bottom left: Juvent pre WBV, bottom right: Juvent post WBV



b)

Figure 4. a) Thermal images taken immediately before and after vibration. b) Boxplots showing comparison of the pre to post vibration temperature change between the groups.

By contrast, five consecutive days of WBV (both groups combined) increased the bone formation marker P1NP by 25.1% and bone resorption marker CTx by 10.9% between the pre-vibration baseline and the day 8 measurement. The second bone formation marker measured, osteocalcin, did not change over the period of observation. This may be because osteocalcin is produced later in the process of endochondral ossification, when mineralisation is taking place. By contrast, P1NP is produced early in the bone formation process, when the osteoid matrix is being formed and deposited.

The greater increase over the 8 day period in the formation marker as opposed to the resorption marker suggests that there is an uncoupling of bone turnover in favour of formation in response to vibration. These changes are consistent with the reported effects of increased activation of the canonical wnt-signalling pathway through LRP5/6 where bone formation is increased and there is increased osteoblastic expression of osteoprotegerin. Our data indicated this with a trend towards an increase in OPG. However increased expression of OPG should also be associated with reduced bone resorption which is not confirmed by our CTx results.

Sclerostin is widely recognised to be a key inhibitor of bone formation by the canonical wnt-signalling pathway through LRP5/6[42] and is an important factor in bone response to mechanical loading[43]. However no significant change in serum sclerostin was detected either over time or between groups and could not therefore explain our observations of increased P1NP. Animal studies have shown down-regulation of sclerostin production at sites of new bone formation following even short periods of mechanical loading[43]. It may be the case that changes in serum sclerostin do not reflect accurately or quickly on changes occurring at a tissue level. An alternative explanation is that the rapid changes observed in bone formation and resorption here were the result of activity either in other pathways, or in other regulators of the wnt-signalling pathway through LRP5/6. Additional inhibitors of the pathway are well described, including DKK1 and WISE, both secreted by osteocytes. PTH inhibits the production of both sclerostin and DKK1 and also interacts via the PTH-PTHR1 complex with LRP6, initiating signalling in the absence of Wnt-ligands[44]. It will be important in future studies to include such factors to understand the underlying physiological basis of acute responses to mechanical loading.

Other studies that have looked at bone turnover marker response to WBV have been conducted in adult populations. Corrie et al[45] found an increase in P1NP of 17.5% in adults aged 64 years and over exposed to either synchronous or side-alternating vibration for 12 weeks, but no change in CTx. This suggests, as shown in our paediatric population, that bone marker response is not dependent on the method of vibration. In contrast to the increase we saw in resorption, Turner et al[46] showed a 34% decrease in urinary NTx following 6 weeks of WBV in postmenopausal women, a third of whom had osteoporosis. Following one episode of vibration and exercise Sherk et al[47] also found a decrease in CTx in healthy young women exposed to one episode of high magnitude synchronous WBV. The de-

crease was greater at 30 minutes post exercise when the women were exposed to WBV immediately prior to resistance exercise than following resistance exercise only (-12.5% vs -1.3%).These studies demonstrate a change in bone turnover markers that suggests an uncoupling of bone turnover in favour of formation as we found in our paediatric population. However, Rubin et al[24] found only a slight decrease of 3% in the resorption marker hydroxyproline in their active vibration group with a much greater decrease in the placebo group of 16%. In accord with our data others have failed to demonstrate a change in the formation marker osteocalcin following WBV[24,27,30].

Our focus on children and the response of bone in the growing skeleton rather than on a fully developed skeleton may explain some differences seen between our results and those of others. The increase in resorption and greater increase in formation in our group, not typically seen in the adult populations, may reflect the enhanced response to loading that has been well reported in the growing as opposed to adult skeleton. Five separate papers[24,27-30] found no change in bone markers following 6-12 months WBV in healthy young adults and postmenopausal women, but this may reflect the adaptation of bone structure and bone remodelling to the continuing stimulus over a prolonged period.

The mechanism of the bone response to WBV is thought to be due to a direct response within the bone tissue to loading or via muscle; either by contractions loading the bone or due to increased muscle mass and/ or force increasing load to bone[48]. Increased muscle activity and blood flow as a result of WBV have been reported in a number of studies[49-55]. To determine if muscle activation following WBV was different between the low and high magnitude groups our study recorded skin surface temperature of the lower leg, as a surrogate measure, pre and post vibration in a sub group of boys. Although we found no difference between groups in bone turnover marker response in the immediate pre to post vibration period, thermal imaging detected a 31% greater increase in skin surface temperature in boys exposed to the high magnitude versus the low magnitude platform. Skin surface temperature has been shown to be increased in passive vibration[56] as well as weight bearing vibration[57] suggesting that some degree of muscle activation occurs regardless of the magnitude or method of the stimulation. Nevertheless, our results suggest muscle activation is not a prerequisite for bone to respond to mechanical stimulation.

In terms of possible future application, we speculate that this approach might have utility in identifying subjects more likely to benefit from vibration exercise-based interventions to increase bone mass; to identify potential adverse "damping" effects from pharmacological therapies targeting bone; and to assess the responsiveness of the skeleton to vibration in different disease states.

Limitations

This study was designed as a pilot study to determine the size and variability of the response of bone turnover markers to vibration and was therefore not powered to detect any specific degree of change. The intervention and control groups had relatively small numbers and so the study findings must be regarded as preliminary. Whilst the study has met its aim of identifying the rate and range of response of bone turnover markers in pre-pubertal boys to WBV it is not clear if the same response would be detected in pre-pubertal girls, or during other stages of growth.

The instructions regarding stance (stand with knees slightly bent) were the same for the subjects irrespective of platform; whilst appropriate for the high magnitude device, this stance may have abrogated the skeletal response to low magnitude stimulation.

The length of time between the last period of vibration and collection of the final samples was different between the boys exposed to 3 or 5 days of vibration, 48 and 72 hours respectively, due to logistical issues. The thermal imaging produced interesting data, however there were some limitations in our methods. A drift in the temperature reading of the camera was observed during the study and the distance of the camera to the point of interest (the boys leg's) was not standardised between participants. Our findings could however be confirmed in future studies using more robust techniques and methods such as use of a thermal imaging suite.

Summary

Five consecutive days of WBV increased the bone formation marker P1NP by 25.1% and the resorption marker CTx by 10.9%, demonstrating an alteration in bone turnover that is in favour of bone formation. The direct mechanism of this acute response is not clear as sclerostin was unchanged by vibration in this study and OPG increased but so did bone resorption. Studies in healthy adult populations have been unable to demonstrate an anabolic effect of WBV on bone over longer periods, but we have shown here that bone in a healthy growing skeleton does have the capacity to respond quickly to WBV irrespective of the magnitude of that vibration. The possible broader application of this approach in other settings requires careful evaluation, but these initial data are encouraging in terms of the size and consistency of response across different types of vibration platforms.

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12.3. Appendix 3 Study 1 Protocol (Final Version)

Sheffield Children's NHS
NHS Foundation Trust

FULL STUDY TITLE A Pilot Study to Investigate the Acute Bone Response to Vibration/Mechanical Stimulation in Pre-pubertal Children

SHORT STUDY TITLE Acute bone response to vibration

STUDY NUMBER CA080017

ORIGINAL DATE AND VERSION NUMBER November 2008 version 1

AMENDMENT DATE AND VERSION NUMBER 19/10/2012 version 6

LAY SUMMARY

Fractures in children are common, accounting for 10% of attendances in Accident and Emergency Departments. Much research in the past has focused on identifying the risk factors for fracture in childhood. It has been found that children who have narrower bones and a low bone mass are more likely to fracture than children with larger bones. Following this work has been undertaken to develop intervention strategies to reduce fracture risk. Studies in postmenopausal women, young women with low bone mass and children with disabling conditions such as cerebral palsy, have shown that 10 minutes a day stood on a vibrating platform can significantly increase bone mass.

However little is known of the immediate response of bone to this form of mechanical stimulation. We want to determine an acute bone response in apparently healthy children to a single period of 10 minutes standing on one of two vibrating platforms. Vibrating platforms can differ in how they work to increase bone mass. We would like to compare the difference between two of the commonly used platforms.

We plan to conduct a two phase study. In phase one we plan to recruit 12 healthy boys aged 9-12 years to stand on one of the two vibrating platforms once only for 10 minutes. Before and twice after the period of vibration we will take a blood sample to measure changes in the bone cells. We will also take two pictures of their legs using a thermal imaging camera. This is to detect differences in muscle temperature as a surrogate for blood flow and muscle activity. In phase two we plan to recruit a further 24 boys aged 9-12 years to stand on one of the two vibrating platforms for 10 minutes on either 3 or 5 consecutive days. We will take a blood sample before and after the period of vibration on days 1, 3, and 5. A control group will also be recruited. Blood samples will be collected at 5 time points over 2 hours to correspond with timing of the samples collected during phases 1 and 2 and a thermal image captured before and after standing for 10 minutes. In addition all children will be asked about the amount of exercise and sport they have participated in over the previous seven days. The findings of this study will be used to direct further research.

GENERAL INFORMATION

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Statistician: Name – Address – Telephone – Fax – Email -	

GLOSSARY

A list of abbreviations and definitions

1.0 BACKGROUND

Fractures account for 10% of childhood Accident and Emergency attendances, approximately 100,000 fractures annually in the UK.[1] The incidence of childhood fractures has increased in the last 30 years.[2] Fractures in children cause pain, may need surgical intervention, may result in further complications such as compartment syndrome, and can result in time off school and subsequently time off work for parents.[3,4]

It has been found that children who have narrower bones and a low bone mass are more likely to fracture than children with larger bones. Much work has been conducted on the effect of mechanical stimulation in the form of exercise on bone health. Studies have found that activities providing the greatest load to bone, such as weight lifting and gymnastics have the greatest effect at increasing bone mass and size.[5,6,7] More recently, however, researchers have found that low impact daily activities also contribute to bone development, with the suggestion that microstrains placed on bone from activity such as standing can also induce bone mass increases.[8,9,10] This has led to the use of vibrating platforms to provide mechanical stimulation. Studies in postmenopausal women, young women with low bone mass and children with cerebral palsy have shown that 2-10 minutes a day standing on a vibrating platform can significantly increase bone mass.[11,12,13,14]

Vibrating platform devices have increased in popularity in gyms and fitness centres as part of adult fitness programmes; they are now sold 'over the counter'. We plan to use two different vibration devices, thought to cause increases in bone mass and muscle function through different mechanisms of action. One is thought to stimulate muscle to load bones; the other causes bone changes through fluid shifts within the bone cells.

Previous studies have focused on the longer term use of vibrating platforms with the aims of increasing bone mass and/or muscle function, however little is known of how soon bone responds to a single exposure period of vibration or consecutive daily short term exposure to vibration. We want to determine the range and rate of acute bone responses in apparently healthy children to standing on a vibrating platform, either following a single exposure of 10 minutes or following 3 or 5 consecutive days at 10 minutes a day. More specifically we plan to focus on the changes in the bone turnover markers CTx (bone resorption) and P1NP (bone formation).

Having established the range and rate of acute responses attributable to each type of intervention, we will undertake further work to determine whether specific factors such as bone mass and architecture, muscle mass, and variation in genes important for the regulation of skeletal homeostasis impact on the acute response. These studies will be the subject of a separate application.

2.0 STUDY OBJECTIVES AND PURPOSE

The aim of this study is to determine the acute response of bone to a 10 minute period of standing on a vibrating platform. It will be undertaken as part of a PhD project. The data will be used to direct further research into the use and applications of vibration therapy in children with fractures and low bone mass, including those with mild osteogenesis imperfecta.

3.0 STUDY DESIGN

We plan to perform a two phase randomised comparative pilot study of vibration therapy in apparently healthy pre-pubertal boys, aged 9-12 years. Initial recruitment will be for participants to be randomly assigned to one single 10 minute period of standing on one of two vibrating platforms, either the Juvent 1000 or Galileo Med M. Following this (phase two) additional participants will be assigned to either 3 or 5 consecutive days of 10 minutes vibration on one of the two platforms. The period of vibration in both phase one and two will be delivered in short bursts with one minutes rest between cycles to a total of 10 minutes of vibration for participant comfort. The vibration is undetectable on the Juvent 1000 platform but can be felt and seen on the Galileo device; therefore neither the researcher nor participants will be blinded to the intervention group. The acute response of bone to the mechanical stimulation will be measured. A control group, not exposed to vibration will also be recruited

The primary outcome will be change in serum bone resorption (CTx) and bone formation (P1NP) markers. CTX and P1NP will be measured at each sampling time point. Serum will also be measured for osteocalcin, osteoprotegerin (OPG), sclerostin and bicarbonate. OPG released by osteoblasts, the bone forming cells, interferes with the formation and survival of osteoclasts which resorb bone. Sclerostin, expressed by the osteocytes in response to mechanical stimulation, is thought to have an effect on bone formation by blocking the Wnt-LRP5/6 signalling pathway within the osteoblasts.

For participants exposed to a single period of vibration, fasted blood samples will be taken immediately prior to standing on the platform and then 10 and 60 minutes post intervention. Those exposed to either 3 or 5 consecutive days of 10 minutes of vibration will have fasted blood samples taken immediately pre- and 10 minutes post vibration and days 1, 3, 5 and 8. 5 blood samples in the control group will occur at half hourly intervals (0, 30, 60, 90, and 120 minutes) over a two hour period on one day only, corresponding with the timing of sampling from phase 1 participants.

A secondary outcome to be measured will be skin surface temperature of the lower limbs measured using an infra-red thermography camera pre- and post intervention in the single exposure group (phase one and controls).

It is expected that the visit will take one and a half hours to complete for the single exposure group and two and a half hours for the control group. No follow-up will be performed as all investigations will be completed within the one visit. Study visits for participants in phase 2 will take 40 minutes when blood samples are collected and 20 minutes on days 2 and 4 when blood samples are not collected. A cannula will be inserted into the arm or hand of the participants for the repeat blood sampling so that they are not exposed to multiple venepuncture. Questions relating to the child's exercise and/or sporting activities over the previous 7 days will be asked and answers recorded doing the 10 minute period of vibration.

4.0 SELECTION OF PARTICIPANTS

In total we will recruit up to fifty-four pre-pubertal boys aged 9-12 years. Pubertal status will be determined during interview by self-assessment using a gender appropriate validated pictorial scale depicting the different stages of puberty.[15]

Inclusion criteria
White Caucasion
Aged 9-12yrs (pre-pubertal)
First language English

Exclusion criteria
Pre-existing chronic illness
Known bone disease
History of one or more fractures
Recent (last 12 months) or current treatment likely to affect bone – this does not include inhaled or intermittent oral therapy with steroids for asthma
Balance problems
Continuing involvement in more than one other research study

Participants will be recruited from local schools, and also youth and activity clubs or groups. The Head teacher or club/group organiser will be approached for permission for letters to be given to the children and parents inviting them to participate in the study. Contact details for the study team will be included in the letters so that parents can contact the team if they wish for their children to participate. With permission an advert will be placed on the notice board at the meeting venue of the clubs/groups. An invitation for participation in the study will also be sent via email to staff working at Sheffield Children's NHS Foundation Trust, the University of Sheffield, and Sheffield Teaching Hospitals NHS Foundation Trust asking for interested persons to contact the research team for further information.

Potential participants attending Sheffield Children's Hospital accident and emergency department and general medical out-patient clinics will be approached by clinical staff responsible for their care. If interest is expressed, they will be given information regarding the study to take away and read, and with

permission will be contacted by a member of the research team. An advert will be displayed at Sheffield Children's Hospital and through the media.

5.0 PARTICIPANT RECRUITMENT

Screening for the inclusion criteria will be done by interview. Information sheets detailing the study procedures and contact details for the study team will be posted out to potential participants and their parents who have expressed an interest in the study prior to their attendance. Age appropriate information sheets will be included for the participants. A minimum of 24 hours will be given for participants to consider their involvement. Parents and participants will have the opportunity to discuss the study further with the research team prior to giving consent.

Consent will be gained by a specialist research nurse with appropriate experience and training in Good Clinical Practice. Enrolment into the study will take in the Clinical Research Facility or the child's school or home where the study procedures (for the 3 and 5 day study) will take place.

Neither the researchers nor participants will be blinded to the intervention groups as the Galileo platform involves a see-saw type motion and it will be possible to see and feel movement when the platform is vibrating. Participants will be randomised to the intervention groups. Allocation will be by successive opening of envelopes containing the individual randomisation codes. This will be done within the CRF by CRF staff.

A member of the research team will be present for the duration of each study visit and will therefore witness compliance to the intervention. Participants are free to withdraw from the study at any time, without giving a reason. As this study involves at the most 5 days exposure to the intervention with no further follow-up, participant withdrawal is expected to be minimal. Study visits for participants allocated to 3 or 5 days of vibration will be conducted in their home or at their school for participant convenience with the aim to reduce the number of withdrawals.

For the single exposure group blood samples to measure bone resorption and formation will be taken immediately prior to the intervention, 10 minutes and 60 minutes post intervention. A validated questionnaire[16] regarding exercise and sport activity over the 7days previous to the study visit will be conducted during the intervention. Thermal images will be taken immediately pre- and post intervention.

For the groups exposed to 3 or 5 days of vibration blood samples will be taken immediately pre- and 10 minutes post- vibration on days 1, 3, and 5. Participants allocated to 3 days vibration will return for repeat blood sampling only on day 5. Participants allocated to 5 days vibration will return for repeat blood sampling only on day 8. (Appendices 1 and 2 show participant involvement in study from initial contact.)

Boys recruited to the control group will have a blood sample collected every half hour over a two hour time period (5 in total), this is to correspond with the timing of the samples from phase 1 of the study. Thermal images will be taken 10 minutes apart to correspond to the timing of standing on the vibration platform.

6.0 DATA HANDLING AND RECORD KEEPING

Data will be entered in the Academic Unit of Child Health and stored in password protected files; all data will be double-entered. 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. Study documents (paper and electronic) will be retained in a secure location during and after the study has finished. All source documents will be retained for a period of 5 years following the end of the study. Where study related information is documented in the medical records – those records will be identified by a "Do not destroy before dd/mm/yyyy" label where date is 5 years after the last patient last visit.

7.0 ACCESS TO SOURCE DATA

The sponsor will permit monitoring and audits by the relevant authorities, including the Research Ethics Committee and the Medicines and Healthcare products Regulatory Agency (MHRA). The investigator will also allow monitoring and audits by these bodies and the sponsor, and they will provide direct access to source data and documents.

8.0 STATISTICAL ANALYSIS

No sample size calculation has been performed for this study as this is a pilot study to determine the range of responses to the period of vibration. We are therefore unable to estimate the number of participants needed. Following advice from Dr Jenny Freeman (statistician) we are recruiting 12 participants to each group (12-18 boys in the control group) as is recommended in current literature regarding sample sizes for pilot studies.[17,18] The data gathered from this study will be used to determine sample size for future studies. We will be seeking further statistical advice once we have started the initial recruitment and if required will recruit further children on the basis of our statisticians advice.

9.0 SAFETY ASSESSMENTS

There are no anticipated safety issues. The platform has been used extensively both in clinical practice and sold 'over the counter' in countries within the EU including the UK without any reported adverse effects. 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 we will ask to hold the back of a chair to steady them self. The Galileo platform has a built in handrail. As the study is unblinded there will be no need to have in place plans for breaking the randomisation codes.

The study will be monitored and audited in accordance with the Monitoring Standard Operating Procedures of the Clinical Research Support Unit. 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 licensing bodies.

10.0 ETHICAL CONSIDERATIONS

The study will be conducted in compliance with a Research Ethics Committee favourable opinion, including any provisions for Site Specific Assessment, and local Research and Development approval. The study will also be conducted in accordance with the International Conference for Harmonisation of Good Clinical Practice (ICH GCP), and the Research Governance Framework for Health and Social Care (2nd Edition).

11.0 FINANCE AND INDEMNITY

Participants will not be paid for their involvement in the study but will be offered a £10 Meadowhall voucher for reimbursement of their time and a voucher towards the cost of breakfast at the investigating site.

This is an NHS sponsored study. For NHS sponsored research HSG (96) 48 reference no. 2 refers. If there is negligent harm during the study when the NHS body owes a duty of care to the person harmed, NHS Indemnity will cover NHS staff, medical academic staff with honorary contracts and those conducting the study. NHS Indemnity does not offer no-fault compensation and is unable to agree in advance to pay compensation for non-negligent harm. Ex-gratia payments may be considered in the case of a claim.

12.0 REPORTING AND DISSEMINATION

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 and in the R&D newsletter. It is the intention that this work should form the basis of a PhD.

TABLES, FIGURES AND REFERENCES

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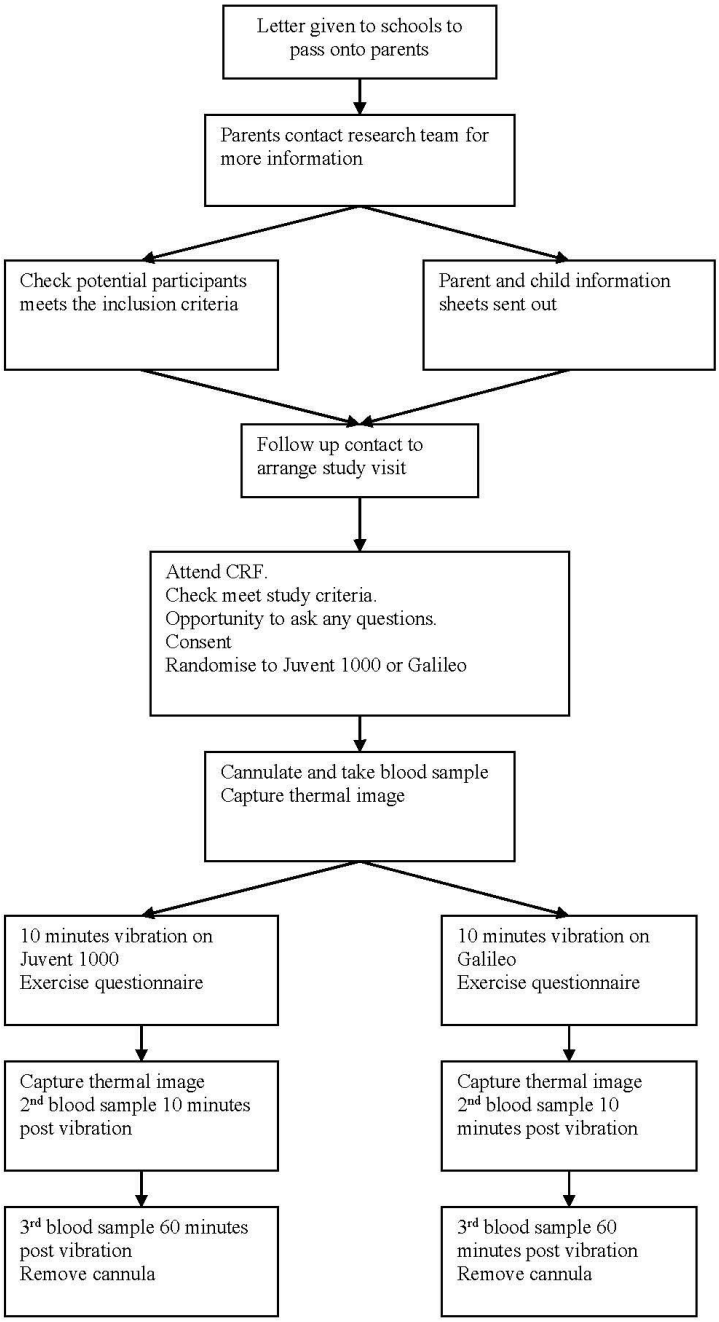
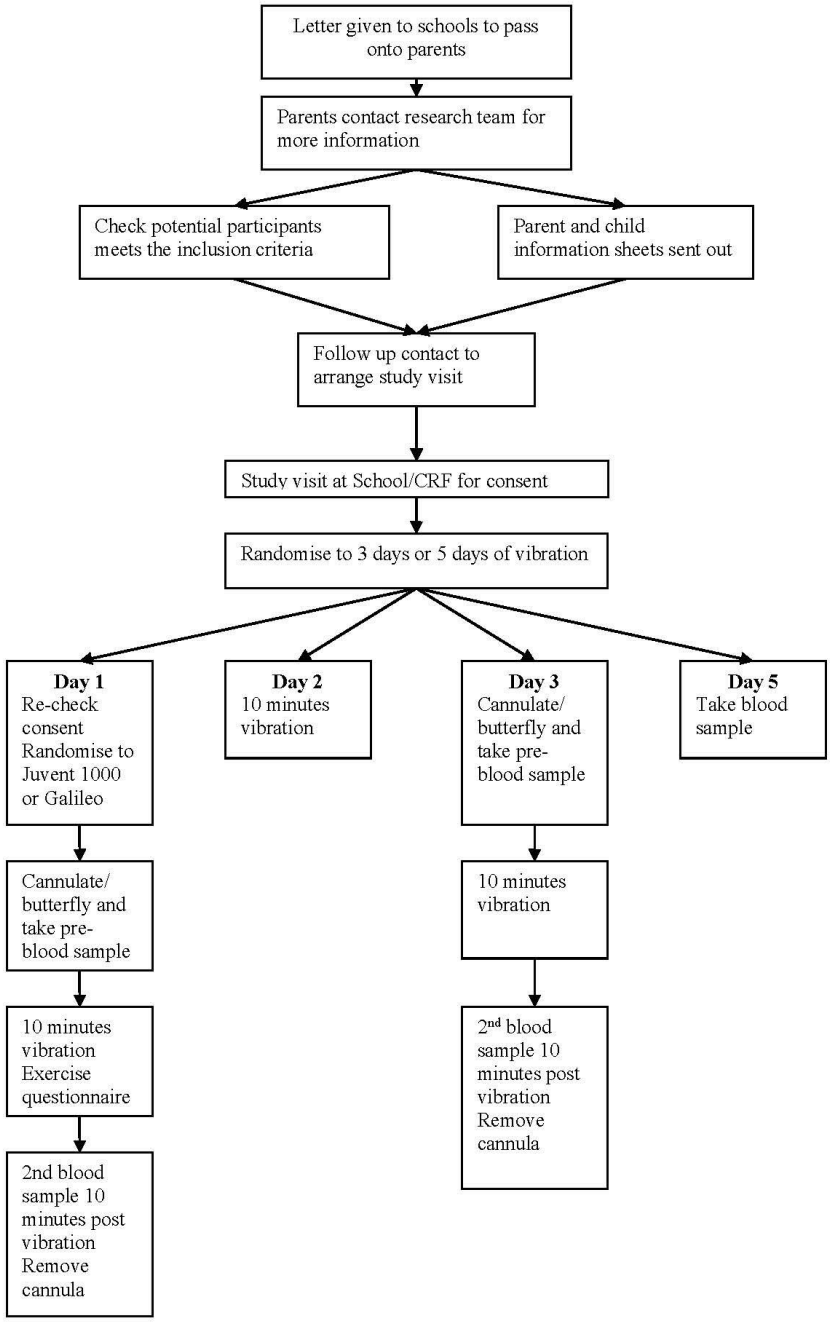
APPENDICES

Appendix 1 Participant Involvement Phase 1 – single vibration exposure
Appendix 2 Participant Involvement Phase 2 – 3 days vibration
Participant Involvement Phase 2 – 5 days vibration
Appendix 3 Participant Involvement Control group

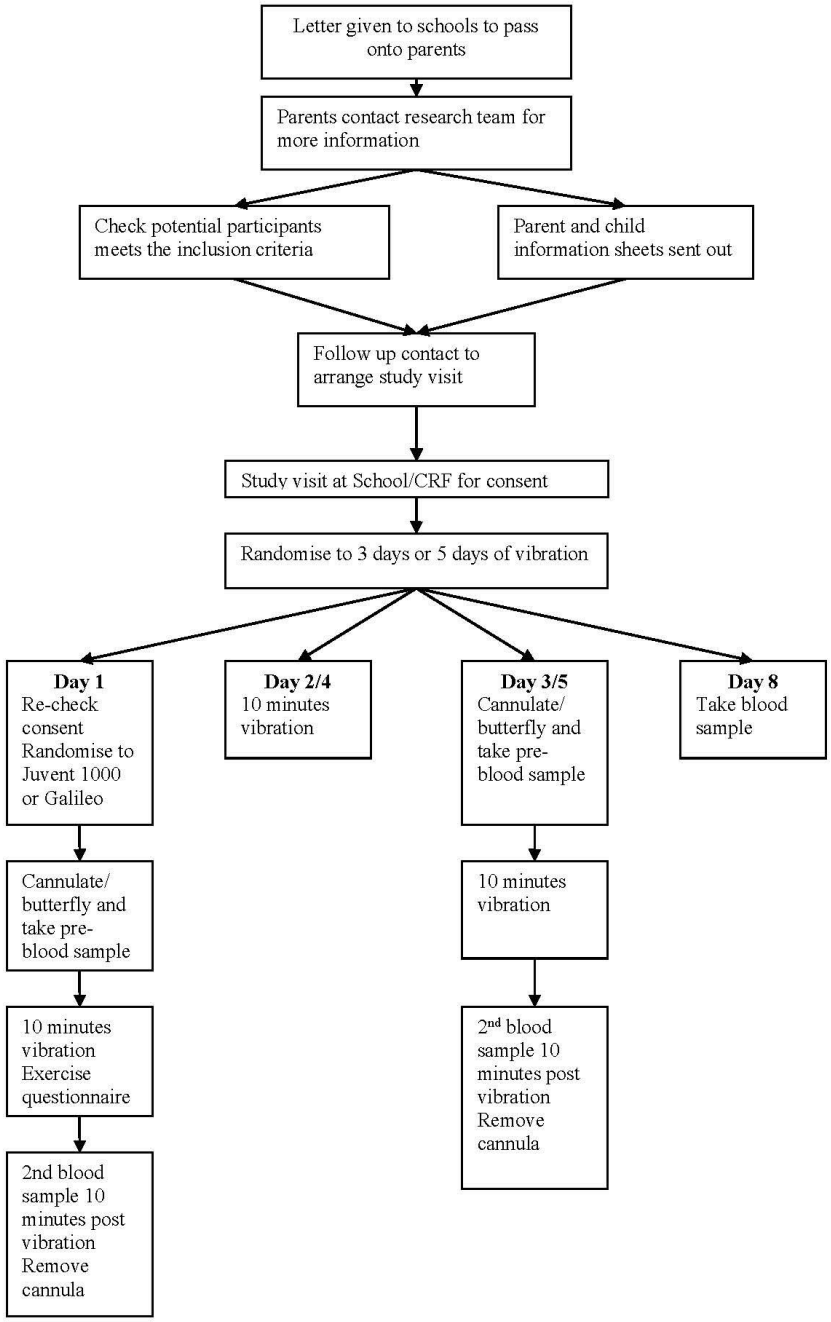
Appendix 1 Participant Involvement Phase 1 – single vibration exposure



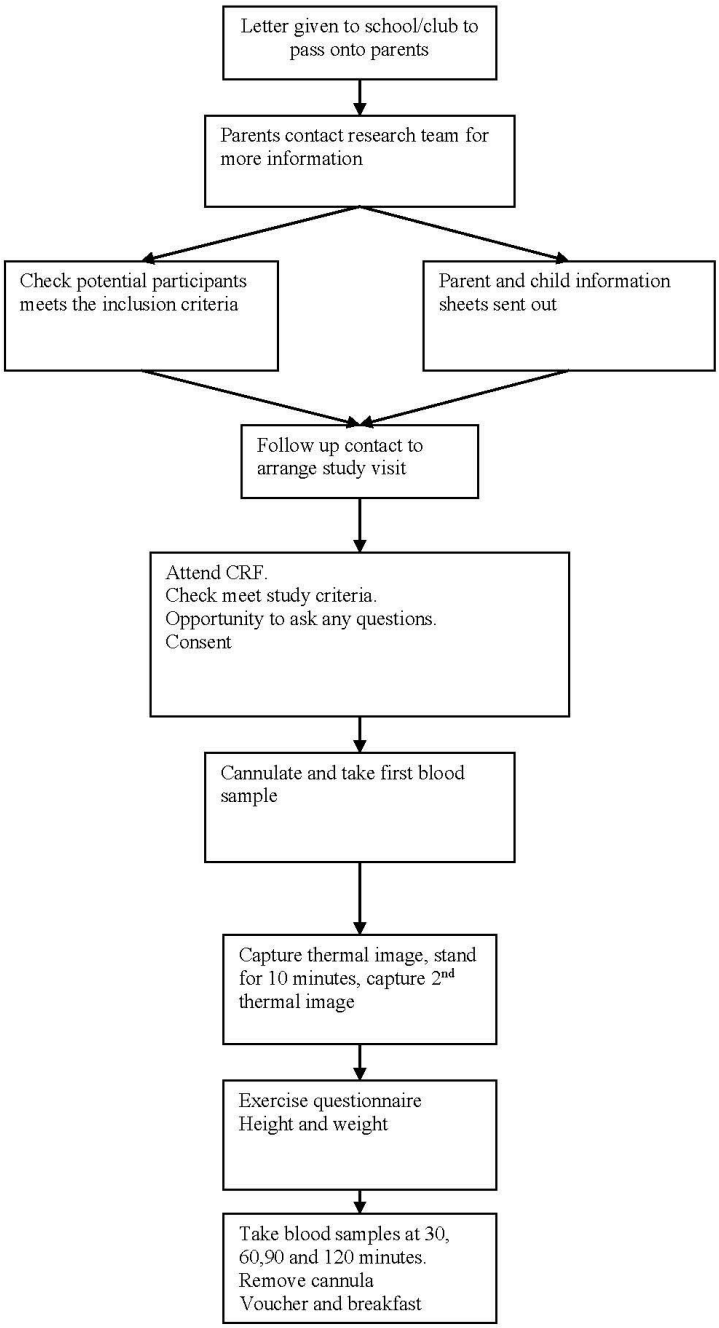
Appendix 2 Participant Involvement Phase 2 – 3 days vibration



Participant Involvement Phase 2 – 5 days vibration



Appendix 3 Participant Involvement Control group



Sheffield Children's NHS
NHS Foundation Trust

PARTICIPANT INFORMATION SHEET FOR YOUNG PEOPLE

To be shown and read by parent/carer if required

Study title
Acute bone response to vibration.

1. What is research?
Research is a careful experiment to find out the answer to an important question

2. Why is this project being done?
Other studies have shown that standing on a vibrating platform for 10 minutes a day for at least 6 months can make bones bigger. Bigger bones are stronger bones, which mean they will break less easily. We want to see how your bones respond when

you stand on a vibrating platform for a total of 10 minutes.

Also this project will help one of the study team to learn about research.

3. Why me?
You have been chosen because you are healthy and haven't broken any bones before and because you are a boy aged 9-12 years. We are asking 12 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 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?

We will ask you to stand on a vibrating platform (that is about the size and shape of typical bathroom weighing scales) for 10 minutes. You will be able to hold onto a rail or back of a chair if you are worried that you might fall off.

Before and twice after you stand on the platform a nurse will take a blood sample from a small plastic tube called a cannula that has been put in a vein in your arm or hand. We will take 2mls (less than half a teaspoon) of blood for each sample.

A special camera that can tell how hot you are will take a picture of your legs. We will ask you to wear a pair of shorts to do this. You will be asked questions about the activities and exercise that you have done in the last week.

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. We will measure your height and weight.

This will all happen in one visit which will last approximately one and a half hours at the Children's Hospital in Sheffield. Your Mum, Dad, or carer will be able to stay with you.

6. Will any of this hurt?

Standing on the vibrating platform will not hurt. The people who make the platform have said that you may feel nausea and dizziness, get blisters where you are in contact with the platform, or itching in the body parts that have been trained, if you use the platform for a long time.

Before we take the blood samples we will put 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. The blood will be taken through a thin plastic tube which will be taped to your skin for about 60 minutes. After the last blood sample has been taken, the tube will be removed.

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 sample. We will give you a voucher to get breakfast from the hospital dining room when the study visit is finished.

7. Will joining in help me?

The study will not help you, but we hope it will help us to understand what happens to bones when children stand on a vibrating platform.

8. What else might happen?

If we find out something that we think is important about you, we will talk to your mum, dad or carer and ask them if they

want to come back and have you checked again at the hospital.

9. What happens when the research study stops?

We will collect all the information together and we will use it to help us design longer studies in the future with children who have problems with their bones.

10. What if something goes wrong?

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.

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

Just tell your mum, dad, carer, doctor or nurse at any time. They will not be cross

with you. You will still have the same care whenever you need to come to hospital.

12. What if I wish to complain about the study?

If you want to complain you or your mum, dad or carer can talk to Rachel Harrison or Mrs Linda Towers at this hospital.

13. Will anyone else know I'm doing this?

The people in our research team will know you are taking part. No one else will know because we will not use your name or address. You will get a number which will be used instead. We will ask you if it is ok to let your GP know that you are taking part.

14. What happens to what the researchers find out?

When we collect your information we will make sure it is stored in a safe place and only the people doing the research study can look at it.

We will use the information to teach doctors about how bone reacts to vibration, put it in medical magazines and on websites that doctors read.

A short summary will also be on the hospital's research website. No-one will know you were in the study.

15. Did anyone else check the study is OK to do?

This study has been checked by several people, to make sure it is alright.

16. How can I find out more about this study?

Your mum, dad, carer or other grownup you trust may be able to answer your questions.

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

Sheffield Children's NHS
NHS Foundation Trust

PARENT/LEGAL GUARDIAN INFORMATION SHEET

Study title
Acute bone response to vibration.

Part 1 – to give you first thoughts about the project

1. Invitation paragraph
You and your child are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully. 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.

Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you want your child to take part.

2. What is the purpose of the study?
It has been found that children who have narrower bones and a low bone mass are more likely to break a bone than children with larger bones. Recent studies have shown that standing on a vibrating platform for up to 10 minutes at a time every day for at least 6 months can increase bone mass. We want to look at the immediate effect of standing for a total of 10 minutes once only on a vibrating platform on the bones of boys aged 9-12 years. If we can understand better how to assess very short term effects and responses to vibration it may help us focus treatment better in the future.

This research is also helping one of the study team to learn more about carrying out research, and the information that is gathered will be used in her final assessment for a qualification (a PhD) from the University of Sheffield.

3. Why has my child been chosen?
Your child has been chosen because he is healthy and hasn't broken any bones before. Also because we are inviting 12 boys aged 9-12 years to take part in the study.

4. Does my child have to take part?
No. It is up to you and your child to decide whether or not to take part. You are both free to withdraw from the research at any time and without giving a reason. Your decisions about this will not influence

how we look after your child whenever they need to come to hospital.

If you are happy to take part, and are satisfied with the explanations from the research team, you will be asked to sign a consent form. Your child will be asked to sign an assent form with you. You will be given a copy of the information sheet and the signed consent/assent forms to keep for your records.

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

We will measure your child's height and weight, and ask you or your child questions about the amount and type of exercise they have done in the 7 days prior to the study visit. We plan for your child to stand on one of two types of platform so that we can compare any differences in how they work. One of the platforms mimics a walking motion, so when standing on the platform your child will feel a wobbling sensation in their legs. The vibration motion in the second platform is undetectable, although you will hear a slight buzzing noise when it is switched on.

Blood samples will be taken from your child before the vibration begins, instantly after, and 1 hour after the vibration has stopped. A thin plastic tube (cannula) will be inserted into a vein in your child's hand or arm and taped in place for the first sample to be taken and until after the third sample is taken.

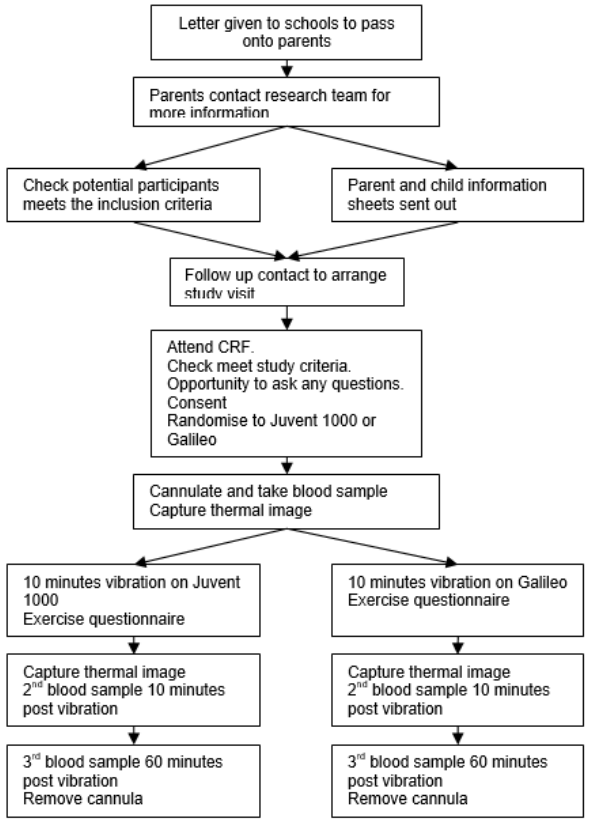
We will use a special cream or spray to numb the area where the cannula is to be inserted so that it doesn't hurt as much. 2mls of blood (less than half a teaspoon) will be taken for each sample. Your child will need to be fasted for all the blood samples, this means nothing to eat or drink except water from midnight the night before the visit. This is really important, because eating and drinking have major effects on the things in the blood we want to measure.

To measure the differences in the muscle activity whilst standing on the vibrating platforms we will take a thermal image using a special camera of your child's lower legs before and after the period of vibration. All of this will happen at one study visit at Sheffield Children's Hospital which is expected to last approximately one and a half hours.

All children who take part in the study will be offered a £10 voucher to spend in Meadowhall as a thank you for helping us with our research. We will also give each child a voucher for a breakfast up to the value of £2.50 to use in the hospital dining room at the end of the visit.

Parents/carers are encouraged to stay with their child during the study visit.

What will happen – single 10 minute vibration



6. What will we have to do?

All study procedures will take place during the study visit. Other than nothing to eat or drink except water after midnight on the day of the study visit no preparation is required.

Before undertaking any study procedures we will ask your child to self-assess their stage of puberty using a pictorial scale.

To enable us to take the thermal image using the special camera we will ask that your child wears a pair of shorts so that the camera can measure the temperature of the lower legs.

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

No adverse events have been recorded in the use of vibrating platforms. However the manufacturer does list the following as possible side effects:

- skin lesions/blisters on contact surface
- itching in trained body parts
- nausea and dizziness
- quick temporary drop of blood pressure
- drop in blood sugar level in diabetics due to high physical activity.

There are no risks associated with using a thermography camera to take a thermal image. Your child will have a cannula placed into a vein for the

taking of blood samples. This can cause discomfort to your child. To reduce this, a local anaesthetic in the form of a cream or a cold spray will be applied to the skin prior to insertion of the cannula.

8. What are the possible benefits of taking part?

We do not anticipate that this study will be of direct benefit to you or your child. We are hoping that this will help us to understand how vibration therapy can cause adaptations in bone.

9. What happens when the research study stops?

We will collect all the information together and we will use this to design longer term studies in the use of vibrating platforms in children with bone health problems.

10. What if there is a problem?

Any complaint about the way you or your child have 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.

11. Will my child's taking part in the research project 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.

12. Contact for further information

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

Name: Rachel Harrison
Designation: Research Nurse
Hospital/Department: Sheffield Children's Hospital, Western Bank, Sheffield, S10 2TH
Tel: 0114 2717120
Email: Rachel.C-Harrison@sch.nhs.uk

This completes Part 1 of the Information Sheet.

If the information in Part 1 has interested you and you are considering participation, please continue to read the additional information in Part 2 before making any decision.

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

13. What if new information becomes available?

Sometimes during the course of a research project new information becomes available. Your child will be involved in the study for one visit only, so it is unlikely that any new information will become available during that time.

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

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

15. What if there is a problem?

Complaints

If you have any cause to complain about any aspect of the way in which you or your child has been approached or treated during the course of this study, the normal National Health Service complaints mechanisms are available to you and are not compromised in any way because you have taken part in a research study. If you have any complaints or concerns please contact either the project co-ordinator:

Name: Rachel Harrison
Designation: Research Nurse
Hospital/Department: Sheffield Children's Hospital, Western Bank, Sheffield, S10 2TH
Tel: 0114 2717120
Email: Rachel.C-Harrison@sch.nhs.uk

Otherwise you can use the normal hospital complaints procedure and contact the following person:

Mrs Linda Towers
Patient Advice & Liaison Co-ordinator
Sheffield Children's NHS Foundation Trust
Tel: 0114 271 7594
Email: Linda.Towers@sch.nhs.uk

Harm

If your child is harmed by taking part in this research project, there are no special compensation arrangements. If your child is harmed due to someone else's fault, then you may have grounds for a legal action – but you may have to pay for it.

16. Will 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 your child cannot be recognised

from it. Once the study is complete all information including questionnaires and blood samples will be kept for 5 years.

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

Access to data will be restricted to members of the research team, the study sponsor and Sheffield Children's Hospital Research and Development department. Monitoring and audit may be carried out by the relevant authorities.

We will also ask for permission to 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?

All samples collected will be anonymised with a study number and will be stored in a freezer in the Clinical Research Facility (CRF) at Sheffield Children's Hospital until they are ready to be analysed in the laboratory. We will ask your permission to use any left over samples for future research and this will only be after further ethical

approval has been given. Access to the samples will be limited to the same people who have access to study data.

18. Will any genetic tests be done?

No.

19. What will happen to the results of the research study?

When the study has finished we will present our findings to other doctors, and we will put the results in medical magazines and websites that doctors 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, in, September 2009, on www.sheffieldchildrenscrf.nhs.uk. The results will also be included as part of the chief investigator's educational qualification. They will be anonymous, which means that your child will not be able to be identified from them.

20. Who is organising and funding the research?

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

21. Who has reviewed the study?

This study was given a favourable ethical opinion for conduct in the NHS by South Humber Local

Research Ethics Committee. It has also been approved by the Research Department at this hospital.

22. How can we find out more about research?

The Clinical Research Facility at this hospital has an **Information for families** section on its website www.sheffieldchildrenscrf.nhs.uk or you could contact the hospital Clinical Research Facility:

Mrs Tracy N'Diaye
R&D Manager
Sheffield Children's NHS Foundation Trust
Tel: 0114 226 7904
Email: Tracy.NDiaye@sch.nhs.uk

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.

Sheffield Children's NHS
NHS Foundation Trust

PARTICIPANT INFORMATION SHEET FOR YOUNG PEOPLE

To be shown and read by parent/carer if required

Study title
Acute bone response to vibration.

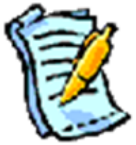
1. What is research?
Research is a careful experiment to find out the answer to an important question

2. Why is this project being done?
Other studies have shown that standing on a vibrating platform for 10 minutes a day for at least 6 months can make bones bigger. Bigger bones are stronger bones, which mean they will break less easily. We want to see how your bones respond when you stand on a vibrating platform for 10

minutes each day for 3 or 5 days in a row. Also this project will help one of the study team learn how to do research.

3. Why me?
You have been chosen because you are healthy and haven't broken any bones before and because you are a boy aged 9-12 years. We are asking 24 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 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?

You will stand on a vibrating platform (that is about the size and shape of typical bathroom weighing scales) for 10 minutes each day for 3 or 5 days in a row. You will be able to hold onto a rail or back of a chair if you are worried that you might fall off. The research team will open an envelope to decide if you will stand on the platform for 10 minutes on 3 or 5 days. Half of the boys taking part will stand on the platform on 3 days, and half will stand on the platform on 5 days.

A nurse will take a blood sample before and once after you stand on the platform from a small plastic tube called a cannula that has been put in a vein in your arm or hand. This will not happen every day.

Day 1:	Take blood sample, stand on vibrating platform for 10 minutes, take a second blood sample.
Day 2:	Stand on vibrating platform for 10 minutes.
Day 3:	Take blood sample, stand on vibrating platform for 10 minutes, take a second blood sample.
Day 4:	Stand on vibrating platform for 10 minutes (only if you have been chosen to stand on the platform for 5 days in a row).
Day 5:	Take blood sample, stand on vibrating platform for 10

minutes, take a second blood sample. If you are chosen to stand on the platform for 3 days in a row, we will ask you to come back on day 5 for a blood sample only.

Day 8: If you are chosen to stand on the platform for 5 days in a row, we will ask you to come back on day 8 for a blood sample only.

This will all take part in your home or in a private room in your school and will take 20-40 minutes each day.

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.

On day 1 you will be asked questions about the activities and exercise that you have done in the last week. 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.

We will measure your height and weight.

6. Will any of this hurt?

Standing on the vibrating platform will not hurt. The people who make the platform have said that you may feel nausea and dizziness, get blisters where you are in contact with the platform, or itching in the body parts that have been trained, if you use the platform for a long time.

Before we take the blood samples we will put 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. The blood will be taken through a thin plastic tube which will be taped to your skin for about 30 minutes. After the last blood sample has been taken, the tube will be removed.

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. We will provide a light breakfast or you can bring something to eat and drink from home for after the blood samples have been taken.

7. Will joining in help me?

The study will not help you, but we hope it will help us to understand what happens to

bones when children stand on a vibrating platform.

8. What else might happen?

This will all happen in your home or school at the start of your school day. When the visits happen in your school we will ask you to come to school a bit earlier if you can and you might be a little bit late for your first lesson, but we will have asked your teacher if this is ok.

If we find out something that we think is important about you, we will talk to your mum, dad or carer and ask them if they want to come back and have you checked again at the hospital.

9. What happens when the research study stops?

We will collect all the information together and we will use it to help us

design longer studies in the future with children who have problems with their bones.

10. What if something goes wrong?

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.

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

Just tell your mum, dad, carer, doctor or nurse at any time. They will not be cross with you. You will still have the same care whenever you need to come to hospital.

12. What if I wish to complain about the study?

If you want to complain you or your mum, dad or carer can talk to Rachel Harrison or Mrs Linda Towers at this hospital.

13. Will anyone else know I'm doing this?

The people in our research team will know you are taking part. Your teacher will also know because this may all happen at school, but they will not see the information that we collect. No one else will know because we will not use your name or address. You will get a number which will be used instead. We will ask your permission to let your GP know that you are taking part.

14. What happens to what the researchers find out?

When we collect your information we will make sure it is stored in a safe place and only the people doing the research study can look at it.

We will use the information to teach doctors about how bone reacts to vibration, put it in medical magazines and on websites that doctors read.

A short summary will also be on the hospital's research website. No-one will know you were in the study.

15. Did anyone else check the study is OK to do?

This study has been checked by several people, to make sure it is alright.

16. How can I find out more about this study?

Your mum, dad, carer or other grownup you trust may be able to answer your questions.

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

Sheffield Children's NHS
NHS Foundation Trust

PARENT/LEGAL GUARDIAN INFORMATION SHEET

Study title
Acute bone response to vibration.

Part 1 – to give you first thoughts about the project

1. Invitation paragraph
You and your child are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully. 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.

Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you want your child to take part.

2. What is the purpose of the study?
It has been found that children who have narrower bones and a low bone mass are more likely to break a bone than children with larger bones. Recent studies have shown that up to 10 minutes standing on a vibrating platform every day for at least 6 months can increase bone mass. We want to look at the short term effect of 10 minute periods of standing on a vibrating platform on the bones of boys aged 9-12 years. If we can understand better how to assess very short term effects and responses to vibration it may help us focus treatment better in the future.

This research is also helping one of the study team to learn more about carrying out research, and the information that is gathered will be used in her final assessment for a qualification (a PhD) from the University of Sheffield.

3. Why has my child been chosen?
Your child has been chosen because he is healthy and hasn't broken any bones before. Also because we are inviting 24 boys aged 9-12 years to take part in the study.

4. Does my child have to take part?
No. It is up to you and your child to decide whether or not to take part. You are both free to withdraw from the research at any time and without giving a

reason. Your decisions about this will not influence how we look after your child whenever they need to come to hospital.

If you are happy to take part, and are satisfied with the explanations from the research team, you will be asked to sign a consent form. Your child will be asked to sign an assent form with you. You will be given a copy of the information sheet and the signed consent/assent forms to keep for your records.

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

We will measure your child's height and weight, and ask you or your child questions about the amount and type of exercise they have done in the 7 days prior to the study. We plan for your child to stand on one of two types of platform so that we can compare any differences in how they work. One of the platforms mimics a walking motion, so when standing on the platform your child will feel a wobbling sensation in their legs. The vibration motion in the second platform is undetectable, although you will hear a slight buzzing noise when it is switched on.

We will ask your child to stand on the vibrating platform for 10 minutes on either 3 or 5 consecutive days. This will be decided at random. Neither the study team nor you will be able to choose.

Bloods samples will be taken from your child before the vibration begins and after the vibration has stopped, except on days 2 and 4. A thin plastic tube (cannula) will be inserted into a vein in your child's hand or arm and taped in place for the first sample to be taken and until after the second sample is taken. We will use a special cream or spray to numb the area where the cannula is to be inserted so that it doesn't hurt as much. 2mls of blood (less than half a teaspoon) will be taken for each sample. Your child will need to be fasted for all the blood samples, this means nothing to eat or drink except water from midnight the night before the visit. This is really important, because eating and drinking have major effects on the things in the blood we want to measure.

Day 1:	Take blood sample, stand on vibrating platform for 10 minutes, take a second blood sample.
Day 2:	Stand on vibrating platform for 10 minutes.
Day 3:	Take blood sample, stand on vibrating platform for 10 minutes, take a second blood sample.
Day 4:	Stand on vibrating platform for 10 minutes (only if selected for 5 days in a row standing on the vibrating platform).
Day 5:	Take blood sample, stand on vibrating platform for 10 minutes, take a second

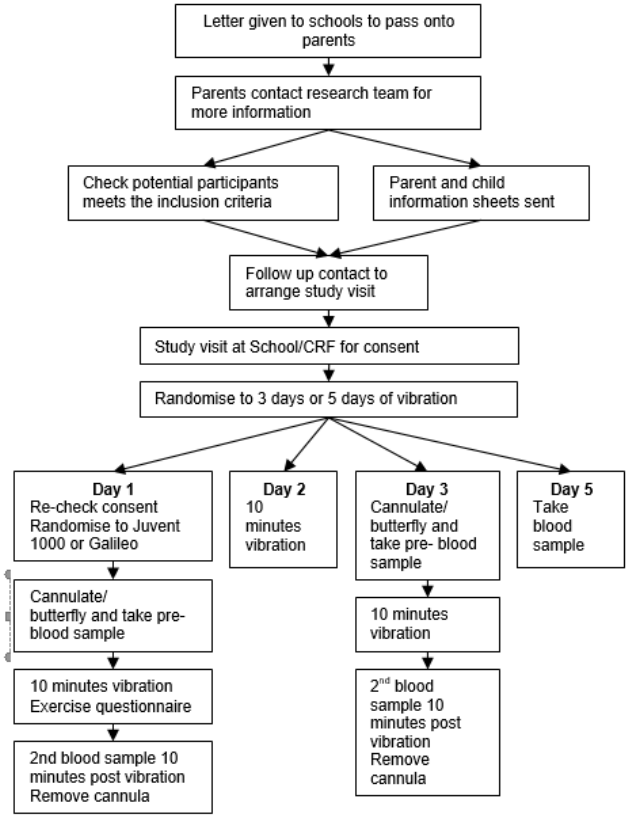
blood sample. If you are chosen to stand on the platform for 3 days in a row, we will ask you to come back on day 5 for a blood sample only.

Day 8: If you are chosen to stand on the platform for 5 days in a row, we will ask you to come back on day 8 for a blood sample only.

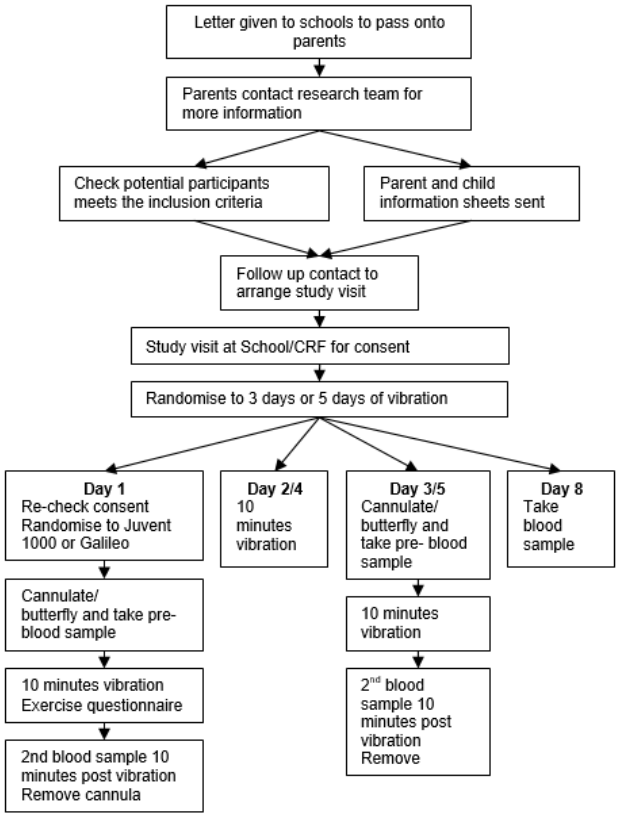
Study visits may take place in your home or with the head teacher's permission may take place at your child's school, first thing in the morning so as to disrupt their school day as little as possible. The visits will take approximately 20-40 minutes to complete. A member of the study team will stay with your child for the whole visit, if you wish to stay with your child too you can. If you are not able to remain with your child and wish us to arrange a chaperone we will do so.

All children who take part in the study will be offered a £10 voucher to spend in Meadowhall as a thank you for helping us with our research. We will provide a light breakfast on the days that blood samples are taken, or alternatively your child could bring in something to eat and drink from home.

What will happen – 3 days vibration



What will happen – 5 days vibration



6. What will we have to do?

All study procedures will take place during the study visits. Other than nothing to eat or drink except water after midnight on the days that the blood samples are taken no preparation is required.

Before undertaking any study procedures we will ask your child to self-assess their stage of puberty using a pictorial scale.

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

No adverse events have been recorded in the use of vibrating platforms. However 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
d. quick temporary drop of blood pressure
e. drop in blood sugar level in diabetics due to high physical activity.

Your child will have a cannula placed into a vein for the taking of blood samples. This can cause discomfort to your child. To reduce this, a local anaesthetic in the form of a cream or a cold spray will be applied to the skin prior to insertion of the cannula.

8. What are the possible benefits of taking part?

We do not anticipate that this study will be of direct benefit to you or your child. We are hoping that this will help us to understand how vibration therapy can cause adaptations in bone.

9. What happens when the research study stops?

We collect all the information together and we will use this to design longer term studies in the use of vibration platforms in children with bone health problems.

10. What if there is a problem?

Any complaint about the way you or your child have 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.

11. Will my child's taking part in the research project 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.

12. Contact for further information

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

Name: Rachel Harrison
Designation: Research Nurse
Hospital/Department: Sheffield Children's Hospital, Western Bank, Sheffield, S10 2TH
Tel: 0114 2717120
Email: Rachel.C-Harrison@sch.nhs.uk

This completes Part 1 of the Information Sheet.

If the information in Part 1 has interested you and you are considering participation, please continue to read the additional information in Part 2 before making any decision.

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

13. What if new information becomes available?

Sometimes during the course of a research project, new information becomes available. As your child is involved for only a week this is unlikely. However if new information relating to vibration and bone health does become available someone from the research team will tell you and your child about it and discuss with you whether you want your child to continue in the study. If you change your mind this will not affect any care your child receives now or in the future whilst in hospital. If you decide to continue in the study you and your child will be asked to sign an updated consent/assent form.

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

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

15. What if there is a problem?

Complaints

If you have any cause to complain about any aspect of the way in which you or your child has been approached or treated during the course of this

study, the normal National Health Service complaints mechanisms are available to you and are not compromised in any way because you have taken part in a research study. If you have any complaints or concerns please contact either the project co-ordinator:

Name: Rachel Harrison
Designation: Research Nurse
Hospital/Department: Sheffield Children's Hospital, Western Bank, Sheffield, S10 2TH
Tel: 0114 2717120
Email: Rachel.C-Harrison@sch.nhs.uk

Otherwise you can use the normal hospital complaints procedure and contact the following person:

Mrs Linda Towers
Patient Advice & Liaison Co-ordinator
Sheffield Children's NHS Foundation Trust
Tel: 0114 271 7594
Email: Linda.Towers@sch.nhs.uk

Harm

If your child is harmed by taking part in this research project, there are no special compensation arrangements. If your child is harmed due to someone else's fault, then you may have grounds for a legal action – but you may have to pay for it.

16. Will 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 your child cannot be recognised from it. Once the study is complete all information including questionnaires and blood samples will be kept for five years.

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

Access to data will be restricted to members of the study team, the study sponsor and Sheffield Children's Hospital Research and Development department. Monitoring and audit may be carried out by the relevant authorities.

We will also ask for permission to 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.

As study visits may take place at your child's school, the teachers may know that your child is

taking part. They will not have access to any of the study data collected.

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

All samples collected will be anonymised with a study number and will be stored in a freezer in the Clinical Research Facility (CRF) at Sheffield Children's Hospital until they are ready to be analysed in the laboratory. We will ask your permission to use any left over samples for future research and this will only be after further ethical approval has been given. Access to the samples will be limited to the same people who have access to study data.

18. Will any genetic tests be done?

No.

19. What will happen to the results of the research study?

When the study has finished we will present our findings to other doctors, and we will put the results in medical magazines and websites that doctors 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, in September 2009, on www.sheffieldchildrenscrf.nhs.uk. The results will also be included as part of the chief investigator's educational qualification. They will be

anonymous, which means that your child will not be able to be identified from them.

20. Who is organising and funding the research?

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

21. Who has reviewed the study?

This study was given a favourable ethical opinion for conduct in the NHS by South Humber Local Research Ethics Committee. It has also been approved by the Research Department at this hospital.

22. How can we find out more about research?

The Clinical Research Facility at this hospital has an **Information for families** section on its website www.sheffieldchildrenscrf.nhs.uk or you could contact the hospital Clinical Research Facility:

Mrs Tracy N'Diaye
R&D Manager
Sheffield Children's NHS Foundation Trust
Tel: 0114 226 7904
Email: Tracy.NDiaye@sch.nhs.uk

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.

Sheffield Children's NHS
NHS Foundation Trust

PARTICIPANT INFORMATION SHEET FOR CHILDREN

To be shown and read by parent/carer if required

Study title
Acute bone response study.

1. What is research?
Research is a careful experiment to find out the answer to an important question

2. Why is this project being done?
Bigger bones are stronger bones, which mean they will break less easily. Not all children have big strong bones.We want to look at bones in normal children to find more out about what is normal.

Also this project will help us to learn more about research.

3. Why me?
You have been chosen because you are healthy and haven't broken any bones before and because you are a boy aged 9-12 years. We are asking up to 18 boys 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 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?

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. We will measure your height and weight.

A nurse will take 5 blood samples from a small plastic tube called a cannula that has been put in a vein in your arm or hand. We will take 3mls (a bit more than half a teaspoon) of blood for each sample.

A special camera that can tell how hot you are will take a picture of your legs before and after standing for 10 minutes. We will ask you to wear a pair of your own

shorts for this. We will ask you about the activities and exercise that you have done in the last week.

This will all happen in one visit which will last approximately two and a half hours at the Children's Hospital in Sheffield. Your Mum, Dad, or carer will be able to stay with you.

6. Will any of this hurt?

Before we take the blood samples we will put 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. The blood will be taken through a thin plastic tube which will be taped to your skin for about 2

hours. After the last blood sample has been taken, the tube will be removed.

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 sample. We will give you a voucher to get breakfast from the hospital dining room when the study visit is finished.

7. Will joining in help me?

The study will not help you, but we hope it will help us to understand more about bones in boys of your age.

8. What else might happen?

If we find out something that we think is important about you, we will talk to your mum, dad or carer and ask them if they want to come back and have you checked again at the hospital.

9. What happens when the research study stops?

We will collect all the information together and we will use it to help us design longer studies in the future with children who have problems with their bones.

10. What if something goes wrong?

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.

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

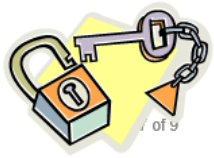
Just tell your mum, dad, carer, doctor or nurse at any time. They will not be cross with you. You will still have the same care whenever you need to come to hospital.

12. What if I wish to complain about the study?

If you want to complain you or your mum, dad or carer can talk to Rachel Harrison or Mrs Linda Towers at this hospital.

13. Will anyone else know I'm doing this?

The people in our research team will know you are taking part. No one else will know because we will not use your name or address. You will get a number which will be used instead. We will ask you if it is ok to let your GP know that you are taking part.



14. What happens to what the researchers find out?

When we collect your information we will make sure it is stored in a safe place and only the people doing the research study can look at it.

We will use the information to teach doctors about what happens to bones in the mornings, put it in medical magazines and on websites that doctors read.

A short summary will also be on the hospital's research website. No-one will know you were in the study.

15. Did anyone else check the study is OK to do?

This study has been checked by several people, to make sure it is alright.

Acute bone response to vibration
Participant Information Sheet control group
Version 1
02/02/2011

© Sheffield Children's NHS Foundation Trust

16. How can I find out more about this study?

Your mum, dad, carer or other grownup you trust may be able to answer your questions.

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

Sheffield Children's NHS
NHS Foundation Trust

PARENT/LEGAL GUARDIAN INFORMATION SHEET

Study title
Acute bone response study.

Part 1 – to give you first thoughts about the project

1. Invitation paragraph
You and your child are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully. 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.

Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you want your child to take part.

2. What is the purpose of the study?
Bone activity can be measured from a simple blood test. Studies in adults have shown that the levels of bone activity detected in our blood can change over a short period of time, especially in the mornings. We want to observe this change over two hours first thing in the morning. If we can understand better how to look at very short term changes it may help children with bone diseases better in the future.

This research is also helping one of the study team to learn more about carrying out research, and the information that is gathered will be used in her final assessment for a qualification (a PhD) from the University of Sheffield.

3. Why has my child been chosen?
Your child has been chosen because he is healthy and hasn't broken any bones before. Also because we are inviting 12 boys aged 9-12 years to take part in the study.

4. Does my child have to take part?
No. It is up to you and your child to decide whether or not to take part. You are both free to withdraw from the research at any time and without giving a reason. Your decisions about this will not influence how we look after your child whenever they need to come to hospital.

If you are happy to take part, and are satisfied with the explanations from the research team, you will be asked to sign a consent form. Your child will be asked to sign an assent form with you. You will be given a copy of the information sheet and the signed consent/assent forms to keep for your records.

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

We will measure your child's height and weight, and ask you or your child questions about the amount and type of exercise they have done in the 7 days prior to the study visit.

A thin plastic tube (cannula) will be inserted into a vein in your child's hand or arm. Five blood samples will be collected from the cannula over a two hours. We will use a special cream or spray to numb the area so that it doesn't hurt as much. 3mls of blood (slightly more than half a teaspoon) will be taken for each sample. Your child will need to be fasted for all the blood samples, this means nothing to eat or drink except water from midnight the night before the visit. This is really important, because eating and drinking have major effects on the things in the blood we want to measure.

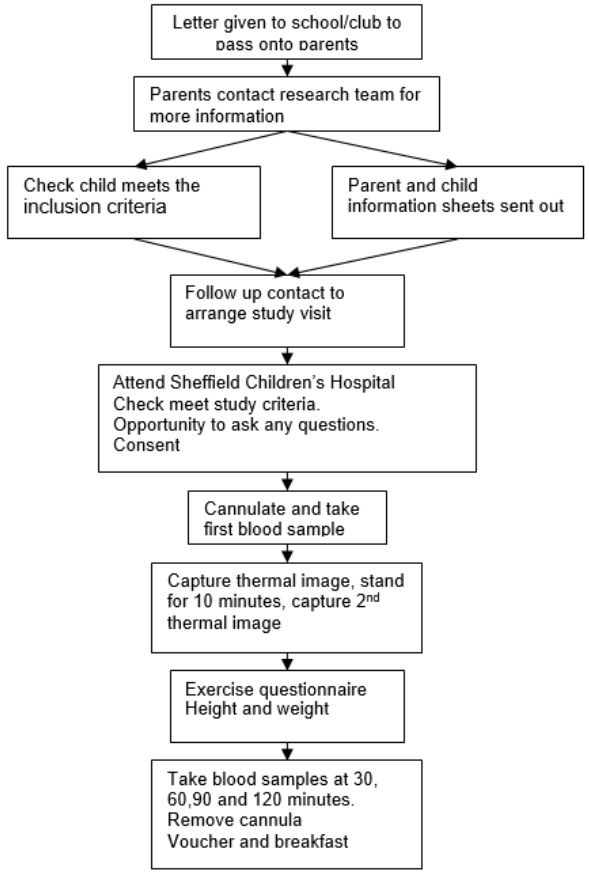
We would also like to take a thermal image using a special camera of your child's lower legs before and after standing for 10 minutes. All of this will happen at one study visit at Sheffield Children's Hospital

which is expected to last approximately two and a half hours.

All children who take part in the study will be offered a £10 voucher to spend in Meadowhall as a thank you for helping us with our research. We will also give each child a voucher for a breakfast up to the value of £2.50 to use in the hospital dining room at the end of the visit.

You will be encouraged to stay with your child throughout the study visit.

What will happen



6. What will we have to do?

Your child will need to have nothing to eat or drink except water from midnight of the day before your visit.

When you arrive we will ask your child to self-assess their stage of puberty using a pictorial scale.

Your child will need to bring a pair of shorts to wear for the thermal image.

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

There are no risks associated with using the thermal imaging camera. Your child will have a cannula placed into a vein for the taking of blood samples. This can cause discomfort to your child. To reduce this, a local anaesthetic in the form of a cream or a cold spray will be applied to the skin prior to insertion of the cannula.

8. What are the possible benefits of taking part?

We do not anticipate that this study will be of direct benefit to you or your child. We are hoping that the information we collect will help us to understand more about changes in the bone.

9. What happens when the research study stops?

We will collect all the information together and we will use this to design longer term studies in the use of vibrating platforms in children with bone health problems.

10. What if there is a problem?

Any complaint about the way you or your child have 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.

11. Will my child's taking part in the research project 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.

12. Contact for further information

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

Name: Rachel Harrison
Designation: Research Nurse
Hospital/Department: Sheffield Children's Hospital, Western Bank, Sheffield, S10 2TH
Tel: 0114 2717120
Email: Rachel.C-Harrison@sch.nhs.uk

This completes Part 1 of the Information Sheet.

If the information in Part 1 has interested you and you are considering participation, please continue to read the additional information in Part 2 before making any decision.

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

13. What if new information becomes available?

Sometimes during the course of a research project new information becomes available. Your child will be involved in the study for one visit only, so it is unlikely that any new information will become available during that time.

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

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

15. What if there is a problem?

Complaints

If you have any cause to complain about any aspect of the way in which you or your child has been approached or treated during the course of this study, the normal National Health Service complaints mechanisms are available to you and are not compromised in any way because you have taken part in a research study. If you have any complaints or concerns please contact either the project co-ordinator:

Name: Rachel Harrison
Designation: Research Nurse
Hospital/Department: Sheffield Children's Hospital, Western Bank, Sheffield, S10 2TH
Tel: 0114 2717120
Email: Rachel.C-Harrison@sch.nhs.uk

Otherwise you can use the normal hospital complaints procedure and contact the following person:

Mrs Linda Towers
Patient Advice & Liaison Co-ordinator
Sheffield Children's NHS Foundation Trust
Tel: 0114 271 7594
Email: Linda.Towers@sch.nhs.uk

Harm

If your child is harmed by taking part in this research project, there are no special compensation arrangements. If your child is harmed due to someone else's fault, then you may have grounds for a legal action – but you may have to pay for it.

16. Will 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 your child cannot be recognised

from it. Once the study is complete all information including questionnaires and blood samples will be kept for 5 years.

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

Access to data will be restricted to members of the research team, the study sponsor and Sheffield Children's Hospital Research and Development department. Monitoring and audit may be carried out by the relevant authorities.

We will also ask for permission to 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?

All samples collected will be anonymised with a study number and will be stored in a freezer in the Clinical Research Facility (CRF) at Sheffield Children's Hospital until they are ready to be analysed in the laboratory. We will ask your permission to use any left over samples for future research and this will only be after further ethical

approval has been given. Access to the samples will be limited to the same people who have access to study data.

18. Will any genetic tests be done?

No.

19. What will happen to the results of the research study?

When the study has finished we will present our findings to other doctors, and we will put the results in medical magazines and websites that doctors 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, in, September 2011, on www.sheffieldchildrenscrf.nhs.uk. The results will also be included as part of the chief investigator's educational qualification. They will be anonymous, which means that your child will not be able to be identified from them.

20. Who is organising and funding the research?

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

21. Who has reviewed the study?

This study was given a favourable ethical opinion for conduct in the NHS by South Humber Local

Research Ethics Committee. It has also been approved by the Research Department at this hospital.

22. How can we find out more about research?

The Clinical Research Facility at this hospital has an **Information for families** section on its website www.sheffieldchildrenscrf.nhs.uk or you could contact the hospital Clinical Research Facility:

Ms Tracy Elliot
R&D Manager
Sheffield Children's NHS Foundation Trust
Tel: 0114 226 7904
Email: Tracy.Elliot@sch.nhs.uk

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.

Patient study number:

PARENT/LEGAL GUARDIAN CONSENT FORM

Title of project: Acute bone response to vibration – phase 1

Names of researchers: Rachel Harrison, Professor N J Bishop

Please initial box

1. I confirm that I have read and understand the information sheet dated 5/08/09 (version 3) for the above study and have had the opportunity to ask questions. ☐
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 sections of any of my child's clinical record may be looked at by responsible individuals from Sheffield Children's NHS Trust 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 give permission for the research team to store any blood that is left over after the initial tests are done, for 5 years, and to use it in further bone related research. After that the sample will be destroyed.
   If you do not want to do this, please leave the box blank. ☐
5. I agree that if I withdraw from this study, any data or samples already collected can be used as described in the information sheet. ☐
6. I give permission for my child's GP to be informed of my child's participation in this study. ☐
7. I agree to my child taking part in the above study. ☐

Name of Parent/Guardian	Date	Signature
Name of Person taking consent (if different from researcher)	Date	Signature
Researcher	Date	Signature

1 copy for parent; 1 copy for researcher; 1 copy to be kept with hospital notes

Patient study number:

PARENT/LEGAL GUARDIAN CONSENT FORM

Title of project: Acute bone response to vibration – phase 2

Names of researchers: Rachel Harrison, Professor N J Bishop

Please initial box

1. I confirm that I have read and understand the information sheet dated 27/04/10 (version 4) for the above study and have had the opportunity to ask questions. ☐
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 sections of any of my child's clinical record may be looked at by responsible individuals from Sheffield Children's NHS Trust 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 give permission for the research team to store any blood that is left over after the initial tests are done, for 5 years, and to use it in further bone related research. After that the sample will be destroyed.
   If you do not want to do this, please leave the box blank. ☐
5. I agree that if I withdraw from this study, any data or samples already collected can be used as described in the information sheet. ☐
6. I give permission for my child's GP to be informed of my child's participation in this study. ☐
7. I agree to my child taking part in the above study. ☐

Name of Parent/Guardian	Date	Signature
Name of Person taking consent (if different from researcher)	Date	Signature
Researcher	Date	Signature

1 copy for parent; 1 copy for researcher; 1 copy to be kept with hospital notes

Patient study number:

PARENT/LEGAL GUARDIAN CONSENT FORM

Title of project: Acute bone response to vibration – control

Names of researchers: Rachel Harrison, Professor N J Bishop

Please initial box

1. I confirm that I have read and understand the information sheet dated 02/02/2011 (version 1) for the above study and have had the opportunity to ask questions. ☐
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 sections of any of my child's clinical record may be looked at by responsible individuals from Sheffield Children's NHS Trust 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 give permission for the research team to store any blood that is left over after the initial tests are done, for 5 years, and to use it in further bone related research. After that the sample will be destroyed.
   If you do not want to do this, please leave the box blank. ☐
5. I agree that if I withdraw from this study, any data or samples already collected can be used as described in the information sheet. ☐
6. I give permission for my child's GP to be informed of my child's participation in this study. ☐
7. I agree to my child taking part in the above study. ☐

Name of Parent/Guardian	Date	Signature
Name of Person taking consent (if different from researcher)	Date	Signature
Researcher	Date	Signature

1 copy for parent; 1 copy for researcher; 1 copy to be kept with hospital notes

ASSENT FORM FOR CHILDREN
(to be completed by the child and their parent/guardian)

Project title: Acute bone response to vibration

Child to circle all they agree with please:

Have you read (or had read to you) about this project?	Yes/No
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, please write your name and today's date

Your name _____

Date _____

Your parent or guardian must write their name here too if they are happy for you to do the project

Print Name _____

Sign _____

Date _____

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

Print Name _____

Sign _____

Date _____

Thank you for your help.

When completed, 1 for participant, 1 for researcher site file, 1 (original) to be kept in medical notes

Date

Dear Parent/Guardian

Here at the Sheffield Children's Hospital, we are investigating the response of bone to mechanical stimulation in healthy boys aged 9-12 years of age. The research is being led by Rachel Harrison and supervised by Professor Nick Bishop. This research will form part of a research training programme for Rachel Harrison that will lead to a qualification (a PhD) from the University of Sheffield. The study will involve one visit to Sheffield Children's Hospital that will take approximately one and a half hours. At this visit your child will be asked to;

- Stand on a vibrating platform for ten minutes.
- Have a blood sample taken before and twice after the vibration to measure biochemical changes in bone.
- Answer some questions about the amount and type of exercise he has taken part in, in the last seven days.
- Have 2 pictures taken of his lower legs using a special (thermography) camera to determine the change in surface body temperature as a result of standing on the vibrating platform.
- Have his height and weight measured.

Participation in this study is entirely voluntary. If you are interested in taking part, and would like more information please contact Rachel Harrison at Sheffield Children's Hospital on 0114 2717120. Alternatively complete the reply slip below, detach and send back to me in the attached stamped addressed envelope.

Yours Faithfully

Rachel Harrison
Research Nurse
rachel.c-harrison@sch.nhs.uk

I/We _____ (name) are interested in the acute bone response to mechanical stimulation study.
Please contact me/us via:
Telephone_____ Email _____

Or Post _____
_____

so that I/we can be given more information about the study.

Date

Dear Parent/Guardian

Here at the Sheffield Children's Hospital, we are investigating the response of bone to mechanical stimulation in healthy boys aged 9-12 years of age. The research is being led by Rachel Harrison and supervised by Professor Nick Bishop. This research will form part of a research training programme for Rachel Harrison that will lead to a qualification (a PhD) from the University of Sheffield. The study will take place over the course of one week at your child's school or in your home. Your child will be asked to:

- Stand on a vibrating platform for ten minutes on either 3 or 5 consecutive days.
- Have a blood sample taken before and after the vibration to measure biochemical changes in bone.
- Answer some questions about the amount and type of exercise he has taken part in, in the last seven days.
- Have his height and weight measured.

Participation in this study is entirely voluntary. If you are interested in taking part, and would like more information, please contact Rachel Harrison at Sheffield Children's Hospital on 0114 2717120. Alternatively complete the reply slip below, detach and send back to me in the attached stamped addressed envelope.

Yours Faithfully

Rachel Harrison
Research Nurse
rachel.c-harrison@sch.nhs.uk

I/We _____ (name) are interested in the acute bone response to mechanical stimulation study.
Please contact me/us via:
Telephone _____ Email _____

or Post _____
_____

so that I/we can be given more information about the study.

12.5. Appendix 5 Pubertal Assessment

Images taken from Illustrated Textbook of Paediatrics. T Lissauer and G Clayden, 1997, Mosby.

12.6. Appendix 6 Exercise Questionnaire

Exercise Questionnaire

Study No. _____ Age _____

Height _____ Weight _____

1. Considering a 7-day period (a week) how many times on the average do you do the following kinds of exercise **for more than 15 minutes** during your free time?

   - strenuous exercise (heart beats rapidly): number of times in a week _____
   - moderate exercise (not exhausting): number of times in a week _____
   - mild exercise (minimal effort): number of times in a week _____

2. Considering a 7-day period (a week) during your leisure time how often do you engage in any activity long enough to work up a sweat (heart beats rapidly)? (please tick)

   - often [ ]
   - sometimes [ ]
   - never or rarely [ ]

Strenuous exercise: running, jogging, hockey, football, soccer, squash, basketball, cross country skiing, judo, roller skating, vigorous swimming, long distance bicycling.

Moderate exercise: fast walking, baseball, tennis, easy bicycling, volleyball, badminton, easy swimming, alpine skiing, popular and folk dancing.

Mild exercise: yoga, archery, fishing from river bank, bowling, horseshoes, golf, snow mobiling, easy walking.

Acute bone response to vibration
Exercise questionnaire Godin G and Shephard RJ (1985)
Version 1
17/07/08

Page 1 of 1

12.7. Appendix 7 Study 2 Protocol (Final Version)

Sheffield Children's NHS
NHS Foundation Trust

FULL STUDY TITLE Acute response of bone to whole body vibration in boys who have previously fractured

SHORT STUDY TITLE Vibration in boys who have a history of fracture

STUDY NUMBER SCH/11/061

DATE AND VERSION NUMBER 05/12/2015 version 3

LAY SUMMARY

Fractures in children are common, accounting for 10% of attendances in Accident and Emergency Departments. Research into risk factors for fracture has found that children who have narrower bones and a low bone mass are more likely to fracture than children with larger bones. Studies in postmenopausal women, young women with low bone mass and children with disabling conditions such as cerebral palsy, have shown that up to 10 minutes a day stood on a vibrating platform can significantly increase bone mass.

However little is known of the immediate response of bone to whole body vibration (WBV). We previously investigated the acute bone response in healthy pre-pubertal boys to standing on one of two vibrating platforms for 10 minutes on 1, 3 or 5 consecutive days. This showed a platform dependent reduction in the bone resorption marker CTx pre to post vibration, with an increase in the bone formation marker P1NP over the 8 day study. We would like to look at the response of bone to WBV in pre-pubertal boys who have a history of fracture(s). We will compare this to our previous data to determine if bone in boys who have previously fractured responds in the same way as bone in boys who have not.

We plan to recruit 24 boys aged 7-13 years who have had a previous fracture to stand on one of two vibrating platforms for 10 minutes on 5 consecutive days. Before and after the period of vibration on days 1, 3, 5, and on days 8 and 12 we will take a blood sample to measure changes in the bone cells. The boys will be asked about the amount of exercise they have participated in over the previous seven days. The findings of this study will be used to direct further research.

Sheffield Children's Hospital Charity

The University Of Sheffield.

GENERAL INFORMATION

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GLOSSARY

A list of abbreviations and definitions

CRF – Clinical Research Facility
CTx – C-terminal cross-linked telopeptide of type 1 collagen
P1NP – Pro-collagen type 1 N-terminal propeptide
WBV – Whole body vibration
OPG – Osteoprotegerin
R&D – Research and Development

1.0 BACKGROUND

Fractures account for 10% of childhood Accident and Emergency attendances, approximately 100,000 fractures annually in the UK [1]. The incidence of childhood fractures has increased in the last 30 years [2] and 20-30% of children who fracture are likely to sustain a subsequent fracture(s) [3-5]. Fractures cause pain, may need surgical intervention, can result in further complications such as compartment syndrome, deformity and reduced mobility, lead to absence from school and subsequently time off work for parents [6, 7].

It has been found that children who have narrower bones and a low bone mass are more likely to fracture than children with larger bones [8, 9]. Bone responds to the loads placed upon it, with increased loads resulting in increased bone size and mass (which ultimately reduces risk of fracture). This response to loading has repeatedly been shown in studies investigating the effects of habitual or interventional exercise on bone in children [10]. In the last few years whole body vibration (WBV) delivered via vibrating platforms has been considered as a technique to load bone. It has been suggested as an alternative to traditional exercise or as an adjunct to therapy in populations that are unable to participate in high impact activities.

Animal studies investigating the effects of WBV have shown an increase in bone strength [11, 12], formation [13-18], volume [12, 19], and area [13, 20], demonstrating adaptation of bone tissue into a structurally stronger organ. Positive outcomes have also been observed over a period of months in postmenopausal women, young women with low bone mass and children with disabling conditions, showing that up to 10 minutes a day stood on a vibrating platform can significantly increase bone mass [21-24]. The clinical studies to date have only investigated the longer term effects of WBV and the intervention protocols have differed greatly in the vibration parameters used. Few studies have investigated paediatric populations and only 2 of these have looked at effects of WBV on bone [24, 25]. Hence further study is needed to fully understand the effect of WBV on bone and particularly the effects of WBV on the growing skeleton.

We want to determine the range and rate of acute bone responses in boys who have a history of fracture, to standing on a vibrating platform on 5 consecutive days for 10 minutes a day. More specifically we plan to focus on the changes in the bone turnover markers CTx (bone resorption), P1NP (bone formation), and in other bone cell derived factors osteoprotegerin (OPG), and sclerostin. Bicarbonate will also be measured to observe the effect of acid balance on bone resorption.

Previous work by our group on WBV in healthy pre-pubertal boys has shown a platform dependent response in the bone resorption marker CTx. Boys exposed to the low magnitude platform followed the diurnal trend of a decrease in the resorption marker whereas the high magnitude platform did not. Additionally over the 8 day study the formation marker P1NP increased in both groups. This demonstrates that even as little as 5 consecutive days of WBV can have an affect on bone turnover.

A variety of devices are now manufactured delivering either high or low magnitude WBV (above or below 1g) either in a side-alternating, vertical and/or horizontal direction. Very few studies have directly compared the different magnitude or methods of vibration. Our previous work compared two of the commercially available platforms and we would like to continue this to determine the suitability and effectiveness of the different modes of WBV.

We have shown that a study investigating the acute response of bone for up to 5 consecutive days of WBV is feasible. It is important that we now investigate a population that has the potential to benefit from the intervention. Assessing the difference in response of healthy boys and boys who have a history of fracture may help us better focus future research and clinical care. WBV could provide a non-pharmaceutical treatment for low bone mass and other musculoskeletal conditions.

2.0 STUDY OBJECTIVES AND PURPOSE

The primary aim of this study is to determine the acute response of bone to whole body vibration (WBV) in boys who have a history of having sustained at least one fracture. A second purpose is to determine if bone in this group of children responds to vibration in the short term in the same way as it does in boys who have not previously fractured. Data collected from this fracture cohort will be compared to data collected in our previous study investigating the bone response to WBV in apparently healthy pre-pubertal boys.

Measurement of the bone formation and resorption markers will allow us to identify if the response of bone to vibration in the short term is due to a change in bone formation or in bone resorption or both. Slight changes in either formation or resorption could cause disruption of the closely coupled bone turnover cycle resulting in a cycle that favours either bone gain or loss. Measurement of OPG and bicarbonate may help us to understand the mechanism by which acute change in bone resorption might be initiated. The osteocyte derived factor sclerostin is a candidate factor in transducing mechanical strain to cellular activity. If bone formation is increased, as occurred in the healthy boys over the previous 8 day study, we would expect to see sclerostin fall in response to the mechanical strain if current paradigms are correct.

This study will be undertaken as part of a research training programme (PhD).

The data will be used to direct further research into the use and applications of vibration therapy in children with fractures and low bone mass, including those with mild osteogenesis imperfecta.

3.0 STUDY DESIGN

This will be a randomised comparative study of vibration therapy in 24 pre-pubertal boys, aged 7-13 years who have previously sustained at least one fracture. Participants will be randomly assigned to stand on either a low magnitude vertical vibrating platform (Juvent) or a high magnitude side-alternating platform (Galileo), 12 boys on each platform in total, for 10 minutes on 5 consecutive days. Blood samples will be collected immediately pre- and 10 minutes post vibration on days 1, 3, and 5, with additional samples collected on day 8 and 12.

The period of vibration will be delivered in 4 repeated cycles of 2 minutes 30 seconds on and 30 seconds off the platform to a total of 10 minutes of vibration. Delivering vibration in this pattern will allow the participants to become accustomed to the platform in a more comfortable manner. Additionally it has been demonstrated that insertion of rest periods enhances the anabolic effect of loading on bone [26-28].

Due to the diurnal variation that is seen in bone turnover markers, blood samples will be collected in the mornings following an overnight fast. Participants will be asked to stand on the vibrating platforms in the morning each day so that the pre- and post samples can be collected on the days as scheduled. A cannula will be inserted into the arm or hand of the participants for the pre- and post blood sampling so that they are not exposed to multiple venepuncture on repeat sampling days.

As the platforms are transportable study visits will take place in the participants own home or school. Study visits involving vibration and blood sampling (days 1, 3 and 5) will take 45 minutes to complete, 20 minutes on days 2 and 4 when the visit includes only vibration, and 15 minutes on days 8 and 12 when blood samples only are collected. Each participant will complete 7 visits over 12 days (Appendix 1 details participant involvement in study from initial contact). Consent for the study may be obtained at an appointment prior to commencing the intervention. A validated questionnaire [29], regarding exercise and sport activity will be conducted to determine the amount of physical activity undertaken over the 7 days prior to day 1 of the study. Height and weight will be measured using calibrated weighing scales and a height stadiometer designed for portable use. Fracture history will be recorded to include date, site, and cause of injury, and subsequent treatment.

The researcher will be present at all study visits to instruct participants in the use of the devices and to ensure compliance to the protocol. As the platform design and mode of action (vertical or side-alternating)

are very different neither the researcher nor participant will be blinded to the intervention group. However this is not of concern as knowing which intervention group each boy has been assigned to will not alter the bone turnover markers. The laboratory staff who perform the serum assays will be blinded to intervention group.

A control group will not be recruited for this study as the objective is to detect a change from baseline in the bone markers of boys who have sustained a previous fracture. Data has already been collected from healthy pre-pubertal boys in an earlier study and this will be incorporated into the data analysis to compare any differences in the change from baseline between the two groups of boys

4.0 SELECTION OF PARTICIPANTS

In total we will recruit twenty-four pre-pubertal boys aged 7-13 years. Pubertal status will be determined during interview by self-assessment using a gender appropriate validated pictorial scale depicting the different stages of puberty [30].

Inclusion criteria
Caucasian
Aged 7-13yrs (pre-pubertal)
First language English
History of one or more fracture(s) – this does not include fractures resulting from severe trauma, based on a modified Landin's description [31]

Exclusion criteria
Pre-existing chronic illness
Known bone disease
Current or healing fracture
Recent (last 12 months) or current treatment likely to affect bone – this does not include inhaled or intermittent oral therapy with steroids for asthma
Balance problems
Continuing involvement in more than one other research study

Participants will be recruited from local schools and youth/activity clubs. The Head teacher/club leader will be approached for permission to display an advertisement for the study and for letters to be given to pupils and parents inviting them to participate in the study. Contact details for the study team will be included so that parents can contact the team if they wish for their child/children to participate. An invitation for participation in the study will also be sent via email to staff working at Sheffield Children's NHS Foundation Trust, the University of Sheffield, Sheffield Hallam University, and Sheffield Teaching Hospitals NHS Foundation Trust asking for interested persons to contact the research team for further information. Boys will also be recruited from Out-patient and Accident and Emergency (A&E) departments at Sheffield Children's Hospital. Boys aged 7-13 years who attended fracture or A&E clinics more than 6 months ago will be identified; their GP will be asked to forward on a letter to them inviting them to participate in the study. An advertisement for the study will be displayed in the Hospital and through the media including social media and on Sheffield Children's NHS Foundation Trust website.

Approximately 66 boys expressed an interest in our previous study of whom 24 were recruited over a ten month period. Interest/recruitment was slow to start with but picked up at the later stage of the study. The changes made to our recruitment strategy over the course of that study will be incorporated into this study.

5.0 PARTICIPANT RECRUITMENT

Screening for the inclusion criteria will be done by interview. Information sheets detailing the study procedures and contact details for the study team will be sent out to potential participants and their parents who have expressed an interest in the study prior to their attendance. Age appropriate information sheets will be included for the participants. A minimum of 24 hours will be given for

participants to consider their involvement. Parents and participants will have the opportunity to discuss the study further with the research team prior to giving consent.

Consent will be gained by a specialist research nurse/research officer with appropriate experience and training in Good Clinical Practice. Enrolment into the study will take in the Clinical Research Facility (CRF) at Sheffield Children's Hospital, the child's school or home where the study procedures will take place. After signing consent/assent pubertal stage will be self-assessed and the child allocated to the study arm, i.e. the type of platform. Vibration will occur at subsequent visits.

Neither the researchers nor participants will be blinded to the intervention groups as the devices are clearly different in appearance and mode of vibration delivery. Participants will be randomised to either the Juvent or Galileo platforms. Allocation will be by successive opening of sealed envelopes containing the individual randomisation codes. These will be prepared by CRF staff.

A member of the research team will be present at each study visit and will witness compliance to the intervention. Participants are free to withdraw from the study at any time, without giving a reason. As this study involves 7 study visits over a 12 day period and no further follow up, participant withdrawal is expected to be minimal. Study visits will be conducted in the participants' home or school for their convenience with the aim to reduce the number of withdrawals. Where consent was withdrawn in the previous study by two participants, this was due to cannulation difficulties. Replacement of withdrawn participants will only occur when no data (other than pubertal stage and age) has been collected.

6.0 DATA HANDLING AND RECORD KEEPING

Data will be entered in the Academic Unit of Child Health 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. Study documents (paper and electronic) will be retained in a secure location during and after the study has finished. All source documents will be retained for a period of 5 years following the end of the study. Where study related information is documented in the medical records – those records will be identified by a "Do not destroy before dd/mm/yyyy" label where date is 5 years after the last patient last visit.

7.0 ACCESS TO SOURCE DATA

The sponsor will permit monitoring and audits by the relevant authorities, including the Research Ethics Committee and the Medicines and Healthcare products Regulatory Agency (MHRA). The investigator will also allow monitoring and audits by these bodies and the sponsor, and they will provide direct access to source data and documents.

8.0 STATISTICAL ANALYSIS

Due to the number of time points that samples are being collected over the initial analysis will be to detect difference from baseline within and between vibration groups. A two sample T-test will be used to compare for differences between the groups and paired t-test to compare pre and post vibration values.

Due to recruiting a specific population to the study, boys aged 7-13 years, pre-pubertal, history of fracture, and no underlying health problems, we expect the groups to be of similar characteristics. However height, weight, exercise frequency and intensity, and fracture history will be recorded and accounted for in the analysis (ANCOVA) if these are found to be different between the two groups.

No sample size calculation has been performed for this study as it is a pilot study to determine the range of responses to the period of vibration. The focus of this study is on assessing the feasibility of the study and of gaining a preliminary estimate of the effect, rather than hypothesis testing. We propose to recruit 12 participants to each group as is recommended in current literature regarding sample sizes for pilot

studies [32, 33] and as agreed for the previous study investigating the acute response of bone to vibration in boys who do not have a history of fracture.

9.0 SAFETY ASSESSMENTS

There are no anticipated safety issues. The 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. The Galileo platform has a built in handrail.

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

The study will be monitored and audited in accordance with the Monitoring Standard Operating Procedures of the Sheffield Children's NHS Foundation Trust R&D department. 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 licensing bodies.

10.0 ETHICAL CONSIDERATIONS

The study will be conducted in compliance with a Research Ethics Committee favourable opinion, including any provisions for Site Specific Assessment, and local Research and Development approval. The study will also be conducted in accordance with the International Conference for Harmonisation of Good Clinical Practice (ICH GCP), and the Research Governance Framework for Health and Social Care (2nd Edition).

11.0 FINANCE AND INDEMNITY

Participants will not be paid for their involvement in the study but will be offered a £10 Meadowhall voucher for reimbursement of their time. If required, breakfast will be provided for the participants on the days that fasted blood samples are collected.

This is an NHS sponsored study. For NHS sponsored research HSG (96) 48 reference no. 2 refers. If there is negligent harm during the study when the NHS body owes a duty of care to the person harmed, NHS Indemnity will cover NHS staff, medical academic staff with honorary contracts and those conducting the study. NHS Indemnity does not offer no-fault compensation and is unable to agree in advance to pay compensation for non-negligent harm. Ex-gratia payments may be considered in the case of a claim.

12.0 REPORTING AND DISSEMINATION

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 and in the R&D newsletter. It is the intention that this work should form the basis of a PhD.

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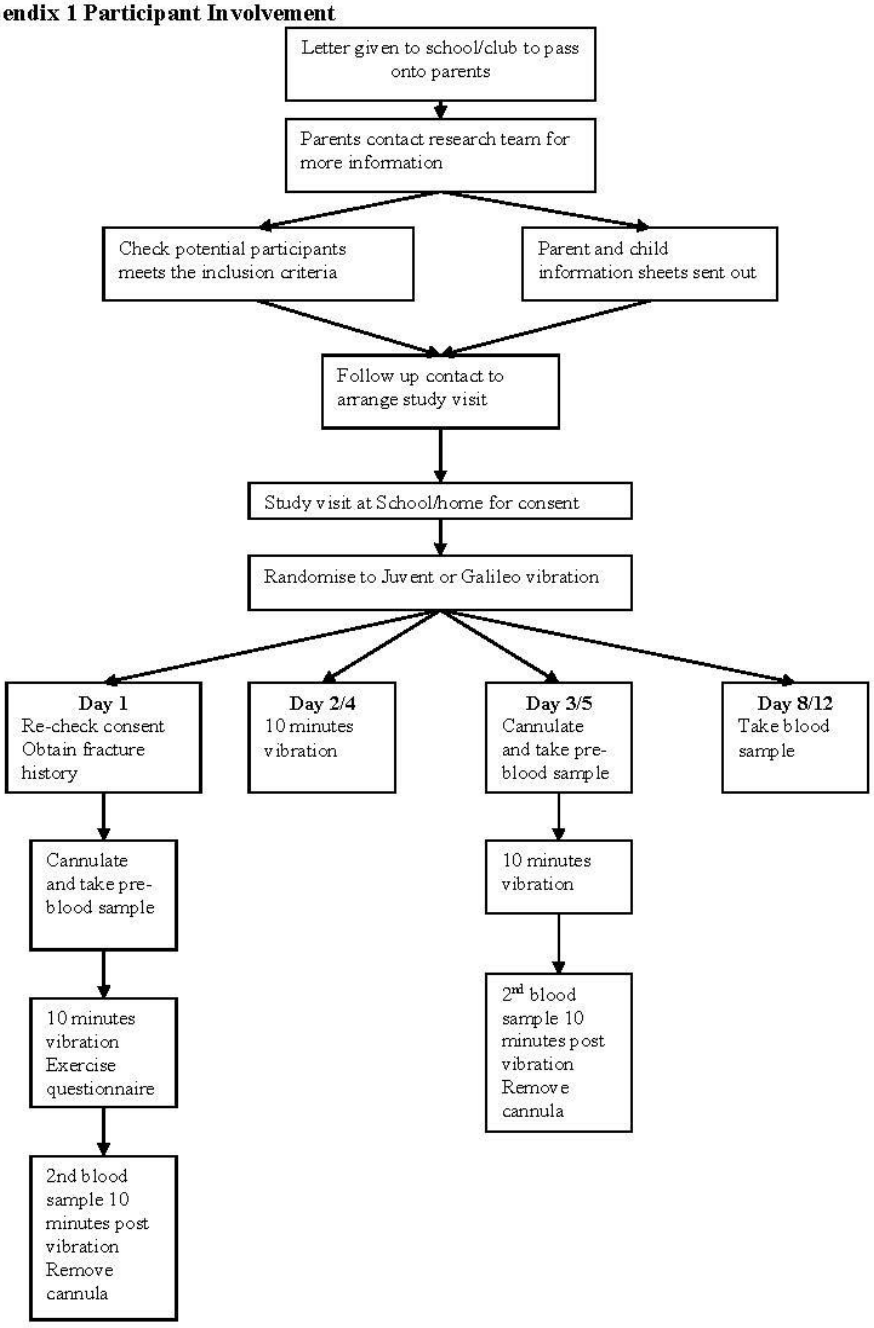
APPENDICES

Appendix 1 Participant Involvement



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PARTICIPANT INFORMATION SHEET FOR YOUNG PEOPLE

Study title
Vibration in boys who have a history of fracture

1. What is research?
Research is a careful experiment to find out the answer to an important question.

2. Why is this project being done?
Other studies have shown that standing on a vibrating platform for 10 minutes a day for at least 6 months can make bones stronger, which means they will break less easily. We want to see how your bones respond when you stand on a vibrating platform for 10 minutes each day for 5 days in a row. Also this project will help one of the study team learn how to do research.

3. Why me?
You have been chosen because you are healthy, have broken at least one bone before and because you are a boy aged 7-13 years. We are asking 24 children all together.

4. Do I have to take part?
No you do not! It is up to you. If you agree to take part, we would like you to write your name on a form. 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?
You will stand on a vibrating platform (that is about the size and shape of typical bathroom weighing scales) for 10 minutes each day for 5 days in a row.

A nurse will take a blood sample before and after you stand on the platform from a small plastic tube called a cannula that has been put in a vein in your arm or hand. We will take 2-4mls (around ½ - 1 teaspoon) of blood for each sample. This will not happen every day.

Days 1, 3, and 5:
Take blood sample, stand on vibrating platform for 10 minutes, take a second blood sample

Days 2 and 4:
Stand on vibrating platform for 10 minutes

Days 8 and 12:
Take a blood sample only

This will all take part in the morning in your home or in a private room in your school and will take 15-45 minutes each day. When the visits happen in your school we will ask you to come to school a bit earlier if you can and you might be a little bit late for your first lesson, but we will have asked your teacher if this is ok.

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.

On day 1 you will be asked questions about the exercise that you have done in the last week. 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. We will measure your height and weight.

6. Will any of this hurt?

Standing on the vibrating platform will not hurt. The people who make the platform have said that you may feel slightly sick or dizzy, get blisters on the bottom of your feet, or feel itchy in your legs, if you use the platform for a long time.

Before we take the blood samples we will put 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. The blood will be taken through a thin plastic tube

which will be taped to your skin for about 30 minutes. After the last blood sample has been taken, the tube will be removed.

7. Will joining in help me?

No, but we hope it will help us to understand what happens to bones when children stand on a vibrating platform.

8. What else might happen?

If we find out something that we think is important about you, we will talk to your mum, dad or carer and ask them if they want to come back and have you checked again at the hospital.

9. What happens when the research study stops?

We will collect all the information together and we will use it to help us design other studies with children who have problems with their bones.

10. What if something goes wrong?

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.

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

Just tell your mum, dad, carer, doctor or nurse at any time. They will not be cross with you. You will still have the same care whenever you need to come to hospital.

12. Will anyone else know I'm doing this?

The people in our research team will know you are taking part. Your teacher may know because this may all happen at school, but they will not see the information that we collect. We will also tell your GP. No one else will know because we will not use your name or address.

13. Did anyone else check the study is OK to do?

This study has been checked by several people, to make sure it is alright.

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

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PARENT/LEGAL GUARDIAN INFORMATION SHEET

Study title
Acute response of bone to whole body vibration in boys who have previously fractured

Part 1 – to give you first thoughts about the project

1. Invitation paragraph
You and your child are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully. 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.

Ask us if there is anything that is not clear or if you would like more information. Take

time to decide whether or not you want your child to take part.

2. What is the purpose of the study?
It has been found that children who have narrower bones and a low bone mass are more likely to break a bone than children with larger bones. Recent studies have shown that up to 10 minutes standing on a vibrating platform every day for at least 6 months can increase bone mass.

In a previous study we found that a measure of the amount of bone being formed, taken from a blood sample, was increased by 25%, after standing on a vibrating platform for 10 minutes on 5 consecutive days. This was in boys aged 9-12 years who had not broken a bone before. We want to know if the same will happen in boys who do have a history of having broken a bone. If we can understand very short term effects and responses to vibration it may help us focus treatment better in the future.

This research is also helping one of the study team to learn more about carrying out research, and the information that is gathered will be used in her final assessment for a qualification (a PhD) from the University of Sheffield.

3. Why has my child been chosen?
Your child has been chosen because he is healthy and has broken at least one bone before. Also

because we are inviting 24 boys aged 7-13 years to take part in the study.

4. Does my child have to take part?

No. It is up to you and your child to decide whether or not to take part. You are both free to withdraw from the research at any time and without giving a reason. Your decisions about this will not influence how we look after your child whenever they need to come to hospital.

If you are happy to take part, and are satisfied with the explanations from the research team, you will be asked to sign a consent form. Your child will be asked to sign an assent form with you. You will be given a copy of the information sheet and the signed consent/assent forms to keep for your records.

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

We will measure your child's height and weight, and ask you or your child questions about the amount and type of exercise they have done in the 7 days prior to the study. Also we would like to know what bone(s) your child has broken before, when, and how it happened. We plan for your child to stand on one of two types of platform so that we can compare any differences in how they work. One of the platforms mimics a walking motion, so when standing on the platform your child will feel a wobbling sensation in their legs. The vibration

motion in the second platform is undetectable, although you will hear a slight buzzing noise when it is switched on.

Blood samples will be taken from your child before the vibration begins and after the vibration has stopped, except on days 2 and 4. A thin plastic tube (cannula) will be inserted into a vein in your child's hand or arm and taped in place for the first sample to be taken and until after the second sample is taken. We will use a special cream or spray to numb the area where the cannula is to be inserted so that it doesn't hurt as much. 2 - 4mls of blood (approximately ½ - 1 teaspoon) will be taken for each sample. Your child will need to be fasted for all the blood samples, this means nothing to eat or drink except water from midnight the night before the visit. This is really important, because eating and drinking have major effects on the things in the blood we want to measure.

Day 1:	Take blood sample, stand on vibrating platform for 10 minutes, take a second blood sample.
Day 2:	Stand on vibrating platform for 10 minutes.
Day 3:	Take blood sample, stand on vibrating platform for 10 minutes, take a second blood sample.
Day 4:	Stand on vibrating platform for 10 minutes.

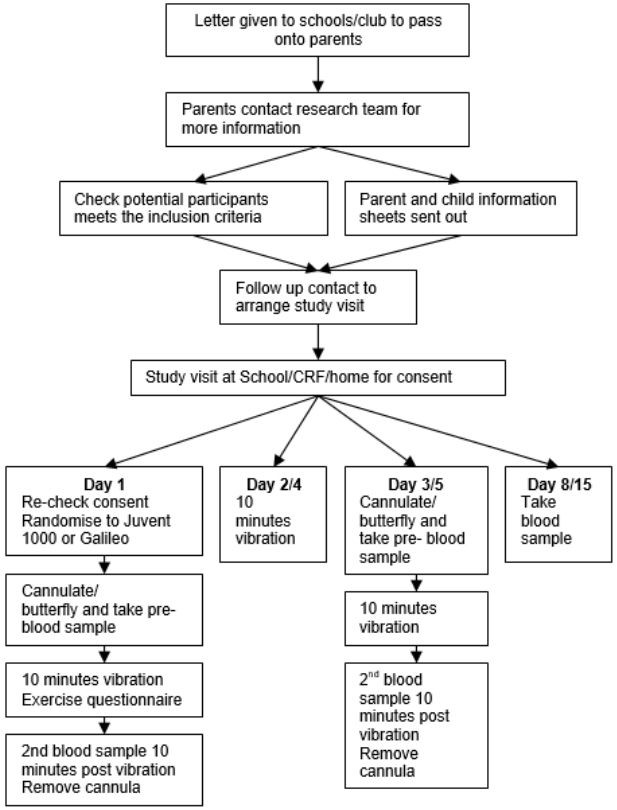
Day 5: Take blood sample, stand on vibrating platform for 10 minutes, take a second blood sample.

Days 8 and 15: Take a blood sample only.

Study visits may take place in your home or with the head teacher's permission may take place at your child's school, first thing in the morning so as to disrupt their school day as little as possible. The visits will take approximately 15-45 minutes to complete. A member of the study team will stay with your child for the whole visit, if you wish to stay with your child too you can. If you are not able to remain with your child and wish for us to arrange a chaperone we will do so.

All children who take part in the study will be offered a £10 voucher to spend in Meadowhall as a thank you for helping us with our research. We will provide a light breakfast on the days that blood samples are taken, or alternatively your child could bring in something to eat and drink from home.

What will happen



6. What will we have to do?

All study procedures will take place during the study visits. Other than nothing to eat or drink except water after midnight on the days that the blood samples are taken no preparation is required.

Before undertaking any study procedures we will ask your child to self-assess their stage of puberty using a pictorial scale.

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

No adverse events have been recorded in the use of vibrating platforms. However 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
d. quick temporary drop of blood pressure
e. drop in blood sugar level in diabetics due to high physical activity.

Your child will have a cannula placed into a vein for the taking of blood samples. This can cause discomfort to your child. To reduce this, a local anaesthetic in the form of a cream or a cold spray will be applied to the skin prior to insertion of the cannula.

8. What are the possible benefits of taking part?

We do not anticipate that this study will be of direct benefit to you or your child. We are hoping that this will help us to understand how vibration therapy can cause adaptations in bone.

9. What happens when the research study stops?

We collect all the information together and we will use this to design longer term studies in the use of vibration platforms in children with bone health problems.

10. What if there is a problem?

Any complaint about the way you or your child have 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.

11. Will my child's taking part in the research project 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.

12. Contact for further information

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

Name: Rachel Harrison
Designation: Research Nurse
Hospital/Department: Sheffield Children's Hospital, Western Bank, Sheffield, S10 2TH
Tel: 0114 2717417
Email: Rachel.C-Harrison@sch.nhs.uk

This completes Part 1 of the Information Sheet.

If the information in Part 1 has interested you and you are considering participation, please continue to read the additional information in Part 2 before making any decision.

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

13. What if new information becomes available?

Sometimes during the course of a research project, new information becomes available. As your child is involved for only 2 weeks this is unlikely. However if new information relating to vibration and bone health does become available someone from the research team will tell you and your child about it and discuss with you whether you want your child to continue in the study. If you change your mind this will not affect any care your child receives now or in the future whilst in hospital. If you decide to continue in the study you and your child will be asked to sign an updated consent/assent form.

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

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

15. What if there is a problem?

Complaints

If you have any cause to complain about any aspect of the way in which you or your child has been approached or treated during the course of this study, the normal National Health Service

complaints mechanisms are available to you and are not compromised in any way because you have taken part in a research study. If you have any complaints or concerns please contact either the project co-ordinator:

Name: Rachel Harrison
Designation: Research Nurse
Hospital/Department: Sheffield Children's Hospital, Western Bank, Sheffield, S10 2TH
Tel: 0114 271 7417
Email: Rachel.C-Harrison@sch.nhs.uk

Otherwise you can use the normal hospital complaints procedure and contact the following person:

Mrs Linda Towers
Patient Advice & Liaison Co-ordinator
Sheffield Children's NHS Foundation Trust
Tel: 0114 271 7594
Email: Linda.Towers@sch.nhs.uk

Harm

If your child is harmed by taking part in this research project, there are no special compensation arrangements. If your child is harmed due to someone else's fault, then you may have grounds for a legal action – but you may have to pay for it.

16. Will 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 your child cannot be recognised from it. Once the study is complete all information including questionnaires and blood samples will be kept for five years.

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

Access to data will be restricted to members of the study team, the study sponsor and Sheffield Children's Hospital Research and Development department. Monitoring and audit may be carried out by the relevant authorities.

We will also ask for permission to 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.

As study visits may take place at your child's school, the teachers may know that your child is

taking part. They will not have access to any of the study data collected.

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

All samples collected will be anonymised with a study number and will be stored in a freezer in the Clinical Research Facility (CRF) at Sheffield Children's Hospital until they are ready to be analysed in the laboratory. We will ask your permission to use any left over samples for future research and this will only be after further ethical approval has been given. Access to the samples will be limited to the same people who have access to study data.

18. Will any genetic tests be done?

No.

19. What will happen to the results of the research study?

When the study has finished we will present our findings to other doctors, and we will put the results in medical magazines and websites that doctors 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, in December 2016, on www.sheffieldchildrenscrf.nhs.uk. The results will also be included as part of the chief investigator's educational qualification. They will be

anonymous, which means that your child will not be able to be identified from them.

20. Who is organising and funding the research?

The research is being organised by Sheffield Children's NHS Foundation Trust and paid for by Orthopaedic Research UK.

21. Who has reviewed the study?

This study was given a favourable ethical opinion for conduct in the NHS by Yorkshire and the Humber – Leeds West Research Ethics Committee. It has also been approved by the Research Department at this hospital.

22. How can we find out more about research?

The Clinical Research Facility at this hospital has an **Information for families** section on its website www.sheffieldchildrenscrf.nhs.uk or you could contact the hospital Clinical Research Facility:

Mrs Wendy Swann
R&D Manager
Sheffield Children's NHS Foundation Trust
Tel: 0114 226 7904
Email: wendy.swann@sch.nhs.uk

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.

Patient study number:

PARENT/LEGAL GUARDIAN CONSENT FORM

Title of project: Vibration in boys who have a history of fracture

Names of researchers: Rachel Harrison, Professor N J Bishop

Please initial box

1. I confirm that I have read and understand the information sheet dated 05/12/2015 (version 2) for the above study and have had the opportunity to ask questions. ☐
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 sections of any of my child's clinical record may be looked at by responsible individuals from Sheffield Children's NHS Trust 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 give permission for the research team to store any blood that is left over after the initial tests are done, for 5 years, and to use it in further bone related research. After that the sample will be destroyed.
   If you do not want to do this, please leave the box blank. ☐
5. I agree that if I withdraw from this study, any data or samples already collected can be used as described in the information sheet. ☐
6. I give permission for my child's GP to be informed of my child's participation in this study. ☐
7. I agree to my child taking part in the above study. ☐

Name of Parent/Guardian	Date	Signature
Name of Person taking consent (if different from researcher)	Date	Signature
Researcher	Date	Signature

1 copy for parent; 1 copy for researcher; 1 copy to be kept with hospital notes

ASSENT FORM FOR CHILDREN
(to be completed by the child and their parent/guardian)

Project title: Vibration in boys who have a history of fracture

Child to circle all they agree with please:

Have you read (or had read to you) about this project?	Yes/No
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, please write your name and today's date.

Your name _____

Date _____

Your parent or guardian must write their name here too if they are happy for you to do the project.

Print Name _____

Sign _____

Date _____

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

Print Name _____

Sign _____

Date _____

Thank you for your help.

When completed, 1 for participant, 1 for researcher site file, 1 (original) to be kept in medical notes

Dear Parent/Guardian

Here at the Sheffield Children's Hospital, we are investigating the response of bone to whole body vibration in boys aged 7-13 years who have previously broken a bone. The research is being led by Rachel Harrison and supervised by Professor Nick Bishop. This research will form part of a research training programme for Rachel Harrison which will lead to a qualification (a PhD) from the University of Sheffield. The study will take place over the course of two weeks at your child's school or in your home. Your child will be asked to:

- Stand on a vibrating platform for ten minutes on 5 consecutive days.
- Have a blood sample taken before and after the vibration to measure biochemical changes in bone.
- Answer some questions about the amount and type of exercise he has taken part in, in the last seven days.
- Tell the researcher about the number of broken bones he has had, when and how they happened.
- Have his height and weight measured.

Participation in this study is entirely voluntary. If you are interested in taking part, and would like more information, please contact Rachel Harrison at Sheffield Children's Hospital on 0114 2717417. Alternatively complete the reply slip below, detach and send back to me in the attached stamped addressed envelope.

Yours Faithfully

Rachel Harrison
Research Nurse
rachel.c-harrison@sch.nhs.uk

I/We _____ (name) are interested in the acute bone response to mechanical stimulation study.
Please contact me/us via:
Telephone _____ Email _____

or Post _____

_____

so that I/we can be given more information about the study.

12.9. Appendix 9 Trauma Levels

Categories of Landin's Modified Trauma Levels

Descriptives indicating slight trauma

- Falling to the ground from <0.5m (standing height)
- Falling to a resilient surface (rubber or sand) from 0.5 – 3m
- Falling from a bed or a cot
- Playing injuries including playground scuffles
- Low-energy sporting injuries such as ball sports, wrestling, judo, karate, and gymnastics

Descriptives including moderate trauma

- Falling to concrete or other non-resilient surface from 0.5 – 3m
- Falling from a bunkbed
- Baby being dropped to the floor by an adult
- Falling downstairs
- Falling from a bicycle or horseback
- Falling from swings or slides or similar playground equipment
- Child being hit by a bicycle
- Falls while moving on skateboards, skis, rollerblades, or skates

Descriptives indicating severe trauma

- Falling from a height exceeding 3m
- All traffic accidents not already mentioned
- Being hit by a moving heavy object

Clark EM, Ness AR, Tobias JH (2008) Bone fragility contributes to the risk of fracture in children, even after moderate and severe trauma. JBMR 23; 2, 173-179

12.10. Appendix 10 Study 1 P1NP, OCN, CTX, OPG and Sclerostin Values by participant

Table 18 Phase 1 and 2 WBV groups P1NP and osteocalcin by participant

			P1NP ng/ml								Osteocalcin ng/ml	
	Study no	**Platform**	**Day 1 Pre**	**10 mins**	**60 mins**	**Day 3 Pre**	**Day 3 Post**	**Day 5 Pre**	**Day 5 Post**	**Day 8**	**Day 1**	**Day 8**
I day WBV	3	J	655.3	511.6	551.3	NA	NA	NA	NA	NA	NA	NA
	4	G	642.5	856.0	826.5	NA	NA	NA	NA	NA	NA	NA
	6	G	588.2	554.6	552.6	NA	NA	NA	NA	NA	NA	NA
	7	G	634.4	671.9	593.6	NA	NA	NA	NA	NA	NA	NA
	9	J	299.1	282.6	262.5	NA	NA	NA	NA	NA	NA	NA
	10	J	1,356.8	1,228.8	1,159.6	NA	NA	NA	NA	NA	NA	NA
	11	G	1,179.6	1,115.6	1,153.6	NA	NA	NA	NA	NA	NA	NA
	13	J	241.8	255.4	242.4	NA	NA	NA	NA	NA	NA	NA
	14	J	654.3	565.7	557.3	NA	NA	NA	NA	NA	NA	NA
	15	G	1,106.0	784.2	941.9	NA	NA	NA	NA	NA	NA	NA
	16	J	396.1	433.9	479.2	NA	NA	NA	NA	NA	NA	NA
3 days WBV	201	J	539.7	443.2	NA	535.1	476.8	473.3	NA	NA	NA	NA
	202	G	442.1	452.9	NA	591.9	-	494.4	NA	NA	NA	NA
	206	G	968.2	860.3	NA	885.2	657.8	944.8	NA	NA	NA	NA
	210	J	985.5	871.3	NA	873.2	802.7	953.3	NA	NA	NA	NA
	211	G	824.4	918.1	NA	772.5	681.7	989.8	NA	NA	NA	NA
	215	J	922.6	719.1	NA	783.7	622.0	632.3	NA	NA	NA	NA
	216	G	942.9	902.2	NA	759.9	771.8	819.1	NA	NA	NA	NA
	217	J	701.1	-	NA	-	-	-	NA	NA	NA	NA
	219	J	666.0	487.8	NA	552.2	489.5	642.6	NA	NA	NA	NA
	220	J	617.5	536.7	NA	556.6	487.9	-	NA	NA	NA	NA
	222	G	691.3	344.5	NA	564.0	515.4	664.7	NA	NA	NA	NA
	225	G	1,030.0	635.8	NA	830.4	657.0	641.4	NA	NA	NA	NA
	226	J	606.0	672.3	NA	833.0	677.1	835.2	NA	NA	NA	NA
5 Days WBV	203	G	846.2	626.2	NA	971.0	783.8	1,008.0	888.0	1,102.0	139.30	113.60
	205	J	652.0	584.8	NA	533.2	431.3	507.8	384.5	738.6	83.20	76.30

207	G	753.9	678.2	NA	638.7	540.5	691.9	670.5	872.7	70.40	72.80
208	J	416.8	455.4	NA	587.2	400.0	583.2	423.0	613.0	78.90	83.00
213	G	621.9	622.1	NA	679.8	534.0	621.0	562.2	705.2	-	77.30
214	J	1,027.0	913.5	NA	856.3	680.3	1,049.0	925.8	1,050.0	112.00	141.20
218	G	700.0	639.3	NA	678.0	-	733.9	603.3	742.4	98.80	90.70
221	G	616.6	785.2	NA	663.1	564.8	-	-	817.5	75.50	87.10
227	J	893.2	805.1	NA	1,063.0	766.2	779.3	735.5	1,068.0	87.20	101.50
228	J	456.9	469.7	NA	562.1	497.6	643.9	575.6	590.2	70.90	87.10
229	G	917.5	-	NA	733.6	761.2	-	-	1,534.4	57.40	120.60

G - Galileo, J - Juvent, NA - not applicable, - missing sample

Table 19 Phase 3 control group P1NP by participant

	P1NP ng/ml				
Study no	**0 mins[a]**	**30 mins[b]**	**60 mins**	**90 mins[c]**	**120 mins**
301	532.9	495.1	436.3	414.8	414.2
302	799.9	703.6	638.5	590.3	657.4
303	726.0	599.7	440.1	497.4	507.0
305	1,128.0	930.1	1,022.0	1,135.0	1,129.0
306	714.0	671.6	595.8	563.8	-
307	437.7	506.3	379.4	434.2	434.3
309	665.1	641.5	603.7	574.3	611.4
310	521.5	462.8	448.9	470.1	469.1
312	673.4	710.5	898.2	676.8	633.7
314	659.7	644.7	661.7	705.5	726.0
316	723.7	682.8	696.5	591.2	642.8
317	387.1	341.3	398.3	397.1	372.1
318	664.2	560.0	533.5	483.8	564.2
319	879.7	704.3	763.2	807.2	741.1

[a]- corresponds to pre WBV, [b] - corresponds to 10 mins post WBV, [c] - corresponds to 60 mins post WBV

Table 20 Phase 1 and 2 WBV groups CTX by participant

			CTX ng/ml							
	Study no	**Platform**	**Day 1 Pre**	**10 mins**	**60 mins**	**Day 3 Pre**	**Day 3 Post**	**Day 5 Pre**	**Day 5 Post**	**Day 8**
I day WBV	3	J	1.71	1.49	1.39	NA	NA	NA	NA	NA
	4	G	2.78	3.07	2.78	NA	NA	NA	NA	NA
	6	G	1.80	1.87	1.77	NA	NA	NA	NA	NA
	7	G	1.87	1.86	1.84	NA	NA	NA	NA	NA
	9	J	0.98	0.92	0.95	NA	NA	NA	NA	NA
	10	J	2.84	2.60	2.60	NA	NA	NA	NA	NA
	11	G	3.18	2.83	3.04	NA	NA	NA	NA	NA
	13	J	1.03	0.94	0.84	NA	NA	NA	NA	NA
	14	J	2.26	2.11	1.88	NA	NA	NA	NA	NA
	15	G	2.39	2.07	2.26	NA	NA	NA	NA	NA
	16	J	1.44	1.37	1.57	NA	NA	NA	NA	NA
3 days WBV	201	J	1.73	1.60	NA	1.37	1.40	1.23	NA	NA
	202	G	1.42	1.38	NA	1.73	-	1.67	NA	NA
	206	G	2.17	2.21	NA	2.32	2.18	2.50	NA	NA
	210	J	2.59	2.05	NA	2.70	2.70	2.81	NA	NA
	211	G	1.98	2.07	NA	1.87	1.81	1.70	NA	NA
	215	J	2.41	2.36	NA	2.39	2.22	2.21	NA	NA
	216	G	1.95	1.89	NA	1.88	2.00	1.81	NA	NA
	217	J	2.09	-	NA	-	-	-	NA	NA
	219	J	1.38	1.30	NA	1.26	1.24	0.82	NA	NA
	220	J	1.52	1.43	NA	1.45	1.47	-	NA	NA
	222	G	1.36	0.98	NA	1.35	1.31	1.30	NA	NA
	225	G	2.39	1.74	NA	2.33	1.94	1.84	NA	NA
	226	J	1.83	1.42	NA	1.90	1.69	2.02	NA	NA
5 Days WBV	203	G	1.98	1.90	NA	2.15	2.24	2.21	2.46	2.20
	205	J	1.79	1.46	NA	1.62	1.47	1.47	1.35	1.78
	207	G	2.07	2.01	NA	1.87	1.90	1.84	1.89	2.24
	208	J	1.59	1.62	NA	2.06	1.80	1.91	1.76	1.84
	213	G	1.58	1.55	NA	1.96	1.80	1.94	1.98	1.56

214	J	2.70	2.36	NA	2.68	2.39	3.06	2.82	3.19
218	G	1.68	1.67	NA	1.86	-	1.97	1.69	1.92
221	G	1.46	1.72	NA	1.62	1.54	-	-	1.45
227	J	2.08	1.91	NA	1.84	1.55	1.64	1.55	2.14
228	J	1.38	1.37	NA	1.51	1.45	1.49	1.33	1.62
229	G	1.91	-	NA	1.97	1.89	-	-	2.57

G - Galileo, J - Juvent, NA - not applicable, - missing sample

Table 21 Phase 3 controls CTX by participant

	CTX ng/ml				
Study no	**0 mins[a]**	**30 mins[b]**	**60 mins**	**90 mins[c]**	**120 mins**
301	1.77	1.75	1.60	1.59	1.59
302	2.18	1.93	1.69	1.61	1.68
303	2.12	1.95	1.85	2.05	2.13
305	2.84	2.60	2.40	2.56	2.49
306	1.57	1.09	1.02	1.22	-
307	1.35	1.32	1.17	1.46	1.56
309	1.88	1.97	1.81	1.76	1.92
310	1.60	1.61	1.56	1.60	1.62
312	2.36	2.39	2.73	2.47	2.10
314	1.73	1.81	1.79	1.87	1.79
316	2.13	2.24	2.25	1.92	1.71
317	1.41	1.11	1.20	1.45	1.54
318	1.75	1.52	1.50	1.31	1.36
319	1.99	1.85	1.69	1.81	1.74

[a]- corresponds to pre WBV, [b] - corresponds to 10 mins post WBV, [c] - corresponds to 60 mins post WBV

Table 22 Phase 1 and 2 WBV groups OPG by participant

			OPG pmol/L					
	Study no	**Platform**	**Day 1 Pre**	**10 mins**	**60 mins**	**Day 3 Pre**	**Day 5 Pre**	**Day 8**
I Day WBV	3	J	4.05	3.95	4.29	NA	NA	NA
	4	G	2.97	2.96	2.97	NA	NA	NA
	6	G	3.20	3.01	2.50	NA	NA	NA
	7	G	3.04	3.67	3.31	NA	NA	NA
	9	J	3.73	3.75	4.01	NA	NA	NA
	10	J	3.33	3.55	3.24	NA	NA	NA
	11	G	3.49	3.32	3.18	NA	NA	NA
	13	J	3.96	3.89	3.51	NA	NA	NA
	14	J	3.75	3.10	3.60	NA	NA	NA
	15	G	3.43	2.95	3.04	NA	NA	NA
	16	J	3.86	3.88	3.86	NA	NA	NA
3 Days WBV	201	J	3.07	NA	NA	3.20	3.07	NA
	202	G	2.20	NA	NA	2.16	1.92	NA
	206	G	4.15	NA	NA	3.07	3.61	NA
	210	J	3.36	NA	NA	3.65	3.79	NA
	211	G	4.46	NA	NA	4.13	4.40	NA
	215	J	4.55	NA	NA	3.95	3.98	NA
	216	G	3.24	NA	NA	2.88	3.05	NA
	217	J	-	NA	NA	-	-	NA
	219	J	4.77	NA	NA	4.47	4.12	NA
	220	J	-	NA	NA	-	-	NA
	222	G	6.55	NA	NA	7.01	6.96	NA
	225	G	4.31	NA	NA	3.76	3.41	NA
	226	J	3.22	NA	NA	3.29	3.51	NA
5 Days WBV	203	G	3.13	NA	NA	3.46	3.52	3.84
	205	J	3.65	NA	NA	2.71	2.83	3.22
	207	G	4.11	NA	NA	4.19	4.48	4.24
	208	J	3.11	NA	NA	3.78	3.37	3.55
	213	G	3.25	NA	NA	3.13	2.99	3.03
	214	J	3.24	NA	NA	3.39	3.72	3.88
	218	G	3.11	NA	NA	2.95	3.26	3.03
	221	G	3.64	NA	NA	3.73	-	4.53
	227	J	2.98	NA	NA	3.22	3.14	2.87
	228	J	4.08	NA	NA	5.22	4.30	4.76
	229	G	4.73	NA	NA	3.95	-	4.89
Controls*	301	C	3.15	2.69	3.54	NA	NA	NA
	302	C	2.76	2.44	2.55	NA	NA	NA
	303	C	3.15	2.77	2.97	NA	NA	NA
	305	C	2.97	2.68	2.99	NA	NA	NA
	306	C	4.12	3.38	3.00	NA	NA	NA
	307	C	2.97	2.26	2.66	NA	NA	NA
	309	C	2.59	2.72	2.88	NA	NA	NA
	310	C	3.05	2.80	2.74	NA	NA	NA
	312	C	2.48	2.50	2.57	NA	NA	NA
	314	C	2.68	2.78	3.13	NA	NA	NA
	316	C	3.68	3.26	2.72	NA	NA	NA
	317	C	3.62	3.29	3.36	NA	NA	NA
	318	C	2.98	3.66	2.59	NA	NA	NA
	319	C	2.71	3.02	3.08	NA	NA	NA

G - Galileo, J - Juvent, C – control, NA - not applicable, - missing sample, * - samples at 0,30 and 90 minutes on day 1

Table 23 Phase 1 and 2 WBV groups sclerostin by participant

			Sclerostin pmol/L					
	Study no	**Platform**	**Day 1 Pre**	**10 mins**	**60 mins**	**Day 3 Pre**	**Day 5 Pre**	**Day 8**
I Day WBV	3	J	33.25	34.32	39.11	NA	NA	NA
	4	G	28.36	36.29	40.74	NA	NA	NA
	6	G	30.42	27.83	27.86	NA	NA	NA
	7	G	44.08	49.05	42.27	NA	NA	NA
	9	J	16.53	15.72	15.11	NA	NA	NA
	10	J	31.01	25.96	29.51	NA	NA	NA
	11	G	27.23	26.12	29.85	NA	NA	NA
	13	J	21.14	22.82	21.81	NA	NA	NA
	14	J	21.65	19.13	21.78	NA	NA	NA
	15	G	19.27	17.90	19.87	NA	NA	NA
	16	J	32.80	28.70	32.35	NA	NA	NA
3 Days WBV	201	J	18.69	NA	NA	21.46	22.39	NA
	202	G	20.42	NA	NA	24.21	18.38	NA
	206	G	28.49	NA	NA	25.76	24.54	NA
	210	J	25.34	NA	NA	24.49	32.97	NA
	211	G	38.19	NA	NA	42.27	43.46	NA
	215	J	38.47	NA	NA	42.32	37.45	NA
	216	G	32.86	NA	NA	31.46	31.66	NA
	217	J	-	NA	NA	-	-	NA
	219	J	28.51	NA	NA	23.53	27.40	NA
	220	J	-	NA	NA	-	-	NA
	222	G	32.15	NA	NA	36.92	31.65	NA
	225	G	31.76	NA	NA	29.40	27.60	NA
	226	J	30.39	NA	NA	33.63	34.14	NA
5 Days WBV	203	G	28.74	NA	NA	27.00	29.87	22.92
	205	J	24.16	NA	NA	23.90	22.73	21.86
	203	G	28.74	NA	NA	27.00	29.87	22.92
	205	J	24.16	NA	NA	23.90	22.73	21.86
	213	G	26.95	NA	NA	30.99	26.54	24.77
	214	J	23.49	NA	NA	22.38	26.89	29.93
	218	G	30.45	NA	NA	32.86	30.76	27.73
	221	G	24.01	NA	NA	24.58	-	26.86
	227	J	23.83	NA	NA	22.30	19.19	22.00
	228	J	18.39	NA	NA	24.39	27.73	24.62
	229	G	31.02	NA	NA	27.91	-	43.56
Controls*	301	C	25.11	30.20	25.86	NA	NA	NA
	302	C	29.20	25.59	26.62	NA	NA	NA
	303	C	30.37	29.71	25.96	NA	NA	NA
	305	C	32.08	26.66	25.61	NA	NA	NA
	306	C	18.34	16.51	15.90	NA	NA	NA
	307	C	13.68	21.98	15.04	NA	NA	NA
	309	C	27.60	25.27	24.61	NA	NA	NA
	310	C	19.95	20.77	24.21	NA	NA	NA
	312	C	34.15	34.76	34.93	NA	NA	NA
	314	C	15.20	17.88	18.48	NA	NA	NA
	316	C	17.42	17.44	17.31	NA	NA	NA
	317	C	34.64	33.03	32.74	NA	NA	NA
	318	C	21.37	16.67	15.75	NA	NA	NA
	319	C	20.42	24.03	20.89	NA	NA	NA

G - Galileo, J - Juvent, C – control, NA - not applicable, - missing sample, * - samples at 0, 30 and 90 minutes on day 1

12.11. Appendix 11 Study 2 P1NP, CTX, OPG and Sclerostin Values by Participant

Table 24 No fracture and fracture groups P1NP by participant

			P1NP ng/ml							
	Study no	**Platform**	**Day 1 pre**	**Day 1 post**	**Day 3 pre**	**Day 3 post**	**Day 5 pre**	**Day 5 post**	**Day 8**	**Day 12**
No fracture	203	G	846.2	626.2	971.0	783.8	1008.0	888.0	1102.0	NA
	205	J	652.0	584.8	533.2	431.3	507.8	384.5	738.6	NA
	207	G	753.9	678.2	638.7	540.5	691.9	670.5	872.7	NA
	208	J	416.8	455.4	587.2	400.0	583.2	423.0	613.0	NA
	213	G	621.9	622.1	679.8	534.0	621.0	562.2	705.2	NA
	214	J	1027.0	913.5	856.3	680.3	1049.0	925.8	1050.0	NA
	217	J	701.1	-	-	-	-	-		NA
	218	G	700.0	639.3	678.0	-	733.9	603.3	742.4	NA
	221	G	616.6	785.2	663.1	564.8	-	-	817.5	NA
	227	J	893.2	805.1	1063.0	766.2	779.3	735.5	1068.0	NA
	228	J	456.9	469.7	562.1	497.6	643.9	575.6	590.2	NA
	229	G	917.5	-	733.6	761.2	-	-	1534.4	NA
Fracture	401	J	574.3	531.8	557.2	520.0	546.6	536.3	539.6	470.5
	402	G	534.9	429.2	449.0	378.6	559.9	444.4	639.7	503.4
	403	G	475.6	465.6	-	-	637.3	529.8	559.9	608.0
	404	G	725.8	798.7	738.6	620.0	722.7	-	-	807.1
	405	J	444.4	343.6	409.8	330.0	450.2	326.3	428.3	399.8
	406	G	558.3	534.2	-	-	-	-	-	-
	408	G	554.1	561.8	645.0	576.3	535.3	505.9	591.0	565.2
	409	J	447.8	417.6	543.9	407.3	-	-	421.7	443.3
	410	G	577.0	541.7	608.4	622.3	555.0	587.0	652.5	603.7
	411	J	697.0	604.9	726.7	603.8	714.2	517.0	-	819.4
	412	G	576.2	460.6	537.4	445.6	518.1	436.8	550.9	546.7
	413	J	524.7	552.2	598.5	486.6	616.2	552.7	607.7	626.0
	414	G	621.0	536.3	527.1	496.7	535.1	514.2	432.1	492.9
	415	J	300.5	286.5	336.9	293.8	263.7	226.4	316.7	369.4
	416	J	828.3	680.6	867.4	771.0	856.4	-	916.3	730.8
	418	J	417.6	409.5	469.3	357.1	263.5	352.4	383.7	444.5
	419	J	873.5	575.5	1200.0	621.9	-	-	833.7	820.7
	420	J	846.3	-	570.0	503.8	-	-	793.1	612.2

G - Galileo, J - Juvent, NA - not applicable, - missing sample

Table 25 No fracture and fracture groups CTX by participant

			CTX ng/ml							
	Study no	**Platform**	**Day 1 pre**	**Day 1 post**	**Day 3 pre**	**Day 3 post**	**Day 5 pre**	**Day 5 post**	**Day 8**	**Day 12**
No fracture	203	G	1.98	1.90	2.15	2.24	2.21	2.460	2.20	NA
	205	J	1.79	1.46	1.62	1.47	1.47	1.350	1.78	NA
	207	G	2.07	2.01	1.87	1.90	1.84	1.890	2.24	NA
	208	J	1.59	1.62	2.06	1.80	1.91	1.760	1.84	NA
	213	G	1.58	1.55	1.96	1.80	1.94	1.980	1.56	NA
	214	J	2.70	2.36	2.68	2.39	3.06	2.820	3.19	NA
	217	J	2.09	-	-	-	-	-	-	NA
	218	G	1.68	1.67	1.86	-	1.97	1.690	1.92	NA
	221	G	1.46	1.72	1.62	1.54	-	-	1.45	NA
	227	J	2.08	1.91	1.84	1.55	1.64	1.550	2.14	NA
	228	J	1.38	1.37	1.51	1.45	1.49	1.330	1.62	NA
	229	G	1.91	-	1.97	1.89	-	-	2.57	NA
Fracture	401	J	1.63	1.45	2.02	1.80	1.86	1.670	1.70	1.51
	402	G	1.99	1.73	1.92	1.78	2.13	1.890	2.08	1.89
	403	G	2.31	2.24	-	-	2.13	1.870	2.44	2.14
	404	G	1.99	2.05	2.36	2.22	2.20	-	-	2.33
	405	J	1.91	1.59	1.91	1.66	1.90	1.670	1.88	1.80
	406	G	1.96	1.74	-	-	-	-	-	-
	408	G	1.86	1.88	2.15	1.97	1.92	1.800	2.36	2.00
	409	J	1.94	1.81	1.71	1.54	-	-	1.68	1.75
	410	G	2.06	2.00	2.15	2.10	2.24	2.000	2.27	2.31
	411	J	2.15	1.98	2.02	1.82	2.19	1.800	-	1.96
	412	G	1.93	1.72	1.66	1.51	1.60	1.510	1.73	1.48
	413	J	2.27	1.96	1.91	1.76	1.90	1.760	2.25	1.96
	414	G	2.05	1.76	1.94	1.70	1.91	1.760	1.35	1.80
	415	J	1.18	1.23	1.27	1.17	1.06	1.020	1.10	1.41
	416	J	2.24	1.97	2.17	1.92	2.21	-	2.35	2.27
	418	J	1.25	1.09	1.71	1.32	1.15	1.180	1.19	1.07
	419	J	2.57	2.22	2.77	2.21	-	-	2.61	2.18
	420	J	1.82	-	1.69	1.47	-	-	1.84	1.45

G - Galileo, J - Juvent, NA - not applicable, - missing sample

Table 26 No fracture and fracture groups OPG by participant

			OPG pmol/L				
	Study no	**Platform**	**Day 1 pre**	**Day 3 pre**	**Day 5 pre**	**Day 8**	**Day 12**
No fracture	203	G	3.130	3.460	3.520	3.840	NA
	205	J	3.650	2.710	2.830	3.220	NA
	207	G	4.110	4.190	4.480	4.240	NA
	208	J	3.110	3.780	3.370	3.550	NA
	213	G	3.250	3.130	2.990	3.030	NA
	214	J	3.240	3.390	3.720	3.880	NA
	217	J	-	-	-	-	NA
	218	G	3.110	2.950	3.260	3.030	NA
	221	G	3.640	3.730	-	4.530	NA
	227	J	2.980	3.220	3.140	2.870	NA
	228	J	4.080	5.220	4.300	4.760	NA
	229	G	4.730	3.950	-	4.890	NA
Fracture	401	J	5.346	NA	5.198	5.933	4.826
	402	G	3.937	NA	4.845	5.089	4.499
	403	G	3.556	NA	3.663	3.468	3.403
	404	G	4.729	NA	4.489	-	4.572
	405	J	3.576	NA	3.828	4.665	3.934
	406	G	5.297	NA	-	-	-
	408	G	4.111	NA	4.143	2.964	2.921
	409	J	4.249	NA	-	4.960	3.465
	410	G	4.592	NA	4.598	4.938	4.188
	411	J	3.273	NA	3.682	-	3.715
	412	G	4.919	NA	4.011	4.063	4.624
	413	J	3.124	NA	3.045	3.369	3.335
	414	G	4.622	NA	4.541	4.827	4.824
	415	J	3.402	NA	3.769	3.887	3.173
	416	J	3.773	NA	4.093	4.093	3.241
	418	J	3.803	NA	3.282	4.161	4.595
	419	J	3.591	NA	-	3.256	3.982
	420	J	-	NA	-	-	3.781

G - Galileo, J - Juvent, NA - not applicable, - missing sample

Table 27 No fracture and fracture groups sclerostin by participant

			Sclerostin pmol/L				
	Study no	**Platform**	**Day 1 pre**	**Day 3 pre**	**Day 5 pre**	**Day 8**	**Day 12**
No fracture	203	G	28.74	27.00	29.87	22.92	NA
	205	J	24.16	23.90	22.73	21.86	NA
	207	G	20.88	20.08	22.14	22.54	NA
	208	J	20.93	21.02	24.21	25.55	NA
	213	G	26.95	30.99	26.54	24.77	NA
	214	J	23.49	22.38	26.89	29.93	NA
	217	J	-	-	-	-	NA
	218	G	30.45	32.86	30.76	27.73	NA
	221	G	24.01	24.58	-	26.86	NA
	227	J	23.83	22.30	19.19	22.00	NA
	228	J	18.39	24.39	27.73	24.62	NA
	229	G	31.02	27.91	-	43.56	NA
Fracture	401	J	35.35	NA	33.86	29.80	30.51
	402	G	53.37	NA	47.93	59.56	44.54
	403	G	24.72	NA	27.70	29.34	34.10
	404	G	36.53	NA	34.68	-	41.69
	405	J	47.28	NA	37.00	34.78	36.01
	406	G	22.40	NA	-	-	-
	408	G	70.57	NA	62.27	58.96	66.38
	409	J	22.23	NA	-	24.28	25.96
	410	G	35.49	NA	34.68	41.96	36.63
	411	J	24.17	NA	31.81	-	27.96
	412	G	48.18	NA	47.24	67.62	45.81
	413	J	39.10	NA	28.15	35.85	37.61
	414	G	39.34	NA	39.34	27.81	33.73
	415	J	31.75	NA	43.19	39.77	52.85
	416	J	33.31	NA	37.61	31.06	31.96
	418	J	29.54	NA	22.05	33.57	31.64
	419	J	34.39	NA	-	43.28	38.48
	420	J	-	NA	-	-	35.80

G - Galileo, J - Juvent, NA - not applicable, - missing sample