Whole body vibration training in chronic disease: muscle, bone, function.

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Declaration

This is to verify that to the best of my knowledge, the content of this thesis is my own work. This thesis has not been submitted for any degree or other purposes.

I certify that the intellectual content of this thesis is the product of my own work and that all the assistance received in preparing this thesis and sources have been acknowledged.

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Abstract

Muscle pull from regular physical activity is crucial for optimal development of the skeleton during growth and maintenance of bone mineral density (BMD) throughout life. Mitochondrial Respiratory Chain Disorders (MRCD) and Cystic Fibrosis (CF) are two chronic diseases that exhibit reduced lean tissue mass and impaired exercise capacity, which negatively impacts bone health in these populations. Whole body vibration training (WBVT) is an emerging therapeutic modality that has been successful in improving BMD and muscle mass and function in heath and disease. Aim: To evaluate whether 6 months of home-based WBVT improves BMD, muscle function, exercise capacity and quality of life (QoL) in people with MRCD or CF. Methods: Participants were enrolled for 15-18 months: 3-6 months observation; 6 months home-based WBVT (3 x 3mins daily at 20Hz on a Galileo® Home vibration platform); 6 months follow-up. Participants attended four study visits and completed: dual energy x-ray absorptiometry (DXA) and peripheral quantitative computed tomography (pQCT) to assess BMD; muscle function testing on the Leonardo Jumping Platform (LJP); 6-minute walk test (6MWT) and/or formal exercise testing to assess exercise capacity; and disease specific QoL questionnaires. Linear mixed models analysis was used to assess changes between visits. Results: The MRCD cohort had 23 participants (13 male) mean (SD) age 31.0 (19.8) years and the CF cohort, 16 participants (8 male) mean (SD) age 12.8 (3.5) years. Statistically significant improvements in BMD of the legs were seen in the MRCD and CF cohorts for both DXA and pQCT. Muscle force during hopping and co-ordination during the chair rise test on the LJP improved significantly post WBVT in the CF.

Exercise capacity did not change in the MRCD or CF cohorts after WBVT. QoL showed improvements in both cohorts. Conclusions: WBVT was well tolerated. WBVT improved BMD, aspects of muscle function, and QoL in people with MRCD or CF and may be a useful adjunct to physiotherapy exercise programs.

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1 Introduction

This study aims to evaluate the effects of six-months home-based whole-body vibration training on bone and muscle structure and function in young people with chronic diseases. In order to put this study into context, bone structure and function, bone health, whole-body vibration training, mitochondrial respiratory chain disorders and cystic fibrosis will be discussed.

1.1 Bone structure, function and biology

Bone is a biologically active tissue that is involved in several important physiological processes. The adult human body has 206 bones that together form the skeleton. It provides a structural scaffold for muscle attachments enabling movement and upright locomotion. It also protectively houses the vital organs and, within the marrow compartment, is involved in haematopoiesis. It also has an important role in calcium and phosphorus mineral homeostasis and acid-base balance and is a reservoir of growth factors and cytokines [1].

Bone is composed of 50-70% mineral, 20-40% organic extra-cellular matrix, 5-10% water and less than 3% fat [1]. Bone mineral is predominantly hydroxyapatite ($Ca_5(PO_D)_3OH$), this inorganic compound providing the bone with load bearing strength and mechanical rigidity [1]. The organic extra-cellular matrix is comprised of collagenous and non-collagenous proteins which make up approximately 85% and 15%

of all bone protein respectively [1-3]. The collagenous component primarily consists of type 1 collagen which provides the structural scaffold upon which bone mineralisation occurs and the non-collagenous component is believed to help regulate mineralisation of the matrix [1]. This organic extra-cellular matrix gives bone it's elasticity and flexibility [1]. The arrangement of these organic and inorganic components determines bone type [4]. The adult skeleton consists of two main bone types, cortical bone which accounts for 80% of the skeleton, and trabecular bone, which accounts for the remaining 20% [1]. Both cortical and trabecular bone are composed of osteons organised in a lamellar pattern, where collagen fibres are arranged in alternating directions to give lamellar bone its strength [1], however their structure is quite different.

Trabecular osteons are organised into parallel lamellae to form a honeycomb type network of plates and rods within the marrow cavity. Trabecular bone is the primary type of bone found in the vertebrae and in the metaphyses and epiphyses at the ends of long bones e.g. the humerus and tibia. Cortical bone is denser and more solid compared to trabecular bone. Cortical osteons form branching networks in cortical bone called Haversian systems, the walls of which are formed by concentric lamellae [1]. Haversian canals contain a blood vessel which is connected to neighbouring Haversian blood vessels by Volkmann's canals. The walls of the Haversian canals have rectangular shaped lacunae embedded within them in which osteocytes reside. Cortical bone forms a thin shell around the boundary of trabecular bone and constitutes the shaft or diaphysis of long bones [1].

Long bones are designed to have a hollow shaft, or diaphysis, with flared ends that participate in articulation with their adjacent bones. The proximal and distal ends of long bones are lined with cartilage under which lies the bony epiphysis. Adjacent to the epiphysis is the metaphysis which transitions into the diaphysis through metaphyseal in-wasting. The metaphysis and epiphysis are separate compartments during skeletal growth due to the presence of the growth plate, but become fused in the mature adult skeleton. Cortical bone in the diaphysis has an outer periosteal surface which is attached to a two-layered fibrous connective tissue, called the periosteum. This periosteal surface has an important role in fracture repair and is the surface upon which appositional growth occurs. The inner surface of the hollow diaphysis, adjacent to the marrow cavity, is the endosteal surface. It is lined with a vascular connective tissue called the endosteum which is in contact with the bone marrow cavity and blood vessels. Endosteum tissue is also present in trabecular bone and in Volkmann's canals [1].

1.2 Bone Cells

1.2.1 Osteoblasts

Osteoblasts are single nucleated cells responsible for the synthesis and mineralisation of new extra-cellular bone matrix [1, 3]. During the formation of new bone, osteoblasts secrete type 1 collagen which becomes the scaffold upon which mineralisation occurs. Osteoblasts also secrete several non-collagenous proteins believed to be integral in regulating the mineralisation process [2]. Two of the most abundant non-collagenous protein secreted by osteoblasts are osteonectin and osteocalcin which are involved in

osteoblast growth and proliferation, and calcium binding and stabilisation of hydroxyapatite respectively [1, 3]. Bone matrix mineralisation is achieved by osteoblasts through the secretion of specialised vesicles. Alkaline phosphatase in osteoblast membranes helps to transport phosphate into the vesicles and create a protected alkaline environment in the vesicles allowing the accumulation of calcium and phosphate and the precipitation of hydroxyapatite crystals. Together with the non-collagenous proteins, alkaline phosphatase is believed to be involved in regulating the amount and size of hydroxyapatite crystal formed and it's ordered deposition in the matrix [1, 5].

Growth and proliferation of the hydroxyapatite crystals eventually ruptures the vesicle, exposing the hydroxyapatite crystals to the matrix. Bone mineralisation commences approximately two weeks after the release of bone matrix from the osteoblasts. The mineralisation process appears to have two stages, primary mineralisation, where nearly three quarters of the bone matrix is mineralised within a few days, followed by a secondary mineralisation process, which occurs over 6 months [6]. As the bone matures the hydroxyapatite crystals enlarge as mineral continues to be added to the matrix resulting in crystal growth and aggregation [1]. Vitamin D is an important co-factor in bone mineralisation as it is involved in the intestinal absorption of calcium and phosphorus and promotes osteoblast differentiation and the expression of osteocalcin, osteonectin, alkaline phosphatase, osteoprotegerin (OPG) and other cytokines involved in bone formation [1]. Serum concentrations of osteocalcin, secreted by osteoblasts, and alkaline phosphatase, released from osteoblast membranes during vesicle rupture, are markers of bone formation activity [1].

Osteoblast differentiation and activity is dependent on the Wnt/beta catenin pathway, which appears to be controlled by the osteocyte (discussed later) [7, 8]. Osteoblasts from the axial and appendicular skeleton differentially express genes and respond differently to hormonal, mechanical and cytokine exposure [1]. After completing their role in the formation of new bone, the majority of osteoblasts undergo apoptosis with smaller percentages becoming osteocytes, trapped within the bone matrix they have laid down, or bone lining cells, which form the endosteum in trabecular bone and line the endosteal and periosteal surfaces of cortical bone [1, 3, 8, 9].

1.2.2 Osteoclasts

Osteoclasts are large multinucleated cells capable of resorbing both the organic and inorganic components of bone [3]. Bone resorption is achieved by the formation of a sealed micro-environment where the osteocyte binds to the bone matrix. This site becomes an isolated acidified compartment where resorption can take place [10]. The osteocyte secretes the cathepsin K enzyme, which digests the organic matrix consisting primarily of type 1 collagen, and hydrogen ions that dissolve the inorganic hydroxyapatite crystals [3, 11]. Osteoclast differentiation and activity is dependent on the expression of receptor activator of nuclear factor-κB ligand (RANKL). This cytokine is expressed by osteocytes, as is its antagonist, osteoprogerin (OPG), which binds to RANKL and inhibits its action at the receptor activator of nuclear factor-κB (RANK) receptor and thus osteoclast activity [1]. The RANKL/OPG ratio, regulated by the osteocyte, ultimately determines osteoclast activity and is integral in the maintenance of

bone mass [11]. After bone resorption is completed osteoclasts undergo apoptosis or return to an inactive state.

1.2.3 Osteocytes

Osteocytes reside in regularly distributed lacunae within the bone matrix and constitute over 90% of all bone cells in the adult skeleton. It is believed that 5-20% of the osteoblasts involved in bone formation become entrapped in the bone matrix during bone deposition and terminally differentiate into osteocytes. During the process of differentiation, changes in gene expression direct a change in osteoblast shape and the formation of osteocyte dendritic processes and canaliculi, the channels through which the dendritic processes run, which forms a branching system to connect the new osteocyte with neighbouring osteocytes, the periosteal and endosteal bone surfaces, bone marrow and blood vessel in the marrow compartment and Haversian canals [1, 8, 12, 13]. The canaliculi from neighbouring osteocytes are believed to establish intracellular communications through gap junctions [8] and the interaction of dendritic processes with other bone cells suggest osteocytes may have the ability to direct other bone cell functions and regulate mesenchymal stem cell differentiation [12, 14]. Osteocytes have major roles in mechano-sensation, maintenance of bone mass and integrity, regulating bone mineralisation [12], hormonal interactions with bone [8] as well as detecting micro-damage and directing the repair process [15, 16].

Osteocytes are considered to be the primary bone cell responsible for the sensation and transduction of mechanical strains imparted on bones [12, 14]. Osteocytes are believed to

sense strains in their cell bodies, dendritic processes and cilia [12] due to bone matrix tissue deformation, changes in fluid flow dynamic pressure, changes in intramedullary cavity pressure [17] or perturbation in canalicular fluid flow causing shear stress [18] in response to mechanical loads imparted upon the skeleton [1, 14]. The osteocyte's surrounding bone matrix including osteons, lacunae and canaliculi are involved in mechano-sensation, and may be responsible for magnifying strains experienced in the bone matrix [19]. Mechano-sensory information from osteocytes is thought to be shared between osteocytes and other bone cells via gap junctions between adjacent dendritic processes [20]. These gap junctions are integral to osteocyte viability and are comprised primarily of gap junction channel protein Connexin 43 (Cx43) which is involved in the release of signalling factors for the control of osteoblast and osteoclast activity [21, 22] [12, 14].

Osteocytes also seem to have a pivotal role in transducing strain signals and interpreting both local strains and the distribution of strains throughout the whole bone to effect a response which ultimately controls bone turnover (formation and resorption signals) [1, 13, 14]. Bone turnover, including preference for bone formation or resorption, appears to be achieved through the osteocyte's expression of sclerostin and resultant modulation of the Wnt/β-catenin pathway by the osteocyte [8]. Wnt's are a family of growth factors [23] and the Wnt/β-catenin pathway is important in bone formation and bone maintenance and osteocyte viability. Sclerostin, encoded by the *SOST* gene, is expressed and secreted by mature osteocytes in the bone matrix. Sclerostin is a negative regulator of the Wnt/β-catenin pathway and thus an inhibitor of bone formation and osteoblast activity

[12, 24, 25]. Osteocytes also express Receptor Activator of Nuclear factor-κB ligand (RANKL) [26] on dendritic processes which interacts with Receptor Activator of Nuclear factor-κB (RANK) receptor to initiate osteoclastogenesis indicating that they are also involved in signalling pathways for bone resorption [12].

Under normal conditions sclerostin expression by the osteocyte keeps the intracellular levels of β-catenin low [14]. However, with increased mechanical loading (from longitudinal bone growth or weight-bearing physical activity), osteocyte expression of sclerostin is reduced [27] and the inhibitory action of sclerostin on the Wnt/ β-catenin pathway is alleviated [8, 12, 14] allowing β-catenin to accumulate in the cell. This accumulation of β-catenin is integral in the maintenance of osteocyte viability and functioning (Bonewald 2011) and influences bone turnover activity. It is involved in the activation of the Extracellular signals-Regulated Kinases (ERK) pathway which preserves osteocyte viability [28, 29]. It also increases the expression of Cx43 which is required for the maintenance of gap junction functioning and osteocyte communication [12, 30]. The presence of β-catenin also increases the expression of OPG by osteocytes which inhibits RANKL and osteoclast activity [8, 12]. The nuclear translocation of accumulated β-catenin influences expression of genes involved in differentiation, proliferation, apoptosis and functionality of bone cells as well as bone formation in response to the mechanical strains [14, 31-33]. Mechanical loading and their associated bone strains are considered to play a significant role in the transport of nutrients and oxygen around the osteocyte-canalicular network [34] and in fluid flow, blood flow and transport of osteocytic products in the canalicular system [8, 35].

Osteocytes have a long life span [8] and in low bone turn-over states can live in the bone for decades [20]. Osteocytes die through apoptosis, which can occur at sites of micro-damage [12], but is also influenced by the activity of the Wnt/β-catenin pathway, whereby increased pathway activity is protective against osteocyte apoptosis. Apoptotic osteocytes appear to send signals for the recruitment of osteoclasts and while the exact mechanism in which the osteocyte does this is unknown, many mechanisms have been postulated including: osteocyte expression of RANKL [8]; osteocyte induced expression of RANKL by osteoblasts [8]; down-regulation of OPG, a RANKL antagonist, in the dying osteocyte [8]; and signalling from apoptotic bodies released by dying osteocytes [36, 37]. Osteocyte apoptosis is considered to be essential for the repair of damaged bone and normal bone turnover processes however it can become accelerated in several suboptimal conditions including ageing, menopause, glucocorticoid therapy, inflammatory cytokines and reduced mechanical loading [12], interrupting the function of the osteocyte network and its ability to sense and respond to mechanical strains imparted on bone.

Aged bone is characterised by accumulation of micro-damage, reduced osteocyte density, accumulation of apoptotic osteocytes and empty lacunae [38, 39]. Hyper-mineralisation of the perilacunar matrix with aging, and the presence of longer lived osteocytes [14], may adversely affect the function and viability [12, 38] of the osteocyte, by altering the flow of fluid through the osteocyte canalicular system and the movement of osteocytic products [8, 12], disrupting dendritic gap junctions between osteocytes [40] interfering

with communication and interrupting the osteocyte's ability to respond to stimuli, which ultimately results in osteocyte apoptosis. The loss of sex steroids is believed to play a significant role in reduced osteocyte viability and increased osteocyte apoptosis with aging [1, 41, 42]. A similar pattern of reduced osteocyte viability and increased osteocyte apoptosis is also seen during treatment with glucocorticoids [1, 43]. It is also likely that the reduced osteocyte density and increased prevalence of apoptotic osteocytes in aging bone is related to the decline in physical activity and therefore mechanical loading of the skeleton [44].

Reduction in the mechanical loading of the skeleton, as is seen with reduced weight-bearing physical activity, ageing, immobility and motor paralysis, is associated with decreased osteocyte viability, increased osteocyte apoptosis [44], increased expression of sclerostin [27] and reduced activity of the Wnt/ β-catenin pathway [38], and increased expression of RANKL by the osteoclast [12, 45], with increased osteoclast activity and increased bone resorption [44]. Together these processes are believed to impair the osteocyte network's ability to sense micro-damage, signal for repair [12], transduce information from mechanical strains and communicate effectively, culminating in reduced integrity of the bone.

It is clear that mechanical loading and unloading of the skeleton influence osteocyte expression of key regulators [27] involved in bone turnover including sclerostin, OPG and RANKL. Mechanical loading of the skeleton is critical for activation of the Wnt/ β-catenin pathway which is crucial for bone formation in response to mechanical loading,

the maintenance of bone mass, the preservation of osteocyte function and viability and inhibition of osteocyte apoptosis [14]. Osteocytes are able to integrate signals from mechanical loading with circulating factors e.g. hormones, glucocorticoids and local stimuli to regulate bone homeostasis and direct modelling and remodelling processes through their ability to regulate bone formation by expression of sclerostin and bone resorption by expression of OPG and RANKL [8, 12].

1.3 Bone Strength

Bone strength refers to the bone's ability to resist mechanical failure (fracture) and is dependent on the interaction between bone quantity and bone quality. Bone quantity refers to the mass of bone, which is responsible for 50-70% of bone strength [1]. Across the life span, bone mass is determined by the peak bone mass achieved after the pubertal growth spurt and the rate and amount of bone loss during adulthood [46]. Bone quality includes several bone features including material properties, amount of micro-damage, geometry, turnover and tissue quality [1, 46-48]. Material properties of bone refer to the quality and assembly of the mineral component (hydroxyapatite) and collagen rich organic matrix component. These properties demonstrate minimal variance between individuals, and along with the amount of micro-damage present, can only be measured using invasive techniques e.g. bone biopsy [47, 48]. The bone's geometric attributes, including longitudinal shape and cross-sectional area, and the distribution of the bone tissue in space, also contribute significantly to overall bone strength [47, 48], with greater strength achieved if a given amount of material is located further from the centre of the structure [49]. Tissue quality refers to the mechanical behaviour of the bone, usually

reflecting the combination of bone mass and geometric factors e.g. the stress strain index (SSI) [48]. Bone mass, geometric features and parameters of tissue quality can be measured non-invasively using densitometric techniques. From a biological perspective, bone strength *in-vivo* is modified throughout life by three important biological processes, longitudinal bone growth, modelling and remodelling [35, 47]. Together these processes optimise bone strength to habitual mechanical loads imposed on the skeleton to optimise function.

1.3.1 Bone remodelling

Bone remodelling is a process that occurs continuously throughout life to maintain bone strength and integrity and mineral homeostasis [1]. Remodelling occurs on the periosteal, endosteal and Haversian surfaces of cortical bone and on trabecular bone surfaces [35]. At the completion of a bone remodelling cycle, bone formation generally exceeds bone resorption on the periosteal surface. On Haversian surfaces, bone turnover is equivocal, playing a major role in preventing accumulation of microdamage and excessive bone age [50]. On endosteal and trabecular surfaces bone resorption exceeds formation [51]. Bone remodelling sites may develop randomly, in response to reduced mechanical loading or through the identification of old or damaged areas of bone by osteocytes [16, 51, 52]. The bone remodelling unit is a tightly coupled group of osteoclasts and osteoblasts that remove discrete packets of old bone and synthesis new bone matrix [1, 35, 51, 53]. The bone remodelling cycle has 4 stages: (i) activation, where osteoclasts are recruited to the site of repair and lift the bone lining cells, under which they create sealed micro-environments around the site of repair where resorption can take place; (ii) resorption,

where osteoclasts take 2-4 weeks to remove the bone matrix, creating Howship's lacunae in trabecular bone and Haversian canals in cortical bone, after which the osteoclasts undergo apoptosis; (iii) reversal, where bone resorption is complete and bone formation commences; and (iv) formation, where osteoblasts take 4-6 months to synthesise new bone matrix and regulate mineralisation, after which osteoblasts undergo apoptosis or form osteocytes or bone lining cells [1]. The final outcome of this cycle is the formation of a new osteon or partially remodelled trabecular in cortical and trabecular bone respectively [1, 53]. Osteocytes regulate the remodelling cycle through expression of receptor activator NF-kappaB ligand (RANKL) and OPG [54].

Bone remodelling continuously occurs throughout life. During childhood and adolescence, the growing skeleton is characterised by modelling and remodelling processes where bone formation exceeds the rate of bone resorption resulting in a net increase in bone [47]. In young healthy adults, after the cessation of growth, remodelling becomes the primary process by which bone is adapted to replace old or damaged bone. Bone formation is usually coupled closely with bone resorption and there is no net change in bone. In post-menopausal and aging bone, as well as pathological bone conditions, the rate of bone resorption exceeds bone formation during remodelling cycles and a net loss of bone occurs [3]. In healthy aging adults, there are subtle increases in periosteal and endosteal circumferences of long bones, due to bone formation exceeding bone resorption on periosteal surfaces, and bone resorption exceeding bone formation on endosteal surfaces, respectively [1, 35]. The endosteal surface has a higher remodelling activity compared to the periosteal surface, so there is also thinning of the cortex and

increased cortical porosity with ageing [1]. In adults, cortical bone turnover occurs at a rate of 2-3% each year. Trabecular bone turnover is higher as it is more metabolically active however it is also believed that the trabecular bone compartment is more involved in mineral homeostasis than the cortical compartment [1, 4].

1.3.2 Bone Modelling

During childhood and adolescence long bones grow in both length and width [1] with total skeletal calcium increasing more than 1000g in the first two decades of life [50, 55]. This accrual in bone mineral content contributes towards increasing bone size to a far greater extent than increasing bone density [3]. Long bones change their length and geometry during growth through a process known as bone modelling which acts on trabecular surfaces and the periosteal and endosteal surfaces of cortical bone [3, 56]. During modelling there is extensive and prolonged bone formation and bone resorption occurring on bone surfaces [4] which are not tightly coupled [1, 50, 56]. Bone modelling results in the changes in the long bone's physical dimensions seen between childhood and adulthood and ensures the growing bone is adapted to maintain bone strength and optimal function of the skeleton, so as it can resist damage caused by strains on bones from habitual mechanical loading. Bone modelling occurs during growth in children and adolescents, however it subsides in adulthood once skeletal maturity has been reached [50, 56, 57].

Longitudinal bone growth occurs at the growth plate, where endochondral ossification replaces cartilage with bone in the metaphyseal region adjacent to the growth plate [58].

The metaphysis is composed primarily of trabecular bone [1, 4, 50]. Newly deposited bone along cartilage septa is referred to as primary trabecular bone and exhibits little structural difference between individuals [59]. Primary trabecular bone is remodelled into secondary trabecular bone which is thicker and exhibits diverse architectural differences between individuals depending on mechanical loads applied to the bone. At the distal tibial metaphysis, trabecular density (trabecular number and thickness), varies very little in females during growth [60, 61], however males have an increase in trabecular density during puberty due to an increase in trabecular thickness but not trabecular number [62]. This increased trabecular thickness in males may provide some protection against osteoporosis with ageing and may partially explain why females are more susceptible to osteoporosis compared to males in later life. Trabecular bone in vertebral bodies demonstrate increases in trabecular volumetric bone mineral density (vBMD) [6, 63] in both sexes during puberty due to increased trabecular thickness but not trabecular number [64]. Males have larger vertebral bodies compared to females but their density (trabecular number and thickness) does not differ to that of females [65].

Longitudinal growth reduces the long bone's ability to resist mechanical loads, as the bending strength of the long bone is inversely related to its length to the third power [66]. To accommodate these increasing mechanical loads associated with longitudinal growth, the diaphyseal bone adapts its mass and geometry, in particular it's outer diameter, as the bending strength of the long bone is related to its diameter raised to the third power [58, 66]. The combined activity of periosteal apposition and endosteal resorption influences the bone's mass, width, cross-sectional area and cortical thickness [4, 56, 57], giving the

bone its load bearing strength [35, 66-68]. Geometric adaptations of the long bone's width are some of the most important determinants of bone strength throughout life [69]. Long bone geometry is also modified in the metaphyseal region, where newly laid down bone in the wider metaphyseal region is reshaped as it transitions into the narrower diaphysis. This is achieved by a process known as metaphyseal in-wasting, where the periosteal surface is resorbed while bone is formed on the endosteal surface by trabecular coalescence [70, 71].

Genetics plays a role in the way a long bone's size and geometry assembles, however bones adapt their size, geometry and the distribution of mineralisation in response to the mechanical loading of bones during growth [57] in order to optimise strength and function [4]. These mechanical loads are a consequence of longitudinal bone growth, increased body weight, increased muscle strength and weight-bearing physical activity [4, 72]. The processes by which the bone adapts its mass and geometry to mechanical loads throughout life, during modelling and remodelling processes are governed by physiological principles including Wolff's Law, the Mechanostat Theory and the Utah Paradigm.

1.3.3 Wolff's Law, the Mechanostat Theory and the Utah Paradigm

Bones adapt their structure including mass, size and geometry, to withstand mechanical loads placed upon them from muscle contractions [73], generated during habitual weight-bearing, body movements and physical activity, to resist mechanical failure (fracture), prevent pain and allow optimal function, a light, yet strong skeletal scaffold that does not

limit mobility or result in excessive energy consumption [74]. This forms the basis of the Mechanostat Theory [47, 75]. The first documentation indicating a link between mechanical loading and bone geometry was proposed by Wolff's Law in 1892 [57]. This law underpinned the development of the Mechanostat Theory proposed by Frost in 1964 and The Utah Paradigm of Skeletal Physiology proposed by Frost in 1998 [75].

The Mechanostat Theory assumes that humans are born with a blueprint of basic skeletal structure and are equipped with biological activities that can effect changes in bone architecture in response to mechanical loading of the skeleton after birth [48, 75]. The Mechanostat Theory postulates that there is a threshold level of minimally effective strains (MES) that determine the osteocyte's response to mechanical loads imposed on bones [35, 72, 76]. There appears to be a higher and lower MES threshold, with an optimal range of MES between the two thresholds. It is believed that these MES thresholds are genetically determined at birth and persist throughout life. The osteocyte directs modelling processes during growth and remodelling processes throughout life to keep bone strains imposed by mechanical loading within the threshold range [35, 56, 72]. Habitual mechanical loading resulting in bone strains within the MES thresholds do not elicit a response from the osteocyte and the bone is considered to be biomechanically adapted to its habitual mechanical loads [57]. However, when the habitual mechanical loading exceeds the MES threshold (overloading), e.g. with longitudinal bone growth or increased weight-bearing physical activity, the osteocyte initiates an adaptive response to make the bone stronger. In contrast, if habitual mechanical loading is consistently below the MES thresholds e.g. reduced weight-bearing physical activity, the osteocyte initiates

remodelling cycles, with bone resorption rates exceeding bone formation rates [35, 47, 51, 56, 57, 72].

The Mechanostat Theory therefore explains how long bones adapt to the more than 50-fold increase in mechanical loads imparted on bones between birth and skeletal maturity in adulthood [35], as well as how bones maintain their shape while increasing over 5-fold in size between birth and adulthood [57]. During growth, modelling increases bone mass, usually on the periosteal surface, and changes geometry (increases cross-sectional area and adapts cortical thickness) to accommodate increasing mechanical loads, and to reduce bone strains so they fall within the acceptable MES thresholds [50, 57, 72, 73]. The Mechanostat Theory also explains the development of age-related and secondary disuse osteoporosis, where bones adapt their mass and strength to the reduced muscle strength and weight-bearing physical activity associated with both conditions [51].

The Mechanostat Theory forms a large part of the Utah Paradigm, however the Utah Paradigm considers the Mechanostat Theory in the context of other biological process and non-mechanical factors that influence bone physiology [47, 75]. The mechanical and biological aspects of the mechanostat together with non-mechanical factors ultimately determine skeletal health. Non-mechanical factors include gender, genetics, growth and sex hormones, nutrition, cytokines, circulating systemic agents, drugs and disease processes [35, 51, 66, 75]. Some non-mechanical factors are required for proper functioning of bone physiological processes and are believed to modulate the mechanical factors acting on bone [73], helping or hindering mechanical control, however non-

mechanical factors cannot replace the function of the mechanical factors and the mechanical factors ultimately guide the biological mechanisms in time and space [75] [47, 66].

1.3.4 The Functional Muscle-Bone Unit

Muscle activity from regular locomotion and weight-bearing physical activity are crucial to bone growth and development and the maintenance of bone health throughout life as their contractions apply the most frequent and greatest mechanical loads to the underlying bone [35, 47, 77-81]. The forces generated by muscles are therefore major determinants of bone strength [68]. This intimate relationship between muscle force and bone strength was coined the "functional muscle-bone unit" [78]. Normal muscle development and the ability of the muscle to generate force therefore has a pivotal role in bone mass accrual and geometric adaptations of bone during growth and the maintenance of bone health throughout adult life.

The measurement of this relationship in a clinical setting requires surrogate measurements of bone strength and muscle force at a corresponding skeletal location [80]. Such measures can be obtained from bone densitometry measurements using dual-energy x-ray absorptiometry (DXA) and peripheral quantitative computed tomography (pQCT). Bone mineral content (BMC) measured in grams (g) or grams/centimetre (g/cm) is considered to be a good surrogate for bone strength, and lean tissue mass (LTM) or muscle cross-sectional area is considered to be a good surrogate of muscle force, when using DXA and pQCT respectively. Strong correlations between total body BMC and

total body LTM have been shown in studies using DXA [82-84] and strong linear correlations between BMC and muscle cross-sectional area have been illustrated by pQCT studies in adults and children at the radius and tibia where muscle cross-sectional area is greatest, the 65% and 66% site for the radius and tibia respectively [61, 78, 85].

A two-step diagnostic algorithm using the functional muscle-bone unit was proposed to determine whether the bone is optimally adapted to muscle activity [78], and to aid in the interpretation of bone disease in individuals by dividing them into 4 groups: normal, primary bone defect, secondary bone defect and mixed bone defect [78]. The first step is to determine whether muscle mass is adequate for height, using LTM for height calculations from DXA measurements and muscle cross-sectional area calculations from pQCT measurements. If the muscle mass is adequate for height, the second step is to determine if BMC is adequate for muscle mass, using BMC for LTM calculations from DXA measurements and the BMC for muscle cross-sectional area ratio from pQCT measurements. If the BMC is adequate for muscle mass the individual has normal bone health. If the BMC is inadequate for the muscle mass, the individual has a primary bone defect. Going back to the first step, if muscle mass is inadequate for height however BMC is adequate for muscle mass, the individual has a secondary bone defect. If muscle mass is inadequate for height and BMC is inadequate for muscle mass the individual is considered to have a mixed bone defect [78].

1.4 Optimising Bone Health

1.4.1 Peak bone Mass

Genetic factors play a major role in determining an individual's potential bone size and mass [3]. Peak bone mass is achieved by the third decade of life [86-89] and 60-80% of the variance in peak bone mass can be genetically explained [55]. Peak bone mass is maintained in the mature adult skeleton while remodelling processes continue to produce a neutral balance, usually till the fifth decade of life, after which bone turnover begins to favour resorption [89] and bone mass begins to decline. Puberty and adolescence is a crucial period of time for the attainment of peak bone mass and the foundation of long term bone health [90]. It is believed that over 50% of peak bone mass is attained during the adolescent growth spurt [50] with peak height velocity a particularly salient stimulus, as 25% of peak bone mass is attained in the two years surrounding this period of rapid growth [91]. Childhood and adolescent years are therefore critical in establishing the foundations of long term bone health and the bone mass acquired during the pubertal growth spurt is believed to be similar to the amount of bone lost in later life [90-92], so it is an important factor in the lifetime risk of developing osteoporosis [86].

Successful attainment of peak bone mass to reach an individual's genetic potential, and the maintenance of bone mass throughout life, is only possible when environmental and other factors are favourable [4, 55]. Any disturbances in the ability to provide optimal nutrition, to participate in regular weight-bearing physical activity, or exposure to endocrine regulating factors and hormones, the presence of a chronic illness and/or the

medical management of that illness can compromise the attainment of peak bone mass and interfere with long term bone health, with increased risk of developing osteoporosis [4, 55, 89].

1.4.2 Nutrition

Lifestyle factors including optimising nutrition and participating in weight-bearing physical activity are important influences on bone health throughout life [55]. Adequate nutrition and normal body weight, including the proportion of muscle and fat mass, is essential for normal growth and the development of bone health during childhood and adolescence and the maintenance of bone health throughout life [3, 93, 94]. Adequate caloric and protein intake, as well as optimal calcium, vitamin D and vitamin K intake, are particularly crucial for bone health. The nutritional requirements for these key dietary factors peaks during the adolescent growth spurt, when bone mass accrual is at its greatest but they are also important in preventing bone loss during adulthood [3, 95]. Calcium is integral for bone mineralisation [94] and vitamin D facilitates intestinal absorption of calcium [3]. Vitamin D also works together with vitamin K, which is involved in the gamma carboxylation of osteocalcin, to improve BMD [96].

1.4.3 Weight-bearing Physical Activity

Weight-bearing physical activity is recognised as the most effective non-pharmacological intervention to optimise bone health across the lifespan, by promoting bone mass accrual during the pubertal growth spurt, reducing bone loss during adulthood, and stimulating

bone mass accrual if bone loss has occurred [88, 97-99]. The type of weight-bearing physical activity employed, and in particular, the mechanical strains imparted on the underlying bone generated by the activity, impact on the adaptation of the bone to the activity. Weight-bearing physical activities that have a high strain magnitude, apply the strain at a rapid rate, have fast acceleration, are multidirectional in nature and produce ground reaction forces in excess of those produced during normal activities of daily living, aiming for 3-9 times body weight, convey the best osteogenic effects. Examples include skipping, hopping, dancing, jumping. Shorter, multiple sessions with rest periods interspersed, which restores the mechano-sensitivity of the osteocytes, that have a cumulative duration of 10-45 minutes and are performed on 3-5 occasions per week appear to result in superior bone adaptations [97, 98, 100]. Bone adaptations in bone mass, geometry and strength are site specific and occur at the sites loaded during the weight-bearing physical activity interventions [97, 100-102].

Weight-bearing physical activity, as explained by the mechanostat theory, increases BMC, BMD and bone strength and therefore makes it an effective strategy in preventing the development of osteoporosis [98, 102]. A meta-analysis conducted by *Behringer et al* in 2014, including 27 randomised controlled trials in children and adolescents with a mean age of 10 years and mean intervention period of 47 weeks found that weight-bearing physical activity interventions significantly increased BMC and BMD, with effect sizes of 0.17 and 0.26 respectively [103]. The effect sizes in pre-pubertal children were larger at 0.28 for BMC and 0.33 for BMD but only remained significant for BMC [103]. In a systematic review and meta-analysis by Nikander *et al* in 2010, investigating

the effect of weight-bearing physical activity interventions of at least 6 months duration on bone strength across the lifespan, it was found that lumbar spine and femoral neck BMD was up to 6% higher in pre-pubertal children and 2% higher in adolescents in the intervention group [97, 102]. Pre-pubertal boys in the intervention group also demonstrated significant improvements in bone strength with an effect size of 0.17 [97, 104]. In the children and adolescents investigated, the weight-bearing physical activity interventions improved cortical bone strength by optimising cortical geometry through periosteal apposition and trabecular bone strength by increasing trabecular number and thickness [97].

In adults, Nikander *et al 2010,* also found benefits of weight-bearing physical activity interventions on BMD and bone strength [97]. In pre-menopausal women, high intensity, progressive resistance training and high impact weight-bearing activities improved lumbar spine and femoral neck BMD 1-2%, with resistance training having a more beneficial effect on lumbar spine BMD and high impact training having a larger effect on femoral neck BMD. There was no significant improvement identified in bone strength, however women who were most compliant with their intervention had greater improvements in bone strength and cortical thickness compared to the women who were least compliant [97]. In post-menopausal women, high intensity, progressive resistance training improved lumbar spine BMD by 2%, and a combination of resistance training and mixed impact exercise including walking, jogging and stair climbing, maintained femoral neck and lumbar spine BMD [97]. Modest, non-significant, site-specific improvements in cortical bone strength were achieved only when adequate exercise

intensity was performed, and maintenance of these improvements required continued participation [97, 105]. In middle aged and older men, high intensity progressive resistance training and moderate intensity weight-bearing activities improved femoral neck BMD and strength by 2% [97, 106]. In another study, older men in the most physically active quartile had greater bone strength, due to larger total bone area, than men in the least active quartile [107]. In another study of adults, physical activity levels in adulthood had a positive linear relationship with BMD measured by DXA, and was associated with higher BMD in later life [108]. Furthermore, healthy adults in the most physically active quartile had significantly lower sclerostin levels than adults in the lowest physical activity quartile [109]. In these adults, weight-bearing physical activity interventions likely improved BMD and bone strength through reduced endosteal bone resorption, rather than changes in bone geometry, as seen in children and adolescents [97].

The bone adaptations that occur during childhood and adolescence appear to be maintained into young adulthood with high levels of weight-bearing physical activity during growth resulting in sustained skeletal benefits in adult bone mass, size and geometry [101]. More physically active adolescents demonstrated an up to 17% greater bone mass accrual during peak height velocity compared to their less active peers [91], and in young adulthood, active adolescent males and females had 10% more bone mass in the total body, total hip and femoral neck measured by DXA [110]. In young adulthood, active adolescent males also demonstrated a 10% larger total area and 13% greater strength at the 66% tibial pQCT site compared to inactive adolescent males, and

active adolescent females had 12% greater cortical BMC and 10% greater cortical area compared to their less active counterparts [101]. These adaptations in bone mass, geometry and strength were independent of the young adult level of weight-bearing physical activity [101, 111, 112].

It is clear, weight-bearing physical activity during growth enhances bone mass accrual, bone geometry and strength, influencing both bone quantity and quality [100], and is an optimal time to introduce interventions to maximise bone health as the skeleton adapts very efficiently, with the capability of producing large effects over a short time period, and establish the foundations for lifelong bone health [50, 97, 98, 101-103, 113]. Once skeletal maturity has been reached, the influence of weight-bearing physical activity on bone health is blunted, with effects primarily maintaining bone health and preventing age-related bone loss, resulting in minimal changes in bone mass and geometry, which are much slower to take effect [50, 55]. These bone health benefits associated with regular weight-bearing physical activity during childhood and adolescence have the capacity to impact on lifelong bone health, the development of osteoporosis and fracture risk [101]. Children and adolescents who are unable to participate in normal weight-bearing activities are at heightened risk of having poor bone health and developing osteoporosis early in life due to the absence of the osteogenic effects of weight-bearing physical activity on bone mass, geometric adaptations and contribution to bone strength during growth. Similarly, adults who are unable to participate in normal habitual weight-bearing activities are at risk of accelerated bone loss and the early development of osteoporosis. Improving participation in weight-bearing physical activity, muscle

strength, mobility and functional outcomes in people unable to participate in normal habitual weight-bearing activities will have beneficial effects on their bone health by improving bone adaptations during growth, minimising bone loss and slowing the progression towards osteoporosis [114].

1.4.4 Endocrine Factors

Timely and adequate exposure to several endocrine factors are crucial in optimising bone heath during the paediatric years, and for maintaining bone health throughout adulthood [50]. These factors include thyroid and parathyroid hormone, growth hormone, glucocorticoids and sex hormones [3, 50, 55, 66, 114, 115].

Differential exposure to androgens and estrogens during puberty and adolescence, affects skeletal development in males and females with ramifications for lifelong bone health. In males, androgens promote skeletal muscle development and periosteal apposition in the cortical diaphysis of long bones, with its effects lasting much later in adolescence compared to females, resulting in a higher bone mass, larger cortical area and more geometrically efficient distribution of cortical bone mass compared to females, culminating in better bone strength indicated by higher cross-sectional moment of inertia (CSMI) and polar stress-strain index (pSSI) values [61, 66, 116-118]. Trabecular bone in the metaphyseal region of long bones also has a greater trabecular thickness compared to females [62] at the end of puberty and this, along with the more favourable geometric attributes of the male skeleton may provide some protection against age-related

osteoporosis, and may partially explain why females are more susceptible to age-related osteoporosis compared to males.

In females, estrogens do not have the same effect on skeletal muscle development as is seen in males, and the majority of puberty associated bone changes are reached by, mid-adolescence at 15-16 years of age [117, 119], explaining the differences in bone mass and cortical area which are closely associated with muscle mass development [120]. However, after puberty, females demonstrated greater bone mass accrual for a similar muscle mass, reduced endosteal expansion and higher volumetric bone mineral density compared to males [61, 81, 82, 116, 117, 119, 120], which persists into adulthood in pre-menopausal women [118]. These differences are considered to be due to the effects of estrogen which may exert several influences on bone turnover. Estrogen is believed to alter the sensitivity of the mechanostat, lowering the MES set point for the endosteal surfaces, allowing greater bone mass accrual on the endosteal surface [121, 122] by inhibiting intra-cortical and endosteal remodelling [117, 118, 123, 124], and is considered to reduce osteoclast activity [3] and promote osteoblast activity by upregulating OPG expression [125]. These estrogen-mediated effects are likely in preparation for pregnancy and lactation [61], however the higher vBMD and therefore intrinsic stiffness of the bone, reduces bone strength as measured by CSMI in females in comparison to males [126]. During menopause, when endogenous estrogen production reduces dramatically, the MES set point for the endosteal surfaces is believed to be increased, resulting in a rapid increase of remodelling activity on endosteal and trabecular bone surfaces, with

consequent loss of bone mass and development of post-menopausal osteoporosis [35, 48, 121].

1.5 Osteoporosis

Osteoporosis is defined as "a skeletal disorder characterised by compromised bone strength predisposing to an increased risk of fracture" [46]. In children osteoporosis is diagnosed when age, gender and body size adjusted BMC or BMD z-scores are more than two standard deviations below the mean and there is a clinically relevant fracture history, which requires two or more long bone fractures under 10 years of age, three or more long bone fractures up to 19 years of age, or a vertebral compression fracture [127]. In adults, osteoporosis is diagnosed if the BMD T-score is more than 2.5 standard deviations below the mean, however pre-menopausal females and males under 50 years of age should have their BMD evaluated using z-scores, and a result more than two standard deviations below the mean indicates BMD is below the expected range for age. In this cohort, osteoporosis should not be diagnosed on BMD results alone [128].

Bone loss is an unavoidable result of the aging process and begins around the 5^{th} decade of life, accelerating thereafter in both males and females [129]. Loss of bone that can be attributed to the natural aging process or hormonal consequences of aging, and are responsible for age-related and post-menopausal osteoporosis [46]. Age-related osteoporosis is associated with decreased muscle mass and strength, increased fat mass [100] and reduced responsiveness of the bone tissue to mechanical loading, resulting in reduced BMD and changes in bone geometry, including endosteal resorption, increased

cortical porosity and periosteal expansion to maintain bone strength [130, 131]. Diminishing postural stability with aging results in increased falls risk which in turn increases fracture risk [100, 130]. The decline in sex hormones in both males and females causes increased bone remodelling and loss of bone mass, and therefore plays an important role in the development of age-related and post-menopausal osteoporosis [130]. The rapid loss of bone mass associated with menopause in females is not seen in males, and they exhibit more trabecular thinning and less trabecular loss in comparison to females [130]. The presence of underlying medical conditions, use of medications, nutritional status and participation in weight-bearing physical activity also impacts on the development of age-related osteoporosis [130].

Secondary osteoporosis refers to bone loss that occurs due to mechanisms independent of aging [46]. It is usually an adverse consequence of an underlying chronic illness, that is not primarily skeletal in nature, or the medical management of that illness, which has negative ramifications on bone mass and bone turnover activity, and may be associated with increased risk of fracture [46, 132]. Secondary causes of osteoporosis are common in paediatrics and were found in two thirds of men, more than one half of pre-menopausal women and one fifth of post-menopausal women diagnosed with osteoporosis [129]. In paediatrics, secondary osteoporosis prevents children from attaining their genetically determined peak bone mass potential [4] and in adulthood it accelerates bone loss. Chronic illness is associated with several factors that may contribute towards the development of secondary osteoporosis, including pubertal delay and hormonal deficiencies, poor nutrition, malabsorption and low body weight, elevated levels of

inflammatory cytokines, bone marrow diseases, immobility and exposure to osteotoxic drugs including glucocorticoids, anticonvulsants and proton pump inhibitors [93, 132].

Primary osteoporosis occurs when an intrinsic skeletal defect is present which can be genetic or idiopathic in origin [94, 114]. The most common genetic cause of primary osteoporosis is Osteogenesis Imperfecta, which occurs due to mutations in the genes encoding the type 1 collagen alpha chains, resulting in bone fragility [133]. The origin of idiopathic Juvenile Osteoporosis is not clear and the mechanisms causing this disease are unknown. It has been proposed that such idiopathic presentations may be due to the inability of the osteocyte to competently perform mechanosensation and mechanotransduction, or due to a disturbance in the Wnt/β-catenin signalling pathway, culminating in abnormal bone turnover and ultimately osteoporosis [114].

1.6 Assessment of Bone Health

1.6.1 Bone Mineral Density and Bone Geometry

The evaluation of bone mineral density (BMD) requires a measurement of the mass of inorganic mineral present in a bone section, and the volume of the bone section measured. In human bone, BMD can be measured at three different levels: material BMD, which directly measures the amount of mineral in the bone matrix; compartment BMD, which measures the amount of mineral in the trabecular or cortical bone compartments; and total BMD, which measures the amount of mineral within the periosteal envelope of a bone or defined section of bone [6]. Material BMD is calculated

by dividing the mass of the extracellular bone matrix by the volume of the bone matrix occupied by inorganic mineral, thus excluding Haversian canals, marrow spaces, lacunae and cancaliculi [6]. Material BMD increases with periosteal expansion during bone modelling, and as the age of the bone matrix increases and is reduced when bone remodelling activity is increased [6]. Material BMD cannot be measured non-invasively using densitometric techniques and requires invasive procedures to obtain bone biopsies which can measure material density using backscattered electron microscopy or the relative ash weight of dried bone [6].

Compartment BMD is calculated by dividing the mass of the extracellular bone matrix by the total volume of the bone matrix [6]. In this calculation the mass is identical to the mass used in the calculation of material BMD, however the volume is larger as it includes both bone and non-bone tissue. As a result, compartment BMD will always be lower than material BMD. In trabecular bone, compartment BMD depends on trabecular number and thickness which is determined by remodelling activity and remodelling balance [6, 64]. In cortical bone, compartment BMD is influenced by the number and size of Haversian canals, which are increased when intra-cortical remodelling activity is high [6]. During childhood, trabecular and cortical compartment BMD increases by ~40%. In the trabecular compartment this is due to increased material BMD and trabecular thickness, and in the cortical compartment, due to increased material density and changes in the relative bone volume [6]. Compartment BMD can be measured non-invasively by peripheral quantitative computed tomography (pQCT), which is able to analyse the trabecular and cortical compartments separately [6].

Total BMD is calculated by dividing the mass of the extracellular matrix, including trabecular and cortical bone, by the total volume of trabecular and cortical bone [6]. Total BMD increases during childhood at sites where the relative cortical area increases, and decreases with increased remodelling activity resulting in endosteal and trabecular resorption [6]. Total BMD can be measured non-invasively using DXA, which generates an areal BMD calculated by dividing the mass of bone by the projection area, and also by pQCT [6].

1.6.1.1 DXA Scans

The measurement of bone mineral density by DXA was introduced in the 1980s for the evaluation of bone density in post-menopausal women [4]. Since this time, it has evolved into one of the most commonly used densitometric methods to assess bone health across the life span. Its popularity is due to the fact it is a commonly available, low cost, non-invasive, relatively fast and highly reproducible investigation with low radiation exposure [4, 114, 134, 135]. Furthermore, many of the common scanning sites, including total body, lumbar spine and proximal hip have age and sex-specific standard deviation scores available for interpretation [114]. DXA scans can be used to identify an individual at risk of impaired bone health, the degree to which their bone health is compromised and consequent skeletal fragility, as well as monitoring the effects of treatment regimens [135].

DXA scans utilise two different x-ray energies, which are attenuated differently by bone and soft tissue, allowing the machine to distinguish between these two tissue types. This process yields parameters of bone mineral density (BMD), bone mineral content (BMC), bone area (BA), lean tissue mass (LTM) and fat mass for the total body, lumbar spine and other regions of interest. Total body and lumbar spine scans are the preferred scan sites for investigations in children, and give information about cortical and trabecular bone respectively [136, 137]. Highly reproducible information can be obtained from these sites in children and adults, with coefficients of variation for total body scans 0.66-1.20% in children [138] and 0.7% in adults [139], and at the lumbar spine 0.64-1.03% in children [138] and 0.7-1.7% in adults [139]. The effective radiation dose of these scans in children range between 1.5-10μSv [140].

DXA derived parameters can also be used for evaluation of the functional muscle-bone unit [135, 141]. In healthy children lean tissue mass (LTM) is strongly correlated with bone size, height and pubertal stage [135]. To determine whether bone mass is adequately adapted to LTM Crabtree et al 2004 [141], following the algorithm of Schoneau et al [78], used LTM for height and BMC for LTM to identify any deficits in bone health. They were able identify that children with spinal muscular atrophy had a secondary bone defect, and children with OI, a primary bone defect, and in a group of children with fragility fractures they found that the mean BMC for LTM z-score was -1.9, indicating that low BMC for LTM is associated with increased risk of fracture [141].

The use of DXA scanning in the assessment of bone health does have some disadvantages. DXA provides a two-dimensional analysis of a three-dimensional structure, with a major limitation being the inability to account for the depth of the bone, making BMD measures size dependent [136]. This results in individuals with larger bones appearing to have greater BMD [4] and smaller, shorter individuals with underestimated BMD [142]. This is especially pertinent in the paediatric years, where bone size is continually changing, potentially impacting on the interpretation of serial measurements. Another limitation is the inability to distinguish between trabecular and cortical bone compartments or provide information about the geometric attributes of the bone [137]. Furthermore, reference data can only be utilised for scans performed using the same scanning hardware and software used to develop the reference data set.

1.6.1.2 pQCT scans

Peripheral quantitative computed tomography (pQCT) is another densitometric measurement that can be used to evaluate BMD. It was first developed in Switzerland in 1976 [143] and while to date it is used predominantly in the research arena, it has several advantages over DXA measurements and its clinical utility is being increasingly recognised. pQCT is a three dimensional technique that evaluates a cross-section of bone in the peripheral skeleton, providing a true volumetric bone mineral density (vBMD) which is independent of bone size [3, 4, 114, 144]. It also allows separate evaluation of the trabecular and cortical bone compartments reflecting their individual tissue properties and response to mechanical forces, drugs and disease processes [3, 114, 144]. Furthermore, it can evaluate the geometric attributes of the bone, as well as

biomechanical properties, by deriving the bone's strength and ability to resist bending and torsion from pQCT measurements [114, 118, 144, 145]. It can also be used to provide information about muscle mass and muscle cross-sectional area for evaluation of the functional muscle-bone unit [145].

pQCT measurements are most commonly performed at the radius and tibia, two long bones commonly affected by fractures in healthy children and individuals with limited mobility [144]. Common scan sites include 4%, 14%, 20%, 38% and 66% of the bone length measured from the bone's distal end. Scans are fast, taking approximately one minute per slice and have low effective radiation doses of <1uSv per slice [4, 118]. Highly reproducible measurements can be obtained from these sites in children and adults, with coefficients of variation for tibial trabecular vBMD and total and cortical CSA at the 4% and 66% sites in adults being less than 1%, cortical vBMD at the 66% site in adults being 2.5% [61], and for periosteal circumference and cortical thickness at the 20% tibial site in adults being 0.5% and 1.2% respectively [146].

During pQCT scanning, thresholds are nominated to distinguish between the different tissue types in the cross-section of the limb being evaluated, including fat tissue, muscle tissue, trabecular and cortical bone tissue. This process yields several parameters for the investigation of bone health including: bone mass indicators such as total, trabecular and cortical BMC, total, trabecular and cortical cross-sectional area (CSA) and total and trabecular vBMD; the bone tissue quality indicator, cortical vBMD; diaphyseal design indicators, including endosteal and periosteal circumferences, cortical thickness, cross-

sectional moments of inertia (CSMI) and the stress-strain index (SSI); and the muscle strength indicator, muscle cross-sectional area [73, 118].

Analysis of the functional muscle-bone unit can be performed using BMC and muscle CSA measured by pQCT at the 65% or 66% site of the radius or tibia respectively, where the muscle CSA is largest. Using the algorithm proposed by Schoneau et al 2002 [78], the same authors were able to characterise the bone health of children with chronic renal failure and after renal transplant. In chronic renal failure, BMC was adequate for muscle mass and muscle mass adequate for height, despite such patients being shorter than their peers, resulting in normal adaptation of the bone to its mechanical environment. After transplant however, BMC was not adequate for muscle mass despite muscle mass being adequate for height, and a primary bone defect was identified, possibly as a result of post-transplant medical management, for example, the use of glucocorticoids [80]. This example illustrates he clinical utility of pQCT measurements in the analysis of the muscle-bone unit, and the ability of such analysis to distinguish between varying alterations in bone health.

In adults, tibial bone length, which increases anterior-posterior bending forces on the underlying bone [147], and calf muscle mass, which imparts torsional deformation on the underling bone [147], were the strongest influences on the development of cortical bone mass, geometry and strength at the 14%, 38% and 66% tibial sites [73]. In children, height, and therefore tibial bone length, also had the greatest influence on cortical bone mass, geometry and strength at the 66% tibial pQCT site, with height accounting for 60-

85% of the variability in these measures, and boys consistently had higher values than girls [61]. At the 20% tibial pQCT site in pre-pubertal children, cortical bone mass, geometry and strength was highly correlated to muscle mass and muscle performance [148]. In adults, measurements of cortical vBMD at several sites along the tibia demonstrated that it remained essentially consistent for all cortical sites, was not influenced by anthropometric or mechanical factors, and was higher in pre-menopausal women compared to men [73, 118]. In children, cortical vBMD at the 66% tibial site was most strongly influenced by age, which accounted for 55% of the variability in this measure, and was greater in girls than boys, independent of height and Tanner stage [61]. In pre-pubertal children cortical vBMD at the 20% tibial pQCT site was not correlated to measures of muscle mass or muscle performance [148]. In children, muscle CSA measured at the 66% tibial pQCT site increased linearly with height, and was greater in boys compared to girls, however, cortical BMC for muscle CSA at this same site, also increased with age, but was higher in girls than boys [61].

The main disadvantage of using pQCT in the assessment of bone health is the impact of the partial volume effect. The partial volume effect refers to the incomplete filling of voxels at the edge of the bone, which results in underestimation of cortical vBMD [123] [146]. This effect has its greatest impact when cortical thickness is less than 2mm due to the higher surface to volume ratio when cortices are thinner [123]. Presence of the partial volume effect also impacts on the interpretation of age-dependent reference values for cortical vBMD, which give the most accurate reference values when cortical thickness is normal for age [123]. Other disadvantages include the need to remain still, especially

when multiple sites are being scanned, and the reproducibility of locating the same scanning sites on serial measurements and between institutions.

1.6.2 Bone Turnover Markers

Bone turnover markers are peptides released from the bone matrix during bone resorption and bone formation processes that are detectable in the blood and urine. These markers, including the bone formation markers osteocalcin [149] and alkaline phosphatase [150], and the bone resorption marker urinary deoxypyridoline [151], reflect modelling and remodelling processes occurring throughout the skeleton [74] and can be used to identify abnormalities in bone and mineral metabolism [3]. These markers peak during the adolescent growth spurt and decrease once skeletal maturity is reached [74, 151]. Higher than expected levels of bone turnover favouring bone resorption is the main cause of microarchitectural deterioration in bone, and low bone turnover states result in the accumulation of microfractures also impacting on bone health [1].

1.7 Whole-body Vibration Training

1.7.1 How it Works

Whole body vibration training (WBVT) is an emerging therapeutic modality that has gained popularity in recent years for its muscle performance enhancing capabilities and rehabilitative potential when muscle mass and performance, bone mass and density and physical function are compromised. It was first developed in the 1970s in an effort to

counteract the loss of muscle and bone mass of astronauts and to enhance athletic performance [152]. There are two types of whole-body vibration platforms commercially available, vertical and side-alternating vibration platforms. Vertical vibration platforms generate synchronous vertical vibrations where the whole platform moves up and down in a vertical direction resulting in symmetrical movement of the lower limbs [153]. Side-alternating vibration platforms rotate around a central fulcrum, similar to the action of a seesaw, generating side-alternating vertical vibrations that are applied alternatively to the left and right side of the body [153]. In contrast to the vertical platforms, side-alternating platforms produce rotational movements around the pelvis and lumbar spine [154]. The training load imposed by WBVT depends on four different parameters: frequency, amplitude, acceleration and duration [153]. Frequency refers to the number of vibration cycles applied per second, normally applied between 0-45Hz, and amplitude, to the displacement height of the oscillation, which is dependent on the distance feet are placed away from the central fulcrum in side-alternating vibration, but independent of foot position in vertical vibration and generally between 1-12mm [155]. Acceleration refers to the product of amplitude and angular velocity, and is proportional to the amount of force applied to the body during WBVT, usually ranging between 0-18g [155]. Increasing frequency and amplitude increases acceleration [156]. Duration refers to the amount of time exposed to the vibration stimulus, and is commonly applied intermittently for 30-60s intervals or continuously for 3-5 minutes [153, 157, 158]. The best settings for each of these parameters to achieve optimal gains in muscle performance, bone adaptations and functional ability is unknown, and likely differs between individuals [155].

Vibration platforms generate a forced oscillation, sinusoidal in shape, that is transferred from the platform to the human body carrying with it mechanical energy which results in acceleration of the body, eliciting reactive forces in muscles [159]. The sinusoidal oscillation causes rapid changes of direction that shorten and stretch the lower limb and trunk muscles and their tendons [159] resulting in rapid repeated concentric and eccentric muscle contractions [159, 160]. The repeated stretching of the muscle during WBVT is believed to enhance muscle spindle discharge [161] which in turn elicits excitation of the α-motor neurone via primary 1α-afferents, resulting in faster contractions of the homonymous muscle units [162], generating a reflex contraction in the muscle known as the tonic vibration reflex (TVR) [163]. The TVR may improve neuromuscular performance by increasing motor-unit synchronisation [164] and recruitment of previously inactive motor units [165] to enhance voluntary muscle contractions as well as increasing the efficiency of agonist-antagonist pairs [166]. While the mechanisms are not fully understood, it is also believed that the reflex activity of the muscle is acutely enhanced immediately after training [159], and may also contribute to heightened muscle performance after WBVT.

The effects of WBVT and the TVR on muscle activity can be observed on an electromyogram (EMG) of lower limb muscles, including the gastrocnemius, soleus and vastis lateralis [160, 167-169]. EMG activity increased as vibration frequency was increased from 15-30Hz [170], and the literature suggests that muscle activation is greatest at a frequency of 30Hz [168, 171]. EMG activity was higher when a static squat position of ~20 degrees of knee flexion was adopted compared to dynamic squatting.

Shallower dynamic squatting, up to 15 degrees knee flexion, had greater EMG activity than dynamic squats up to 35 degrees knee flexion [167], and shallow single leg squats increased EMG activity more than two leg squats [172]. Side-alternating vibration platforms increased EMG activity of the vastis lateralis and gastrocnemius muscles significantly more than vertical vibration platforms, however vertical vibration elicited greater EMG activity in the tibialis anterior muscles compared to side-alternating vibration [167]. There was lower transmission of the vibration stimulus to the upper body and head with side-alternating vibration compared to vertical vibration, and this was considered to be due to significant absorption of vibration with rotation of the pelvis and lumbar spine in side-alternating vibration [154].

The mechanical dampening properties of human muscles, which is individualised and dependent on the compliance of the lower limb joints [167], absorbs the mechanical energy, or force, generated during WBVT, and determines how it is dissipated and transmitted cephalad in the body [159, 173]. The greatest force and acceleration occurs in the muscles closest to the vibration platform and are dissipated as the vibration stimulus travels cephalad up the body [174]. The greatest absorption of force in the lower limbs occurred when WBVT was performed in 10-15 degrees of knee flexion [167], and when side-alternating vibration platforms were used [154]. Due to the influence of individual characteristics on EMG responses during WBVT, it is unlikely that a “one size fits all” approach can be taken when participating in WBVT. A study comparing WBVT performed at a frequency determined by optimal EMG activity compared to WBVT at

30Hz found jumping performance to be increased by 18% when the frequency was individually tailored [175].

The muscular contractions elicited by WBVT and the TVR impact on energy metabolism in the muscle [153]. ATP consumption in the muscle is increased and this is associated with a decrease in intracellular PCr levels [176] and small increases in oxygen uptake, which increases as frequency and amplitude are increased [177], but is less pronounced in older adults [178]. The increased muscle metabolism also increases intramuscular temperature, which increased more rapidly with WBVT than cycle exercise [179], and together these effects increase muscle perfusion [180, 181], which appears to be frequency dependent [182], and more marked when using side-alternating vibration platforms compared to vertical platforms [183, 184]. Skin blood flow in the lower limbs is also enhanced during WBVT and along with the increased muscle perfusion is likely to be responsible for the erythema and itching, which is commonly described with WBVT [185]. WBVT during a static squat did not elicit any changes in heart rate, or blood pressure [180], however performing exhaustive squatting exercise during WBVT induces muscle deoxygenation [186], an increase in heart rate (~30%), proportional to the increase in oxygen uptake [187], an increase in systolic blood pressure, and decrease in diastolic blood pressure, indicating arterial vasodilation and increased muscle perfusion [180, 185].

WBVT is therefore believed to influence muscle performance through the generation of the TVR, which improves neuromuscular performance and voluntary muscle contraction

as evidenced by augmented EMG activity and changes in muscle energy metabolism. WBVT may improve balance and functional ability due to the repeated, rapid displacements that immediately challenge balance [159] and changes in joint stiffness [188]. Improved neuromuscular performance results in enhanced agonist-antagonist pairing [189, 190], which positively effects joint stiffness [191, 192], resulting in improvements in joint stabilisation, particularly of the ankle and knee [189, 193, 194] and which is likely to contribute to improved balance and function [189, 190, 195].

WBVT is thought to induce its effects on bone indirectly, through mechanical loading of the skeleton by skeletal muscle contractions which have improved neuromuscular efficiency and performance, and directly by mechanical deformation of the underlying bone causing perturbations in canalicular fluid flow and stimulation of the osteocyte and the WNT/β-catenin pathway [196].

1.7.2 Effects on Bone

The first group to investigate the effects of WBVT on bone in children was Ward *et al* in 2004 [197]. A randomised controlled trial (RCT) was performed in 20 children and adolescents with disabling conditions and motor impairment, including cerebral palsy and muscular dystrophy, with half receiving low magnitude WBVT performed 10 minutes a day, 5 times per week for 26 weeks as part of a standing program [197]. Proximal tibial and spine trabecular vBMD measured by QCT increased 6.27mg/ml (6.3%) and 7.29mg/ml (5.5%) respectively with the control group demonstrating reductions in both parameters. Tibial cortical bone parameters including cross-sectional area, periosteal

circumference, vBMD, pSSI, cortical thickness and muscle area were not altered with WBVT [197]. In a study of 20 children with cerebral palsy randomised to WBVT, 9 minutes a day, 5 times a week for 26 weeks, or conventional physiotherapy, lumbar spine BMD measured by DXA increased slightly more in the WBVT group, and lateral distal femur BMD decreased in the WBVT group compared to the physiotherapy group, however these differences between groups were not statistically different [198]. In a randomised cross-over study of 30 children with cerebral palsy participating in a daily standing program with or without the addition of WBVT, pQCT measures of tibial cortical bone area and moment of inertia increased significantly after WBVT however trabecular vBMD in the tibia and lumbar spine did not differ between groups [199]. In a third study of children with CP using WBVT as part of an intensive physiotherapy program also utilising resistance training and treadmill walking, total body BMD and BMC increased 2.3% and 5.74% after 6 months training respectively, and improvements were greater in children less than 10 years of age compared to children over 10 years of age [200]. In a more recent study, 30 children with spastic diplegic CP, 10-13 years of age were randomised to either a conventional exercise program or the same program with the addition of WBVT, 10 minutes a day, 5 times a week for 24 weeks. Children in the WBVT group significantly increased their lumbar spine BMD by 0.16mg/cm^2 and femur BMD by 0.27mg/cm^2, these improvements significantly greater than the improvements seen in the children participating in the conventional training alone [201]. A recent systematic review and meta-analysis for the effectiveness of WBVT for bone density in children and adolescents with cerebral palsy found that femur bone density was increased

significantly with WBVT, reporting a standardised mean difference of 1.32, however there was no significant improvements in lumbar spine bone density with WBVT [202].

WBVT has also been found to have beneficial effects on bone in other cohorts of children. In children with Osteogenesis Imperfecta, 6 months of WBVT, 9 minutes a day, as part of an intensive physiotherapy program also utilising resistance training and treadmill walking, resulted in significant increases in total body BMD and BMC however their corresponding z-scores did not show significant changes [203]. In 6 ambulant boys with Duchenne Muscular Dystrophy, 3 months of WBVT, 6 minutes a day, demonstrated a significant improvement in total body BMC measured by DXA, but no significant improvements in parameters measured by tibial pQCT at the 4% and 66% sites [204]. In eight girls with endocrine disorders, WBVT for 30 minutes, 3 times a week for 8 weeks improved trabecular vBMD of the spine and cortical vBMD of the femur [205], and in adolescent girls with idiopathic scoliosis randomised to WBVT for 20 mins, 5 times a week for 52 weeks, or control, femoral neck BMD and lumbar spine BMC improved significantly with WBVT compared to controls, however no differences between groups in distal tibial high resolution pQCT parameters were found [206]. In young women with low BMD measured by DXA and a history of at least one fracture, WBVT 10 minutes daily for 52 weeks compared to controls showed significant improvements in lumbar spine trabecular vBMD, femoral cortical bone area measured by QCT in those in the WBVT group [207]. In adults, 19-38 years, randomised to WBVT 4 minutes, 3-5 times a week for 8 months or control, there were no significant differences in any lumbar spine or hip DXA parameters or tibial pQCT parameters between groups [208].

In post-menopausal women, a systematic review and meta-analysis including five RCTs [209-213], found a statistically significant treatment effect of WBVT for hip BMD with a standard mean difference of 0.015g/cm^2, however there were no treatment effects of WBVT on lumbar spine BMD or tibial trabecular vBMD [214]. When total hip and femoral neck analyses were analysed separately, the results remained significant for the total hip only with a standard mean difference of 0.014g/cm^2, and the effects of WBVT on hip BMD were significant when WBVT was compared to sham or no treatment, but lost significance when compared to an exercise intervention [214]. In a more recent trial of osteopoenic post-menopausal women randomised to WBVT, 10 minutes, 2-3 times a week for 12 months, or control, there were no differences between groups in femoral neck or lumbar spine BMD or on bone architecture measured by high-resolution pQCT [215].

In the older adult population, recent systematic reviews and meta-analyses found that WBVT significantly improved femoral neck BMD in comparison to conventional exercise interventions with standard mean difference of 0.04g/cm^2 [216], however there were no differences between WBVT and control or exercise intervention groups for total hip BMD [217] and lumbar spine BMD [216, 217], even when osteoporosis was diagnosed [218]. A study investigating the effects of WBVT in older adults using tibial pQCT also found no changes in any pQCT parameters after WBVT [212]. WBVT also does not appear to impact on bone turnover markers in older adults [219], after stroke [220], in healthy young adults [208], and in postmenopausal women [211, 221].

However, another study in post-menopausal women found a reduction in bone resorption markers after 8 weeks of WBVT, and 12 weeks of WBVT in older adults increased bone formation and resorption markers [222].

The lack of consensus in the literature regarding the effects of WBVT on bone mass, geometry and strength is largely due to the widely varying treatment protocols utilised, however it does appear that the bone-related benefits of WBVT are greatest in children and potentially most effective in the pre-pubescent age range when the ability of the skeleton to adapt to mechanical stimuli is at its peak. It is also apparent from the literature that adherence to the training regime influences outcomes in bone parameters, with greater adherence associated with better outcomes at the femoral neck [211] and lumbar spine [207]. A systematic review by Matute-Llorente et al in 2014 investigating WBVT in children and adolescents, suggested that WBVT would be most osteogenic when it was performed for 10-20 minutes, more than 3 times a week, for a minimum of 26 weeks with a frequency of 25-35Hz and an amplitude of 4mm [205].

1.7.3 Effects on Muscle

Several systematic reviews and meta-analysis have been conducted into the effects of WBVT on muscle performance. When compared with a control group performing no additional exercise, WBVT had a significant positive effect on jump height with a standardised mean difference of 0.77, with effects greater in non-athletes, when interventions lasted longer than 3 months, when frequency was over 30Hz, amplitude greater than 3mm, and the duration of exposure each treatment session more than 10

minutes [223]. When compared to an exercise intervention, WBVT had a significant positive effect on jump height with a standardised mean difference of 0.63, with side alternating platforms showing a greater standardised mean difference of 0.81 compared to the average [223]. In another study, WBVT was also found to have a significant influence on jump height, with a standardised mean difference of 0.87, which was greater in the young adult group, (standardised mean difference of 1.00), compared to the older age group, (standardised mean difference of 0.60), likely due the fact the young adult group generally performed exercises during the WBVT, and that in the elderly, a longer training period is required for the same improvements in muscle performance [224]. WBVT frequencies greater than 30Hz had a significant additional effect with a standardised mean difference of 1.38 and amplitudes less than or equal to 4 mm had significant effects on muscle power, with a standardised mean difference of 1.25 [224]. A third systematic review also found long-term WBVT to have a significant effect on muscular power production, with an effect size of 0.99 that was larger in older adults, (2.24) than younger adults (0.43), greater when dynamic exercises where performed during the WBVT, effect size 1.16 compared to 0.72 during isometric exercise, and larger when the WBVT was progressive, with increases in amplitude and frequency during the training period, with an effect size of 1.19, compared to 0.39 with.no progression [225]. This study also found frequencies over 30Hz to be most effective, however they also found amplitudes greater than 6mm to be most effective, with 6-12 minutes of training with shorter training intervals and vertical platforms to be more effective [225].

The addition of WBVT to a conventional training program significantly increased knee extensor strength with a standardised mean difference of 0.76 [224]. When the young adult and older adult groups were analysed separately, the effects on knee extensor strength were not significant for the young adult population but remained significant in the older adult population with a standardised mean difference of 0.47 [224]. WBVT frequencies greater than 30Hz had a greater effect on knee extensor strength compared to frequencies less than 30Hz, with standardised mean differences of 1.12 and 0.41 respectively, and amplitudes up to 4mm had a significant effect on knee extensor strength, with a standardised mean difference of 1.08, whereas amplitudes greater than 4 mm did not have a significant influence [224]. In another study, chronic WBVT significantly improved knee extension strength, with an effect size of 1.24 which, was smaller in young adults compared to older adults, with effect sizes of 1.18 and 1.83 respectively [225]. Females demonstrated greater effect sizes than men (1.53 compared to 0.75), untrained participants had greater effect sizes than trained participants (1.78 and 0.54 respectively), and WBVT protocols including dynamic exercises had a greater effect size, (1.40), in comparison to isometric muscle contraction, (0.78) [225]. Effect sizes increased with higher frequencies, amplitudes and dose, with 12-15 minutes of WBVT each session associated with maximum gains in knee extensor strength [225]. These improvements in knee extensor strength associated with WBVT are considered to be similar to those seen in conventional resistance training programs, however these effects appear to only occur with vertical platforms and not side-alternating platforms [225].

The effects of WBVT on muscle in children has been investigated in several disease cohorts including Cerebral Palsy (CP), Osteogenesis Imperfecta (OI), Duchenne Muscular Dystrophy (DMD), Spinal Muscular Atrophy (SMA), Down syndrome and Cystic Fibrosis. In children with CP who undertook 6 months of WBVT, nine minutes a day, as part of an intensive physiotherapy program also utilising resistance training and treadmill walking, total body lean tissue mass increased 3.11% [200]. In another study of children with CP randomised to WBVT, 18 minutes, 3 times a week for 8 weeks, or control, tibialis and soleus muscle thickness increased significantly in the WBVT group [226]. In contrast, WBVT did not increase lean tissue mass or muscle cross sectional area in children with disabilities [197], CP [199], DMD [204] and Down syndrome [227].

The effects of WBVT on muscle strength in children have also demonstrated variable results. In boys with DMD, three months of WBVT, 6 minutes, 2-3 times a week, did not improve knee extensor, plantar flexion or dorsiflexion strength [204], and another study of children with DMD and SMA, WBVT 9 minutes a day, 5 times a week for 8 weeks, also found no improvement in leg muscle strength after WBVT [228]. A similar result was seen for leg muscle strength after 4 weeks of WBVT, 10-15 minutes, 3 times a week, in children with cystic fibrosis [229], and in children with CP no difference in calf muscle strength was found between groups after 6 months of WBVT for 10 minutes daily or a standing program [199]. In contrast, muscle force increased significantly after six months of WBVT, 18 minutes daily, in children with OI [230, 231] and in young adults with cystic fibrosis, 6 minutes of daily WBVT for six months significantly increased muscle force during one-leg hopping [232], however in another study of WBVT, 6 minutes, 5

times a week for three months, in young adults with cystic fibrosis muscle force during one-leg hopping did not improve [233]. In both of these studies in young adults with cystic fibrosis, jump performance increased significantly after WBVT [232, 233]. Muscle force also increased in children with CP who performed WBVT, 9 minutes a day for six months, as part of an intensive physiotherapy program also utilising resistance training and treadmill walking [200], and significantly more in children with CP who participated in WBVT, 9 minutes, 5 times a week for three months, compared to children participating in a conventional physiotherapy program [234]. In young adults with CP, WBVT for 2-6 minutes, or resistance training 3 times a week for eight weeks significantly improved muscle strength, however there was no difference between groups [235]. A recent systematic review and meta-analysis investigating the effects of WBVT in CP in children did not find that WBVT had a significant effect on muscle strength when compared with a control group [202]. Much of the divergence in strength outcomes after WBVT in children and young adults can be attributed to the small number of studies, their small sample sizes, the lack of RCTs and the variability in vibration training protocols, including type of plate, frequency of training, intensity and volume. A systematic review of the effects of WBVT for strength in children and adolescents suggests that muscle strength gains occur when WBVT is performed daily for 10-20 minutes, at a frequency of 15-30Hz, an amplitude of 1-4mm and a duration of 26 weeks [205].

In healthy young adults, a progressive WBVT protocol for 4 minutes, 3-5 times a week for four [236] and eight months [208] duration, in comparison to a control group, showed

no improvement in isometric knee extension strength, however jumping performance improved significantly in the WBVT groups in both studies, with jump height increasing 8.5% [236] and 7.8% [208] respectively. In young adult women, 12 weeks of WBVT, 3 times a week with knee extension exercises compared to the same exercises without vibration, a lower limb resistance training group and control, found that jump performance increased significantly in the WBVT training group only, with a 7.6% increase in jump height, and knee extension strength increased significantly in the WBVT and resistance training group compared to placebo and control groups, however there were no differences in the effects of training between these two groups [169]. In a study of young men comparing the effects of nine weeks of training, 2-3 times a week, between WBVT with weighted squatting exercises, WBVT and unweighted squatting exercises, and floor based weighted squatting exercises, similar improvements in leg strength and jump performance were demonstrated in all groups [237]. In two further studies of physically fit students, WBVT for 5-8 minutes, 3 times a week for 11 weeks compared to control demonstrated no changes in muscle strength or jump performance after WBVT or between groups [238], and WBVT with progressive exercises compared to the same exercises performed on the floor 3 times a week for four weeks, found no differences in jump performance after the intervention in either group, however muscle endurance increased significantly in the WBVT group compared to the conventional training group [188]. These studies suggest that WBVT may be equivalent to conventional resistance training in young healthy adults, but does not appear to consistently provide an additive effect to conventional training programs.

In post-menopausal women, WBVT for 3-6 mins, 2 times a week for 24 weeks compared to control significantly improved jump performance by ~5%, due to increases in power but not force [212]. In another study, 24 weeks of progressive WBVT with exercises for 3-20mins, 3 times a week compared to conventional resistance training and control demonstrated significant improvements in jump performance (~20%) and knee extensor strength (~15%) in both the WBVT and resistance training groups, but not in the control groups, with the majority of the improvement seen in the first three months of WBVT [239]. A similar study comparing WBVT for 30 minutes, 3 times a week for 24 weeks, with conventional resistance training and control also found significant improvements in knee extensor strength for the WBVT and resistance training groups but not the control group [213]. WBVT, 6 minutes, 3 times a week for eight months compared to a 60 minute walking program at 70% maximum heart rate did not find any improvements in knee strength in either group, however vertical jump performance improved significantly (~7%) in the WBVT group [240]. In a more recent study, WBVT 5 mins, 2-3 times a week for twelve months vs control did not show any improvements in jump height or knee extensor strength after WBVT [215]. Similar to the studies in young adults, WBVT in post-menopausal women indicate that WBVT may be as effective as conventional resistance training protocols in improving knee extensor strength and jump performance, however the improvements with WBVT appear to be accomplished with much shorter time requirement compared to conventional training regimes [213].

In older adults, two recent systematic reviews and meta-analyses have sown that when WBVT was compared to conventional exercise programs there were no between group

differences in the improvements in knee extensor muscle strength of jump performance [217, 241]. However, when WBVT was compared to the control state, isometric knee extension strength was significantly improved with WBVT, with a standardised mean difference of 2.15 [241], and dynamic knee extension strength was also significantly improved with WBVT, with a standardised mean difference of 0.63 [217]. Power generated during dynamic exercises was also significantly higher after WBVT, with a mean difference of 10W [241], and leg press performance improved significantly after WBVT, with a standardised man difference of 0.57 [242]. In a study of older men, WBVT with execises for 40 minutes, 3 times a week compared to 90 minutes of conventional exercise, including cardiovascular, resistance and balance exercises, or control, femoral muscle mass increased ~3% in both the exercise and WBVT groups but not in the control group [243]. A more recent study, comparing WBVT to sham in combination with a falls prevention program in elderly participants at risk of falling, found that leg power increased significantly with WBVT compared to control [222]. Another study WBVT in older adults reported that standing position on the platform and posture adopted likely influences the response to WBVT, as improvements in muscle strength and power appeared to be greater when participants stood on the plate with flexed knees [244]. The same authors also reported that significant gains in muscle performance occurred when studies were a minimum of eight weeks duration and used side-alternating platforms with frequencies of 26-28Hz [244].

The effects of WBVT on muscle function have also been investigated in neuromuscular diseases including stroke and multiple sclerosis. A systematic review and meta-analysis

in stroke patients found that WBVT compared to control or another intervention did not have a significant treatment effect on knee extensor muscle strength [245]. Another systematic review [246] found that when WBVT in stroke patients was compared to an identical program without WBVT there were no significant treatment effects for knee extension strength [247] and there was no change in rectus femoris, vastus lateralis or medial gastrocnemius thickness [248], however, when WBVT was compared to convention physiotherapy, WBVT induced significantly greater improvements in knee extension strength [249, 250]. In multiple sclerosis, a systematic review [251] found that progressive WBVT, 50 sessions over 20 weeks, 2.5-16.5 minutes, compared to control, did not improve knee extension strength [252], whilst four weeks of WBVT, 3 times a week for 7 minutes, compared to the same exercises without WBVT significantly improved knee extensor strength, however there was no difference between groups [253]. In a case report of the use of WBVT in a young adult with late onset Pompe disease, 4 minutes, 3 times a week for 15 weeks, resulted in increased jump performance, and was a favourable alternative to conventional exercise regimes which cause fatigue and shortness of breath and require high levels of motivation and effort [254].

1.7.4 Effects on Function

In children with CP, a systematic review and meta-analysis [202] of two studies [198] [255] found that WBVT significantly increased performance in the Gross Motor Function Measure (GMFM) walking, running and jumping domain, with a mean difference of 2.97 [202], however the improvement in the standing domain did not reach significance [202]. Another study in children with CP using six months of WBVT, 9 minutes a day, as part

of an intensive physiotherapy program also utilising resistance training and treadmill walking, a 10% improvement in the GMFM sitting domain was found, which remained significant in children less than 10 years but not over 10 years of age, a 14% increase in the crawling and kneeling domain, a 35% increase in the standing domain, which was only significant in children under 10 years of age, and an 8% improvement in the walking, running and jumping domain [200]. WBVT was found to significantly improve gait speed by 0.13m/s in a systematic review and meta-analysis [202] of two studies [198] [226], however in young adults with CP, a progressive WBVT 4-6 minutes, 3 times a week for eight weeks, compared to resistance training, did not improve gait function measured by the six minute walk test and timed up and go and there was no improvement seen in the GMFM [235]. In children with CP randomised to WBVT, 9 minutes, 5 times a week for three months, as well as routine physiotherapy or routine physiotherapy alone, the children receiving the WBVT had significantly more improvement in their stability indices in the anterio-posterior and medio-lateral directions as well as overall [234].

In children with OI participating in six months of WBVT, 9 minutes a day, as part of an intensive physiotherapy program also utilising resistance training and treadmill walking, one-minute walking distance increased 43% [203]. Other studies in children with OI have also found improvements in walking ability and independence [256, 257]. In children with spina bifida, WBVT for 9 minutes twice a day, 5 times a week for six months with ongoing physiotherapy intervention improved gait speed by 0.11m/s, and demonstrated significant improvements in the GMFM total score, and in the standing and walking, running and jumping domains [258]. In children with DMD and SMA, WBVT 9 minutes

twice daily, 5 times a week for four weeks demonstrated improvements in the distance walked in the six-minute walk test, however this was only significant in the children with SMA, who had an 8% improvement [228]. Another study of WBVT in DMD, 2 minutes, 3 times a week for four weeks, also found no improvement in mobility [259]. In young adults with cystic fibrosis, WBVT 6 minutes a day, 5 times a week for three months significantly improved chair rise test performance [233].

In older adults, a systematic review and meta-analysis comparing WBVT to conventional exercise or control did not find a significant effect of WBVT for the timed up and go test [241], however another systematic review and meta-analysis found that when WBVT was compared to control, timed up and go performance was significantly improved after WBVT, with a standardised mean difference of 0.34, but there was no difference between WBVT and conventional exercise programs in improving timed up and go performance [260]. Similarly, another group found that chair rise test performance was significantly improved after WBVT compared to control, with a standardised mean difference of 0.72, however WBVT compared to an exercise intervention had equivalent effects [217]. Conflicting results for the effect of WBVT on walking speed were found in a systematic review and meta-analysis [260].

The effect of WBVT on function has also been investigated in neuromuscular diseases including Parkinson's Disease (PD), Multiple Sclerosis (MS) and stroke. A Cochrane review on the effect of WBVT for PD found no difference in disease score, balance or gait performance between WBVT and exercise interventions [261], and this was

supported by another systemic review [262]. PD patients randomised to WBVT, 30 minutes, 5 days a week for three weeks or balance training, demonstrated significant improvements in balance, however these improvements were not significantly different to the conventional balance training group [263]. There is some evidence in the literature that long term WBVT may have an impact on health related quality of life in patients with PD [263, 264]. In MS, WBVT did not demonstrate a superior effect on gait [262] when compared to an exercise program [265] or control [252], and no differences in quality of life were found when WBVT was compared to an exercise intervention [252]. However, eight weeks of WBVT in patients with MS resulted in significant improvements in 10m walk test, timed up and go and standing balance [266]. A systematic review and meta-analysis of the effects of WBVT on muscle function after stroke found that long term WBVT significantly improved timed up and go performance in comparison to a control or exercise intervention, however there was no difference in the improvements between WBVT and other interventions for the 10m and six-minute walk tests [245]. The same authors did not find any benefit of WBVT in comparison to control or exercise interventions on balance, measured by the Berg Balance Scale, after stroke [245], however another systematic review [246] suggested that WBVT compared to no intervention significantly improved postural control [250], but this superior effect of WBVT was not maintained when WBVT was compared to an exercise intervention [247, 267]. When WBVT with exercises was compared to the same exercises without WBVT after stroke, there were no between group differences in the incidence of falls [268]. Furthermore, WBVT in polio sufferers, 10 minutes, twice a week for five weeks, did not improve timed up and go or six-minute walk performance [269].

In summary, WBVT appears to be equally effective in improving muscle strength and mobility as conventional exercise interventions, however it has the benefits of achieving these outcomes in less time [262, 270, 271], in a small space and without requiring technical abilities [272], making it an alternative treatment strategy for improving muscle performance, balance and function, and optimising bone health in exercise intolerant or mobility limited individuals who are unable to participate in more conventional high impact, high intensity exercise interventions [260, 270, 273-275]. WBVT is easily integrated into daily life [228], simultaneously exercises muscles in the lower limbs and trunk, does not generate the fatigue and lack of motivation associated with participating in conventional aerobic and resistance interventions [271], and as a result is an attractive modality for use as an adjunct in physical rehabilitation programs [261].

1.7.5 Adverse Events

No serious adverse events associated with WBVT have been reported in the literature. Common mild adverse effects include muscle soreness [195, 228, 245, 260, 271], itching in lower limbs [214, 225, 241, 245, 260, 271], erythema [214, 225, 228, 241, 260, 271], foot, groin and knee pain [195, 225, 241, 245, 260, 271], joint effusion [195, 225, 260], headache [195, 241, 260, 271] and mild dizziness [245]. These adverse effects are generally mild and transient, subsiding after the first few sessions as training progresses [195, 228, 241, 260].

1.8 Mitochondrial structure, function and genetics

Mitochondria are small, cytoplasmic, membrane bound organelles, measuring 0.5-1.0μm in diameter, found in the majority of eukaryotic cells [276]. A primary role of the mitochondria is the generation of cellular adenosine triphosphate (ATP) from the metabolic substrates, glucose and fatty acids, through oxidative phosphorylation (OXPHOS). The generation of ATP provides eukaryotic cells with a useable form of energy that fuels intracellular processes [276-281]. The mitochondrial content of a cell is dependent on the tissue's requirement for ATP [277]. Mitochondria are also involved in a number of other processes required for cellular functioning [279, 282, 283] including the citric acid cycle (TCA), parts of the urea cycle, calcium homeostasis [284, 285], regulation of apoptosis [277, 286], intracellular signalling [287], iron sulphur biogenesis, the formation of free radicals [288] and reactive oxygen species [289], and they are believed to contain over 1000 proteins [290, 291].

1.8.1 Mitochondrial Genetics

Mitochondria are eukaryotic organelles that are under dual genetic control from both nuclear deoxyribonucleic acid (nDNA) as well as mitochondrial DNA (mtDNA), for may cellular functions, including OXPHOS [281-283, 292-295]. The origin of this dual genetic control dates back nearly two billion years, when early eukaryotic cells were colonised by bacteria. These early eukaryotic cells derived their energy from anaerobic glycolysis, a much less efficient pathway than the aerobic metabolic pathways utilised by the bacterial cells. A symbiotic relationship developed between the eukaryotic cells and

the bacteria, and with evolution this relationship became permanent, with bacteria evolving into mitochondria, providing a pathway for aerobic metabolism within eukaryotic cells [280, 296]. The mitochondria however, retained their own DNA from their bacterial origins. Throughout evolution, the mitochondrial genome has lost the majority of its genes and autonomy to the nuclear genome. Modern day mitochondria are dependent on nuclear genes and their products for all their basic functions including mtDNA replication and translation, the synthesis of mitochondrial respiratory chain (MRC) subunits, and the synthesis of phospholipids, the structural components of the inner mitochondrial membrane in which the MRC resides and where OXPHOS takes place [283, 293, 296-298].

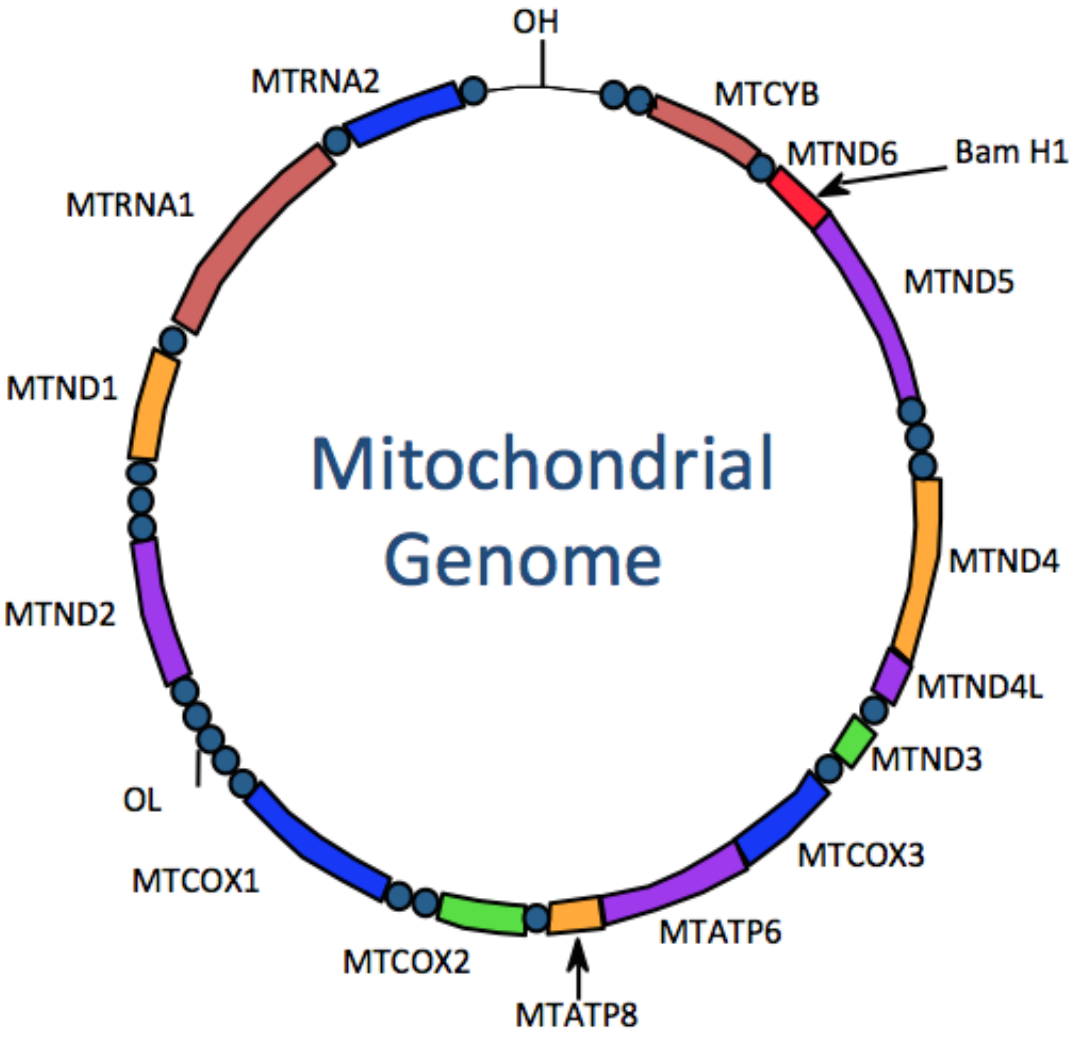

Figure 1.1: Mitochondrial genome.

The mitochondrial genome (Figure 1.1) was first discovered in 1963 [299, 300] and has many features akin to its bacterial heritage [298]. It is a double stranded, circular molecule consisting of 16,569 base pairs, predominantly composed of exons, with no introns, infrequent non-coding bases between genes and no termination codons [301]. There is one small, non-coding region in the outer strand, the displacement loop, where mtDNA transcription and replication regulatory factors bind [282, 288]. Replication of

the mitochondrial genome, similar to bacterial replication, is bidirectional and asynchronous, with the outer, heavy-stand, of the double stranded circular molecule, undergoing replication first, followed by replication of the inner, light-strand [290]. The mitochondrial genome encodes 37 gene products: 13 MRC subunits contributing to complexes I, III, IV and V; 2 ribosomal ribonucleic acids (rRNA), a large one, 16S, and a small one, 12S; and 22 transfer RNA (tRNA), with the rRNA and tRNA required for mtDNA translation [279, 281, 295-298, 302]. The majority of the mtDNA encoded gene products are located on the heavy-strand, however one MRC subunit and 8 of the 22 tRNA are located on the light-strand. All other MRC subunits, proteins required for the maintenance and expression of mtDNA and mitochondrial protein synthesis are encoded by the nuclear genome, and are believed to number around 1700 [281, 295, 298, 302-305], indicating significant interdependence between the mitochondrial and nuclear genomes and the importance of competent inter-genomic signalling [290]. Nuclear encoded MRC subunits are synthesised in the cell cytoplasm, transported into the mitochondria and assembled with mtDNA encoded subunits, synthesised within the mitochondrial matrix, to form the MRC complexes [282, 306, 307].

Mitochondrial genetics, like mitochondrial replication, transcription and translation, also have characteristics of their bacterial ancestors and differ from mendelian genetics and nDNA mechanisms [290, 295, 304]. In contrast to the laws of mendelian genetics, all mtDNA is maternally inherited [308], thousands of copies of mtDNA coexist in a single cell and not all copies of mtDNA are identical [281, 283, 288, 297, 309, 310]. The coexistence of wild-type and mutant mtDNA is known as heteroplasmy [311], and next

generation sequencing has suggested that heteroplasmy exists in low levels, 0.2-2%, in all individuals [312], and only becomes pathogenic when a critical threshold of mutant mtDNA accumulation is reached, the threshold effect [281, 297, 309, 313]. Disease manifestation usually occurs when mutant mtDNA constitute in excess of 80% of all mtDNA in a cell [297], however the pathogenic threshold varies between tissue types depending on their utilisation of OXPHOS and dependence on aerobic metabolism [281, 282] and provides an explanation for tissue selectivity in MRCD [314]. Paradoxically, mutation load, and therefore genotype and clinical phenotype, can change over time [315]. During replicative segregation of the mitochondrial genome, the allocation of wild-type or mutant mtDNA to daughter cells is random, resulting in fluctuations around the pathogenic threshold, explaining pathologic variation within and between tissues. Variation in mutant load also underpins the clinical variability seen in individuals with the same disease [281, 282, 288, 297, 309, 316]. Finally, shifts in the frequency of pathogenic mtDNA mutations between generations may be explained by the bottleneck theory. This theory postulates that during oogenesis, a bottleneck occurs which may cause the segregation of a subsample of mtDNA carrying a high mutation load, resulting in increased frequency of pathogenic disease in the subsequent generation despite absence of disease in the previous generations [278, 317-319].

1.8.2 Mitochondrial Structure

Mitochondria are divided into four compartments, the outer membrane, the inner membrane, the inter-membrane space and the matrix. The outer membrane is smooth and is separated from the inner membrane by the inter-membrane space. The inner membrane

encapsulates the matrix and contains many folds, known as cristae, which protrude into the matrix. The mitochondrial respiratory chain and enzymes required for OXPHOS are embedded in the inner mitochondrial membrane. The matrix contains enzymes required for the citric acid cycle, the pyruvate dehydrogenase complex and β-oxidation of fatty acids as well as chaperones for mitochondrial protein import and the assembly of OXPHOS enzymes and finally, mtDNA [276, 279]. Each compartment and their respective functions have critical roles in ATP generation.

1.8.3 ATP Generation - The Mitochondrial Respiratory Chain

The majority of cellular ATP is generated by OXPHOS, which is achieved through a series of biochemical events that occur along the mitochondrial respiratory chain (MRC) [296, 302]. The MRC is comprised of five functionally coupled multimeric protein complexes (Figure 1.2). Four of these complexes and two mobile electron carriers make up the electron transport chain, which provides links to the TCA and access to reducing equivalents from intermediary metabolism, and catalyses electron transfer to oxygen to form water, creating with it a proton gradient which is used by the fifth and final complex to generate ATP [276, 278, 281, 297, 298].

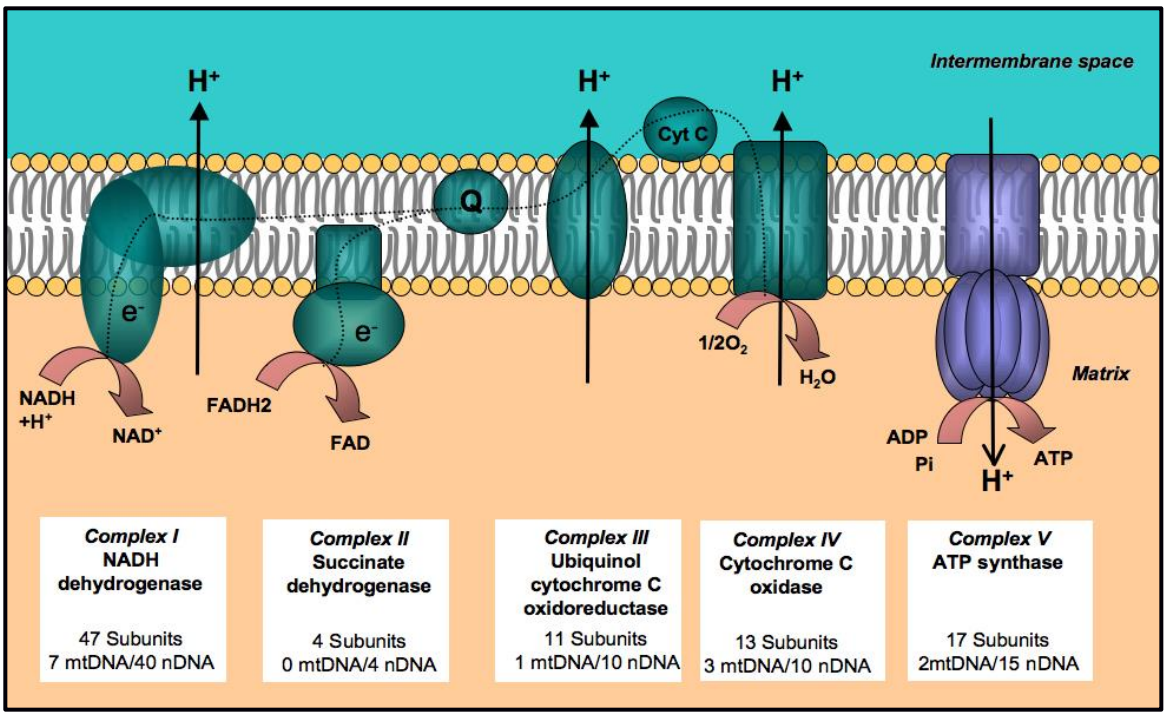

Figure 1.2: Mitochondrial Respiratory Chain. Source: www.bioscience.org/2009/v14/af/3509/fig5.jpg.

1.8.3.1 Complex I

Complex I, reduced nicotinamide adenine-dinucleotide (NADH) dehydrogenase-ubiquinone (CoQ10) reductase, is the largest and heaviest of the 5 MRC complexes and consist of at least 46 subunits, 7 or which are encoded by mtDNA, ND1-4, ND4L, ND5 and ND6 [280, 292, 302]. Pyruvate, generated from the glycolysis of glucose in the cytoplasm of the eukaryotic cell, is carried into the mitochondrial matrix where it is converted into acetyl-CoA by the pyruvate dehydrogenase complex. In another pathway, cytoplasmic fatty-acyl-CoA is carried into the mitochondrial matrix and converted into acetyl-CoA via β-oxidation. The acetyl-CoA then enters the TCA and NADH is generated [279]. NADH enters complex I which catalyses the transfer of electrons from

NADH to CoQ10 and expels protons across the inner mitochondrial membrane into the inter-membrane space [290, 320]. The electron carrier CoQ10 is encoded solely by nDNA [280, 298].

1.8.3.2 Complex II

Complex II, succinate-ubiquinone reductase, is the smallest of all the MRC complexes, consisting of only 4 subunits which are all located on the matrix side of the inner mitochondrial membrane functioning as part of the TCA as well as the electron transport chain, with all subunits are encoded by nDNA. Reduced flavin adenine-dinucleotide ($FADH_2$), generated in the TCA, enters complex II which catalyses the transfer of electrons from $FADH_2$ to CoQ10 via the complex's iron-sulphur cluster. As Complex II does not span the inner mitochondrial membrane, it is the only complex of the electron transport chain that does not expel protons from the matrix to the inter-membrane space [280, 290, 292, 298, 302, 320-322].

1.8.3.3 Complex III

Complex III, ubiquinone-cytochrome c reductase, consists of 11 subunits of which only one is encoded by mtDNA, cytochrome b. Electrons transferred from complexes I and II to CoQ10, are transferred from the fully reduced CoQ10 to cytochrome c via the catalytic action of complex III. Energy from the transfer of these electrons expels protons from the matrix to the inter-membrane space [280, 290, 292, 298, 302, 321].

1.8.3.4 Complex IV

Complex IV, cytochrome c oxidase, consists of 13 subunits, 3 of which are encoded by mtDNA, COX I, COX II and COX III, which contribute to the catalytic core. Complex IV is the final complex in the electron transport chain and catalyses the transfer of electrons, from cytochrome c, to oxygen to form water, producing energy which expels protons from the matrix to the inter-membrane space [280, 292, 302, 320-322]. The electron carrier cytochrome c is encoded by nDNA [280, 298].

1.8.3.5 Complex V

Complex V, ATP synthase, consists of 16 subunits, 2 of which are encoded by mtDNA, ATPase 6 and ATPase 8 [280, 292, 302]. The protons expelled from the matrix to the inter-membrane space by complexes I, III and IV create a proton gradient which is then used by complex V to generate ATP as the protons flow back into the matrix, driven by the proton gradient, through this complex [280, 281, 292, 297, 298, 302].

1.9 Mitochondrial Respiratory Chain Disorders

Mitochondrial respiratory chain disorders (MRCD) occur when the ability of the mitochondria to perform OXPHOS and generate ATP is impaired [296, 304]. MRCD can be caused by mutations in either the mitochondrial or nuclear genomes due to the bi-genomic regulation of the MRC. Pathogenic mutations in mtDNA can be sporadic large-scale deletions, or maternally inherited point mutations, affecting mitochondrial protein

synthesis. Mutations in nDNA follow mendelian genetics and can be autosomal dominant, autosomal recessive or X-linked. Nuclear DNA mutations causing MRCD include mutations in genes encoding MRC subunits, genes encoding MRC assembly proteins, genes associated with mtDNA translation, genes involved in the structural composition of the inner mitochondrial membrane, genes affecting mitochondrial dynamics and genes interfering with mitochondrial DNA maintenance [281, 290, 296, 297, 302, 305, 309, 321, 323-325].

1.9.1 Mutations in mtDNA

Mutations in mtDNA were first identified in 1988 [311, 326] and since then over 120 large-scale deletions, 260 pathogenic point mutations and numerous non-pathogenic polymorphisms have been identified [281, 282, 288, 298, 321, 327]. MRCD caused by mutations in mtDNA are generally heteroplasmic, with pathogenic features and severity reflective of the tissues affected, their pathogenic thresholds and dependence on oxidative metabolism and the individual's mutation load [282]. Mutations in mtDNA are the most common cause of adult onset mitochondrial disease [328].

1.9.1.1 Large-scale deletions

Large-scale deletions, or rearrangements, in mtDNA are generally sporadic, occurring early in foetal development, and involve a single, large deletion, the size and position of which can differ, but which appear to occur in regions of the mitochondrial genome adjacent to repeated sequences [329]. One proposal is that these single large-scale

deletions may occur during the repair of damaged mtDNA [330] and often affect multiple genes and their associated proteins. MRCD caused by single large-scale deletions include Kearns-Sayre Syndrome (KSS), Pearson Syndrome and Progressive External Opthalmoplegia (PEO) [281, 282, 290, 296-298, 321, 324, 325].

1.9.1.2 Point mutations

Point mutations in mtDNA are usually maternally inherited and affect genes responsible for the synthesis of proteins essential for the formation of MRC subunits or tRNA and rRNA function, resulting in heteroplasmic disorders and multi-system disease [281, 290, 296, 297, 309, 323, 331]. Point mutations in mtDNA are 10 fold higher than that seen in nDNA [281, 332, 333] and this increased frequency of mutations is considered to be due to an increased susceptibility of the mitochondrial genome to damage as a result of: the proximity of the mitochondrial genome to the MRC which generates reactive oxygen species [296, 332]; the low repair capacity of mtDNA [296]; the absence of histone-mediated protection [281, 333]; and the accumulation of mutations with age [281]. Pathogenic point mutations have been found in all of the mtDNA encoded MRC subunits in complexes I, III, IV and V, with over 100 different mutations currently identified [281]. In complex I, point mutations in the mtDNA encoded subunits are the most numerous of all the MRC complexes, accounting for 50 of the known mtDNA point mutations in MRC subunits [296] and cause Leber Hereditary Optic Neuropathy (LHON) [326, 331, 334, 335], Mitochondrial Encephalomyopathy, Lactic Acidosis and Stroke-like episodes (MELAS) and Leigh Syndrome (LS) [296]. In complex III, point mutations in mtDNA encoded subunit cytochrome b, account for 20 of the known mtDNA MRC

subunit point mutations, the largest number in any single subunit, and cause LS, MELAS and myopathy. Complex IV subunits are the second most common site for mtDNA point mutations accounting for 23 of the known point mutations, which cause myopathy. The two mtDNA encoded subunits of in complex V account for 15 of the known pathogenic mtDNA point mutations and cause LS, LHON, Neuropathy, Ataxia and Retinitis Pigmentosa (NARP) and encephalopathy [281, 296, 298, 305, 321, 324, 325]. More than 160 pathogenic point mutations have been found in mtDNA encoding rRNA and tRNA and over 50% of all identified pathogenic point mutations are located in the mitochondrial tRNA genes, despite these genes comprising less than 5% of the mitochondrial genome [282]. Pathogenic point mutations in mitochondrial rRNA and tRNA genes result in impaired overall mitochondrial protein synthesis [281, 282]. Point mutations in the mtDNA genes encoding the small rRNA, 12S, cause deafness, and in the large rRNA, 16S, point mutations cause cardiomyopathy. Pathogenic point mutations have also been identified in all of the 22 tRNA genes, with the most common sites involving the tRNA Leu, tRNA Ile and tRNA Lys genes [296]. These pathogenic point mutations cause various clinical presentations including myopathy, encephalomyopathy, Myoclonus Epilepsy and Ragged-Red Fibers (MERRF), deafness, cardiomyopathy, PEO, LS, MELAS, LHON and epilepsy [281, 298, 305, 321].

1.9.2 Mutations in nDNA

There are approximately 1500 mitochondrial proteins encoded by nDNA [336-338] and mutations in these nuclear encoded proteins likely account for the majority of MRCD [321]. The first pathogenic nDNA mutation was identified in a nuclear encoded subunit

of the MRC complex II in 1995 [339]. Since then, more than 220 pathogenic nDNA protein encoding genes have been identified [279], with the majority following an autosomal recessive inheritance pattern and parental consanguinity increasing the incidence of autosomal recessive inheritance of MRCD [296, 321, 337, 340]. Many of these disorders are multi-systemic with disease onset in infancy and childhood [302, 328]. Adult onset MRCDs caused by nDNA mutations are generally caused by the progressive loss of mtDNA integrity [328].

1.9.2.1 Mutations in genes encoding MRC subunits

Mutations in nDNA genes encoding MRCD subunits have been identified in all of the 5 MRC complexes, however they are most common in complex I, where mutations in at least 16 nDNA encoded subunits have been identified [296, 328]. These nDNA mutations in complex I are often associated with autosomal recessive LS [328, 341]. Nuclear mutations in the structural subunits of complexes II, III, IV and V are also most commonly associated with LS which have similar clinical presentations causing severe infantile disease [281, 296, 297, 323, 328].

1.9.2.2 Mutations in genes encoding MRC assembly or ancillary proteins

MRC assembly and ancillary proteins are essential in the synthesis of both nuclear and mitochondrial encoded MRC subunits, the transport of nuclear encoded MRC subunits and other essential nuclear encoded proteins required for mitochondrial functioning from the cell cytoplasm into the mitochondria, and assembly of mitochondrial and nuclear

encoded subunits into the MRC multimeric complexes. They are responsible for proper protein folding, the addition of co-factors, protein stabilisation and quality control, ensuring optimised structural integrity and function [290, 321]. Mutations in any of the genes involved in these processes cause MRCD and are known to effect the proper functioning of complexes I, III, IV, V and CoQ10 [281, 296, 297]. To date, few mutations in genes involved with protein transport into the mitochondria have been identified, however one such mutation in the *TIMM8A* gene causes Mohr-Tranebjaerg deafness-dystonia syndrome [296]. The first mutation in a nuclear encoded MRC assembly gene, *SURF1*, was identified in 1998 [342, 343]. There are now over 30 known mutations in the *SURF1* gene [344], which is associated with assembly of MRC complex IV, and one of the most common causes of LS. More recently, mutations in at least seven more genes associated with complex IV assembly have been identified [296]. Mutations in assembly genes associated with complex III were first identified in 2002 in the *BCS1L* gene, which encodes a mitochondrial chaperone involved in the assembly of the Rieske iron-sulphur subunit into complex III as well as complexes I and II. Mutations in this nDNA encoded protein is associated with defects in multiple MRC complexes and causes Growth Retardation, Aminoaciduria, Cholestasis, Iron overLoad, and Early death (GRACILE) syndrome [345]. More recently, mutations in complex I assembly factors have been identified causing LS [346]. LS caused by mutations in nuclear encoded assembly and ancillary proteins commonly present as multi-systemic disorders in infancy with leukodystrophy and cardiomyopathy and more diverse clinical pathology compared to LS due to mutations in MRC subunits [296, 347]. Mutations in nuclear encoded genes causing CoQ10 deficiency were identified in 2006 [348, 349] and cause cerebellar

atrophy, stroke like lesions, encephalomyopathy and nephrosis [283, 296, 297, 347] which manifests as 5 predominant phenotypes: encephalomyopathy with recurrent myoglobinuria, seizures, ataxia, mental retardation and ragged red fibers; severe infantile multisystemic disease; cerebellar ataxia; LS; and isolated myopathy [350].

1.9.2.3 Mutations in genes associated with mtDNA translation

Mitochondrial DNA encodes 9 monocistronic and 2 bicostronic messenger RNA (mRNA) that are translated by mitochondrial ribosomes into the 13 mtDNA encoded subunits of the MRC. The synthesis of functioning MRC subunit proteins requires the correct and co-ordinated functioning of the mitochondrial ribosome, mitochondrial rRNA and tRNA and each of the 4 phases of mitochondrial mRNA translation (initiation, elongation, termination and recycling), as well as the ancillary factors involved in each phase, translational activators and tRNA and rRNA modifiers [281, 290, 296, 297, 304, 321, 351-353]. All of these components involved in mtDNA translation are nuclear encoded and mutations in any of these genes can cause MRCD, generally characterised by multiple MRC complex defects, with severe neurological involvement, often presenting as LS in infancy, however clinical heterogeneity in these disorders has been reported [297, 298, 304, 328, 354]. Mutations in ribosomal protein assembly genes, e.g. *MRPS16* and *RMND1,* causes fatal neonatal lactic acidosis and mutations in nuclear encoded elongation factors cause infantile hepatocerebral syndrome (*GFM1* gene) and infantile encephalomyopathy (*TUFM* gene). Nuclear mutations in translational activator genes, e.g.*TACO1*, cause LS and nDNA mutations in tRNA modifying genes, e.g.

YARS2, cause Myopathy, Lactic Acidosis and Sideroblastic Anaemia (MLASA) [296, 297, 355].

1.9.2.4 Mutations in genes involved in the structural composition of the inner mitochondrial membrane

The phospholipid inner mitochondrial membrane is composed primarily of cardiolipin and acts a scaffold upon which the multimeric complexes of the MRC embed themselves. It has an active role in the formation of the MRC complexes, interacts with them, actively participates in OXPHOS and depends on intact MRC functioning for the synthesis of cardiolipin. The inner mitochondrial membrane is also in physical contact with matrix-dwelling endoplasmic reticulum via the mitochondrial-associated endoplasmic reticulum membrane. This functional association is believed to have a role in the phospholipid composition of the inner mitochondrial membrane and impact on mitochondrial dynamics [281, 296, 297, 309, 323]. Nuclear mutations in genes involved in phospholipid composition impair the integrity of the inner mitochondrial membrane and alter MRC architecture and function. The first mutations in phospholipid composition were identified in 2000 and 2002 and involved the *TAZ* gene causing cardiolipin deficiency and Barth Syndrome, an X-linked disorder characterised by myopathy, growth retardation, neutropenia and cardiomyopathy [356, 357]. Another gene, *AGK*, encoding a phospholipid precursor to cardiolipin, causes Sengers Syndrome, characterised by myopathy, cardiomyopathy, lactic acidosis and congenital cataracts [358]. Mutations in the *SERAC1* gene, whose protein is involved in phospholipid transfer across the

mitochondrial-associated endoplasmic reticulum membrane, caused alterations in the composition of cardiolipin and MEDGEL syndrome, 3-methylglutaconic aciduria with sensorineural deafness, encephalopathy, and Leigh-like syndrome [359, 360]. Mutations in the *CHKB* gene that encodes a phospholipid precursor synthesized in the mitochondrial-associated endoplasmic reticulum membrane, causes encephalomyopathy with giant displaced mitochondria, the abnormal structure of these mitochondria adversely impacting mitochondrial mobility and their ability to distribute normally in cells [281, 296, 361].

1.9.2.5 Mutations in genes affecting mitochondrial dynamics

Mitochondrial dynamics refers to mitochondrial mobility, fusion and fission [281, 296]. Mitochondria are constantly moving and undergoing repeated cycles of fission and fusion, characteristics related to their bacterial origins. These features allow mitochondria to form extensive tubular networks throughout the cell which ensures energy is distributed adequately within the cell, and contributes towards mitochondrial quality control by providing a mechanism for the elimination of damaged mitochondria [297, 362] [281]. These tubular networks are especially important in cells of the central and peripheral nervous system as mitochondria have to travel along nerve cell axons to adequately distribute aerobic energy sources essential for proper neuronal functioning [296]. Mitochondrial fission and fusion rely heavily on proper mitochondrial structure, function and the maintenance of mtDNA. Fission requires an intact outer mitochondrial membrane, several cytosolic proteins e.g. DRP1 and mitochondrial fission factors e.g. MFF, and fusion requires coordination between mitofusions of the outer mitochondrial

membrane e.g. MFN2 and inner-mitochondrial membrane e.g. OPA1 as well as scaffolding proteins of the inner-mitochondrial membrane [296]. Proteins involved in mitochondrial dynamics are nuclear encoded and mutations in any of the genes encoding these proteins results in mitochondria of abnormal shape and size and alters their distribution within cells causing MRCD, inevitably involving the nervous system causing neurological sequelae [296, 297]. Mutations in proteins involved in fission are uncommon however mutations in the *DRP1* and *MFF* genes have been reported in the literature and cause fatal infantile encephalomyopathy [296]. Mutations in mitochondrial fusion proteins are more common, with mutations in the *OPA1* gene encoding an inner mitochondrial membrane mitofusion, crucial for maintaining the inner-mitochondrial membrane architecture [363, 364], causing dominant optic atrophy (DOA), and mutations in the outer mitochondrial membrane mitofusion *MFN2* gene causing multiple mtDNA deletions and depletion and multisystemic manifestations [296].

1.9.2.6 Mutations in genes interfering with mitochondrial DNA maintenance

The nuclear genome encodes the genes responsible for mtDNA maintenance, including mtDNA replication and integrity. These genes therefore control the abundance and quality of mtDNA, and interference in the inter-genomic signalling between nDNA and mtDNA impairs mtDNA maintenance, resulting in mtDNA depletion, multiple mtDNA deletions and site-specific mtDNA point mutations thereby causing loss of integrity of the mitochondrial genome [281, 296, 309, 328, 365]. Mitochondrial DNA depletion syndromes usually follow an autosomal recessive inheritance pattern [302, 321, 366,

367], and culminate in quantitative alterations to mtDNA while multiple mtDNA deletions often follow an autosomal dominant inheritance pattern and culminate in qualitative alterations in mtDNA [297, 309, 323, 347]. Mitochondrial DNA depletion syndromes and multiple mtDNA deletions were considered to be two different conditions, recently however, whole-genome sequencing has indicated that these conditions often co-exist. Furthermore, mutations in the same gene can result in the preferential manifestation of mtDNA depletion or multiple mtDNA deletion syndromes [296]. Preferential manifestation of mtDNA depletion syndromes often causes infantile disease while preferential manifestation of multiple mtDNA deletion syndromes generally causes adult-onset disease, with PEO the most common clinical presentation [296, 323, 328, 347, 368, 369].

Mitochondrial DNA maintenance can be impaired due to defects in the mtDNA replication machinery, the replisome, or due to defects in the intra-mitochondrial pool of deoxyribonucleotide triphosphates (dNTPs) [296]. The mtDNA replisome consists of: a catalytic subunit of mtDNA polymerase (mtDNA polymerase gamma, encoded by *POLG*) [370, 371]; an accessory subunit encoded by *POLG2* [372]; and the replicative helicases Twinkle (encoded by *PEO1*) [373] and DNA2 [296]. *POLG* encodes 3 domains within the catalytic subunit: the polymerase domain involved in mtDNA replication; the exo-nuclease domain involved in proofreading; and the linker region that links the replication and proofreading domains [296, 297, 309, 370]. Mutations in the *POLG* gene can result in autosomal dominant or recessive inheritance patterns, mtDNA depletion or multiple mtDNA deletions and clinical heterogeneity, likely a reflection on which domain

of the catalytic subunit has been affected [296, 309]. Mutations in *POLG* can cause Alpers Syndrome, a severe childhood disorder with autosomal recessive inheritance causing mtDNA depletion [374], and adult onset disorders due to multiple mtDNA deletions including autosomal dominant and autosomal recessive PEO and the multi-systemic disorder, SANDO, characterised by Sensory Ataxic Neuropathy, Dysarthria and Opthalmoparesis [296]. Mutations in *POLG2* which encodes the accessory subunit of POLG causes multiple mtDNA deletions and adult onset autosomal dominant PEO [372] [296]. Mutations in the gene *PEO1* which encodes the replicative helicase "Twinkle" usually results in multiple mtDNA deletions causing adult onset autosomal dominant PEO however it has also been found to result in mtDNA depletion syndromes causing infantile hepatocerebral syndrome and infantile onset spinocerebellar ataxia [296].

Disruption to mtDNA maintenance also occurs due to defects in the intra-mitochondrial pool of dNTPs. There are 4 dNTPs whose availability is crucial for the replication and resynthesis of mtDNA. The pool of dNTPs is regulated by 6 identified enzymes, and defects in these enzymes alter the availability and balance of the dNTP pool, generally resulting in mtDNA depletion syndromes, characterised by 4 major syndromes with marked tissue specific deficiency in mtDNA: infant or adult onset myopathy caused by mutations in the *TK2* gene [375]; infantile hepatoencephalopathy caused by mutations in the *DGUOK* gene [376]; infantile encephalomyopathy caused by mutations in the *SUCLA2* gene [377]; and Mitochondrial NeuroGastroIntestinal Encephalomyopathy (*MNGIE*) caused by mutations in the *TYMP* gene [296, 378, 379]. Mitochondrial DNA maintenance can also be impaired by mutations in the *OPA1* gene. As previously

discussed, the *OPA1* gene encodes an inner mitochondrial membrane mitofusion, which when mutated, causes a non-syndromic form of DOA. The *OPA1* gene is also involved in mtDNA maintenance through its role in tethering nucleotides required for mtDNA replication to the inner mitochondrial membrane. Mutations in the *OPA1* gene affecting this nucleotide tethering role causes multiple mtDNA deletions presenting as DOA-plus, a syndrome characterised by optic atrophy, PEO, sensorineural hearing loss, ataxia, myopathy and polyneuropathy [296]. Mutations in the *MGME1* gene, which encodes Mitochondrial Genome Maintenance Exonuclease 1, integral in mtDNA replication, also impairs mtDNA maintenance, resulting in mtDNA depletion and multiple deletions in muscle causing PEO, emaciation and respiratory failure [296].

1.9.3 MRCD Clinical Presentation

The first reported case of mitochondrial disease was identified in 1962 by Luft and colleagues [380]. Over two decades later the first pathologic mtDNA mutations were discovered, large scale mitochondrial deletions causing KSS, and the point mutations m.3243A>G and m.1178G>A causing MELAS and LHON respectively [311, 326]. A decade later, the first pathogenic nDNA mutation was identified in a nuclear encoded subunit of the MRC complex II [339]. Currently there are in excess of 100 known distinct MRCD [305] and they are considered to be the most common, and often the most complex, of all inherited genetic diseases [295, 381] with the highest incidence of all congenital metabolic disorders [336, 382] and the most prevalent of all inherited neuro-metabolic disorders [283, 383]. As the most common group of inborn errors of metabolism, it is believed that 1 in 5000 births will be diagnosed with a MRCD at some

point in their lifetime, with approximately 50% having an onset in the first 5 years of life and 50% having an onset at some point during their child or adult years [321, 336, 382, 384, 385].

1.9.3.1 Prevalence of MRCD

The exact prevalence of MRCD is difficult to establish due to difficulties with the confirmation of diagnosis, the clinical and genetic heterogeneity characteristic of MRCD, and the ever expanding phenotypic spectrum of MRCD [295, 382, 386]. In the largest study of the prevalence of MRCDs in an adult population in the UK, 16 years and older, performed in a predominantly white cohort, with English ethnicity, and a low rate of parental consanguinity, the prevalence of all pathogenic mutations was 23/100,000, and the minimum point prevalence of affected adults with MRCD as a result of mtDNA or nDNA mutations was 12.5/100,000 [386]. The prevalence of clinically affected adults with mtDNA mutations was 9.6/100,000, accounting for over 75% of all clinically affected adults [386], consistent with previous reports [387, 388]. Mitochondrial DNA encoded point mutations in tRNA accounted for the majority of mtDNA mutations with a prevalence of 4.3/100,000, the m.3243A>G mutation being the most common with a prevalence of 3.5/100,000 [386]. Mutations in mtDNA causing LHON were the next most common with a prevalence of 3.7/100,000, followed by single mtDNA deletions with a prevalence of 1.5/100, 000, consistent with a previous report in the adult Finnish population of 1.6/100,000 [389], and the mtDNA encoded point mutation m.8993T>C with a prevalence of 0.1/100,000 [386]. Individuals at risk of developing MRCD as a result of mtDNA mutations had a prevalence of 10.7/100,000 [386]. The prevalence of

clinically affected adults with nDNA mutations was 2.9/100,000, accounting for approximately 25% of all clinically affected adults, a third of those affected by mtDNA mutations, however the prevalence of nDNA mutations is likely to increase in the future with improved diagnostic techniques and increased recognition of pathogenic nDNA mutations [386]. Some of the most prevalent nDNA mutations were in genes interfering with mitochondrial DNA maintenance causing multiple deletions of mtDNA and gradual accumulation of mutant mtDNA [296] including: the progressive external ophthalmoplegia 1 protein (*PEO1*) gene with autosomal dominant inheritance resulting in a prevalence of 0.7/100,000; the optic atrophy 1 (*OPA1*) gene with autosomal dominant inheritance resulting in a prevalence of 0.4/100,000; and the *POLG* gene with autosomal recessive inheritance resulting in a prevalence of 0.3/100,000 [386]. Individuals at risk of developing MRCD as a result of nDNA mutations had a prevalence of 5.9/100,000 [386].

In contrast to adults, where diagnosis of MRCD is generally made by the identification of a recognised pathogenic mtDNA mutation [388], the diagnosis of MRCD in infants and children is based on mitochondrial respiratory chain enzyme defects [382] with molecular diagnoses only obtained in approximately 20% of patients presenting with MRCD [382, 390, 391]. MRCDs presenting in childhood are more likely to have nDNA mutations [296, 328, 382, 387, 392, 393] with only 10-30% of children presenting with MRCD having a mtDNA defect [290, 324, 382, 390, 393, 394], with MELAS the most common presentation caused by a mtDNA mutation [393]. The vast majority of these nDNA defects follow an autosomal recessive inheritance pattern with a mean age of onset between 3 and 4 months, much earlier than those with mtDNA mutations [328, 382, 390,

392, 395]. MRCD in childhood are often associated with severe, progressive disease and higher mortality, 10-50% per year after diagnosis, compared to diagnosis and clinical onset occurring in adulthood, with a mortality of 5-20% per year [279, 321, 384, 385]. In children, mitochondrial respiratory chain enzyme defects are most commonly found in Complex I (32% of presentations), followed by multiple respiratory chain complexes (26% of presentations), Complex IV (19% of presentations), Complex III (16% of presentations) and Complex II (7% of presentations) [382, 391, 393]. The minimum birth prevalence of childhood onset MRCD in an Australia cohort was found to be between 5-6.2/100,000 [382], higher than the 3.2/100,00 reported in British Columbia [396] but lower than the 8.9/100,000 reported in Sweden [390]. The Australian study proposed an estimate for the prevalence of MRCD presenting in childhood of 10/100,000 [382]. The minimum birth prevalence of MRCD among the Lebanese population in the Australian study was significantly higher at 58/100,000, likely due to the high parental consanguinity in this ethnic group [382].

1.9.3.2 Tissue Selectivity in MRCD

Mitochondria are considered to be ubiquitous, present in essentially all eukaryotic cells, hence MRCD can result in disruption to the function of any tissue in the body [281, 321]. Tissues with high metabolic demands and a greater reliance on OXPHOS are more commonly and severely affected. These includes the brain, central and peripheral nervous systems, skeletal muscle, heart muscle, the retina and optic musculature, the cochlea, bone marrow, kidneys, liver, pancreas and gastro-intestinal system [281-283, 298, 305, 319, 321, 379, 383, 397]. The lack of available ATP from OXPHOS requires increased

dependence on the anaerobic generation of ATP through the conversion of pyruvate to lactate resulting in systemic lactic acidosis and elevated lactate and pyruvate levels [282]. The frequent involvement of the brain, nervous systems and skeletal muscle in MRCD highlights the selective vulnerability of these tissues due to their reliance on OXPHOS, and in 1977 they were termed "encephalomyopathies" by Shapira et al [398], a description still used today to describe many presentations of MRCD [296, 321, 328, 399]. MRCD appear to target five mains areas of the brain: the cortex; white matter; brain stem; cerebellum; and basal ganglia, with many well defined syndromes having characteristic lesions within the brain [347]. Individuals with MRCD may present with central and/or peripheral neurological features. Central nervous system sequelae include stroke like episodes, migraines, seizures, psychomotor regression, ataxia, encephalopathy and cognitive impairment [283, 379]. Stroke like episodes generally occur as a result of a sudden reduction in ATP availability and are most typically seen in MRCD as a result of mtDNA point mutations or nDNA mutations causing mtDNA depletion and multiple deletions e.g. *POLG* mutations [379, 400]. Seizures occur in 35-60% of individuals diagnosed with a MRCD and commonly occur in *POLG* mutations and other mutations interfering with mtDNA maintenance, Complex I deficiencies, mutations in genes encoding MRC assembly or ancillary proteins, mutations in MRC subunits, especially of Complex V, mutations interrupting CoQ10 availability and mutations associated with mtDNA translation [401]. Psychomotor regression can be slowly progressive or can occur acutely in response to a metabolic stressor, seizure or stroke like episode [379]. Ataxia often presents as part of a cerebellar syndrome, also including dysarthria and nystagmus, and often in conjunction with seizures, stroke like episodes and other multi-

systemic manifestations [379]. Cognitive impairment or mitochondrial dementia can be slowly progressive, generally starting with specific, focal cognitive deficits, or can be triggered acutely by seizures or stroke like episodes. It can cause frank psychosis, confusion, behavioural changes, or Alzheimer and Parkinson Disease-like dementias. Progressive or acute presentations are often associated with weakness, ataxia, spasticity and migraines. Cognitive impairment is often seen in nDNA mutations affecting *POLG*, *PEO1*, *TK2*, *TYMP* and *SURF* genes [379, 402]. Depression is also a common feature of individuals with MRCD and cognitive impairments. Depression was found to be present in 42% of those with symptomatic MELAS and occurs frequently in individuals with mutations in genes interfering with mitochondrial DNA maintenance including *POLG* and *PEO1* [297]. Peripheral nervous system sequelae include myopathy, ophthalmoplegia and peripheral neuropathy [283].

The incidence of skeletal muscle involvement in MRCD is common due to the highly oxidative nature of this tissue [403-405]. Symptoms include muscle weakness, predominantly of the hip and shoulder girdle [406], exercise intolerance, cramps and myalgia [407]. The loss of muscle strength can be cause by the impairment of muscle function or due to denervation associated with peripheral neuropathy which causes muscle atrophy and weakness distally [408]. Exercise intolerance in MRCD was first described in the 1960s [409] and is considered to be a hallmark of MRCD with many individuals experiencing muscle fatigue, muscle pain, dyspnoea and lactic acidosis with minimal exertion, [403, 405, 406, 410-412] impacting significantly on the performance of activities of daily living and quality of life [413]. As a consequence of the uncomfortable

side effects of exertion, many individuals with MRCD avoid exercise, adopting a sedentary lifestyle, which in turn results in deconditioning, reduced aerobic capacity and exercise intolerance [406, 414, 415]. Exercise tolerance in MRCD exhibits wide variation depending on the level of OXPHOS deficiency between individuals and exercise testing is considered to be a useful screening and evaluation tool in the management of MRCD [288, 405, 406].

Studies utilising formal cardiopulmonary exercise testing with gas analysis have been pivotal in understanding the mechanism underlying exercise intolerance in MRCD. Mitochondrial respiratory chain defects interrupt the function of OXPHOS and during exercise this is reflected by the exercising muscle's inability to extract and utilise the available oxygen from the circulating blood [288, 412, 416-418]. This impaired oxygen extraction is indicated by a low peak arteriovenous oxygen (a-vO_2) difference, which measures the amount of oxygen that is removed from blood in the capillaries supplying the skeletal muscle [416]. Exercise studies in MRCD have found oxygen extraction at peak exercise to range between 3-10ml/dl, less than 50% of that seen in matched controls [405, 406, 412, 414, 416, 419]. This disruption to the normal close relationship between oxygen delivery and utilisation by the exercising muscle, where cardiac output (Q) normally increases 5L for every 1L increase in oxygen uptake (VO_2) [420], results in hyperkinetic circulation, where oxygen delivery relative to oxygen utilisation (Q/VO_2) is at least 3 fold higher than normal [405, 411, 412, 414, 416, 417, 419, 421, 422]. The inability of the muscle to extract and utilise the available oxygen is believed to be sensed by metaboreceptors in the exercising muscle which are believed to have a focal role in

driving the hyperkinetic circulatory response seen in MRCD [405]. In a study of 40 adults with MRCDs and age matched controls, the hyperkinetic circulation was characterised by a strong negative exponential relationship between peak Q/VO_2 and peak systemic a-vO_2 difference and between peak Q/VO_2 and peak VO_2, indicating that the mismatch increases as the severity of the OXPHOS deficit increases [405]. A study investigating capillary area on muscle biopsy found that abnormalities in capillary growth in MRCD may help explain the hyperkinetic circulatory response seen in this population [416]. Capillary growth, which is considered to be stimulated by hypoxia in exercising muscle and closely correlated with peak VO_2 and a-vO_2 difference in the control population, did not follow the same relationship in individuals with mtDNA defects, who demonstrated a 3 fold higher capillary area compared to controls, with capillary growth correlating highly with Q/VO_2 indicating that those with the lowest VO_2 had the highest levels of capillary growth and that the impairment in OXPHOS stimulates capillary growth independent of tissue hypoxia [416]. Furthermore, muscle biopsies showed that these capillaries were concentrated around the OXPHOS deficient fibres which paradoxically promotes the mismatch between oxygen delivery and oxygen utilisation, as more blood is delivered to the most deficient muscle fibres, consequently augmenting the hyperkinetic circulation which is a characteristic response to exercise in MRCD [416].

Peak oxygen uptake is determined by the delivery of oxygen to the exercising muscle and extraction of available oxygen by the muscle [408]. In MRCD the delivery of oxygen to the muscle is normal [406], however oxygen extraction is significantly impaired and the degree of impairment is closely related to the severity of the OXPHOS deficit [405].

Consequently, peak VO_2 is dramatically reduced in MRCD and highly correlated with peak a-vO_2 difference, a relationship that is not seen in healthy controls [405]. Exercise studies in MRCD have found peak VO_2 ranging between 6 and 22 ml/kg/min, less than 50% of that seen in matched controls [405, 406, 412, 414, 416, 417, 419, 423]. Peak work rate has also been shown to be severely reduced [405, 406]. When activities of daily living require a VO_2 of between 15-25 ml/kg/min [424, 425], and two thirds of individuals with MRCD have a VO_2 less than 18ml/kg/min [405], it is clear that many individuals with MRCD will be limited in performing their ADLs, and why exercise intolerance reduces their quality of life. Studies investigating mutation load and exercise capacity found strong negative exponential relationships between mutation load and peak VO_2 [405, 426], peak work rate [426], and peak a-vO_2 difference [405] indicating that the more severe the MRC deficit, the more compromised the aerobic capacity. Exercise tolerance is further compromised in MRCD due to reduced basal levels of Phosphocreatine (PCr) and impaired PCr resynthesis. PCr is a readily mobilised but very short lasting source of energy that contributes to the first 10 seconds of exercise, and both basal levels of PCr and the speed of resynthesis of PCr may be reduced by over 2/3 compared to matched controls [288, 414, 427].

Exertional dyspnoea is a common cause of exercise limitation in MRCD [412, 419, 428]. In a small study of 5 adults with mtDNA mutations without respiratory muscle weakness and age-matched controls, the mechanisms behind exertional dyspnoea in MRCD were investigated [419]. Impairments in OXPHOS caused by the MRCD increase the reliance on anaerobic glycolysis during exercise, which causes a rapid increase in the production

of lactic acid, the magnitude of which was found to correlate with mtDNA mutation load [426]. In turn, blood lactate increases causing a metabolic acidosis and a drop in pH, often at low exercise intensities [288, 405, 411, 417, 427, 429]. The elevated blood lactate is sensed by the carotid bodies which initiate a ventilatory response to compensate for the metabolic acidosis and prevent further decline in pH levels. The resultant progressive increase in respiratory drive results in exaggerated ventilatory responses during exercise, most evident in the respiratory equivalents of oxygen (VE/VO_2) and carbon dioxide (VE/VCO_2) which demonstrated abnormally steep increases relative to workload and at peak exercise were double that seen in matched controls [419]. Another study of 40 adult patient with MRCD found a moderate negative exponential relationship between peak VE/VO_2 and peak systemic $a\text{-}vO_2$ difference and between peak VE/VO_2 and peak VO_2, indicating that the mismatch increases as the severity of the OXPHOS deficit increases [405]. The disproportionate production of carbon dioxide in relation to VO_2 results in an elevated respiratory exchange ratio (RER) relative to the workload [288, 423, 430] casing the anaerobic threshold to be exceeded earlier in exercise [428] and grossly exaggerated peak RER, often 50% higher than controls [405, 419]. Blood gases during exercise also showed evidence of exaggerated ventilatory responses with $PaCO_2$ and HCO_3^- lower and PaO_2 higher than matched controls [419]. Metaboreceptors in the exercising muscle are also considered to have a role in the exaggerated ventilatory response to exercise, which are independent of metabolic acidosis, but likely induce an exaggerated catecholamine response, evidenced by a two fold increase in adrenaline and noradrenaline in MRCD compared to controls, which augments the utilisation of intra-muscular glycogen stores and stimulates ventilatory responses during exercise [405, 417].

This is illustrated by the responses that occur in recovery once exercise has ceased. Blood lactate continues to increase and pH decreases, which in the controls, maintained respiratory drive resulting in increases in VE/VO_2 and RER and a decrease in $PaCO_2$ on blood gases. However, the opposite is seen in MRCD where minute ventilation (VE), VE/VO_2 and RER decrease rapidly and $PaCO_2$ increases when exercise is ceased. This paradoxical response can only be explained by withdrawal of input from the exercising muscle [419]. An earlier study in 28 adults with MRCD found an association between weakened respiratory muscles and participants reporting exertional dyspnoea as their limitation to exercise, suggesting that exertional dyspnoea limiting exercise in MRCD may be caused by the increased ventilatory response and respiratory muscle weakness in some individuals [428].

Heart muscle can be affected in MRCD causing hypertrophic cardiomyopathy, dilated cardiomyopathy, conduction block and arrhythmias [305, 393]. The eyes are also commonly affected in MRCD with manifestations including optic nerve atrophy and neuropathy, retinal degeneration and pigmentary retinopathy, ptosis and ophthalmoplegia [379]. Sensorineural hearing loss with impaired function of the inner hair cells of the cochlea commonly occurs in MRCD but rarely in isolation [282]. The bone marrow may be affected causing haematological manifestations including anaemia, which is seen in mutations of the *YARS2* gene associated with mtDNA translation. The renal tubules of the kidneys can be affected in MRCD, as can the β-cell of the pancreas resulting in diabetes mellitus. Gastro-intestinal dysmotility is another common manifestations of MRCD [282] as well as failure to thrive, growth retardation and short stature [305, 383].

1.9.3.3 Clinical Heterogeneity in MRCD

Mitochondrial respiratory chain disorders are not only characterised by genotypic variability, with mutations passed from generation to generation by all known inheritance patterns, sporadic, mendelian and mitochondrial, they also display diverse phenotypic heterogeneity and variability in presentation [278, 283, 290, 295, 305, 326, 336, 337, 347, 383, 431]. Disease onset can occur at any age from infancy to late adulthood [298, 321, 379]. Individuals with MRCD may present with tissue specific involvement or multi-systemic manifestations, which may be analogous with well-defined syndromes or largely non-specific disorders, precipitated by metabolic stressor or as a result of progressive or recurrent symptoms [281, 282, 295-298, 327, 384]. There is also a large spectrum of disease severity with some MRCD being asymptomatic or oligosymptomatic and others causing chronic disability, significant morbidity and premature death [283, 305, 432, 433]. The complexities underlying clinical phenotype are influenced by many factors including the differing demand for ATP and rate of OXPHOS that exists between different tissues, making some tissues more vulnerable to mitochondrial dysfunction than others, and the intricacies of mitochondrial genetics including heteroplasmy and the threshold effect, where disease manifestations only become apparent when a certain threshold of mutant mitochondria or demand on OXPHOS is exceeded [279, 281]. This threshold also varies for different genetic abnormalities, with tRNA point mutations causing disease manifestations at a threshold of greater than 90% mutant load compared to a threshold of 50-60% mutant load when mtDNA deletions are present [282]. Phenotype can change rapidly from normal to abnormal and once the pathogenic

threshold is exceeded, small increases in the percentage of mutant DNA present in cells can result in significant progression of phenotypic severity [319], however phenotypic severity can fluctuate throughout life [305, 379]. MRCD also exhibit poor genotype-phenotype correlations with identical clinical phenotypes caused by different genetic abnormalities and the same genetic abnormality resulting in a spectrum of phenotypes, generally attributed to heteroplasmy [296, 297, 305, 379, 434, 435]. Similarly, there can be poor correlation between mutation load and symptom and phenotypic severity, especially in muscle [305].

The predominant features of childhood presentation of MRCD were found to be myopathy, encephalopathy and cardiomyopathy but also include hearing and visual impairments, lactic acidosis, failure to thrive, diabetes, liver and kidney disease [305, 321, 393, 397, 401]. In a study of over 100 paediatric patients with mitochondrial disease, all those presenting with MRCD had moderate to severe developmental delay or motor regression at presentation [393] with hypotonia and lethargy other common myopathic symptoms [321, 397]. MRI scans of the brain revealed abnormalities in 70% of children with MRCD including cerebellar and cortical atrophy, brain stem and basal ganglia lesions, white matter abnormalities, agenesis of the corpus callosum, posterior fossa abnormalities and cerebellar volume loss [393]. Seizures are the most common manifestation of encephalopathy in childhood [401] and were present in 50% of children diagnosed with MRCD, 32% had ophthalmologic problems including optic atrophy, ptosis, nystagmus, ophthalmoplegia and pigmentary retinopathy, 21% exhibited sensorineural hearing loss [393]. Cardiac involvement was identified in 40% of children

presenting with MRCD, 60% of these with hypertrophic cardiomyopathy, 30% with dilated cardiomyopathy and 10% with arrhythmias [393]. The presence of cardiac involvement on presentation is associated with a significantly reduced life expectancy, with less than 20% of children having cardiac involvement surviving to 16 years of age compared to 95% of children who did not present with cardiac involvement [393]. Movement disorders, especially dystonia, were apparent in 12% of children and ataxia in 6% [393]. Cognitive deficits, while difficult to accurately measure, have been identified in 60% of children with MRCD in one study [436], and in another study 75% of children with encephalomyopathies were found to have an intellectual disability or cerebral dysfunction [437]. Another study found that children with MRCD have reduced visual-spatial abilities, nonverbal cognitive impairments and low performance and verbal IQ [438].

Adult presentations of MRCD are characterised by exercise intolerance and muscle weakness usually affecting the proximal muscles. With more severe disease, muscle weakness may also cause dysarthria and dysphagia. Involvement of the central nervous system is also common manifesting as seizures, migraines and focal neurological deficits. Many will exhibit sensorineural hearing loss, ophthalmologic problems including optic atrophy, ptosis, progressive external ophthalmoplegia and pigmentary retinopathy. Cardiac involvement is commonly associated with arrhythmias, conduction block and hypertrophic cardiomyopathy. Gastrointestinal dysmotility is common causing constipation and pseudo-obstruction and endocrine problems include diabetes and short stature [305].

1.9.3.3.1 Progressive External Opthalmoplegia (PEO)

Progressive External Opthalmoplegia is an example of a MRCD that exhibits large genetic variability such that identical clinical phenotypes are caused by different genetic abnormalities. It can be caused by sporadic mutations or mutations in either the nuclear or mitochondrial genomes. Sporadic mutations generally cause large-scale deletions of mtDNA and maternally inherited PEO is usually associated with tRNA point mutations. PEO caused by mutations in nDNA are a result of mutations in gene interfering with mtDNA maintenance and can follow an autosomal dominant or autosomal recessive inheritance pattern. PEO is the most common clinical presentation when mutations in these nuclear encoded genes e.g. *POLG, POLG2* and *PEO1* cause multiple mtDNA deletions [296, 305]. The characteristic features of PEO, including bilateral ptosis and reduced eye movements limiting peripheral vision, demonstrate the vulnerability of the extra-ocular muscles in MRCD due to their dependence on OXPHOS [296, 439]. Other features include slowly progressive skeletal muscle weakness and disease onset in early adulthood, generally between 20-30 years of age [321, 439].

1.9.3.3.2 Mutations in the Nuclear Encoded POLG gene

POLG-related disorders are an example of phenotypic variability and weak genotype-phenotype correlations seen in MRCD, despite a common underlying genetic cause [440]. There are over 150 known mutations in the *POLG* gene [379] which interfere with mtDNA maintenance causing mtDNA depletion or multiple mtDNA deletions [296, 309].

In a review of 38 presentations with *POLG* mutations, 10% presented with an autosomal dominant pattern of inheritance with mutations predominantly affecting the polymerase domain, 24% with an autosomal recessive pattern of inheritance, including all children of consanguineous parents, and the remaining 66% presenting as sporadic compound heterozygotes [440]. Nearly 30% had more than 2 substitutions in *POLG* and only one third had a family history of disease [440]. Half of the cohort presented in childhood and half in their teenage and adult years. Over 60% of those presenting in childhood had mutations in the linker region, 20% of those being homozygous in nature, with the remaining 40% most commonly seen in the polymerase domain and most childhood presentations occurring in males [440]. Individuals presenting in their teenage and adult years usually had mutations in the exo-nuclease proofreading domain [440].

Clinical phenotype, age at presentation and disease progression associated with *POLG* mutations is highly variable [379, 440] however POLG-related disorders do cause several well defined and overlapping phenotypes including: Alpers Syndrome, childhood Myo-Cerebro-Hepatopathy Syndrome (MCHS); Myoclonic Epilepsy, Myopathy and Sensory Ataxia syndrome (MEMSA); Spino-Cerebellar Ataxia and Epilepsy (SCAE); Sensory Ataxic Neuropathy, Dysarthria and Opthalmoparesis (SANDO) and autosomal dominant and autosomal recessive PEO [347, 379]. Infant and childhood presentation are generally severe and progressive with common symptoms including encephalopathy, seizures, cognitive impairment, sensorineural hearing loss, lactic acidosis, myopathy, hepatic failure, renal failure, failure to thrive and vomiting [347, 379, 440]. Presentation in the teenage and adult years is commonly associated with PEO, ataxia and myopathy [379]

and the study by *Howarth et al 2006* found that all those presenting at this time had PEO and ptosis, 79% had myopathy and 42% ataxia. Other common symptoms in this cohort of teen and adult onset disease were dysphagia in 37%, peripheral neuropathy in 32% and diabetes, sensorineural hearing loss and dementia in 11% [440]. In adult patients, characteristic neuroimaging in those with *POLG* mutations involved stroke-like lesions, basal ganglia involvement and cerebral and cerebellar atrophy [347]. In teenage and adult onset disease there was no gender bias towards males as was seen in childhood presentations [440].

1.9.3.4 Mitochondrial Respiratory Chain Complex Involvement in MRCD

The majority of individuals with MRCD present with deficiencies in multiple MRC complexes [295], with the literature suggesting these presentations account for 30-51% of all presentations, and up to 25% of paediatric presentations [441]. At least 50% of presentations involving multiple MRC complex deficiencies are caused by mutations in genes interfering with mtDNA maintenance [320, 352, 441] but can be caused by any mtDNA or nDNA mutation [395]. In a cohort of children with MRCD involving multiple MRC complexes, 24% were due to large scale mtDNA deletions or rearrangements, 19% due to mutations in the *POLG* gene, 18% due to mtDNA depletions, 18% due to mutations in the *DGUOK* gene and 12% due to mutations causing MELAS [395]. Multi-system disease manifestations are common including encephalopathy, myopathy, cardiomyopathy, visual and hearing impairments, kidney and liver disease, hypotonia and growth retardation [290]. Deficiencies in Complex I are the most commonly seen deficiencies in MRCDs, with pathogenic mutations identified in all of the 7 mtDNA

encoded subunits and 16 of the nDNA encoded subunits as well as 6 assembly factors [347]. Complex I deficiencies generally occur in combination with other complex deficiencies however isolated Complex I deficiencies account for 22% of presentations with MRCD [290, 295]. Complex I deficiencies are most commonly associated with LS and are the most frequent cause of MRCD in children [321, 347]. Complex I deficiencies almost universally present with bilateral, symmetrical brain stem lesions, and necrotising leukoencephalopathy is generally found in individuals with nDNA mutations, and cerebellar atrophy in those with mtDNA mutations [347]. Clinically, individuals often present with developmental delay, hypotonia, seizures, visual sequelae and cardiomyopathy [347]. Deficiencies in Complexes II and III are rare, in presentations involving multiple MRC complexes and also as isolated complex deficiencies, accounting for up to 4% of all presentations of MRCDs [321, 442]. Deficiencies in these complexes demonstrate high clinical heterogeneity with Complex II deficiencies generally presenting as LS and Complex III deficiencies as LS or encephalomyopathy [297, 321]. Complex IV deficiencies are more common and isolated complex IV deficiencies account for 13% of MRCD presentations. These deficiencies are generally maternally inherited and their heteroplasmic nature results in diverse clinical heterogeneity [290]. Pathogenic mutations have been identified in mtDNA and nDNA encoded subunits as well as assembly factors for Complex V, however it is considered to be a rare cause of MRCD with deficiencies primarily caused by mutations in the mtDNA encoded ATP Synthase 6 subunit. Clinically, Complex V deficiencies present as LS, with basal ganglia lesions, cerebellar atrophy, brain stem lesions, ataxia, neuropathy and retinitis pigmentosa [347].

1.9.3.5 Leigh Syndrome

Leigh Syndrome was first described by Denis Leigh in 1951 [443] and the first pathogenic mutation was identified four decades later in 1991 [444]. Leigh Syndrome is the most commonly seen paediatric presentation in MRCD and over 80% of all LS cases present by 2 years of age [320, 445]. The estimated prevalence of LS is 1/40,000 births [446]. Like many MRCD, LS displays marked genetic heterogeneity with over 75 pathogenic mutations identified in both the nuclear and mitochondrial genomes which are transmitted by autosomal recessive, X-linked and maternal inheritance patterns and encode structural subunits of the MRC complexes, proteins required for their assembly and stability, proteins involved in the mtDNA maintenance or proteins involved in mtDNA translation, however structural subunits of the MRC complexes and their associated assembly factors are most commonly affected [283, 320, 340]. Mutations in the nuclear genome are the most common cause of LS with mutations in the mitochondrial genome only accounting for up to a quarter of all LS presentations [446-448]. Complex I deficiencies are the most common cause of LS with up to 50% of individuals with Complex I deficiency presenting with LS [320, 449, 450]. Over one third of all pathogenic mutations causing LS affect Complex I [320, 450] and in 80% of individuals with Complex I deficiencies, the underlying genetic cause is due to mutations in nDNA [451] with mutations in the NADH dehydrogenase iron-sulphur protein 4 (*NDUFS4*) subunit the most frequent autosomal recessive cause of Complex 1 deficiencies resulting in LS [320, 449, 450]. Complex IV deficiencies are the next most common cause of LS, accounting for around 15% of LS presentations [320, 446, 447].

Mutations in the Complex IV assembly factor gene *SURF1* accounts for 75% of presentations with Complex IV deficient LS [320]. Complex V deficiencies cause 5-10% of LS syndrome presentations with mutations in the ATP Synthase 6 subunit of Complex V the most common cause of maternally inherited LS, the most frequently occurring of which is the mt.8993T>G mutation [320, 434, 452, 453]. Deficiencies in complexes II, III and CoQ10 are quite rare, causing less than 10% of LS presentations [320, 446, 447]. Deficiencies in multiple respiratory chain complexes causing LS are most frequently caused by mutations in genes involved in mtDNA translation [450] [320].

Leigh syndrome is also characterised by clinical heterogeneity with regards to age of onset, symptom presentation and progression, severity and life expectancy [320, 450]. Individuals with LS will exhibit progressive neurodegeneration which is a hallmark of the disease [320, 446, 450]. The neurodegeneration is characterised by subacute necrotising encephalomyopathy with episodes of psychomotor regression which may be associated with illness or infections where partial recovery may occur, or follow a stepwise deterioration with intermittent periods of stability or even improvement of varied duration [445]. Neuroradiology reflects characteristic features including bilateral, symmetrical lesions in the brainstem and basal ganglia, spinal cord, thalamus and cerebellum [296, 297, 320, 328, 347]. Other neurological symptoms reflect brainstem and basal ganglia dysfunction and intellectual and motor delay and include pyramidal and extrapyramidal symptoms with movement disorders including choreoathetosis, dyskinesia, dystonia, tremor and ataxia, hypotonia, spasticity, abnormal tendon reflexes, dysphagia, feeding difficulties, vomiting and failure to thrive, central respiratory

dysfunction including apnoeas, hypo- or hyper-ventilation neuro-ophthalmological problems including nystagmus, optic atrophy, ptosis, retinitis pigmentosa, ophthalmoparesis, neuropathy, myopathy and thermoregulation [290, 296, 320, 347, 379]. Other non-neurological symptoms include cardiac abnormalities including hypertrophic or dilated cardiomyopathy, arrhythmias, conduction block, anaemia, kidney and liver disease, gastrointestinal immotility, diabetes and short stature, [320]. Infantile onset LS is most commonly associated with hypotonia, developmental delay, vomiting and failure to thrive [445]. Presentation during the toddler years are characterised by gross motor delay, especially in walking, dystonia and dysarthria while presentation during the later childhood and adolescent years is characterised by ataxia and extrapyramidal features including dystonia and rigidity [320]. Adult onset LS does not follow a typical presentations and can be associated with headaches, hallucination, memory loss, intellectual deterioration, ataxia, spasticity, dysarthria, dyspnoea or ophthalmological problems [320]. Almost half of all individuals with LS will be hospitalised during acute episodes, primarily due to infections or respiratory compromise [320]. Peak mortality occurs prior to 3 years of age with death a consequence of respiratory dysfunction [447] or cardiac failure [445].

1.9.3.6 Mitochondrial Encephalomyopathy, Lactic Acidosis and Stroke-like episodes (MELAS) Syndrome

MELAS was first discovered in 1991 by *Tanaka et al* [454] and is one of the most common MRCD. MELAS is caused by maternally inherited pathogenic point mutations

in mtDNA genes encoding MRC subunits and tRNA [281, 290, 296-298, 309, 321, 323, 331, 455]. Pathogenic mtDNA point mutations encoding MRC subunits are most commonly found in the subunits of Complex I and Complex III [296, 455] however the most frequent pathogenic mtDNA point mutation associated with MELAS is the m.3243A>G transition of the gene encoding the mitochondrial Leucine $tRNA^{(UUR)}$ which is identified in around 80% of individuals presenting with MELAS symptoms [281, 298, 321, 455-460]. This specific mutation, along with other pathogenic point mutations in mitochondrial tRNA genes, results in impaired overall mitochondrial protein synthesis [281, 282]. The minimum point prevalence of MELAS in the adult population caused by the m.3243A>G mutation was found to be 3.5/100,000 in a recent UK study [386], however earlier reports suggest a much higher point prevalence of 16.3/100,000 [461, 462].

MELAS displays features of mitochondrial genetics with replicative segregation, heteroplasmy and the threshold effect underlying the phenotypic heterogeneity characteristic of the disease [280, 281, 296, 298, 321]. Symptoms usually manifest once mutant mtDNA exceed the 90% threshold, however symptoms can be transient or recurrent with approximately 70% if individuals experiencing their initial symptoms between the age of 2 and 20 years, after a period of normal development [281, 296, 455, 463]. MELAS is multisystemic in nature and a hallmark of the disease is progressive neurodegeneration that has devastating cumulative effects [281, 290, 305, 455]. Common neurological symptoms include repeated stroke like cerebral episodes commonly affecting the temporal, parietal and occipital lobes, often causing migraines, seizures,

hemiparesis, hemianopia, cortical blindness, ptosis, ophthalmoplegia, dementia, encephalopathy, ataxia and sensorineural hearing loss [281, 298, 321, 455]. Neuroimaging reflects lesions in the basal ganglia, cerebellar and cortical atrophy, neuronal loss and stroke like episodes [282, 347]. Other symptoms include systemic lactic acidosis, myopathy and proximal muscle weakness, fatigue, myalgia, peripheral neuropathy, vomiting and other gastro-intestinal disturbances, cardiomyopathy and cardiac conduction defects, renal impairment, diabetes and short stature [281, 290, 305, 347, 455, 456, 459, 464].

1.9.3.7 Kearns-Sayre Syndrome (KSS)

Kearns-Sayre Syndrome is a multisystemic disorder caused by mtDNA deletions of various sizes in over 90% of presentations [439, 465]. The vast majority of these deletions occur between the two origins of replication on the circular mtDNA, which includes over two thirds of the mitochondrial genome. These mitochondrial DNA deletions can remove between 9-50% of the genome, with the accumulation of deletions within tissues underlying the progressive nature of the syndrome [290, 315]. KSS is characterised by a diagnostic triad of disease onset during childhood, progressive external ophthalmoplegia and pigmentary retinopathy. This triad generally occurs with other at least one of the following: a cardiac conduction defect; cerebellar syndrome; or elevated protein content in cerebrospinal fluid [290, 321, 439, 465]. Other symptoms include cognitive impairment, seizures, sensorineural deafness, gastrointestinal dysmotility, short stature, diabetes, endocrine and renal impairments, myopathy with predominant proximal and bulbar weakness, dysphagia, sensory and motor neuropathies, ptosis, optic atrophy,

hypertrophic or dilated cardiomyopathy, cardiac arrhythmias and lactic acidosis [290, 305, 321, 347, 465]. Hallmark features on neuroimaging include symmetrical lesions white matter regions of the spinal cord, brainstem, thalamus, globus pallidus, cerebrum and cerebellum reflecting severe spongiform degeneration [290, 347].

1.9.4 Treatment of MRCD

The treatment of MRCD poses a major challenge to the multidisciplinary teams involved in the management of individuals with these disorders. There is currently no known cure or established treatment regimens as no clear evidence for effective interventions have been identified [283, 295, 386, 466-468] which imposes significant costs on the healthcare system as the burden of MRCD is usually extensive with multisystem derangements requiring medical management [386]. Therapeutic approaches are aimed at managing secondary complications, ameliorating symptoms and improving quality of life [295, 321, 468, 469]. Symptomatic therapy includes the management of epilepsy with anticonvulsants, diabetes with insulin and/or dietary modification, the insertion of a pacemaker for conduction block and cochlear implants for sensorineural hearing loss and the surgical correction of ptosis [468]. The use of small molecule therapies and metabolic manipulation, along with dietary and exercise regimes have been focused on enhancing the capacity for ATP synthesis, bypassing the mitochondrial defect, stimulating mitochondrial biogenesis and reducing the levels of reactive oxygen species [279]. These therapies include pharmacological adjuncts that attempt to boost the level of metabolites and co-factors including carnitine, creatinine, thiamine, riboflavin, succinate, CoQ10, vitamin E, vitamin C and folic acid [283, 295, 321, 468]. Aerobic exercise training has

been shown to increase work and oxidative capacity by up to 30%, oxygen extraction by up to 16%, and the activity of some MRC enzymes up to 50% as well as stimulating mitochondrial proliferation and improving quality of life by up to 28%, so it is an effective treatment to improve exercise tolerance and prevent deconditioning [292, 407, 411, 414, 415, 468, 470-475]. Resistance exercise training has also been shown to increase leg strength by up to 25%, upper limb strength by 60%, improve oxygen extraction and the activity of some MRC enzymes [474, 476]. In a study of five children with MRCD, exercise time and oxygen uptake remained stable after six months of physical therapy incorporating aerobic and resistance training[477]. Many individuals with MRCD experience muscle pain, fatigue and dyspnoea with exercise making the motivation to participate in and adhere to exercise interventions difficult, especially in children where behavioural difficulties may also interfere with optimal training [476, 477]. While exercise interventions and pharmacological adjuncts have been successful in isolated cases and small clinical trials, the efficacy of these interventions in larger cohorts have not been investigated to provide clear evidence of their usefulness in the management of MRCD [283]. Such studies are often difficult to conduct due to the genetic and phenotypic heterogeneity that is characteristic of MRCD making it difficult to compare the efficacy of interventions between participants [283, 295, 320, 321, 478]. Supportive and preventative therapy involves the provision of prognostic education, genetic counselling and where possible prenatal diagnoses [283, 468].

1.10 Cystic Fibrosis

Cystic fibrosis (CF) is the most common life-limiting inherited disorder in the Caucasian population [479] with an incidence of 1 in every 3700 babies born in Australia [480]. This multi-system disease was first described in 1938 By Dr Dorothy Andersen [481]. Over a decade later, CF was found to be associated with an abnormality in the epithelial glands [482] which triggered studies into sweat electrolyte levels and the development of the sweat test which is widely used as a diagnostic tool [483]. Alteration to chloride permeability and conductance was established in sweat glands [484-486] and later confirmed in the pancreas [487] and airway epithelia cells [488-491].

A decade after being first described, researchers suspected a single-gene defect was responsible for CF due to the autosomal recessive pattern of inheritance [492] and in 1985 it was found to be located on chromosome 7 [493, 494], the gene discovered a few years later in 1989 [495, 496] along with the mutation in F508Del that encoded the cystic fibrosis transmembrane conductance regulator (CFTR) [497]. Over 2000 mutations have since been discovered, with just over 10% considered to be disease-causing [498]. Despite this diversity in mutation type, 85% of Caucasians with CF have at least one F508Del mutation [498, 499].

The CFTR is an ATP binding cassette transport-class protein [498, 500] located in the apical membrane of epithelial cells in the lung, liver, pancreas, intestines, sweat glands and reproductive tract, among others [479, 501]. It is responsible for the transport of chloride ions across the apical membrane, regulating trans-epithelial salt and water flow

via an osmotic gradient [500, 502]. The CFTR is composed of 5 domains [497, 500, 503, 504]: two membrane spanning domains that form the channel pore [505, 506]; two nuclear binding domains that interact with ATP to modulate channel activity; and a regulatory domain that directs the channel to open/close [506-508].

In CF the trans-epithelial transport is disrupted as a result of altered CFTR synthesis and/or function. Six classes of gene mutations have been identified [479, 498, 499, 509-513] and their effects on the CFTR are summarised in Table 1.1. These mutations result in the production of abnormally thick and sticky mucus causing multi-system disease affecting the airway, pancreas, gut, sweat glands and male fertility [513] leading to chronic dysfunction, disability, and a reduced life expectancy [514]. Morbidity and mortality is most commonly caused by lung disease [515, 516], where defective chloride transport causes a reduction in the airway surface liquid layer [517-519] and impaired muco-ciliary clearance, resulting in mucous retention that causes airway obstruction, inflammation and infection [520, 521] and eventually irreversible, fibrotic damage to the surrounding lung parenchyma [479, 500]. Progressive suppurative pulmonary disease, recurrent bronchopulmonary infections and declining lung function are hallmarks of CF [515, 516]. Around 90% of CF patients are pancreatic insufficient due to pancreatic obstruction, requiring pancreatic enzyme replacement therapy to improve protein and fat absorption [479].

While no cure is available, improvements in the clinical management of CF to treat end-organ sequelae [498], including early detection of the disease through newborn screening,

Table 1.1: CFTR gene mutation classes.

Class	Function Interrupted	Consequence
I	No synthesis	Truncation of transcribed mRNA resulting in absent, non-functional or unstable CFTR protein
II	Processing/Trafficking	Mis-folded CFTR protein degraded/removed by intracellular quality control mechanisms while migrating to cell surface
III	Gating	CFTR present at cell surface however regulation disrupted interrupting normal flow of chloride ions
IV	Conductance	CFTR present at cell surface however altered conductivity of the channel pore, resulting in abnormal chloride permeability
V	Low synthesis/reduced expression	Reduced number of CFTR at cell surface however those at the cell surface have normal function
VI	High turnover	CFTR present at cell surface however stability reduced resulting in high turnover

Note: mRNA, messenger ribonucleic acid; CFTR, cystic fibrosis transmembrane conductance regulator.

advances in antibiotic therapy [522-524], attempts to reduce inflammation [525-528], more aggressive management of gastro-intestinal complications and targeted, specialised physiotherapy to optimise secretion removal [529-531], has culminated in increases in the average life expectancy of individuals with CF. In Australia, the average life expectancy is now well into the fourth decade of life [480]. This increase in survival, along with improved screening, has seen the emergence of previously under-recognised extra-pulmonary complications including CF bone disease (CFBD) [532-544]. Second to cystic fibrosis related diabetes, CFBD is the second most common extra-pulmonary complication [545] and its prevalence is increasing [532, 546, 547].

CFBD is characterized by low bone mass, compromised bone quality (density, geometry and strength), increased risk of fragility fractures and exaggerated thoracic kyphosis, and un-coupled bone turnover. The underlying pathophysiology is multifactorial involving multiple risk factors [532, 548-552]. Awareness of CFBD as a significant co-morbidity impacting on the effectiveness of airway clearance techniques, exercise participation, health status, quality of life, suitability for transplant, pain and ultimately mortality, was not fully recognised till the 1990s [532, 536, 539, 542-545, 547, 549-569]. Despite advancement in multidisciplinary CF care, a recent study of CF adults found bone density of the lumbar spine and radius to be similar to that seen in a historic cohort 15 years prior, despite significant improvements in lung function and vitamin D status [550], indicating further interventions need to be investigated to optimize CF bone health. The Australian Cystic Fibrosis Data Registry Report, found an overall prevalence of osteopenia of 16% and osteoporosis of 5.9% in Australians with CF of all ages. Children

aged 6-11 years and 12-17 years had a prevalence of osteopenia and osteoporosis of 2.5% and 1.4%, and 15.1% and 5.6% respectively, indicating that CFBD can occur in young Australian children with CF, and that prevalence increases with age [480].

1.10.1 Characteristics of CFBD

1.10.1.1 Bone Density in Cystic Fibrosis

Low bone mineral density in CF was first described in 1979 [570, 571] and has been found at multiple skeletal sites in both children and adults with CF. Studies investigating lumbar spine bone mass across the lifespan in CF, found lumbar spine bone density to be significantly reduced [545, 557, 559, 563, 572-574]. Most studies have found deficits becoming more prevalent in adolescents and adults [563, 574], however others have found similar prevalence across the lifespan [557, 559]. Three longitudinal studies demonstrated minimal overall changes in lumbar spine bone density after 2 years [559, 572, 575]. One study reported changes in lumbar spine bone density to be smaller than expected for age and gender matched controls [559] and two studies reported decreases in lumbar spine bone density in 38% [559] and 35% [572] of their cohorts.

In CF adults, lumbar spine bone density was significantly lower compared to their healthy counterparts [547, 548, 552, 557, 563, 574-588]. Several studies measuring lumbar spine bone density by DXA, found standard deviation scores (SDS) to be at least 2 standard deviations below the norm in 6-41% of their cohorts [548, 552, 553, 559, 569, 576, 581-583, 586-589] and osteopoenia at the lumbar spine ranged between 10-69%

[548, 576, 582, 583, 589], the lowest scores seen in cohorts with end-stage disease referred for transplant [552, 569]. A study measuring lumbar spine bone density using quantitative commuted tomography (QCT) found 25% of the cohort had SDS at least 2 SDS below the norm [590]. Longitudinal studies in CF adults did not find lumbar spine bone density measured by DXA [583, 591, 592] or quantitative commuted tomography (QCT) [591], to be significantly changed over follow-up periods up to 24 months [583, 591, 592]. In contrast, two studies reported significant annual declines in lumbar spine bone density of 0.86% [580] and 0.18% [543].

In paediatric cohorts lumbar spine bone density was significantly reduced when measured by DXA [557, 559, 563, 567, 574, 575, 593-603] and quantitative commuted tomography [604]. Lumbar spine bone density SDS were in the osteopoenic range in 25-62% of the cohorts studied [559, 567, 600, 602, 605-607] and more than 2 SDS below the norm in 9-27% [563, 567, 594, 596, 600, 602, 607]. Longitudinal studies in paediatric cohorts with CF found significant reductions lumbar spine bone density in females but not males [592]. Another study, with a follow-up period of two years, found lumbar spine bone density in children to decrease by 0.18 SDS in males, mean age 13 years, and to increase 0.23 SDS in females [572]. In another cohort of children with CF, lumbar spine bone density decreased 0.15 SDS annually [595]. Longitudinal follow-up of two years in females, found no lumbar spine bone density SDS [605]. Despite the lack of significance, 50% of the cohort demonstrated declining SDS over the follow-up period [605].

Total body bone density SDS [547, 559, 563, 576-578, 585, 608, 609] and T-scores [543, 606] were found to be significantly reduced in the majority of the adult literature. Total body bone density at least one SDS below the norm occurred in 12-67% of adults studied [606, 608, 610] and over two SDS below the norm occurred in 12-38% [553, 563, 608]. No significant change in total body bone density was found in follow-up studies [543, 580, 592]. In CF children, total body bone density was generally found to be normal [563, 564, 593, 594, 598, 601, 611-614], however others found significant reductions compared to control populations [559, 592, 615], particularly in adolescents [563] and females [608, 612]. Longitudinal follow-up did not find any change in total body bone density after 2 years [559, 592].

Bone density measured at the hip was significantly reduce in CF adults [539, 552, 553, 563, 573, 575-581, 585, 606] with 28-53% of cohorts with bone density at least one SDS below the norm [552, 573, 576, 582, 586, 606] and 7-41% reporting bone density two SDS below the norm [552, 553, 576, 581, 582, 586]. The literature on CF children was divided with some reporting significantly reduced hip bone density [575, 593, 595], and others no deficits [564, 616, 617]. Longitudinal follow-up studies reported decreases in hip bone density [539, 580] [543, 595], however others found no change [572, 575, 592].

Analysis of the distal radius using high resolution peripheral quantitative computed tomography (HR-pQCT) demonstrated significantly reduced trabecular thickness [561], this was in contrast to pQCT findings at the 4% distal radius sit where trabecular volumetric bone mineral density (vBMD) was found to be normal [615, 618, 619],

however total vBMD was found to be significantly reduced [545, 615]. At the 66% radius site cortical vBMD was found to be normal [545, 618] however cortical cross-sectional area (CSA) and thickness were significantly reduced [545, 618] as was muscle CSA [545] and polar stress-strain index (SSI) [615]. Another study found bone mass to be significantly reduced at the mid-shaft [571] and bone density measured by DXA was found to be significantly reduced at the radius [552, 570, 620].

Analysis of the tibia by HR-pQCT found adults with CF to have significantly reduced total vBMD and trabecular thickness in the distal tibia [561]. Similar deficits were seen at the 4% tibial pQCT site with significantly decreased total and trabecular vBMD in female children and adolescents [621]. Another study found BMC to be significantly reduced at the 4% and 66% pQCT sites in pubertal females [622]. Muscle CSA was found to be decreased in males and females in one study [621] but only pubertal males and females in another [622]. Cortical CSA was reduced at the 20% pQCT site in males and females [621] and at the 66% site in pubertal females [622] and cortical polar SSI was decreased in pubertal females [622].

These studies indicate that in CF children with well-preserved lung function and nutritional status, bone health is preserved and does not deviate from their healthy counterparts. However, impairments in bone health emerge during adolescent with deficiencies in bone mass accrual and geometric adaptations during bone modelling. Deficits in bone density are established in adults with CF and accelerated bone loss is seen.

1.10.1.2 Increased risk of fragility fractures

In adults with CF, most studies have reported elevated rates of fractures, with 13-50% of cohorts reporting a previous fracture [561, 582, 586, 623, 624], and a pooled prevalence of non-vertebral fractures of 20% [544]. Vertebral fractures were also reported with increased frequency in CF adults, with 5-53% of cohorts having vertebral fractures [539, 540, 543, 547, 552-554, 561, 575, 576, 588, 623-625], and a pooled prevalence of 14% [544]. These rates are similar to those seen in older adults and untreated post-menopausal women [544, 547]. The majority of vertebral fractures occurred in the thoracic spine [544, 553, 554, 575, 623], and were mild in nature [588]. Multiple vertebral fractures were found in 8-17% [543, 553, 588]. The presence of vertebral fractures was associated with increasing age [543] and lower lung function [588, 625, 626]. Vertebral fracture incidence was found to be between 5-8.5% [543, 588] and those with new fractures were found to have a greater rate of bone loss compared to those without new fractures [543]. Rib fractures were found in 8-15% of adult CF cohorts [547, 623] and one study found fractures to be associated with lower bone density [623]. The majority of studies found that bone density was unable to distinguish between those with a fracture history and those without [539, 540, 547, 552, 553, 561, 575, 582, 623, 625, 627]. Similarly, high-resolution pQCT studies of the distal radius in adults with CF found no difference in trabecular bone architecture between those with and without fractures [561, 566]. These findings indicate that fractures are not solely a consequence of low bone mass, and that bone quality, including geometric attributes of the bone, are likely involved in the increased fracture rates described in adults with CF [544, 554, 625].

There are conflicting reports in the literature about fracture rates in children. In general fracture rates have been found to be similar between CF children and controls [563, 565, 594, 628, 629], however some studies have found increased rates compared to control [545, 629, 630]. While some studies have shown no difference in bone density between those with fractures and those without [563, 565], others have found that children with a previous fracture had significantly lower total body bone mineral content for height SDS [594], had bone density values in the osteopenia range [606], and in those with more than one fracture, total body and lumbar spine bond density and trabecular vBMD measured at the forearm were significantly lower than those with only one fracture [545].

In CF adults, exaggerated thoracic kyphosis has been reported at rates between 22-62% [547, 625, 631, 632], in adolescents between 15-31% [576, 606, 629, 633], and in children younger than 10 years, less than 15% [631], indicating that the prevalence of thoracic kyphosis increases with age [629, 631, 633]. In general the development of thoracic kyphosis was associated with the presence of vertebral fractures [547, 625]. Some studies found the presence of a thoracic kyphosis to be positively correlated with bone density [547, 606] however others did not [625, 627]. No correlation between the presence of a thoracic kyphosis and lung function was found by some studies [631, 632] however other studies found thoracic kyphosis to be negatively correlated with lung function [625, 633] and weight-bearing physical activity (WBPA) [625]. The development and progression of a thoracic kyphosis imparts abnormal stresses on the spine [634, 635] further increasing the risk of future vertebral fractures [636]. The

development of thoracic kyphosis and presence of vertebral and rib fractures has negative ramifications on effectiveness of airway clearance, pulmonary function tests and participation in WBPA.

1.10.1.3 Un-coupled bone turnover

Both paediatric and adult studies have found an imbalance in bone turnover. In studies of children with CF, bone resorption markers have in most cases remained within normal limits [574, 575, 600, 616, 617, 637-639], however some studies have reported elevated resorption [592, 601, 607, 630] that was correlated with age [607] and inversely correlated with bone density [559, 601, 607]. In contrast, few paediatric studies found bone formation to be normal [574, 639], the majority finding bone formation markers, especially osteocalcin, to be significantly lower than expected [575, 598, 600, 601, 603, 607, 616, 617, 630, 638] and often found to be significantly correlated with lung function [574, 598, 603] but not bone density [559, 598, 603]. One study also found significantly reduced amounts of OPG and a significant reduction in the OPG:RANKL ratio [638], providing further evidence of reduced bone formation activity. This un-coupling of normal bone turnover in the paediatric years provides support for the failure to accrue peak bone mass that becomes apparent during the pubertal growth spurt in adolescent with CF [539, 540, 547, 549, 552, 556, 557, 559, 560, 563, 570-572, 576, 585, 590, 592, 595, 596, 604-606, 613, 619, 623, 630, 640-643] with ramifications for long term bone health.

A slightly different disruption to bone turnover was seen in CF adults. Markers of bone resorption were increased in the vast majority of studies [539, 553, 568, 574-576, 579, 582, 586, 623, 630, 640, 644], with only a few studies reporting normal bone resorption [550]. There was a positive correlation between bone resorption markers and age [574] and a negative correlation with lung function [640] and bone mineral density [550, 601, 624], however others found no correlation with bone density [553, 566, 574, 586]. One study found osteoprotegerin (OPG) to be significantly reduced compared to control [645], which would have contributed to increased osteoclast activity providing further support for the increased bone resorption seen in the adults with CF. Most studies found bone formation markers to be in the normal range [550, 624, 630], however levels were found to be reduced by some [582, 630]. No association between bone formation markers and bone density was found [553, 568, 574], however others found significant correlations between the two [540, 548, 566, 586] and some significant negative correlations between bone formation markers and bone density [539, 589, 601, 624] suggesting a higher rate of bone turnover in those with lower bone density. This un-coupling in bone turnover in the CF adults provides support for the accelerated bone loss seen [576, 591, 592, 630, 646, 647].

Bone histomorphometry studies in adults have also found evidence of interruptions to normal bone turnover. In post-mortem lumbar spine bone specimens, there was a significant reduction osteoblast activity reflected by reduced osteoblast number and biosynthetic potential as well as increased osteoclast activity, evidenced by increased osteoclast number and resorptive activity [558]. Another study of rib bone specimens

taken during transplant compared to healthy specimens taken from donors, bone formation activity was significantly reduced however osteoblast numbers were normal suggesting a problem with osteoblast function or maturation [569] consistent with animal studies [648]. Histomorphometry of trans-iliac crest biopsies found significant reductions in osteoblast activity and bone formation rate due to a shortened lifespan and impaired function of mature osteoblasts. Osteoclast numbers were not significantly increased, suggesting bone deficits due to reduced formation rate and not increased resorption [579].

1.10.2 Risk factors involved in the pathogenesis of CFBD

1.10.2.1 Influence of the CFTR protein

In 2007 Shead et al [649] identified the presence of CFTR in osteocytes, osteoblast and osteocytes [649], which was supported by later studies [648-655] indicating that CFBD could be directly related to abnormal CFTR function in bone cells. When compared to control samples, the expression of OPG [533, 653] and prostaglandin E2 receptor (PGE2) [653] was significantly decreased however receptor activator for nuclear factor kappa-B ligand (RANKL) [533, 653] and the RANKL:OPG ratio [653] expression was significantly increased in human F508Del osteoblasts, indicating that bone loss in CF is likely caused by a reduction in osteoblastic bone formation and increased osteoclastogenesis and resorption [653]. These alterations to the expression of bone mass regulators were found to be associated with reduced chloride transport in the CFTR [533, 653]. There did not appear to be an effect of CFTR dysfunction on osteoblast

differentiation however osteoblasts did not progress through maturation processes normally, delaying mineralisation of the extracellular matrix [533].

When human F508Del osteoblasts were treated with a corrector, PGE2 [656] and OPG [533] production was increased, and RANKL [657] and the RANKL:OPG ratio was decreased [533, 656]. Furthermore, when CFTR activity was inhibited in normal human osteoblast cultures, there was a significant reduction in OPG production [652] and a significant increase in the RANKL:OPG ratio [533]. These findings confirm a pathological link between the CFTR, reduced osteoblast activity and increased bone resorption and that CFTR function has a role as a negative regulator in the expression of RANKL and the RANKL:OPG ratio.

In CFTR-null mice, histomorphometry revealed significantly reduced trabecular bone volume [650, 658], [655], cortical thickness [650, 658], cortical bone density[655] and bone apposition rate [650, 655] compared to heterozygous and wild-type litter mates [650], these findings consistent with impairments seen in humans with CF, indicating that loss of CFTR activity has direct actions on bone. Similar impairments in trabecular and cortical bone were seen in the F508Del mouse model [654].

Osteoblasts from CFTR-null and F508Del mice demonstrated significantly reduced number [648], impaired differentiation [648], function [654, 655, 659] and WNT-signalling [648], significantly reduced OPG expression [648, 659] and β-catenin [659], resulting in depressed WNT-signalling [659], a significantly elevated RANK expression

[659], RANKL:OPG ratio [648] and significantly increased osteoclasts [648, 654] and bone resorption [654], [648]. When given a F508Del corrector, trabecular bone volume and bone formation and apposition rate increased significantly and osteoclast number and activity significantly decreased [654], and in another study OPG expression significantly increased [659]. These finding supporting a direct effect of altered CFTR function on bone health in CF.

Furthermore, some studies found that F508Del homozygotes had significantly lower bone density compared to other genotypes in adults [538, 572] and children [559, 621] and adults with homozygous of heterozygous for F508Del had significantly lower bone density compared to other genotype [624]. However others have found no correlation with genotype in CF adults [539, 540, 550, 560, 561, 563, 566, 568, 573, 581, 602, 606, 623, 627] or children [563, 594, 595, 602, 603, 612, 614].

1.10.2.2 Disease severity and chronic inflammation

Several studies have found a strong correlation between bone density and measures of disease severity including lung function, disease severity scores, chest radiograph scores and bone mass index (BMI). The majority of studies in adults with CF have found bone density to be significantly associated with lung function [539, 540, 548, 553, 554, 560, 563, 566, 568, 572, 573, 581, 584-587, 589, 595, 606, 608, 609, 614, 620, 623-625, 627, 640, 660] and that bone density changes over time are significantly correlated to lung function [583], however some studies found no association between bone density and

lung function [547, 550, 552, 561, 578, 585, 590, 599]. Similar finding were found in children and adolescents, with the majority of the existing literature reporting significant associations between bone density and lung function [556, 557, 559, 563, 565, 567, 572, 573, 594, 595, 597, 600, 602, 603, 606, 608, 621, 661-665] however, some studies in well children with mild disease did not [575, 578, 598, 613].

Disease scores were found to be correlated with bone density in adult [560, 592, 627, 630]and paediatric cohorts [557, 575, 594, 600, 602, 604, 606, 661, 665], however others found no correlation [575, 578, 585, 590]. Similarly chest radiograph scores were commonly associated with bone density in both children [594, 595, 602, 606, 661] and adults [587, 627, 630] with CF, however a few studies found no link [590, 598, 614]. BMI was also found to be significantly correlated with bone density in CF adults [539, 540, 547, 548, 550, 552-554, 559-561, 563, 572, 576-578, 581, 584, 586, 587, 595, 597, 606, 608, 623-625, 666] and children [557, 559, 563, 567, 572, 594, 597, 600, 602, 605, 607, 608, 611, 621, 661, 664, 665], with few studies finding no correlation [552, 585, 599, 613, 616, 624].

Worsening disease severity is accompanied by chronic pulmonary infection and inflammation [667-669]. Recurrent pulmonary exacerbations cause further spikes in pro-inflammatory cytokines, including interleukin-1 (IL-1), interleukin-6 (IL-6) and tumour necrosis factor (TNF) alpha [561, 645, 669-671], establishing a climate for osteoclastogenesis and increased bone resorption [672]. Despite intravenous antibiotic treatment for infective exacerbations, there is often only partial resolution of elevated

pro-inflammatory cytokines [669, 673, 674], suggesting a role of inflammation in CFBD. To further support this, the inhibition of CFTR activity in normal human osteoblast cultures in induced inflammatory conditions, significantly increased RANKL and the RANKL:OPG ratio [533]. Human F508Del osteoblasts also demonstrated significantly increased RANKL expression [533] and production [657] and significantly lower OPG expressions [533] compared to control osteoblasts, under inflammatory conditions. However, when treated with a corrector the F508Del osteoblasts demonstrated significant reductions in RANKL [657], the RANKL:OPG ratio [533] and significant increases in OPG [533]. These studies confirm a direct link between inflammation and bone resorption and an exaggerated response of this pathway in the presence of CFTR dysfunction.

In adults with CF inverse correlations with pro-inflammatory cytokines and bone density have been found for IL-1 [582], IL-6 [669, 670, 675], TNF-alpha [669, 670] as well as C-reactive protein (CRP) [539, 640], number of antibiotic courses [572, 623], number of hospital days [563, 584] and exacerbations [609]. IL-6 was found to be negatively correlated with bone formation [589] and IL-1 significantly associated with bone resorption [644]. In CF children, TNF-alpha, IL-6 and CRP were found to have an inverse relationship with bone density [615] as did the number of intravenous antibiotic days [572, 594] and number of hospital days [563].

Oral and inhaled glucocorticoids are often used with increasing disease severity, however they adversely affect bone health by inhibiting osteoblast activity, promoting bone

resorption and suppressing intestinal calcium absorption and the secretion of gonadal and growth hormones [9, 676]. Most studies found oral glucocorticoid use to be correlated with bone density in CF adults, particularly at the lumbar spine [540, 547, 553, 560, 577, 586, 623, 624], however other did not find correlations [550, 566, 578, 585]. In children there was no consensus in the literature, with some studies finding a correlation [597, 621] [557, 559, 603, 606] and others not [538, 564, 594, 595, 608, 614, 665]. There was also no consensus in the existing literature in regards to the effect on inhaled glucocorticoids on bone density with some studies finding a correlation [563, 603, 606, 624] but the majority not [538, 550, 564, 578, 584, 586, 594, 602, 623, 627, 630, 661].

1.10.2.3 Pubertal delay and hypogonadism

Pubertal delay, with a late and blunted increase in sex hormones and delayed peak height velocity is reported in adolescents with CF [564, 677-679] and has been associated with impaired peak bone mass accrual [532, 536, 547, 560, 575, 641]. In paediatric CF populations, the majority of studies have not found a correlation between tanner stage and bone density [594, 630, 663, 665], however others have found significantly reduced bone density in the setting of delayed puberty [605, 607]. In adults with CF, several studies found that bone density was inversely correlated with age at puberty [547, 553, 560, 590, 620, 630] and age at menarche [560, 624], however other studies have not found a correlation between age at menarche and bone density [552, 606, 623]. One study found testosterone to be significantly correlated with bone density [623] however several others

have not [547, 553, 564, 582, 586]. Despite this, hypogonadism is considered to be associated with accelerated bone loss in CF adults [532, 560, 575, 606].

1.10.2.4 Nutritional factors

Calcium, vitamin D and vitamin K play important roles in bone modelling and remodelling processes. Serum calcium, calcium intake or calcium supplementation was not found to be correlated with bone density in children or adults with CF [540, 559, 560, 572, 574, 584, 595, 606, 614, 624]. Similarly, few studies found evidence of a correlation with parathyroid hormone [553, 574, 581, 582, 584, 586, 589, 598, 624, 640], however those that did found significant negative correlations with bone density [539, 566, 568]. Despite sub-optimal vitamin D level being frequently reported in CF [539, 540, 547, 552, 576, 606, 640, 641], no causal link with bone density has been found in adults [548, 552, 595], [539, 540, 547, 553, 560, 561, 572-574, 576, 581, 582, 584-586, 590, 606, 620, 623, 624, 640] or children [559, 596, 598, 600, 607, 613, 614, 617, 665], with few studies reporting a correlation [548, 552, 557, 566, 594, 595, 624, 666]. Similarly no correlation has been found between vitamin K and bone density [600], however a study of high Vitamin K supplementation found significantly higher concentration of carboxylated osteocalcin and significantly lower concentrations of the under-carboxylated form [637], a climate favourable for bone health.

1.10.2.5 Weight bearing physical activity and lean tissue

Weight bearing physical activity is similar to controls in prepubescent CF children, however from the onset of puberty there is a divergence in reported physical activity between boys and girls with CF, with girls being less active than boys [680, 681], and adults with CF spend significantly less hours performing physical activity compared to their healthy counterparts [563]. In adults with CF bone density was found to be correlated with peak exercise watts [666], peak oxygen uptake [581, 625, 627], peak working capacity [572, 573], time spent in moderate and vigorous activity [583, 666] and other measures of daily physical activity [539, 568, 606, 623] [560]. Time spent in moderate and vigorous activity as well as peak exercise watts were also associated with bone formation markers [666]. In CF children bone density was significantly correlated with peak working capacity [572, 573] and an activity score [563].

Lean tissue mass is often reported to be significantly decreased in CF cohorts compared to controls [559, 576, 614, 619, 621, 663], with negative ramifications on bone health. Positive correlations between leant tissue mass and bone density have been reported in both children [559, 602, 603, 612, 614, 621, 639, 661, 663, 677] and adults [566, 578, 582, 610, 618, 624, 660, 666] with CF, and is positively correlated to bone formation markers [666].

1.11 Summary

Mitochondrial respiratory chain disorders and cystic fibrosis are two chronic diseases that demonstrate impairments in bone mass and density, muscle mass and function, exercise capacity and quality of life. Participating in conventional exercise programs is often difficult due to functional restrictions imposed by their underlying disease. Whole-body vibration training does not elicit a significant cardiovascular response or require a high level of musculoskeletal competency. It has been found to be well tolerated and beneficial in improving bone mass and density as well as muscle mass and performance across the lifespan and in people with underlying respiratory and neuromuscular diseases. Whole-body vibration training may therefore be an effective training modality to improve impairments in bone, muscle, exercise and quality of life parameters commonly seen in people with MRCD and CF.

1.12 Aims

To evaluate whether a six-month home-based whole-body vibration training program in people with MRCD or CF influences:

1. Bone mass, density and geometry
2. Bone biochemistry and markers of bone turnover
3. Muscle mass
4. Muscle force, power and efficiency
5. Exercise capacity

6. Quality of Life

1.13 Hypotheses

Six-months home-based whole-body vibration training in children with MRCD or CF will improve musculoskeletal structure, function and quality of life.

2 Methods

2.1 Ethics

The studies presented in this thesis were approved by the Human Research Ethics Committee at The Children's Hospital at Westmead (CHW). Vibration training in children with cystic fibrosis: function, power, bone (Ethics Code: 2007/018) and Whole-body vibration training for Mitochondrial Respiratory Chain Disorders and Rett Syndrome (Ethics Code: 08/CHW/89). Informed, written consent was obtained from all participants and their care-givers.

2.2 Participant Recruitment

In the CF cohort, participant recruitment occurred between April 2010 and August 2011. Patients attending the Cystic Fibrosis Clinic at CHW were approached and invited to participate in the study. In the MRCD cohort, participant recruitment occurred between June 2009 and February 2012. Patients referred to the Western Sydney Genetic Program (WSGP) at the CHW were identified by investigators as potential participants. During management clinics held at the CHW, potential participants and their families were approached and invited to participate in the study.

Families showing interest in participating were provided with child and parent information sheets and a follow-up phone call was made to families allowing the opportunity for detailed discussion about the study protocol and to have any queries addressed. If they wished to participate in the study a mutually convenient time was scheduled for their baseline visit.

In the MRCD cohort, ethics was initially sought for participants aged between 8-18 years however early in the recruitment process it became evident that we would not reach our recruitment goal of 25 participants with this age restriction. An ethics amendment was submitted to recruit children and adults referred to the WSGP from other health services who were interested in participating in the study. Several adult participants were recruited from the Neurogenetics Clinic at Royal North Shore Hospital under the care of Professor Carolyn Sue. These amendments to the original protocol allowed for more timely recruitment and completion of the study.

2.3 Inclusion Criteria

- Age $\geq$ 6 years
- Baseline FEV1 $\geq$ 25% in the CF cohort
- Proven mitochondrial respiratory chain disorder based on positive enzymology in muscle biopsies or known pathogenic variant of a relevant nuclear or mitochondrially encoded gene

2.4 Exclusion Criteria

- Long bone or vertebral fracture within the last 6 months
- Past or present history of arthritis
- CF liver disease with portal hypertension
- Co-existent neuropathy or myopathy preventing participants being able to stand on a vibration platform for at least 10 minutes
- Cognitive impairment which would impede the ability of participants to comply with testing
- Untreated vitamin D deficiency (25-hydroxyvitamin D Level < 50nmol/L)
- History of using any of the following medications known to interfere with bone metabolism, regardless of dose, for at least 1 month, within 3 months of enrolment:
    - Anabolic agents
    - Oestrogen (except contraceptives)
    - Progestogens (except contraceptives)
    - Calcitriol
    - Calcitonin
    - Fluoride (except dental health products)
    - Bisphosphonates
    - Growth hormone
    - Parathyroid hormone (PTH)
    - Strontium

2.5 Study Design

The studies were divided into three periods: observation, WBVT and follow-up. During the observation period participants were monitored to gauge the progression of their chronic illness, each acting as their own control to compare with the WBVT and follow-up periods. This design was chosen due to the significant phenotypic variability displayed in both MRCD and CF and due to the relatively small pool of eligible participants. A design where each participant acted as their own control was considered optimal as the likelihood of being able to successfully recruit for a randomised-controlled study able to compensate for phenotypic variability was minimal. In the MRCD cohort, the observation period was 3 months in duration and in the CF cohort, 6 months.

The WBVT period was 6 months in duration in both the MRCD and CF cohorts. This duration was chosen for WBVT as significant improvements in tibial trabecular bone density had previously been found using this duration of WBVT in ambulant children with disabling conditions [197]. During this phase of the study participants were provided with a Galileo® Home vibration platform (Stratec, Pforzheim, Germany), which provided a side-alternating vibration stimulus. Participants were asked to complete three 3-minute sessions of vibration training daily. A minimum of 3 minutes rest was required between each session to allow the muscles of the lower limbs and trunk to rest. This training protocol was chosen as it had been previously used and well tolerated in children and adolescents with motor impairments and bone fragility [256, 257], and in adults with cystic fibrosis [233], with favourable results on mobility and muscle function

respectively. The participant was allowed to structure their daily training to best fit in with their daily activities e.g. all in the morning or some in the morning and some at night. Vibration training was only conducted under the direct supervision of a responsible adult and was for the sole use of the study participant.

Participants were instructed to stand on the platform with their feet between markers "2" and "3" on the vibration platform (Figure 2.1) corresponding to an amplitude between 4-6mm. They were asked to adopt a static semi-squat position by standing with a slight knee bend during training sessions. If desired they could place their hands on their knees. In this position the knees were comfortably bent between 10-45 degrees, a recommendation of a previous study [257] to optimise absorption of the vibration stimulus to the legs and trunk and minimise caudal transmission of the vibration to the head which could limit tolerability. Some participants in the MRCD cohort who felt they would be unable to maintain their balance on the vibration platform were provided with a pick-up frame (Figure 2.1) to use for stability during training. These participants were instructed to use the frame only when absolutely necessary and to try and limit the amount of weight transferred to the frame through the upper limbs so as to maximise the amount of weight through the lower limbs to enhance the effectiveness of the vibration training.

Figure 2.1: Child on Galileo® Home vibration platform (left). Pick-up frame with Galileo® Home vibration platform (right).

Participants trained at a vibration frequency of 20Hz for the duration of the study. This frequency was chosen as it should theoretically promote the maintenance of, and potentially augment, muscle mass as the vibration stimulus at this frequency does not allow the muscle to fully relax between vibration cycles resulting in a tonic contraction of the muscle [682, 683]. This frequency had also been previously used and well tolerated in children and adolescents with motor impairments and bone fragility [256, 257], and in adults with cystic fibrosis [232, 233], with favourable results on mobility and muscle function. The initial training session was performed under the supervision of the research physiotherapist. This allowed for the identification of any safety concerns, potential adverse events and to ensure the participant was competent in the use of the vibration

platform. Participants were slowly built up to a vibration frequency of 20Hz over the first week of training and the speed at which they reached this frequency was participant-driven. The research physiotherapist was in regular telephone contact with participants during the WBVT period.

The follow-up period was 6 months in duration in both the cohorts. This phase allowed for the assessment of any de-training effects or whether benefits persisted after completion of the intervention period.

Throughout the study, participants continued to receive their standard treatments. Nutritional and physiotherapy reviews were performed at least annually with more frequent follow-up attended as clinically indicated on an individualised basis.

2.5.1 Visit Structure

All participants attended The Children's Hospital at Westmead on 4 occasions for study visits: baseline; 3-6 months later (Start WBVT); after 6 months of WBVT (End WBVT); and after 6 months follow-up (Follow-up). At each visit participants underwent assessments of bone density, blood and urine analysis, muscle function, exercise capacity and Quality of Life, as well as pulmonary function testing in the CF cohort. The order in which assessments were performed at the first visit were recorded and every effort was made to keep the order of assessments, especially those requiring physical exertion, in a similar order at subsequent visits.

2.6 Outcome measures

2.6.1 Anthropometric Measures

Height and weight were measured to the nearest 0.1cm and 0.1kg respectively, in light clothing without shoes, using a stadiometer (Seca Model 220, Seca GmbH, Germany) and digital scale (Seca Model 703, Seca GmbH, Germany). LMSgrowth software version 2.74 was used to calculate height, weight and BMI standard deviation scores (SDS).

2.6.2 Bone Parameters

2.6.2.1 DXA

DXA scans of the total body and lumbar spine were performed in the Nuclear Medicine Department at the Children's Hospital at Westmead (CHW) using the Lunar Prodigy (GE Lunar Radiation Corp, Madison, Wisconsin, USA). Total body (Figure 2.2) and posterior-anterior lumbar spine (Figure 2.2) scans were performed using positioning and scanning procedures recommended by the manufacturer and analysed using software version 8.6. Effective radiation from the DXA scans is <1 microSv. The DXA scans directly measured bone mineral content (g) and the bones' two-dimensional projection area (cm^2). Areal bone mineral density was then calculated from these parameters. The total body scan was also used to assess the body composition variables, lean tissue and fat

mass, and to perform analysis on skeletal subregions (arms, trunk, legs) to assess the site-specificity of any changes. Lumbar spine vertebrae 1-4 volumetric bone density was calculated as described by Carter et al [684]. DXA parameters are shown in Table 2.1. Age, height and sex-matched SDS for children were calculated using normative data from CHW, based on extended data of published work [685-687]. Total body and lumbar spine bone mineral density SDS for adults were calculated from a reference data set provided with the Lunar software, which included Australian normative data [688].

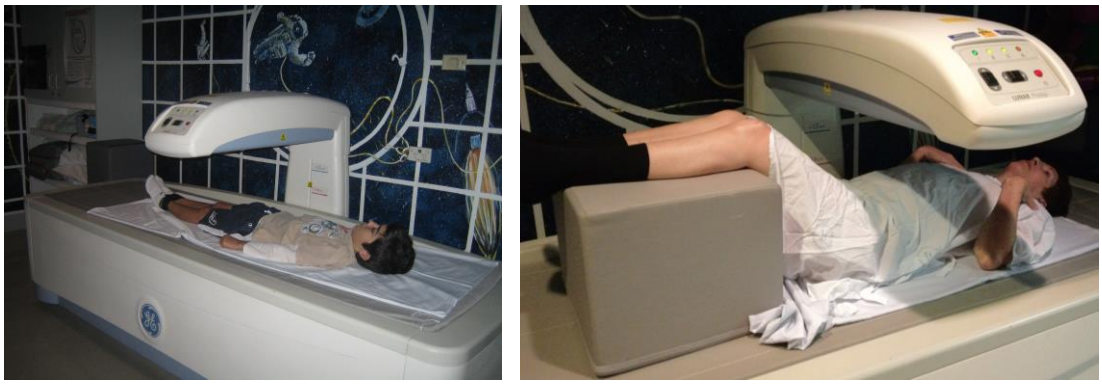

Figure 2.2: Child having total body DXA scan (left). Adult having lumbar spine DXA scan (right).

Table 2.1: DXA parameters, their definitions and associated coefficients of variation.

Parameter	Definition	CV
Total Body DXA Scans		
TBMC (g)	Total Body Bone Mineral Content	1.81%[†]
TBA (cm^2)	Total Body Bone Area	-
TBMD (g/cm^2)	Total Body Bone Mineral Density	1.55%[†]; 1.7%[‡]
LTM (g)	Total Body Lean Tissue Mass	0.80%[†]
Fat (%)	Total Body percentage Fat (calculated by fat/soft tissue)	-
Ancillary Parameters		
LTM for Height SDS	LTM as predicted by Height SDS	-
TBA for Height SDS	Total BA as predicted by Height SDS	-
TBMC for LTM SDS	Total Body BMC as predicted by Lean Tissue Mass SDS	-

TBMC for TBA SDS	Total Body BMC as predicted by Bone Area SDS	-
Parameters of Segmental Analysis of Total Body DXA Scans		
Trunk BMD (g/cm^2)	Bone Mineral Density of the Trunk	-
Arms BMD (g/cm^2)	Bone Mineral Density of the Arms	-
Legs BMC (g)	Bone Mineral Content of the Legs	-
Legs BA (cm^2)	Bone Area of the Legs	-
Legs BMD (g/cm^2)	Bone Mineral Density of the Legs	-
Legs LTM (g)	Legs Lean Tissue Mass	-
Legs Fat (%)	Legs percentage Fat (calculated by fat/soft tissue)	-
Lumbar Spine DXA Parameters		
LS BMC (g)	Lumbar spine vertebrae 2-4 bone mineral content	0.48%†
LS BMD (g/cm^2)	Lumbar spine vertebrae 2-4 bone mineral density	0.43%†; 1.7%‡

L14 BMC (g)	Lumbar spine vertebrae 1-4 bone mineral content	-
L14 BA (cm^2)	Lumbar spine vertebrae 1-4 bone area	-
L14 vBMD (g/cm^3)	Lumbar spine vertebrae 1-4 volumetric bone mineral density	-

Note: CV, coefficient of variation; DXA, dual-energy x-ray absorptiometry; †based on paediatric data from the department of Nuclear Medicine at CHW; ‡ based on adult data from Crabtree et al 2009 [134].

2.6.2.2 pQCT

pQCT scans were performed on the non-dominant tibia using the XCT-2000 scanner (Stratec Medizintechik, GmbH, Pforzheim, Germany). Tibial length was measured from the medial malleolus to the superior margin of the medial condyle using a tape measure while the patient rested their non-dominant ankle on the opposite knee. This length was entered into the scanner software version 6.0B and used to calculate relative distances of the three analysis sites, 4%, 20% and 66% of the tibial length. The participant's leg was extended into the machine and secured distally to a footplate and proximally by a clamp just above the knee (Figure 2.3). Participants were asked to remain as still as possible for the duration of the scan. Young children were given a portable DVD player to help distract them and keep them still during the scan.

Figure 2.3: pQCT scanner showing distal end (left) and proximal end (right).

Once positioned correctly in the scanner, a scout scan was performed to identify the distal tibial growth plate, or end plate if final height had been reached. The proximal boundary

of this growth plate/endplate was considered the reference line. The scanner software then re-positioned the gantry to scan the three analysis sites. Each slice took about 2 minutes to complete and incurred minimal radiation to the participant (<0.2microSv/ slice). Scans were acquired with a voxel size of 0.4mm and scanning speed of 20mm/s with a slice thickness of 2.4mm. To avoid errors due to the "partial volume effect", only images with a cortical thickness greater than three times the voxel size were analysed. All measurements were performed by the same experienced technician. The scan generated a cross-sectional image of the bone and surrounding tissues at the 4% distal metaphyseal site and 20% and 66% diaphyseal sites (Figure 2.4). Parameters at each of these sites and their corresponding analysis settings are shown in Table 2.2.

Reference data for the 4%, 20% and 66% sites for adult and children have been sourced from available published papers and personal communications. In children, reference data for the 4% and 66% sites was obtained from the published height interval data of Moyer-Mileur et al [61] and used to generate SDS. Dr Teresa Binkley (South Dakota State University, USA) kindly provided her paediatric reference data for the 20% site and this was prepared in height intervals (Table 2.3), similar to the Moyer-Mileur et al [61] data, and SDS were calculated. pQCT data for the adult MRCD cohort was sent to Dr Ferretti (National University of Rosario, Argentina) who analysed parameters from the 4%, 20% and 66% sites, using his reference database of healthy adults.

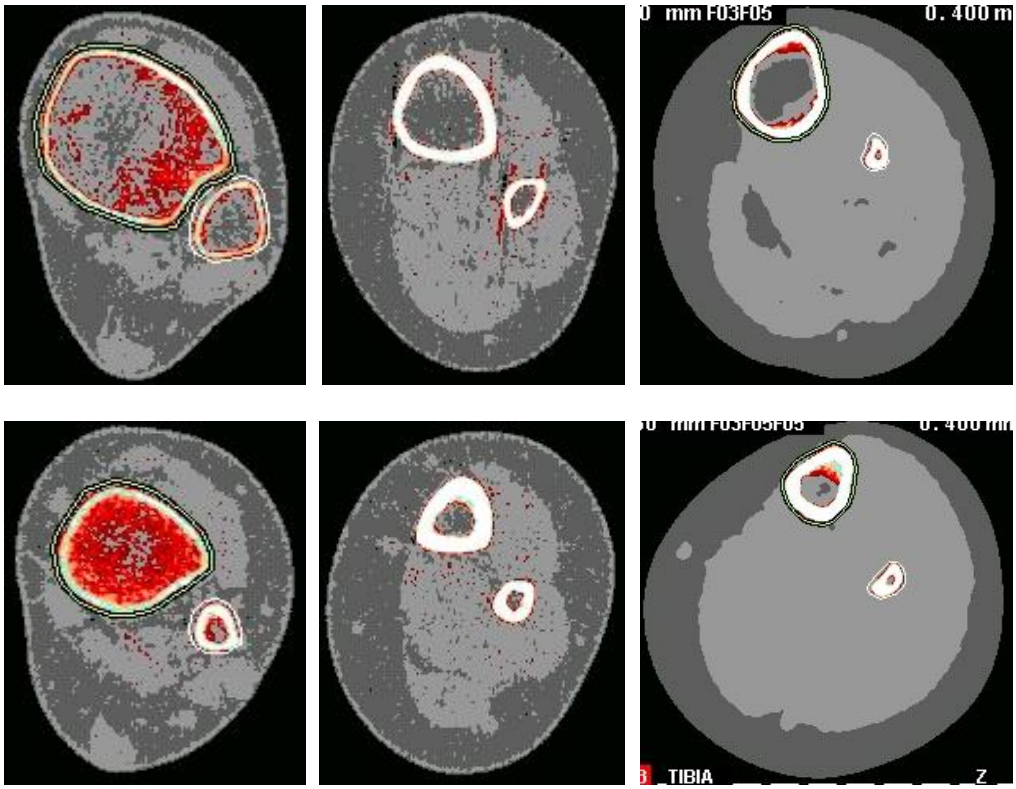

Figure 2.4: pQCT cross-sections at the 4%, 20% and 66% site in a MRCD patient (top) and CF patient (bottom).

2.6.2.3 Bone Turnover Markers

Bone mineral homeostasis and turnover markers were assessed at each visit. Parameters and their analysis methods can be found in Table 2.4. Blood samples were collected at the Children's Hospital at Westmead Pathology Department and urine samples were collected from the second void of the day on the day of their visit, wrapped in foil and refrigerated till their review.

Table 2.2: pQCT settings for bone and muscle parameters at the 4%, 20% and 66% tibial sites.

Site	Variable	Analysis parameter	Threshold mg/cm^3	Mode			CVs
				Contour	Peel	CORT	
4%	Total BMC	CALCBD	180[a]; 169[b]	1	1	-	1%[d]
	Total CSA						
	Total vBMD						
	Trabecular BMC						
	Trabecular CSA	*NB: Trabecular parameters calculated on inner 45% of the cross-section*					
	Trabecular vBMD						1%[d]
20%	Total BMC	CALCBD	180[a]; 169[b]	1	2	-	<2%[e]
	Total CSA						
	Total vBMD						
	Total SSIp	CALCBD	280	1	1	-	<5%[e]
	Cortical BMC	CORTBD	711[a]; 700[b]	-	-	1	

	Cortical CSA	*Determined using circular ring model described by Louis 1995 in Binkley 2000*					<2%[e,f]
	Cortical vBMD						<2%[e,f]
	Cortical thickness						1.2%[c]
	Periosteal circumference						0.5 - 1.4%[c,f]
	Cortical CSMIp						
	Cortical SSIp						
66%	Total BMC	CALCBD	180[a]; 169[b]	1	2	-	
	Total CSA						1%[d]
	Total vBMD						
	Cortical BMC	CORTBD	711	-	-	1	
	Cortical CSA						1%[d]
	Cortical vBMD						2.5%[d]
	Cortical thickness	*Determined using circular ring model described by Louis 1995 in Binkley 2000*					
	Periosteal circumference						
	Cortical CSMIp						

	Cortical SSIp					
	Muscle CSA	CALCBD	50	3	1	
		Filter F03F05 used for analysis				
	Fat CSA	CALCBD	-51	3	1	

Note: BMC, bone mineral content; CSA, cross-sectional area; vBMD, volumetric bone mineral density; SSIp, polar stress-strain index; CSMIp, polar cross-sectional moment of inertia; CVs, coefficients of variation; [a]paediatric parameters; [b]adult parameters; [c]Binkley et al [146]; [d]Moyer-Mileur et al [61]; [e]Binkley et al [148]; [f]Binkley et al [117].

Table 2.3: Reference ranges for bone mass, geometry, density and strength by height intervals for boys and girls.

Height (cm)	N	Age (years)	Cortical BMC	Cortical CSA	Cortical vBMD	Periosteal Circumference	Cortical Thickness	Polar SSI
Females								
105 – 114	3	5.4 ± 0.3	58.1 ± 14.7	60.1 ± 13.2	961.9 ± 35.5	53.0 ± 1.9	1.2 ± 0.3	293.3 ± 80.7
115 – 124	9	7.1 ± 0.6	80.7 ± 7.1	82.3 ± 7.1	981.3 ± 29.6	52.6 ± 4.1	1.8 ± 0.3	386.1 ± 50.5
125 – 134	21	8.5 ± 1.3	91.1 ± 13.7	91.3 ± 12.8	996.5 ± 22.4	56.8 ± 3.3	1.8 ± 0.3	478.8 ± 71.0
135 – 144	12	10.2 ± 1.1	105.1 ± 18.1	103.8 ± 16.2	1010.6 ± 42.7	60.5 ± 3.6	1.9 ± 0.3	605.1 ± 113.6
145 – 154	15	11.4 ± 2.1	139.7 ± 26.2	134.7 ± 20.7	1033.9 ± 52.5	63.8 ± 5.0	2.4 ± 0.5	803.7 ± 141.7
155 – 164	26	15.4 ± 2.8	179.8 ± 32.4	163.3 ± 23.0	1095.1 ± 61.8	66.8 ± 5.7	2.9 ± 0.7	1033.6 ± 176.7
165 – 174	18	15.9 ± 2.6	210.3 ± 28.1	187.8 ± 22.5	1118.1 ± 35.6	69.4 ± 5.1	3.2 ± 0.5	1265.9 ± 216.9
175 – 184	4	17.2 ± 1.9	214.4 ± 21.7	188.2 ± 18.7	1139.3 ± 32.6	75.0 ± 5.4	2.9 ± 0.3	1479.5 ± 193.9

Males								
115 – 124	9	6.5 ± 1.1	73.4 ± 15.9	74.5 ± 14.2	967.5 ± 44.2	52.8 ± 4.0	1.6 ± 0.3	364.9 ± 87.5
125 – 134	12	8.3 ± 1.6	98.4 ± 15.7	100.1 ± 13.5	980.0 ± 42.2	58.1 ± 3.4	1.9 ± 0.3	527.6 ± 84.7
135 – 144	17	9.6 ± 1.4	113.7 ± 12.7	114.0 ± 12.8	998.2 ± 27.3	61.9 ± 5.1	2.1 ± 0.3	656.3 ± 114.3
145 – 154	15	10.9 ± 1.4	139.9 ± 21.5	139.4 ± 19.3	1001.8 ± 35.9	66.2 ± 4.9	2.4 ± 0.5	825.5 ± 128.5
155 – 164	8	13.3 ± 1.2	188.5 ± 38.7	184.9 ± 35.9	1017.7 ± 37.1	73.8 ± 3.9	2.9 ± 0.5	1249.8 ± 259.6
165 – 174	10	14.7 ± 2.9	207.5 ± 52.4	194.2 ± 38.4	1058.6 ± 66.6	72.3 ± 5.9	3.2 ± 0.6	1341.5 ± 363.6
175 – 184	9	16.5 ± 1.5	246.7 ± 30.5	224.2 ± 26.2	1100.7 ± 44.6	77.2 ± 5.7	3.4 ± 0.7	1627.2 ± 241.9
185 – 194	6	18.0 ± 1.4	272.9 ± 34.7	253.9 ± 28.2	1073.2 ± 45.0	82.2 ± 5.1	3.6 ±0.6	1840.9 ± 260.2

Note: BMC, bone mineral content; CSA, cross-sectional area; vBMD, volumetric bone mineral density; SSI, stress-strain index. Data provided by Dr Teresa Binkley.

Table 2.4: Bone mineral homeostasis and turnover markers, analysis methods and reference data used.

Parameter	Unit	Analysis Method	Reference Data
Alkaline Phosphatase	(U/L)	"Dry chemistry" technique (Vitros Fusion 5.1, Ortho-Clinical Diagnostics)	Tate et al [689]
Calcium	(mmol/L)		
Phosphate	(mmol/L)		
Osteocalcin	(nmol/L)	Sold phase chemiluminescent immunoassays (Immulite 1000, Siemens, Los Angeles, USA)	As per the Children's Hospital at Westmead Endocrine Laboratory Policy and Procedures Manual
Parathyroid Hormone	(ng/l)	Two site chemiluminescent enzyme labelled immunometric assay (Immulite, DPC, California, USA)	
Urinary Deoxypyridinoline: Creatine Ratio	(nmol/mmol creatinine)	Pyrilinks-D assay (Immulite, DPC, California, USA)	
25-hydroxyvitamin D	(pmol/L)	Radioimmunoassay (Diasorin, Stillwater, Minn, USA)	Normal range > 50

2.6.3 Muscle Function Testing

Functional muscle testing was performed on the Leonardo Mechanography Ground Reaction Force Plate (Novotec, Medical GmbH, Pforzheim, Germany) (Figure 2.5). Two adjacent force plates (left and right), each with four strain gauge force sensors and a resonance frequency of 150Hz, measure vertical ground reaction forces exerted on the platform. Signals from the platform are sampled at a frequency of 800Hz and connected to a computer by a USB 2.0 connection. The platform was calibrated according to the manufacturers recommendations and zeroed prior to the performance of each test. Leonardo Mechanography GRFP Research Editions software was used to analyse manoeuvres performed on the plate.

Three commonly used mechanographic tests measuring force and their derivatives were chosen due to their proven correlation with motor capacity that is relevant to activities of daily living and functional decline [690-694]: the multiple one-leg jump, single two-leg jump and chair-rise test. These manoeuvres allowed for a range of functional abilities among participants [692]. They have established validity and reliability and reference data exists for both paediatric and adult populations [692, 694-696]. Prior to each test, participants stood stationary on the platform with one foot on each of the force plates to measure body mass. After a single-tone, participants commenced the manoeuvre, the vertical force applied to the platform used to calculate vertical acceleration and thus power. After completing the manoeuvre, participants stood stationary on the platform till a double tone was heard, indicating test completion. At least 3 trials of each manoeuvre

were completed. Parameters, reference data and coefficients of variation (CV) are presented in Table 2.5.

Table 2.5: Force plate parameters, reference data and coefficients of variation.

Parameter	Cohort	Unit	Definition	Reference Data for SDS calculation	CV[a]
Multiple One-leg Jump (M1LJ)					
F max	Paediatrics	kN	Maximum voluntary ground reaction force		4.7-5.9%
	Adults				3.7-4.8%
F max rel	Paediatrics	g	Maximum ground reaction force in relation to body weight	Lang et al [695]	4.2-6.4%
	Adults			Leonardo Software Package	3.8-5.2%
Single Two-leg Jump (S2LJ)					
EFI	Paediatrics and Adults	%	Esslinger Fitness Index - Maximum relative power as a percentage of the mean value of the gender- and age-matched reference group. 100% is equivalent to the 50th centile	Maximum relative power normalized to age and gender - Tsubaki et al [697]	
FE	Paediatrics and Adults	%	Force Efficiency of movement pattern – maximum relative power output in relation to normalized maximum force	Leonardo Software Package	

P max	Paediatrics	kW	Maximum power during the lift-off phase (combined right and left legs)		3.6%
	Adults				5.5%
P max rel	Paediatrics	W/kg	Maximum power in relation to body mass	Busche et al [696]	3.4%
	Adults			Dietzel et al [694]	5.5%
F max	Paediatrics	kN	Maximum ground reaction force during lift off phase (combined right and left legs)		12.7%
	Adults				6.0%
F max rel	Paediatrics	g	Maximum force in relation to body weight	Busche et al [696]	13.1%
	Adults	N/kg	Maximum force per kg body mass	Dietzel et al [694]	6.6%
Chair-Rise Test (CRT)					
Time per test	Paediatrics	s	Average time per repetition	Busche et al [696]	
	Adults				
V max	Adults	m/s	Average maximum vertical velocity during rise of all repetitions	Dietzel et al [694]	6.2%
P max	Paediatrics	W	Maximum power (combined right and left legs)		14%

	Adults				8.1%
P max rel	Paediatrics	W/kg	Average maximum power during the rise phase of each repetition in relation to body mass	Busche et al [696]	15.6%
	Adults	W/kg	Maximum power during the rise phase of each repetition per kg body mass	Dietzel et al [694]	8%
F max	Paediatrics	kN	Maximum ground reaction force during the rise phase of each repetition per kg body mass		7%
	Adults				8.3%
F max rel	Paediatrics	g	Average maximum ground reaction force during the rise phase of each repetition as a multiple of body weight	Busche et al [696]	7.9%
	Adults	N/kg	Maximum force during the rise phase of each repetition per kg body mass	Dietzel et al [694]	7.9%

Note: SDS, standard deviation score; CV, coefficient of variation; [a]Referring to CVs from Veilleux et al [692]; F, force; EFI, Esslinger Fitness Index; FE, force efficiency; P, power; V, velocity.

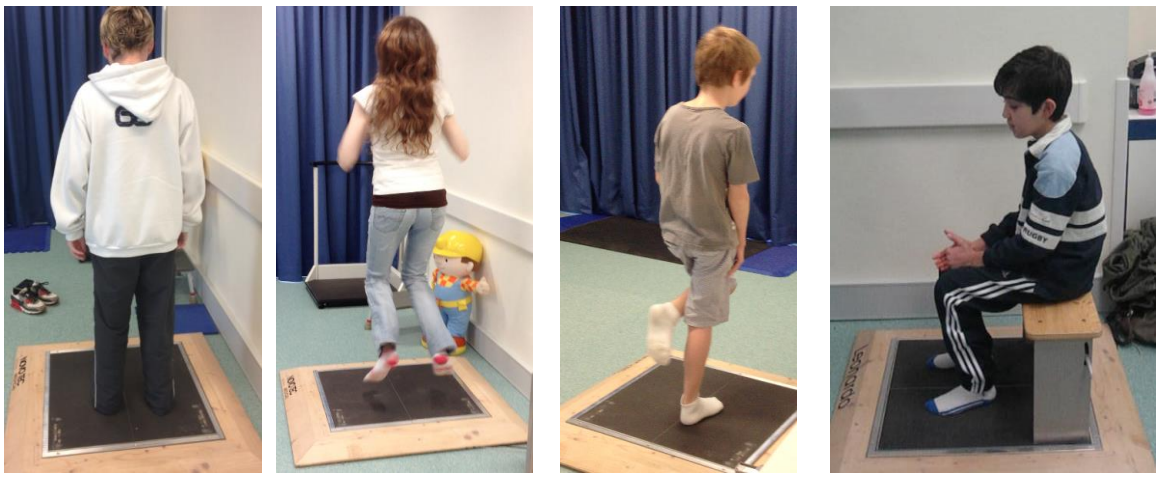

Figure 2.5: Muscle function testing on the Leonardo Mechanography Ground Reaction Force Plate.

2.6.3.1 Multiple One-leg Jump (M1LJ)

Participants were instructed to perform 10 consecutive hops as high as possible on the ball of the non-dominant foot, trying to keep the heel off the ground by keeping the knee stiff (Figure 2.5). The trial with the highest maximum force was selected for analysis (Figure 2.6). This manoeuvre allows quantification of maximal ground reaction forces a participant can generate and is a surrogate measure for the maximum forces the tibia is exposed during habitual physical activity. Performance has been correlated with tibial bone parameters assessed using pQCT [698, 699].

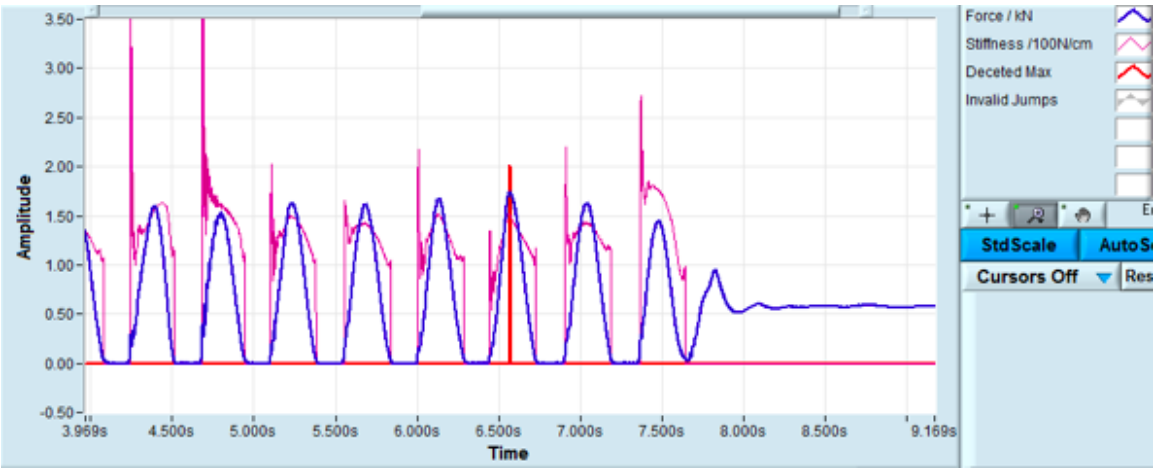

Figure 2.6: Multiple one-leg jump output.

2.6.3.2 Single Two-leg Jump (S2LJ)

Participants were instructed to perform one jump as high as possible using both legs (Figure 2.5). Three trials were performed and the trial with the highest maximum power was selected for analysis (Figure 2.7). This manoeuvre requires muscle coordination and balance and allows quantification of maximal power generated by the lower limbs, a surrogate measure of anaerobic capacity [700].

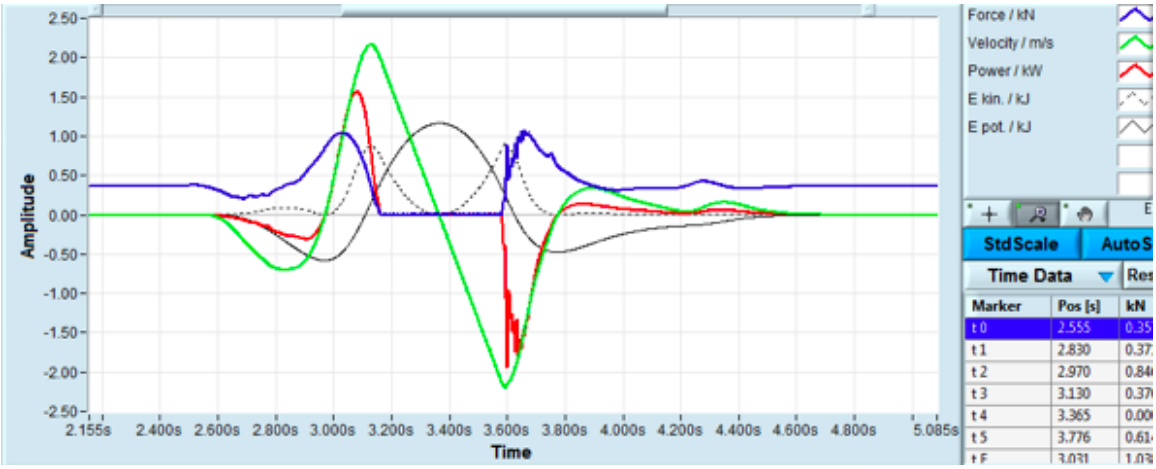

Figure 2.7: Single two-leg jump output.

2.6.3.3 Chair-rise Test (CRT)

Participant were instructed to perform 5 repetitions of a sit-to-stand as fast as possible, ensuring a full standing position is achieved with each repetition. A specifically designed bench (34 or 46cm) was anchored to the Ground Reaction Force Plate for this manoeuvre, at a height aiming for hips and knees to be near 90 degrees when in the seated position (Figure 2.5). The same height chair was used on all testing occasions. The trial with the highest maximum power was selected for analysis (Figure 2.8). This manoeuvre is relevant for daily activities and provides a measurement of power when jumping manoeuvres are unable to be performed [692].

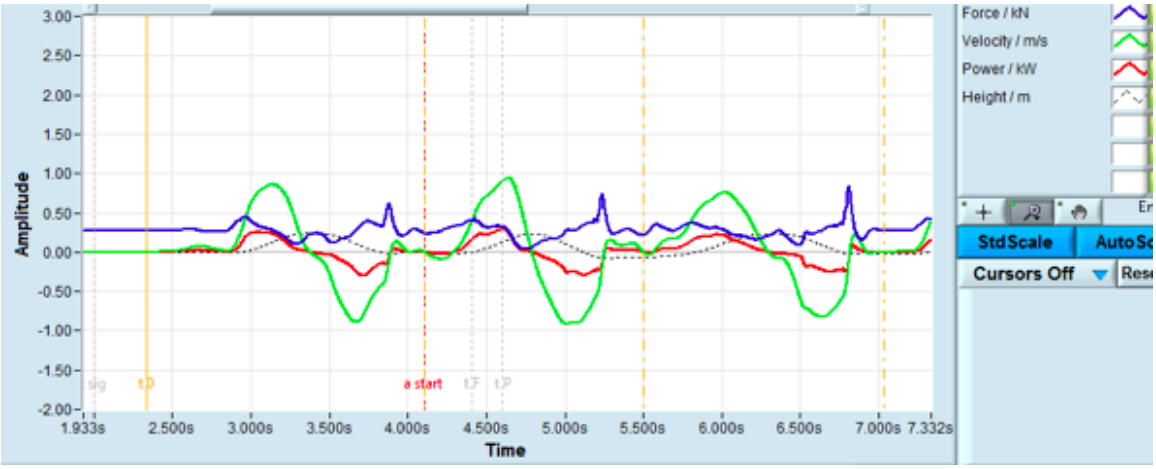

Figure 2.8: Chair-rise test output.

2.6.4 Exercise Testing

2.6.4.1 Six Minute Walk Test

This test was performed by all participants in the MRCD cohort. It was not performed by the CF cohort. The six minute walk test (6MWT) was performed on a 25m track according to ATS criteria [701]. Prior to the test participants were seated alongside the walking track. Systolic and diastolic blood pressure, heart rate and oxygen saturations were recorded using a digital sphygmomanometer (Vital Signs Monitor 300 Series, Welch Allyn, USA). A modified rate of perceived exertion (RPE) scale (Cox ref) was used to get participants to subjectively rate their degree of physical exhaustion. Participants were given instructions about the test according to ATS criteria [701]. In brief, participants were asked to walk as far as possible in the 6 minutes and given the option to take standing rests if they became exhausted or out of breath however they were encouraged to start walking again as soon as possible. The total distance completed was

recorded. After completion of the test participants were seated alongside the track. Repeat measurements of blood pressure, heart rate, oxygen saturations and RPE were performed and recorded. After a five-minute rest period these were collected again. Reference data for children was sourced from Ulrich et al [702], for adults < 40 years of age from Chetta et al [703] and for adults $\geq$ 40 years from Enright et al [704].

2.6.4.2 Formal Cardiopulmonary Exercise Testing

All children in the CF cohort and capable children and young adults in the MRCD cohort performed formal exercise testing in the Respiratory Function Unit at the Children's Hospital at Westmead using the Bruce Protocol [705] on an electronic treadmill (TMX 55, Trackmaster Treadmills, Newton, USA) and breath-by-breath gas analysis (Vmax Encore 229, Care Fusion, CA, USA) by a soft facemask (7900 Series Silicone Facemask, Hans Rudolph, Missouri, USA). Using Vmax Series Version 21-1A software (CareFusion, CA, USA), breath-by-breath gas analysis was used to determine peak oxygen uptake (VO_2), exercise ventilation (VE), carbon dioxide production (VCO2), ventilatory equivalents of oxygen (VE/VO2) and carbon dioxide (VE/VCO2) and the respiratory exchange ratio (RER). Heart rate and oxygen saturations were measured throughout the test using a 4 lead ECG (Cardiosoft 12-Lead ECG, GE Healthcare, Chicago, USA) and pulse oximeter (Massimo Radical, Irvine, Canada) respectively, and was integrated into the Care Fusion Vmax system. Equipment was calibrated according to the American Thoracic Society guidelines prior to each test.

Once participants were set up and breath-by-breath data acquisition had commenced a baseline period of 2-5 minutes was recorded. During this time participants had blood pressure measured using a digital sphygmomanometer (Vital Signs Monitor 300 Series, Welch Allyn, USA) and rated their degree of physical exhaustion using the Borg scale [706]. After the baseline measurement period the Bruce Treadmill Protocol was commenced (Figure 2.9). Every three minutes the speed and incline of the treadmill increased according to the Bruce Protocol. At the end of each stage participants were asked to rate their degree of physical exhaustion on the Borg scale. The test was terminated when the participant reported physical exhaustion or was unable to maintain a safe cadence on the treadmill. Participants were seated and blood pressure and RPE were recorded. Recovery data were collected for 5 minutes. A final measure of blood pressure and RPE was recorded and the participant disconnected from the equipment.

Adults in the MRCD cohort capable of performing a formal exercise test had their tests performed at Royal North Shore Hospital (RNSH), an accredited Pulmonary Function Laboratory for adults. These participants had breath-by-breath analysis during exercise using the same equipment, hardware and software however they performed their tests on an electronically braked cycle ergometer (CareFusion, MS-CPX, CA, USA) using a 10 Watt/minute protocol. These participants underwent the same baseline and recovery conditions however during the exercise test they were instructed to cycle at a cadence of 60 revolutions per minute. Cycle ergometry testing was terminated when the participant reported physical exhaustion or was unable to maintain the required cadence of 60

revolutions per minute. All exercise tests at both CHW and RNSH were performed with at least two people present and under the supervision of a Respiratory Physician.

Reported values for each variable at baseline, anaerobic threshold (AT), peak exercise and recovery were calculated by taking an average of the breath-by-breath data over a 30 second time frame. Anaerobic threshold was identified using the ventilatory equivalents method [707], peak exercise was when peak VO_2 was achieved and recovery data were the last 30-second interval of the 5-minute recovery period. AT and Peak VO_2 were selected under the guidance of a Respiratory Physician. Reference data for children was obtained from Cooper et al [708], and for adults, from a reference data set provided with the CareFusion software using data recommended by the American Thoracic Society and European Thoracic Society. Maximum predicted heart rate was calculated by subtracting the participant's age from 220.

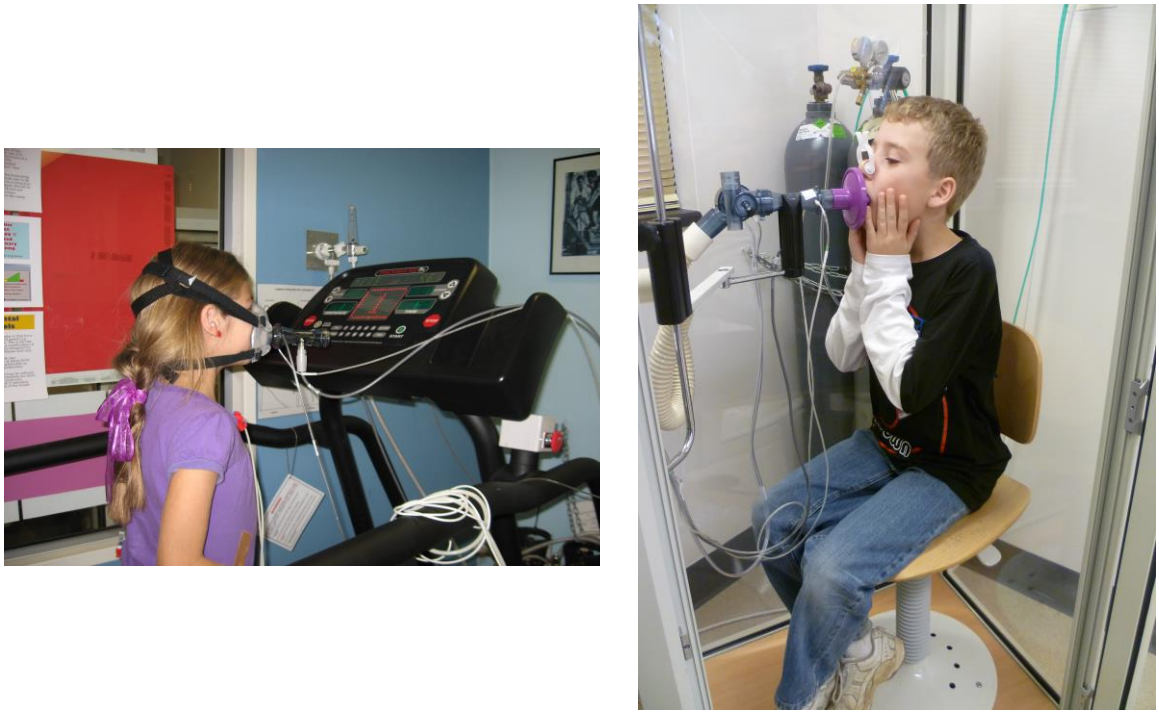

Figure 2.9: Child performing CPET (left) and pulmonary function (right).

2.6.5 Disease Specific Quality of Life Questionnaire

2.6.5.1 Newcastle Paediatric Mitochondrial Disease Scale (NPMDS) and Newcastle Mitochondrial Disease Scale (NMDS)

The quality of life of child participants in the MRCD group was assessed using the Newcastle Paediatric Mitochondrial Disease Scale (NPMDS) [709]. This rating scale addresses different aspects of mitochondrial disease with 4 domains: Current Function; System Specific Involvement; Current Clinical Assessment and Quality of Life. Most questions were rated using a four-point scale from 0-3: 0 representing normal, 1- mild, 2- moderate and 3- severe, with examples of each given. Higher scores indicating more severe disease and poorer quality of life. Two age-specific versions of the NPMDS were used: 2 – 11 years and 12 – 18 years. Adult participants in the MRCD group completed the Newcastle Mitochondrial Disease Adult Scale (NMDAS) [710]. It included the Current Function; System Specific Involvement; Current Clinical Assessment domains used in the NPMDS, however most questions were rated using a six-point scale from 0-5, higher scores indicating more severe disease. Quality of Life was measured using the validated SF12v2 and QualityMetric Health Outcomes Scoring Software 3.0 (QualityMetric Incorporated, Lincoln, USA) which generates SDS for 8 quality of life domains: Physical Functioning; Role Physical; Bodily Pain; General Health; Vitality; Social Functioning; Role Emotional; and Mental Health, as well as physical and mental component scores. Both the NPMDS and NMDAS have excellent inter-rater reliability and established validity with higher scores correlated with greater disease severity in both

adult [710, 711] and paediatric [709] patients. Designed primarily to assess the natural history of mitochondrial disease and monitor progression in patients [709, 710], no meaningful clinically important difference has been identified, however studies have found correlations between disease progression measured by the NMDAS and muscle mitochondrial DNA heteroplasmy, DNA deletion location and size [711] and increases in cerebral blood flow correlated with improvements in NMDAS and NPMDS scores [712].

2.6.5.2 The Cystic Fibrosis Questionnaire (CRQ-R)

The quality of life of participants in the CF cohort was assessed using the CFQ-R. The CFQ-R is a disease specific, health related quality of life measure for individuals with CF. The questionnaire has developmentally appropriate versions; *Child*, for children aged 6-13 years, with an interviewer format for those aged 6-11 years and a self-report format for those 12-13 years; *Parent*, for parents of children with CF aged 6-13 years; and a *Teen/adult* for those 14 years and older. The questionnaire encompasses general domains of health-related quality of life: physical functioning, role functioning, vitality, health perception, emotional functioning and social functioning, as well as domains specific to CF: body image, eating disturbances, treatment burden, respiratory and digestive symptoms. Most questions were rated using a four-point scale that was standardised to a 100-point scale, with higher scores reflecting better quality of life. The teen/adult version of the questionnaire has been validated as a reliable measure of health related quality of life for individuals with CF [713]. Domain scores were generated using the CFQ-R

Scoring Calculator (Version 2.0). Normative means for each domain the three versions have been published [714]. The questionnaire takes about 10 minutes to complete.

2.6.6 Adherence to training

The Galileo® Home Vibration Platform has an inbuilt counter that measured the time that the platform was operating. This was used to calculate the treatment time and expressed as a percentage of perfect adherence (9 minutes daily for 6 months).

2.6.7 Cystic Fibrosis Specific Outcome Measures

2.6.7.1 Pulmonary Function Tests

Spirometry, Body Plethysmography and Multiple Breath Nitrogen Washout (MBNW) test were performed by the CF cohort (Figure 2.9). All tests were performed according to standard ATS techniques and at least 3 satisfactory tests were recorded for each measure, the best result used for analysis. Spirometry was measured using the Vmax Encore 229 equipment (Care Fusion, CA, USA) and body plethysmography by the V62J Autobox (Care Fusion, CA, USA) using Vmax Series Version 21-1A software (CareFusion, CA, USA) in accordance with American Thoracic Society Guidelines [715]. Children sat upright with a nose peg (SureGuard nose clip, BIRD Healthcare, Port Melbourne, Australia and mouthpiece with a bacterial filter (SureGuard bacterial viral filter, BIRD Healthcare, Port Melbourne, Australia). Spirometry parameters included forced vital

capacity (FVC), forced expiratory volume in one second (FEV_1), the ration of FEV_1/FVC and forced expiratory flow ($FEF_{25-75\%}$). The ratio of residual volume (RV) and total lung capacity (TLC) was used from plethysmography. Results were expressed as a percentage of predicted values with the use of reference data from Wang et al [716].

MBNW allowed the calculation of the lung clearance index (LCI), a measure of abnormal ventilation distribution. Children were encouraged to adopt a regular, comfortable breathing pattern, and once this was achieved, the patient began to breathe 100% oxygen. Expired volume was recorded until the nitrogen concentration was $1/40^{th}$ of the starting concentration, and LCI was calculated by dividing this expired volume by functional residual capacity (FRC). Tests were separated by at least twice the washout time. Studies in healthy children have demonstrated that the upper limit for normal LCI is 7.77 with higher values indicating more severe dysfunction. Tests were performed in the same order at every visit, MBNW – spirometry – plethysmography – CPET.

2.6.8 Search Strategy

Key search terms (Table 2.6) were entered into the Medline and Pubmed Databases. Articles available in full text and the English language, investigating the effects of at least 4 weeks of vibration training in standing in humans were reviewed.

Table 2.6: Key search terms used in search strategy.

Participants	**Intervention**	**Outcome Measures**
Humans	Vibration	DEXA/DXA
Paediatric	Vibration training	pQCT/QCT
Child	Whole-body vibration	Bone
Adult	Oscillating	Bone density
Neuromuscular	Side-alternating	Bone mass/bone mineral content
Cystic fibrosis	Vertical	Bone loss
Respiratory disease		Bone area
Mitochondrial disease		Bone strength
Mitochondrial respiratory chain disorders		Bone turnover markers
Mitochondrial myopathy		Function
		Muscle
		Muscle area
		Muscle mass/lean mass
		Muscle force
		Muscle power
		Muscle strength

	Hop/Jump
	Single-leg jump/One-leg jump
	Chair-rise test/Sit-to-stand
	Force plate
	Walk test
	Six-minute walk test
	Exercise
	Exercise test
	Exercise capacity
	Oxygen uptake
	Quality of life
	Lung function
	Spirometry/FEV1
	Lung volumes/ plethysmography
	Lung clearance index
	Nitrogen multiple-breath washout

2.6.9 Power Calculation

To detect a 0.75 standard deviation difference in the primary outcome measure (bone mass) with a significance of 95% and power of 80%, 16 subjects would be required. The aim was to recruit 20 participants into the CF study and 25 into the MRCD study.

2.6.10 Statistical Analysis

To estimate whether between time point differences were statistically significant, a linear mixed model [717] with an autoregressive covariance matrix was used for each outcome measure. An autoregressive covariance structure provided the best fit for the main outcomes as indicated by the lowest -2 log likelihood ratio. This structure allows data closer together in time to be more correlated than data points further apart. Least significant difference (LSD) tests were used to test whether there were significant differences between the follow-up time points. A P value of <0.05 was considered statistically significant. The mean differences and 95% confidence intervals (95% CI) reported are predicted mean values from the model. The residuals from each linear mixed model were plotted to verify that they were approximately normally distributed and to confirm that there were no influential outliers. For parameters were SDS were calculated, one sample *t*-tests were used to examine for differences from zero. Statistical analysis was performed using IBM SPSS Statistics Version 25 (IBM Corporation).

3 CF Results

3.1 Participants

Nineteen participants, 10 male, 7-18 years of age were recruited in to the study (Table 3.1). Two participants were withdrawn from the study during the observation period due to the development of septic arthritis in one patient and the development of allergic broncho-pulmonary aspergillosis (ABPA), requiring medium-term oral steroid use, in the other. A third participant was lost to follow-up.

The remaining 16 participants, 10 males, had a mean (SD) age of 12.8 (3.5) years and presented with Class I, II, III and V mutations (Table 3.2). Pancreatic insufficiency was seen in 14/16 participants (87.5%), which was reflective of Australian CF population, in which 82.2% are pancreatic insufficient [480]. The F508del mutation was present in 15/16 participants (93.8%) with 9/16 (56.3%) being homozygous for this mutation. These figures were also consistent with the Australian CF population, where 92.1% carry the F508del mutation and 50.2% are homozygous for this mutation [480].

Participants attended the hospital for four research visits. The observation period occurred between Visits 1 and 2. The mean (SD) time elapsed between these visits was 6.5 (1.0) months. The whole-body vibration training (WBVT) period occurred between Visits 2 and 3. The mean (SD) time between visits was 6.6 (0.7) months. The follow-up

period occurred between visits 3 and 4. The mean (SD) time periods between these visits was 6.6 (1.3) months.

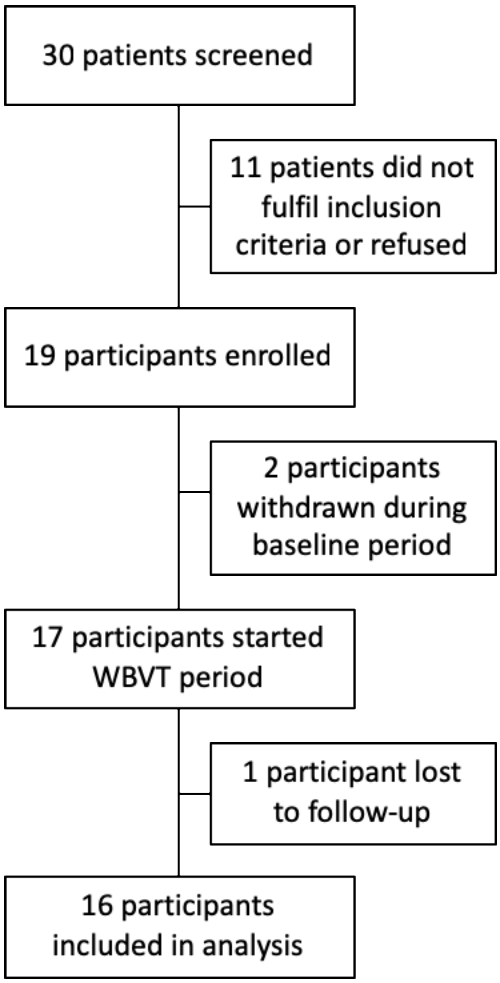

Figure 3.1: Flow diagram of participant recruitment.

3.2 Anthropometric Parameters

The baseline anthropometric data for the cohort are presented in Table 3.2. The CF cohort has a mean (SD) height of 155.5 (16.8) cm and a mean (SD) height standard deviation score (SDS) of 0.0 (0.9), mean (SD) weight of 43.8 (13.3) kg and mean (SD) SDS -0.5 (0.9) which was significantly lower than the reference population (Figure 3.1). BMI and BMI SDS were also low with a mean (SD) of 17.6 (2.3) kg/m^2 and -0.7 (0.9) which was also significantly different to the reference population (Figure 4.1). The lower weight and BMI compared to the reference population a common presentation in CF, especially in those with pancreatic insufficiency.

Analysis of anthropometric variables can be found in Appendix H, Table H.1. There were significant increases in height, weight and BMI during the observation period of 2.6cm, $p<0.001$, 2.7kg, $p<0.001$, and 0.5kg/m^2, $p=0.005$ respectively. However, there were no significant changes in their corresponding SDS, indicating that the growth trajectory was not altered. After WBVT, height increased significantly by 2.2cm, $p<0.001$, however there was no significant change in its corresponding SDS or in weight, BMI, or their corresponding SDS. Height, weight and BMI increased significantly during the follow-up period by 2.2cm, $p<0.001$, 2.1kg, $p<0.001$ and 0.4kg/m2, $p=0.041$ respectively. There were no significant changes in the SDS scores. Anthropometric variables were unaffected during the study, as there were no significant changes in height, weight or BMI SDS.

Table 3.1. Participant characteristics.

Patient	**Gender**	**Age**	**Pancreatic**	**Mutation 1**		**Mutation 2**		**Status**
		(years)	**Status**	***Type***	***Class***	***Type***	***Class***	
1	Male	7	PI	F508del	II	F508del	II	
2	Female	17	PI	1898+1G>A	I	Unknown		
3	Male	14	PI	F508del	II	F508del	II	
4	Female	14	PI	F508del	II	F508del	II	WITHDRAWN - septic arthritis
5	Male	15	PS	F508del	II	Unknown		
6	Female	13	PI	F508del	II	F508del	II	
7	Male	15	PI	F508del	II	F508del	II	
8	Male	16	PI	F508del	II	Unknown		WITHDRAWN - ABPA
9	Female	18	PS	F508del	II	Unknown		

10	Male	17	PI	F508del	II	F508del	II	
11	Male	12	PI	F508del	II	W79X	I	
12	Female	8	PI	F508del	II	F508del	II	
13	Male	8	PI	F508del	II	F508del	II	
14	Female	14	PI	F508del	II	G551D	III	
15	Male	11	PI	F508del	II	L1077P	II	
16	Female	11	PI	F508del	II	F508del	II	
17	Female	12	PI	F508del	II	3849+10kbC>T	V	
18	Male	10	PI	F508del	II	I508del	II	WITHDRAWN - lost to follow-up
19	Female	8	PI	F508del	II	F508del	II	

Note: PI, pancreatic insufficient: PS, pancreatic sufficient.

Table 3.2. Baseline anthropometric data.

Patient	Gender	Age (years)	Height (cm)	Height SDS	Weight (kg)	Weight SDS	BMI (kg/m^2)	BMI SDS
1	Male	7	126.10	0.17	26.50	0.55	16.67	0.63
2	Female	17	166.20	0.44	54.00	-0.39	19.55	-0.56
3	Male	14	172.10	0.47	50.70	-0.42	17.12	-1.10
5	Male	15	169.80	0.04	57.40	0.14	19.91	0.22
6	Female	13	153.60	-0.22	43.00	-0.28	18.23	-0.22
7	Male	15	170.40	0.03	51.30	-0.57	17.67	-0.89
9	Female	18	174.30	1.77	64.70	0.79	21.30	0.03
10	Male	17	179.80	0.44	54.60	-1.39	16.89	-2.10
11	Male	12	140.40	-1.39	32.00	-1.34	16.23	-0.79
12	Female	8	133.10	0.40	27.00	-0.16	15.24	-0.55
13	Male	8	130.00	0.20	23.10	-0.89	13.67	-1.67
14	Female	14	144.30	-2.38	34.10	-2.56	16.38	-1.48
15	Male	11	141.20	-0.50	29.80	-1.10	14.95	-1.30
16	Male	16	142.50	-0.35	41.50	0.64	20.44	1.07
17	Female	12	152.40	-0.09	33.20	-1.51	14.29	-2.37
19	Female	8	130.00	0.43	24.70	-0.33	14.62	-0.84

Note: SDS, standard deviation score; BMI, body mass index.

3.3 Bone Parameters

3.3.1 DXA

3.3.1.1 Baseline

Baseline DXA parameters are presented in Table 3.3 and Figure 3.1. This includes: total body DXA scan parameters, segmental analysis of the trunk, arms and legs from the total body scans; and analysis of the lumbar spine (LS), including lumbar vertebrae 2-4; and analysis of the lumbar vertebrae 1-4 (L14). Total body DXA parameters were further analysed to obtain ratios between measures of BMC, BA, LTM, and height: LTM for height SDS; TBA for height SDS; TBMC for LTM SDS; and TBMC for TBA SDS.

The baseline SD scores for total body BMC, arms BMD, trunk BMD, lean tissue mass for height, total bone area for height and lumbar vertebrae 1-4 vBMD were significantly reduced compared to the reference population, however total body BMD, BMC for lean tissue mass and bone area, legs BMD and lumbar spine BMD and BMC were not (Figure 4.1). These findings were generally consistent with the existing literature. The height-adjusted total body BMC SDS was found to be significantly reduced in other child and adolescent CF populations [559, 663, 677] but not significantly different to the reference or control populations in others [594, 605, 639, 663]. The height-adjusted total body

BMD SDS was not different to the reference or control populations in all [564, 594, 611] but one [559] of the previous studies, consistent with that seen in the CF cohort. Total body BMD SDS were < -1 in 4/16 (25%) and less < -2 in 2/16 (13%) of the CF cohort, similar to that seen in the current literature for young people with CF with 4-28% having a SDS <-1 [598, 608] and 4-27% having a SDS <-2 [563, 594]. One study has previously investigated the segmental analysis of the total body DXA scans, and similar to that seen in the CF cohort, height- and weight-adjusted SDS for the arms and trunk BMD were significantly reduced in children and adolescents. They also found a significantly reduced BMD SDS in the legs, which was contrary to that seen in the CF cohort, however their cohort was much larger, and likely a more representative sample [559]. The significant reduction in lean tissue mass for height SDS was consistent with several previous studies [559, 603, 614, 621, 663], however others have found no significant difference between CF patients and controls [612, 619, 639]. Similarly, the significantly reduced bone area for height SDS has also been widely seen in the existing literature [594, 608, 612], with only one existing publication finding bone area to be similar between CF patients and controls matched for height, weight and pubertal status [611]. The SDS for BMC for lean tissue was not significantly different to the reference population, consistent with previous reports [612, 619], however others have reported this SDS to be significantly reduced in children and adolescents [559] and in pubertal females with CF [612]. The BMC for bone area SDS was slightly elevated, but not significantly different to the reference population. One previous study found this SDS to significantly increased in its CF children and adolescents [608].

Similar to that seen in the CF cohort, height-adjusted lumbar spine vBMD was found to be significantly reduced in previous studies of CF children and adolescents [559, 593, 602]. Lumbar spine BMD SDS was not significantly reduced in the CF cohort, which was consistent with an earlier Australian study that adjusted for height and lean tissue mass [564], however in contrast to two international studies which adjusted for height and found lumbar spine BMD to be significantly reduced [559, 603]. Lumbar spine BMD SDS were < -1 in 6/16 (38%) and < -2 in 2/16 (13%) which were similar to the ranges seen in the current literature with 22-52% of CF child and adolescent populations having a SDS <-1 [567, 598, 603, 605, 607] and 9-35% having a SDS < -2 [559, 563, 567, 594, 596, 603, 607]. The lumbar spine BMC SDS was also not significantly reduced compared to the reference population. This was in contrast to the one published study using height- and weight-adjusted SDS that found the same parameter to be significantly reduced in children and adolescents with CF [559].

These findings indicate compromised bone health in both the cortical and trabecular bone compartments. When considering the cortical compartment, analysis of the total body DXA scan using the 4-step algorithm of Hogler et al [687], indicates that the low bone mass seen in the CF cohort is secondary to the significantly reduced lean tissue mass for height. This reduction in lean mass, imparts less muscle pull on the underlying bone, reducing the mechanical stimulus required for optimal bone modelling and in particular, geometric adaptations. This caused the significant reduction in bone area for height, as the required mechanical stimulus did not surpass the threshold required for geometric adaptations to be elicited and deposition of new bone at the same rate as their healthy

counterparts. The normal adaptation of BMC to both lean tissue mass and bone area further supports the reduced lean tissue mass in the CF cohort being the primary culprit for the low bone mass phenotype described.

Table 3.3. Baseline dual-energy x-ray absorptiometry parameters for scans of the total body, lumbar spine and distal femur. Mean (SD).

Total Body DXA Parameters	
TBMC (g)	1611.8 (613.3)
TBMC SDS	-0.7 (1.1)
TBA (cm^2)	1615.6 (452.1)
TBMD (g/cm^2)	0.974 (0.109)
TBMD SDS	-0.5 (1.1)
LTM (g)	31407.1 (10401.5)
Fat (%)	18.6 (7.1)
Fat (%) SDS	-0.2 (0.7)
LTM for Height SDS	-0.7 (0.9)
TBA for Height SDS	-0.9 (1.0)
TBMC for LTM SDS	-0.2 (1.1)
TBMC for TBA SDS	0.1 (1.1)
Parameters of Segmental Analysis of Total Body DXA Scans	

Trunk BMD (g/cm^2)	0.763 (0.097)
Trunk BMD SDS	-0.3 (0.4)
Arms BMD (g/cm^2)	0.694 (0.010)
Arms BMD SDS	-0.7 (1.0)
Legs BMC (g)	599.3 (272.2)
Legs BA (cm^2)	576.2 (173.0)
Legs BMD (g/cm^2)	0.998 (0.182)
Legs BMD SDS	-0.3 (1.2)
Legs LTM (g)	10114.3 (4001.4)
Legs Fat (%)	24.1 (8.7)
Lumbar Spine DXA Parameters	
LS BMC (g)	29.5 (11.8)
LS BMC SDS	-0.3 (1.1)
LS BMD (g/cm^2)	0.863 (0.189)
LS BMD SDS	-0.4 (1.2)
L14 BMC (g)	36.9 (14.8)
L14 BA (cm^2)	42.5 (9.0)
L14 vBMD (g/cm^3)	0.291 (0.059)

L14 vBMD SDS	-0.9 (1.0)

Note: DXA, dual-energy x-ray absorptiometry; TBMC, total body bone mineral content; TBA, total body bone area; TBMD, total body bone mineral density; SDS, standard deviation score; LTM, lean tissue mass; BMD, bone mineral density; BMC, bone mineral content; BA, bone area; LS, lumbar spine vertebrae 2-4; L14, lumbar spine vertebrae 1-4; vBMD, volumetric bone mineral density.

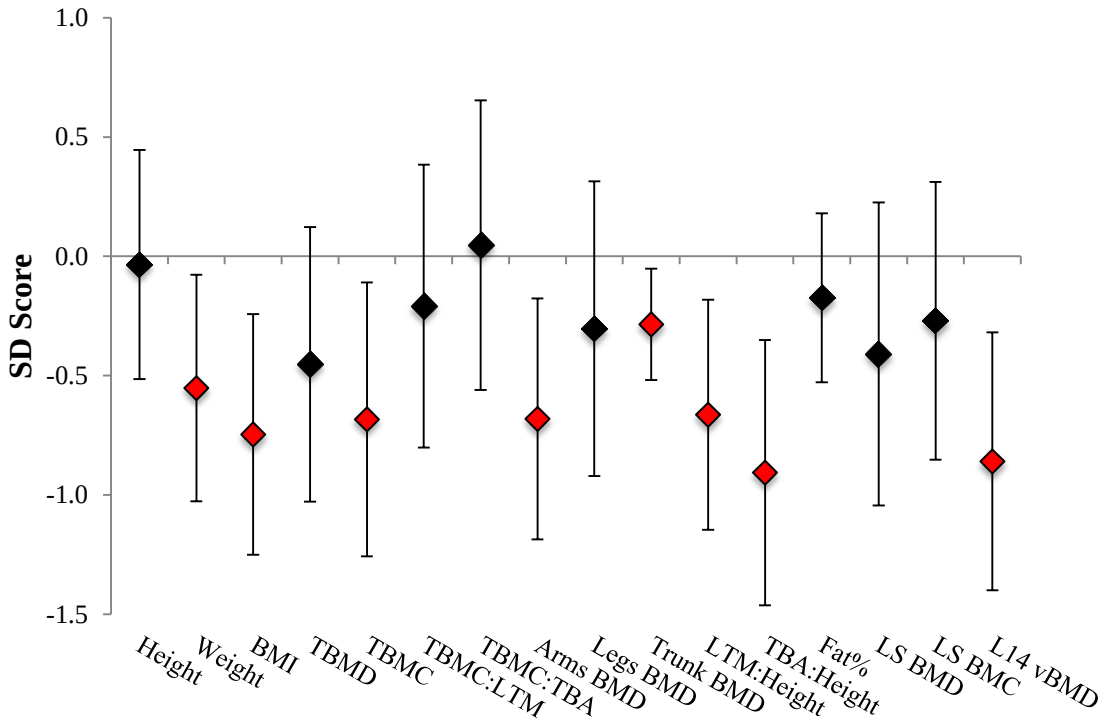

Figure 3.2. Standard deviation (SD) scores for anthropometric and DXA parameters in the paediatric cohort. Mean and 95% confidence intervals. Parameters shown in red were significantly different to the reference population on one-sample t-tests, $p<0.05$.

3.3.1.2 Observation Period

The observation period occurred between Visit 1 (Baseline) and Visit 2 (Start WBVT). Full analysis data for all DXA parameters for the observation period in the CF cohort can be found in Appendix A, Table A.1. There were significant changes in parameters of the total body DXA scan, segmental analysis of the total body DXA scan and the lumbar spine DXA scan (Table 3.4 and Appendix A, Table A.1). Total body BMC, bone area, bone density and lean tissue mass increased significantly from: 1611.8 – 1713.6g ($p<0.001$); 1615.6 – 1689.2 cm^2 ($p<0.001$); 0.974 – 0.992g/cm^2 ($p<0.001$); and 31407.1 – 32653.7g ($p<0.001$) respectively. However, there were no significant changes in the SDS for BMC or BMD or the bone area for height and LTM for height SDS, indicating these changes were associated with normal growth. On segmental analysis, trunk BMD and arms BMD increased significantly from 0.763 – 0.779g/cm^2 ($p=0.001$) and 0.694 – 0.717g/cm^2 ($p<0.001$), however their corresponding SDS did not change significantly, again indicating normal skeletal development with growth. Legs BMC, BA, LTM and BMD increased significantly: 599.3 – 632.8g ($p<0.001$); 576.2 – 597.6cm2 ($p<0.001$); 10114.3 – 10629.5g ($p<0.001$); and 0.998 – 1.021g/cm2 ($p<0.001$) respectively. There was no significant change in the legs BMD SDS, indicating that the changes in the leg bone parameters were likely associated with bone modelling during growth. Similarly, lumbar spine BMC and BMD increased significantly from 29.5 – 31.7g ($p<0.001$) and 0.863 – 0.891g/cm2, however there were no significant changes in their corresponding SDS. In keeping with growth related increases in bone parameters, the BMC and BA of lumbar spine vertebrae 1-4 increased significantly: 36.9 – 39.8g ($p<0.001$) and 42.5 – 44.3cm2 ($p<0.001$) respectively.

Table 3.4. DXA parameters for the CF cohort at each visit, changes between visits and p values. *p<0.05.

		TBMC (g)	TBMC SDS	TBA (cm^2)	TBMD (g/cm^2)	TBMD SDS
Visit 1	Value	1611.809	-0.683	1615.616	0.974	-0.452
	Δ in Observation	101.744	-0.011	73.575	0.018	-0.011
	p	0.000*	0.855	0.000*	0.000*	0.820
Visit 2	Value	1713.553	-0.693	1689.192	0.992	-0.463
	Δ with WBVT	78.243	-0.086	45.105	0.020	0.032
	p	0.000*	0.144	0.000*	0.000*	0.501
Visit 3	Value	1791.796	-0.779	1734. 296	1.011	-0.431
	Δ in Follow-up	88.182	0.031	77.600	0.007	-0.089
	p	0.000*	0.600	0.000*	0.059	0.078
Visit 4	Value	1879.978	-0.748	1811.897	1.018	-0.520

		TLTM (g)	TLTM:Ht SDS	TBA:Ht SDS	TBMC:TBA SDS	Trunk BMD (g/cm^2)
Visit 1	Value	31407.062	-0.664	-0.906	0.047	0.763
	Δ in Observation	1246.666	-0.112	0.027	-0.068	0.016
	p	0.000*	0.172	0.730	0.205	0.001*
Visit 2	Value	32653.729	-0.776	-0.879	-0.021	0.779
	Δ with WBVT	1026.258	-0.134	-0.169	0.121	0.018
	p	0.001*	0.103	0.034*	0.027*	0.000*
Visit 3	Value	33679.986	-0.910	-1.048	0.100	0.796
	Δ in Follow-up	1110.143	-0.043	0.186	-0.264	0.004
	p	0.001*	0.611	0.024*	0.000*	0.400
Visit 4	Value	34790.129	-0.953	-0.862	-0.165	0.800

		Trunk BMD SDS	Arms BMD (g/cm^2)	Arms BMD SDS	Legs BMC (g)	Legs BA (cm^2)
Visit 1	Value	-0.285	0.694	-0.681	599.273	576.178
	Δ in Observation	-0.018	0.023	0.085	33.543	21.401
	p	0.496	0.000*	0.144	0.000*	0.000*
Visit 2	Value	-0.303	0.717	-0.597	632.816	597.579
	Δ with WBVT	-0.002	0.009	-0.108	35.089	16.488
	p	0.946	0.045*	0.065	0.000*	0.000*
Visit 3	Value	-0.304	0.726	-0.705	667.905	614.067
	Δ in Follow-up	-0.061	0.004	-0.122	24.584	17.830
	p	0.025*	0.355	0.044*	0.001*	0.000*
Visit 4	Value	-0.366	0.730	-0.826	692.489	631.897

		Legs BMD (g/cm^2)	Legs BMD SDS	Legs LTM (g)	LS BMC (g)	LS BMC SDS
Visit 1	Value	0.998	-0.304	10114.26	29.501	-0.270
	Δ in Observation	0.024	-0.051	515.272	2.233	-0.024
	p	0.000*	0.414	0.000*	0.000*	0.727
Visit 2	Value	1.021	-0.355	10629.541	31.734	-0.294
	Δ with WBVT	0.031	0.047	283.020	1.711	-0.065
	p	0.000*	0.448	0.034*	0.000*	0.342
Visit 3	Value	1.051	-0.307	10912.560	33.445	-0.359
	Δ in Follow-up	0.016	-0.053	381.908	1.431	-0.067
	p	0.012*	0.410	0.007*	0.003*	0.341
Visit 4	Value	1.068	-0.360	11294.468	34.877	-0.426

		LSBMD (g/cm^2)	LS BMD SDS	L14 BMC (g)	L14 BA (cm^2)
Visit 1	Value	0.863	-0.409	36.928	42.531
	Δ in Observation	0.027	-0.095	2.882	1.769
	p	0.004*	0.256	0.000*	0.000*
Visit 2	Value	0.891	-0.504	39.810	44.300
	Δ with WBVT	0.023	-0.060	2.062	1.353
	p	0.015*	0.470	0.000*	0.001*
Visit 3	Value	0.913	-0.564	41.873	45.653
	Δ in Follow-up	0.006	-0.147	1.720	1.532
	p	0.533	0.090	0.004*	0.001*
Visit 4	Value	0.919	-0.711	43.592	47.185

Note: Δ, change; TBMC, total bone mineral content; TBA, total bone area; TBMD, total bone mineral density; SDS, standard deviation score; TLTM, total body lean tissue mass; TFat, total body fat; BMD, bone mineral density; BMC, bone mineral content;

BA, bone area; LTM, lean tissue mass; LS, lumbar spine vertebrae 2-4; L14, lumbar spine vertebrae 1-4; vBMD, volumetric bone mineral density.

3.3.1.3 WBVT Period

The WBVT period occurred between Visit 2 (Start WBVT) and Visit 3 (End WBVT). Full analysis data for all DXA parameters for the WBVT period for the CF cohort can be found in Appendix A, Table A.1. During the WBVT period there were significant improvements in several parameters for the total body DXA scan, segmental analysis of the total body DXA scan, and the lumbar spine DXA scan (Table 3.4 and Appendix A, Table A.1).

Total body BMC, BA, BMD and LTM increased significantly from: 1713.6 – 1791.8g ($p<0.001$); 1689.2 – 1734.3cm^2 ($p<0.001$); 0.992 – 1.0g/cm^2 ($p<0.001$); and 32653.7 – 33680.0g ($p=0.001$) respectively. Similar to the observation period there were no significant changes in the SDS for BMC or BMD or the LTM for height SDS, indicating the above changes were consistent with bone modelling during growth, however the BA for height SDS, decreased significantly from -0.9 to -1.1 ($p=0.034$), suggesting that the geometric adaptations of bone area were progressing more slowly than expected. As a consequence, the total BMC for BA SDS increased significantly from 0.0 – 0.1 ($p=0.027$).

Segmental analysis of the total body DXA scan demonstrated similar changes as those seen during the observation period. Trunk BMD and arms BMD increased significantly from 0.779 – 0.796g/cm^2 ($p<0.001$) and 0.717 – 0.726g/cm^2 ($p=0.045$). Legs BMC, BA, LTM and BMD increased significantly: 632.8 – 667.9g ($p<0.001$); 597.6 – 614.1cm^2 ($p<0.001$); 10629.5 – 10912.6g ($p=0.034$); and 1.021 – 1.051g/cm^2 ($p<0.001$)

respectively. The lack of significant change in the trunk, arms and legs BMD SDS, likely indicate that the changes in these parameters were associated with skeletal modelling.

Lumbar spine scans also mirrored changes seen during the observation period. Lumbar spine BMC and BMD increased significantly from 31.7 – 33.4g ($p<0.001$) and 0.891 – 0.913g/cm^2, with no significant changes in their corresponding SDS. BMC and BA of lumbar spine vertebrae 1-4 also increased significantly: 39.8 – 41.9g ($p<0.001$) and 44.3 – 45.7cm^2 ($p=0.001$) respectively.

3.3.1.4 Follow-up Period

The follow-up period occurred between Visit 3 (end WBVT) and Visit 4 (follow-up). Analysis data for all DXA parameters for the CF cohort can be found in Appendix A, Table A.1. During the follow-up period, the CF cohort demonstrated significant improvements in several parameters for the total body DXA scan, segmental analysis of the total body DXA scans and the lumbar spine DXA scans (Table 3.4 and Appendix A, Table A.1).

Total body BMC, BA and LTM increased significantly from: 1791.8 – 1880.0g ($p<0.001$); 1734.3 – 1811.9cm^2 ($p<0.001$); and 33680.0 – 34790.1g ($p=0.001$) respectively. There were no significant changes in the corresponding SDS for BMC and LTM however the BA for height SDS increased significantly from -1.1 to-0.9 ($p=0.024$), indicating that the cohort made geometric adaptations to bone are more rapidly than

expected, and returning the cohort to the same status as seen at baseline. In response, the total BMC for BA SDS decreased significantly from 0.1 to -0.2 ($p<0.001$).

Segmental analysis of the total body DXA scans did not find any significant changes in trunk and arm BMD however their corresponding SDS decreased significantly from -0.3 to -0.4 ($p=0.025$) and -0.7 to -0.8 ($p=0.044$), likely indicating a reduction in the accrual rate of bone mass in these areas during the follow-up period, the first representing trabecular bone and the second a non-weight bearing limb. Similar to the observation and WBVT periods, legs BMC, BA, LTM and BMD, but not legs BMD SDS, increased significantly: 667.9 – 692.5g ($p<=.001$); 614.1 – 631.9cm^2 ($p<0.001$); 10912.6 – 11294.5g ($p=0.007$); and 1.051 – 1.068g/cm^2 ($p<0.001$) respectively, indicating changes associated with growth.

Bone parameters in the lumbar spine demonstrated similar changes to those seen in the observation and WBVT periods. Lumbar spine BMC, but not the SDS, increased significantly from 33.4 – 34.9g ($p=0.003$). Significant increases were seen in the BMC and BA of lumbar spine vertebrae 1-4: 41.9 – 43.6g ($p=0.004$) and 45.7 – 47.2cm^2 ($p=0.001$) respectively.

3.3.2 Tibial pQCT

3.3.2.1 Baseline

Bone parameters for the 4% and 20% sites and bone and composition parameters for the 66% site are presented in Table 3.5 and Figure 3.2. At the 4% site, trabecular vBMD was significantly reduced compared to the reference population, with 8/16 (50%) having a SDS < -1.0 and 4/16 (25%) with a SDS < -2.0. The significantly reduced trabecular vBMD is consistent with findings in child and adolescent females with CF, but not males in one study [621], and contrary to the findings in a second study, which did not find significantly reduced trabecular vBMD at the 4% tibial pQCT site in pre-pubertal or pubertal males or females with CF [622]. Two 20% site parameters also reflected compromised bone health in the CF cohort. Cortical vBMD was significantly elevated compared to the reference population, with 9/16 (56%) having a SDS >1 and 5/16 (31%) having a SDS >2. This is in contrast to the findings of Kelly et al [621], who did not find elevated cortical vBMD at the 20% pQCT site in their CF males or females compared to controls adjusted for age, race, height and pubertal stage [621]. The same study found cortical cross sectional area at the 20% pQCT site to be reduced compared to their control population in males and females [621], which was not consistent with the findings in the CF cohort. However, females but not males had a significantly reduced periosteal circumference compared to controls [621], which supported the findings of significantly lower periosteal circumference in the CF cohort compared to the reference population. These findings indicate a potentially lower rate of bone turnover and compromised geometric attributes in the CF cohort, respectively.

At the 66% site, bone and composition parameters were significantly different to the reference population, providing further evidence of compromised bone health. Cortical BMC was significantly reduced, similar to the findings of Brookes et al [622] in their pubertal females. The cortical bone's geometric attributes, cortical CSA and cortical thickness were also significantly reduced. However, the CSA of the total bone was not significantly different to the reference population at the 66% site, consistent with the findings in pre-pubertal males and females and pubertal males in the study by Brookes et al [622]. In keeping with that seen at the 20% site in the CF cohort, cortical vBMD was significantly higher than the reference population with 7/16 (44%) having a SDS >1 and 2/16 (13%) having a SDS >2 however 1/16 (6%) had a SDS < -1. This was in contrast to the study by Brookes et al [622] where cortical vBMD was not elevated compared to controls [622]. To confirm the impact of the compromised cortical geometry, polar CSMI of the cortical bone was significantly reduced, indicating sub-optimal distribution of bone mass around the bone's central axis. This, together with the elevated cortical bone density resulted in significantly reduced cortical bone strength measured by polar SSI. This significant reduction in polar SSI at the 66% site was consistent with the findings of Brookes et al [622] in pubertal females.

Calf muscle CSA in the CF cohort was significantly reduced compared to the reference population, in keeping with the findings of Brookes et al [622] in their pubertal males and females. This finding likely contributed to the impairments in bone mass, geometry and strength seen in tibial pQCT analysis of the CF cohort, as bone adapts these physical

attributes to the muscle forces applied to it. Despite the significantly reduced cortical CSA, the cortical CSA to muscle CSA is normally adapted at 5.1%, indicating that the thinner bone seen in the CF cohort was due to the reduced muscle CSA. The ratio of cortical BMC to muscle CSA was significantly increased and together with the significantly increased cortical vBMD, suggests that the rate of bone turnover is likely reduced in this cohort, potentially as a result of reduced muscle forces not eliciting the geometric adaptations, and hence bone turnover and modelling, seen in the healthy reference population as part of normal increases in stature and lean tissue mass development.

Table 3.5. Baseline peripheral quantitative computed tomography parameters for the tibial 4%, 20% and 66% sites. Mean (SD).

4% Site Parameters	
Total BMC (mg/mm)	226.3 (70.4)
Total CSA (mm^2)	765.4 (249.9)
Total vBMD (mg/cm^3)	300.200 (39.071)
Trabecular BMC (mg/mm)	71.9 (26.0)
Trabecular CSA (mm^2)	344.3 (112.5)
Trabecular vBMD (mg/cm^3)	210.406 (34.436)
Trabecular vBMD SDS	-1.0 (1.4)
Periosteal Circumference (mm)	97.2 (15.5)

20% Site Parameters	
Total BMC (mg/mm)	192.1 (51.9)
Total CSA (mm^2)	318.4 (101.9)
Total vBMD (mg/cm^3)	615.081 (78.494)
Cortical BMC (mg/mm)	162.7 (44.4)
Cortical BMC SDS	0.2 (0.9)
Cortical CSA (mm^2)	147.3 (37.6)
Cortical CSA SDS	-0.3 (0.9)
Cortical vBMD (mg/cm^3)	1099.694 (42.172)
Cortical vBMD SDS	1.3 (1.3)
Cortical Thickness (mm)	2.7 (0.4)
Cortical Thickness SDS	0.4 (1.1)
Periosteal Circumference (mm)	62.5 (9.9)
Periosteal Circumference SDS	-0.9 (1.0)
Cortical CSMI p (mm^4)	11337.3 (6734.4)
Cortical SSI p (mm^3)	877.4 (384.9)
Total CSMI p (mm^4)	14490.0 (8603.3)
Total SSI p (mm^3)	951.2 9427.7)

Total SSI p SDS	-0.4 (0.8)
66% Site Parameters	
Total BMC (mg/mm)	271.4 (80.4)
Total CSA (mm^2)	472.9 (146.2)
Total CSA SDS	0.1 (0.9)
Total vBMD (mg/cm^3)	579.400 (73.893)
Cortical BMC (mg/mm)	227.9 (71.6)
Cortical BMC SDS	-0.6 (1.0)
Cortical CSA (mm^2)	212.6 (62.7)
Cortical CSA SDS	-0.8 (0.9)
Cortical CSA: Total CSA (%)	3.3 (0.5)
Cortical vBMD (mg/cm^3)	1067.406 (43.708)
Cortical vBMD SDS	0.8 (1.1)
Cortical Thickness (mm)	3.2 (0.6)
Cortical Thickness SDS	-1.1 (0.9)
Periosteal Circumference (mm)	76.3 (11.6)
Cortical CSMI p (mm^4)	26296.4 (15681.3)
Cortical CSMI p SDS	-1.3 (0.7)

Cortical SSI p (mm^3)	1417.3 (637.5)
Cortical SSI p SDS	-0.6 (0.9)
Soft Tissue Total CSA (mm^2)	6538.1 (2148.8)
Muscle CSA (mm^2)	4272.6 (1432.1)
Muscle CSA SDS	-0.8 (1.1)
Fat CSA (mm^2)	1773.1 (761.8)
Fat CSA: Muscle CSA (%)	42.1 (12.1)
Muscle CSA: Soft Tissue Total CSA (%)	65.4 (4.9)
Cortical CSA: Muscle CSA (%)	5.1 (0.6)
Cortical BMC: Muscle CSA (mg/mm^2)	0.1 (0.0)
Cortical BMC: Muscle CSA SDS	4.3 (6.5)

Note: BMC, bone mineral content; CSA, cross sectional area; vBMD, volumetric bone mineral density; SDS, standard deviation score; CSMI, cross sectional moment of inertia; p, referring to the polar measurement; SSI, stress strain index.

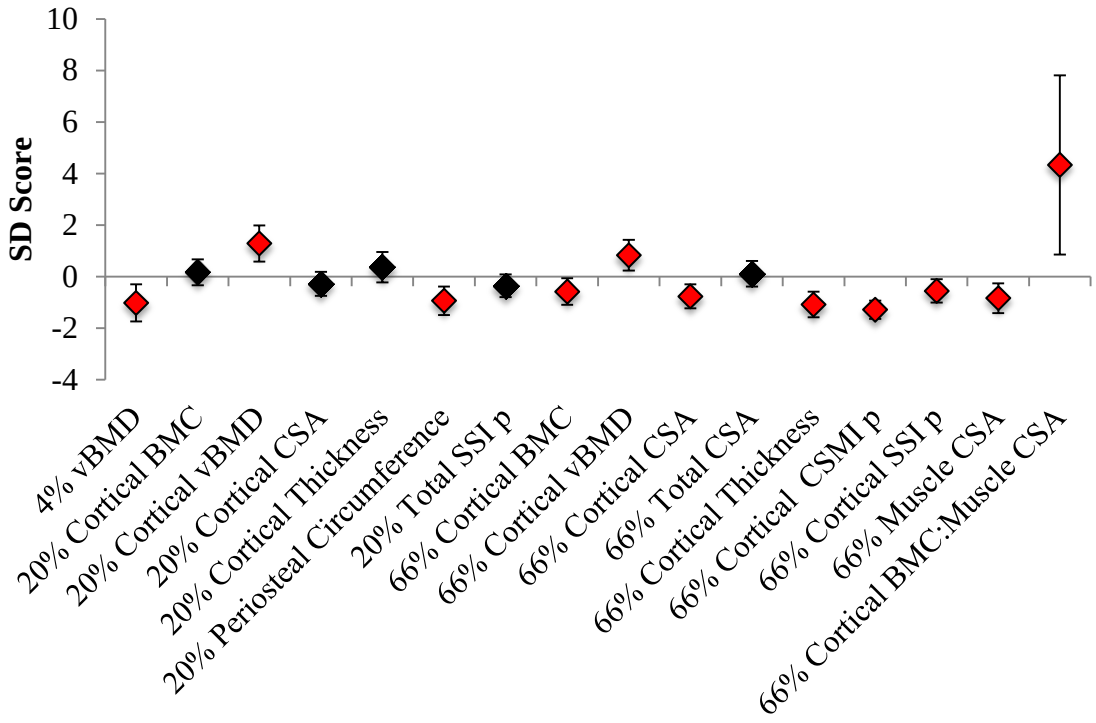

Figure 3.3. Standard deviation (SD) scores for peripheral quantitative computed tomography (pQCT) parameters in the paediatric cohort. Mean and 95% confidence interval. Parameters shown in red were significantly different to the reference population on one-sample t-tests, $p<0.05$.

3.3.2.2 Observation Period

During the observation period there were significant changes in parameters at the 20% and 66% distal tibial pQCT sites, but not the 4% site. (Table 3.6 and Appendix B, Table B.1). At the 20% site, total bone BMC, CSA and vBMD increased significantly: 192.1 – 199.3mg/mm, $p<0.001$; 318.4 – 325.9mm^2, $p<0.001$; and 615.081 – 622.294mg/cm^3, $p=0.013$ respectively. Cortical BMC and vBMD and their corresponding SDS also increased significantly: 162.7 – 170.3mg/mm, $p<0.001$; 0.2 – 0.4, $p<0.001$; 1099.694 –

1107.644mg/cm^3, p=0.004; and 1.3 – 1.5, p=0.007 respectively. Geometric attributes of the cortical bone at the 20% site as well as their corresponding SDS were increased significantly. Cortical CSA increased from 147.3 – 153.1mm^2, p<0.001 and -0.3 to 0.0, p<0.001, cortical thickness from 2.7 – 2.8mm, p<0.001 and 0.4 – 0.5, p=0.003, and periosteal circumference from 62.5 – 63.3mm, p<0.001 and -0.9 to -0.8, p<0.001. Improved BMC and geometrical attributes of the 20% site cortical bone resulted in significant increases in parameters of bone strength, polar CSMI and SSI of 11337.3 – 11997.7mm^4, p<0.001 and 877.4 – 924.3mm^3, p<0.001 respectively. Total bone measures of polar CSMI and SSI as well as the SDS for polar SSI also increased significantly: 14490.0 – 15127.4mm^4, p<0.001; 951.2 – 1002.1mm^3, p<0.001 and -0.4 to 0.0, p<0.001 respectively.

Analysis at the 66% site demonstrated significant changes in total bone, cortical bone and composition parameters. Total bone BMC increased significantly from 271.4 – 282.7mg/mm, p<0.001, and total bone CSA and its corresponding SDS increased significantly from 472.9 – 492.6mm^2, p<0.001 and 0.1 – 0.4, p<0.001. Cortical BMC and CSA and their corresponding SDS as well as periosteal circumference also increased significantly from: 227.9 – 236.1mg/mm, p<0.001 and -0.6 to -0.3, p<0.001; 212.5 – 219.1mm^2, p=0.001 and -0.8 to -0.6, p=0.001; and 76.3 – 77.9mm, p<0.001 respectively. Significant increases were also seen in cortical bone measures of strength, polar CSMI and SSI and their corresponding SDS: 26296.3 – 28134.5mm^4, p<0.001 and -1.3to -1.1, p<0.001; 1417.3 – 1505.6mm^3, p<0.001 and -0.6 to -0.3, p<0.001 respectively. Total soft tissue CSA increased significantly from 6538.1 – 6721.9mm^2, p=0.022, however this was

due to a significant increase in fat CSA from 1773.1 – 1854.5mm^2, p=0.042, with no significant change seen in muscle CSA or its corresponding SDS.

3.3.2.3 WBVT Period

During the WBVT period there were significant improvements in parameters at the 4%, 20% and 66% distal tibial pQCT sites (Table 3.6 and Appendix B, Table B.1). At the 4% site, total bone BMC and vBMD increased significantly from 229.1 – 236.6mg/mm, p=0.005 and 298.187 – 304.244mg/cm^3, p=0.040, however there were no significant changes in trabecular bone parameters after WBVT. These increases in total bone parameters were not seen during the observation period.

At the 20% site, significant changes were seen in total and cortical bone parameters after WBVT. Total bone BMC, CSA and vBMD increased significantly: 199.3 – 207.3mg/mm, p<0.001; 325.9 – 333.3mm^2, p<0.001; and 622.294 – 632.056mg/cm^3, p=0.001 respectively. Cortical BMC and vBMD and their corresponding SDS also increased significantly: 170.3 – 177.6mg/mm, p<0.001; 0.4 – 0.7, p<0.001; 1107.644 – 1114.713mg/cm^3, p=0.011; and 1.5 – 1.6, p=0.013 respectively. Cortical CSA and its SDS increased from 153.1 – 158.7mm^2, p<0.001 and 0.0 – 0.3, p<0.001, cortical thickness and its SDS from 2.8 – 2.9mm, p<0.001 and 0.5 – 0.7, p=0.001, and periosteal circumference and its SDS from 63.3 – 64.1mm, p<0.001 and -0.8 to -0.6, p<0.001, indicating improved geometric attributes after WBVT. Improvements in bone mass and geometry resulted in significant increases in cortical and total bone strength after WBVT. Cortical bone polar CSMI and SSI increased 11997.7 – 12647.2mm^4, p<0.001 and 924.3

– 967.9mm^3, p<0.001, and total bone measures of polar CSMI and SSI as well as the SDS for polar SSI increased 15127.4 – 15935.3mm^4, p<0.001; 1002.1 – 1048.3mm^3, p<0.001 and 0.0 – 0.3, p<0.001 respectively.

Analysis at the 66% site demonstrated significant changes in total and cortical bone parameters as well as composition parameters. Consistent with changes seen in the observation and WBVT periods, total bone BMC and total bone CSA and its corresponding SDS increased significantly from: 293.4 – 301.7mg/mm, p<0.001; 511.6 – 522.0mm^2, p=0.041; and 0.7 – 0.9, p=0.027 respectively. Cortical BMC and CSA and their corresponding SDS as well as periosteal circumference again demonstrated significant increases: 244.5 – 252.0mg/mm, p<0.001 and -0.1 to 0.2, p<0.001; 225.9 – 230.7mm^2, p=0.012 and -0.3 to -0.2, p=0.008; and 79.4 – 80.2mm, p=0.043 respectively. Cortical vBMD and its corresponding SDS increased significantly during the follow-up period from 1077.144 – 1086.686mg/cm^3, p=0.009 and 1.1 – 1.3, p=0.021. Significant increases were also seen in cortical bone measures of strength, polar CSMI and SSI and their corresponding SDS: 30101.5 – 31303.2mm^4, p=0.017 and -0.9 to -0.7, p=0.004; 1583.3 – 1650.8mm^3, p=0.001 and 0.0 – 0.3, p=0.001 respectively.

Table 3.6. pQCT parameters for the CF cohort at each visit, changes between visits and p values. *p<0.05.

		4% Total BMC (mg/mm)	**4% Total vBMD (mg/cm^3)**	**20% Total BMC (mg/mm)**	**20% Total CSA (mm^2)**	**20% Total vBMD (mg/cm^3)**
Visit 1	Value	226.3	300.200	192.1	318.4	615.081
	Δ in Observation	2.8	-2.013	7.3	7.5	7.213
	p	0.268	0.486	0.000*	0.000*	0.013*
Visit 2	Value	229.1	298.187	199.3	325.9	622.294
	Δ with WBVT	7.5	6.056	8.0	7.4	9.762
	p	0.005*	0.040*	0.000*	0.000*	0.001*
Visit 3	Value	236.6	304.244	207.3	333.3	632.056
	Δ in Follow-up	4.4	2.582	6.6	6.2	7.393
	p	0.098	0.387	0.000*	0.000*	0.014*
Visit 4	Value	241.0	306.826	213.9	339.5	639.450

		20% Cortical BMC (mg/mm)	20% Cortical BMC SDS	20% Cortical CSA (mm^2)	20% Cortical CSA SDS	20% Cortical vBMD (mg/cm^3)
Visit 1	Value	162.7	0.2	147.3	-0.3	1099.694
	Δ in Observation	7.6	0.3	5.8	0.3	7.950
	p	0.000*	0.000*	0.000*	0.000*	0.004*
Visit 2	Value	170.3	0.4	153.1	0.0	1107.644
	Δ with WBVT	7.3	0.3	5.6	0.3	7.069
	p	0.000*	0.000*	0.000*	0.000*	0.011*
Visit 3	Value	177.6	0.7	158.7	0.3	1114.713
	Δ in Follow-up	6.2	0.3	4.2	0.2	9.332
	p	0.000*	0.000*	0.000*	0.001*	0.001*
Visit 4	Value	183.8	1.0	162.9	0.6	1124.045

		20% Cortical vBMD SDS	20% Cortical Thickness (mm)	20% Cortical Thickness SDS	20% Periosteal Circumference (mm)	20% Periosteal Circumference SDS
Visit 1	Value	1.3	2.7	0.4	62.5	-0.9
	Δ in Observation	0.2	0.1	0.2	0.8	0.2
	p	0.007*	0.000*	0.003*	0.000*	0.000*
Visit 2	Value	1.5	2.8	0.5	63.3	-0.8
	Δ with WBVT	0.2	0.1	0.2	0.8	0.2
	p	0.013*	0.000*	0.001*	0.000*	0.000*
Visit 3	Value	1.6	2.9	0.7	64.1	-0.6
	Δ in Follow-up	0.2	0.1	0.1	0.6	0.2
	p	0.011*	0.009*	0.018*	0.000*	0.002*
Visit 4	Value	1.8	2.9	0.9	64.7	-0.4

		20% Cortical CSMI p (mm^4)	20% Cortical SS1p (mm^3)	20% Total CSMI p (mm^4)	20% Total SS1p (mm^3)	20% Total SS1p SDS
Visit 1	Value	11337.3	877.4	14490.0	951.2	-0.4
	Δ in Observation	660.2	47.0	637.4	50.9	0.4
	p	0.000*	0.000*	0.000*	0.000*	0.000*
Visit 2	Value	11997.5	924.3	15127.4	1002.1	0.00
	Δ with WBVT	649.7	43.6	807.9	46.2	0.3
	p	0.000*	0.000*	0.000*	0.000*	0.000*
Visit 3	Value	12647.2	967.9	15935.3	1048.3	0.3
	Δ in Follow-up	457.2	44.5	519.4	41.1	0.3
	p	0.001*	0.000*	0.002*	0.000*	0.000*
Visit 4	Value	13104.4	1012.4	16454.6	1089.4	0.6

		66% Total BMC (mg/mm)	66% Total CSA (mm^2)	66% Total CSA SDS	66% Cortical BMC (mg/mm)	66% Cortical BMC SDS
Visit 1	Value	271.4	472.9	0.1	227.9	-0.6
	Δ in Observation	11.3	19.7	0.3	8.2	0.3
	p	0.000*	0.000*	0.000*	0.000*	0.000*
Visit 2	Value	282.7	492.6	0.4	236.1	-0.3
	Δ with WBVT	10.7	19.0	0.3	8.3	0.3
	p	0.000*	0.000*	0.000*	0.000*	0.000*
Visit 3	Value	293.4	511.6	0.7	244.5	-0.1
	Δ in Follow-up	8.3	10.4	0.2	7.6	0.2
	p	0.000*	0.041*	0.027*	0.000*	0.000*
Visit 4	Value	301.7	522.0	0.9	252.0	0.2

		66% Cortical CSA (mm^2)	66% Cortical CSA SDS	66% Cortical vBMD (mg/cm^3)	66% Cortical vBMD SDS	66% Periosteal Circumference (mm)
Visit 1	Value	212.6	-0.8	1067.406	0.8	76.3
	Δ in Observation	6.5	0.2	5.363	0.2	1.6
	p	0.001*	0.001*	0.118	0.135	0.000*
Visit 2	Value	219.1	-0.6	1072.769	1.0	77.9
	Δ with WBVT	6.8	0.2	4.375	0.1	1.5
	p	0.000*	0.000*	0.201	0.361	0.000*
Visit 3	Value	225.9	-0.3	1077.144	1.1	79.4
	Δ in Follow-up	4.8	0.2	9.543	0.3	0.8
	p	0.012*	0.008*	0.009*	0.021*	0.043*
Visit 4	Value	230.7	-0.2	1086.686	1.3	80.2

		66% Cortical CSMI p (mm^4)	66% Cortical CSMI p SDS	66% Cortical SSI p (mm^3)	66% Cortical SSI p SDS
Visit 1	Value	26296.4	-1.3	1417.3	-0.6
	Δ in Observation	1838.2	0.2	88.3	0.3
	p	0.000*	0.000*	0.000*	0.000*
Visit 2	Value	28134.5	-1.1	1505.6	-0.3
	Δ with WBVT	1967.0	0.2	77.7	0.3
	p	0.000*	0.000*	0.000*	0.000*
Visit 3	Value	30101.5	-0.9	1583.3	0.0
	Δ in Follow-up	1201.6	0.2	67.5	0.2
	p	0.017*	0.004*	0.001*	0.001*
Visit 4	Value	31303.2	-0.7	1650.8	0.3

		66% Soft Tissue Total CSA (mm^2)	66% Muscle CSA (mm^2)	66% Muscle CSA SDS	66% Fat CSA (mm^2)
Visit 1	Value	6538.1	4272.570	-0.8	1773.1
	Δ in Observation	183.8	82.530	0.1	81.4
	p	0.022*	0.204	0.125	0.042*
Visit 2	Value	6721.9	4355.100	-0.7	1854.5
	Δ with WBVT	64.6	57.010	0.1	-9.1
	p	0.407	0.378	0.166	0.815
Visit 3	Value	6786.500	4412.1	-0.7	1845.4
	Δ in Follow-up	281.510	186.8	0.2	77.5
	p	0.001*	0.007*	0.004*	0.060
Visit 4	Value	7068.010	4598.9	-0.5	1922.8

Note: Δ, change; 4%, referring to 4% tibial pQCT site; BMC, bone mineral content; CSA, cross-sectional area; vBMD, volumetric bone mineral density; SDS, standard deviation score; 20%, referring to the 20% tibial pQCT site; CSMI, cross-section moment of inertia; p, referring to the polar measurement; SSI, stress strain index; 66%, referring to the 66% tibial pQCT site.

3.3.2.4 Follow-up Period

During the follow-up period there were no significant changes in any parameters at the 4% pQCT site. Significant changes were seen in total and cortical bone parameters at the 20% and 66% sites and in composition parameters at the 66% site (Table 3.6, Appendix B, Table B.1).

At the 20% site, similar to the observation and follow-up periods, significant changes were seen in total bone BMC, CSA and vBMD: 207.3 – 213.9mg/mm, $p<0.001$; 333.3 – 339.5mm^2, $p<0.001$; and 632.056 – 639.450mg/cm^3, $p=0.014$ respectively. Cortical BMC and vBMD and their corresponding SDS also increased significantly: 177.6 – 183.8mg/mm, $p<0.001$; 0.7 – 1.0, $p<0.001$; 1114.713 – 1124.1mg/cm^3, $p=0.001$; and 1.6 – 1.8, $p=0.011$ respectively. Cortical bone geometric attributes and their corresponding SDS increased significantly: cortical CSA increased from 158.7 – 162.9mm^2, $p<0.001$ and 0.3 – 0.6, $p=0.001$; cortical thickness from 2.9 – 2.9mm, $p<0.009$ and 0.7 – 0.9, $p=0.018$, and periosteal circumference from 64.1 – 64.7mm, $p<0.001$ and -0.6 to -0.4, $p<0.002$ respectively. Significate improvement s were also seen in parameters of bone strength: cortical bone polar CSMI and SSI increased 12647.2 – 13104.4mm^4, $p=0.001$ and 967.9 – 1012.3mm^3, $p<0.001$; and total bone measures of polar CSMI and SSI as well as the SDS for polar SSI increased 15935.3 – 16454.6mm^4, $p=0.002$; 1048.3 – 1089.4mm^3, $p<0.001$ and 0.3 – 0.6, $p<0.001$ respectively. Total soft tissue CSA increased significantly from 6786.5 – 7068.0mm^2, $p=0.001$, and was associate with significant increases in muscle CSA and its SDS, 4412.1 – 4598.9mm^2, $p=0.007$ and -0.7 to -0.5, $p=0.004$.

3.3.3 Serum Biochemistry and Bone Turn-over Markers

Table 3.7. Baseline Serum Biochemistry and Bone Turnover Marker Parameters. Mean (SD).

Alkaline Phosphatase (U/L)	213.2 (65.3)
Calcium (mmol/L)	2.32 (0.1)
Phosphate (mmol/L)	1.5 (0.2)
Parathyroid Hormone (ng/l)	4.5 (2.6)
Osteocalcin (nmol/L)	6.1 (1.9)
Vitamin D (pmol/L)	70.7 (13.9)
Urinary Deoxypyridinoline: Creatine Ratio (nmol/mmol creatinine)	17.8 (9.4)

3.3.3.1 Baseline

Baseline parameters for the serum biochemistry are presented in Table 3.7. Calcium, magnesium and phosphate were within normal reference ranges in all participants over the course of the study, except for participant number 10 who had one low calcium value at visit 3. Parathyroid hormone was transiently lower than the reference range in participants 10 and 17, and transiently higher than the reference range in participants 16 and 19, however all other participants had levels within the normal reference range.

Vitamin D was transiently lower than 50pmol/L in 5 participants (participants 2, 7, 10, 11 and 16) at one time-point during the study, which coincided with the winter months. Serum alkaline phosphatase was within normal reference ranges for all participants for the duration for the study except for one, number 10, who had a high level at his baseline visit. Osteocalcin was outside of the reference range for at least one time-point in 11 of the 16 participants. Osteocalcin was below the reference range at least once in participants 1, 2, 5, 6, 9 and 10, and was lower than the reference range at all time points in participants 2 and 9. Osteocalcin was above the reference range at least once over the study duration in participants 3, 5, 11, 14, 15 and 17, with participants 14 and 17 recording elevated levels at 3 of the 4 time-points. The urinary deoxypyridinoline: creatinine ratio was higher than the normal reference range at least once in 7 participants, 2, 7, 10, 12, 13, 14 and 19 with participant 10 demonstrating elevated levels at all visits. Participant 15 had a low at one of the times he was tested. The finding that the bone formation marker, osteocalcin, was below the reference range at least once in 6/16 (38%) of participants and consistently below the reference range in 2/16 (13%), as well as the bone resorption marker, urinary deoxypyridinoline: creatinine ratio, being increased at least once in 7/16 (44%) and at all visits in one participant, is consistent with reduced bone formation and increased bone resorption. This indicates an uncoupling of normal bone turnover in the CF cohort and is consistent with the compromised bone health found on DXA and pQCT analysis.

3.3.3.2 Study Periods

Analysis data for bone turnover markers and serum biochemistry during the observation, WBVT and follow-up periods can be found in Table 3.7 and Appendix C Table C.1. During the observation period osteocalcin decreased significantly, and considering this marker of bone formation was decreased in 38% of the CF cohort at baseline, it may indicate a further reduction in the rate of bone formation. Calcium and magnesium decreased significantly after WBVT, however they also remained within their normal reference ranges. In the follow-up period, calcium increased significantly but remained within its normal reference range.

Table 3.8. Serum biochemistry and bone turn-over marker parameters for the CF cohort at each visit, changes between visits and p values. *p<0.05.

		Calcium (mmol/L)	**Magnesium**	**Osteocalcin (nmol/L)**
Visit 1	Value	2.3	0.8	6.1
	Δ in Observation	0.0	0.0	-2.4
	p	0.714	0.078	0.013*
Visit 2	Value	2.3	0.9	3.8
	Δ with WBVT	-0.1	-0.1	0.8
	p	0.003*	0.001*	0.383

Visit 3	Value	2.2	0.8	4.6
	Δ in Follow-up	0.1	0.0	-0.4
	p	0.000*	0.177	0.661
Visit 4	Value	2.4	0.8	4.2

Note: Δ, change; WBVT, whole body vibration training.

3.4 Force-plate Parameters

3.4.1 Baseline

Baseline parameters for the M1LJ, S2LJ and CRT performed on the force plate are presented in Table 3.9 and Figure 3.3. Maximum force produced by the leg muscles was derived from the M1LJ, maximum power generated derived from the S2LJ and the functional performance of leg muscles was derived from the CRT. The force SDS from the M1LJ was significantly reduced compared to the reference population, -1.8, $p<0.001$. The force SDS from the S2LJ was also significantly reduced compared to the reference population, -0.5, $p=0.037$, however there was no impact on the ability of the muscles to generate power from the force produced and the power, Esslinger Fitness Index and force efficiency SDS were not significantly different to the reference population. The CRT speed, power and force SDS were not significantly different to the reference population indicating good functional performance of the leg muscles. These results indicate that during dynamic manoeuvres, the peak force generated by leg muscle is significantly

reduced in the CF cohort compared to their healthy peers. While the magnitude of this impairment is not impacting on function, it gives some insight to the reduced bone mass and density as well as compromised geometry and strength seen on DXA and pQCT analysis. In the CF cohort, the peak forces imparted on the underlying bone during dynamic manoeuvres are of a significantly reduced magnitude compared to their healthy counterparts. Considering these forces play a central role in directing bone modelling processes, and particularly optimising geometric adaptations to increase strength, these deficiencies in muscle force likely explain some aspects of the compromised bone health seen in the CF cohort.

Table 3.9. Baseline force plate parameters. Mean (SD).

Multiple One-leg Hop (M1LH) Parameters	
F max (kN)	10.9 (3.4)
F max rel (g)	2.8 (0.3)
Force SDS	-1.8 (0.9)
Single Two-leg Jump (S2LJ) Parameters	
EFI (%)	95.9 (14.2)
EFI SDS	-0.3 (1.2)
Force Efficiency (%)	103.6 (12.2)
Force Efficiency SDS	0.3 (1.2)
P max (kW)	1.7 (0.8)

P max rel (W/kg)	41.3 (7.7)
Power SDS	-0.4 (1.1)
F max (kN)	0.9 (0.3)
F max rel (g)	2.3 (0.3)
Force SDS	-0.5 (0.9)
Chair Rise Test (CRT) Parameters	
Time per test (s)	1.1 (0.2)
Speed SDS	0.3 (0.9)
P max (W)	558.1 (188.6)
Ave P max rel (W/kg)	12.6 (1.9)
Power SDS	-0.4 (1.0)
F max (kN)	1.6 (0.2)
Ave F max rel (g)	1.5 (0.1)
Force SDS	0.1 (0.9)

Note: F max, maximum force; F max rel, maximum relative force per force equivalent to body weight; SDS, standard deviation score; EFI, Esslinger Fitness Index; P max, maximum power; P max rel, maximum power in relation to body weight; Ave P max rel, average maximum power in relation to body weight; Ave F max rel, average maximum relative force per force equivalent to body weight.

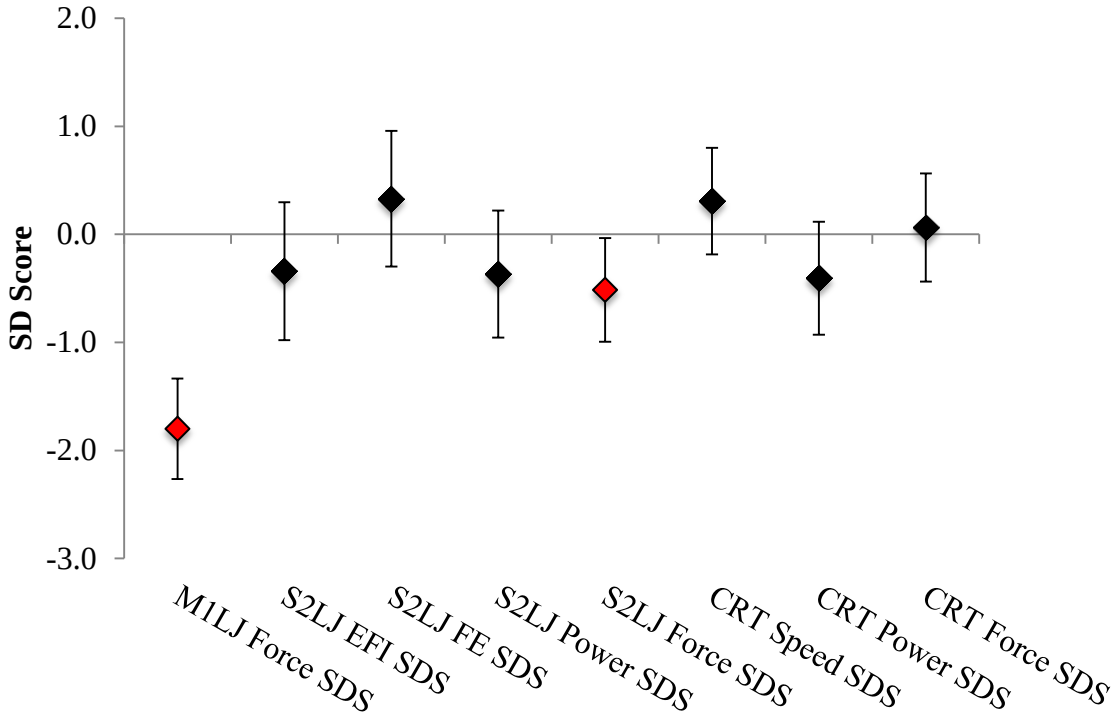

Figure 3.4. Standard deviation (SD) scores for force plate parameters in the paediatric cohort. Mean and 95% confidence interval. Parameters shown in red were significantly different to the reference population on one-sample t-tests, $p<0.05$.

3.4.2 Observation Period

Analysis data for the M1LJ, S2LJ and CRT performed on the force-plate are presented in Table 3.10 and Appendix D, Table D.1. During the observation period, the maximum relative force and force SDS for the M1LJ decreased significantly, 2.8 – 2.6g, p=0.004 and -1.8 to -2.2, p=0.004. Maximum power in the S2LJ increased significantly from 1.7 –

1.9kW, p=0.010, however the maximum relative power and power SDS did not change significantly, indicating that this increase in power was associated with normal musculoskeletal development. The time taken to perform the chair rise test decreased significantly by 0.1s, p=0.025 and the speed SDS increased from -0.3 – 0.00, p=0.025.

3.4.3 WBVT Period

After WBVT, maximum force in the M1LJ increased by 0.5kN, p=0.050, however there was no significant change in the maximum relative force of force SDS, suggesting this increase was associated with normal musculoskeletal development. The maintenance of the latter two parameters was in contrast to the significant decline seen during the observation period and may indicate that WBVT hindered the decline in force production during the M1LJ in the CF cohort. Maximum force also increased significantly in the S2LJ by 0.1kN (10%), p=0.027 however the maximum relative force and force SDS did not change significantly, indicating this increase was likely associated with musculoskeletal development. The time to complete the CRT and the speed SDS improved significantly after WBVT. Time per test decreased 0.1s (9.1%), p=0.001 and the speed SDS increased from 0.0 – 0.5, p=0.001, a larger improvement than that seen during the observation period. The maximum force generated during the CRT also increased significantly after WBVT by 0.1kN (6.7%), p=0.028, however there was no significant change in the maximum relative force or force SDS, indicating a change likely associated with musculoskeletal development.

Three previous studies have investigated muscle function on a force plate in CF populations. In 10 CF adults, 22-27 years, 3 months of progressive home-based side-alternating WBVT (20-25Hz, 0.6mm, 18 minutes, 5 times a week), found a non-significant increase in M1LJ force of 72N (~5%) [233], similar in magnitude to the increase in the CF cohort. There was a significant reduction in maximum S2LJ force of 122N (9%), which correlated to a 0.43 reduction in the force SDS [233]. This significant reduction in maximum force occurred without a significant change in maximum power or its corresponding SDS [233] indicating that the efficiency of muscle function during the S2LJ was significantly improved after WBVT, as less force was required to generate an equivalent amount of power [718]. These improvements in S2LJ muscle efficiency were not seen in the CF cohort, perhaps due to the lower vibration frequency used. There was also a significant reduction in the time to perform the CRT of 1.05 seconds (15%), significant increase in force of 438N or 7N/kg (41%) and significant increase in power output of 135W or 2.38W/kg (19%) during the CRT [233]. The increases in CRT speed and force larger than those seen in the CF cohort. In the second study of young adults with CF, 21-41 years, 6 months of home-based side-alternating WBVT (12Hz, 6 minutes with trunk bends, rotations and extensions, 5 times a week, as well as 26Hz, 6 minutes, with the progressive addition of weights up to 9kg, 3 times a week), increased M1LJ force by a median of 6.7%, however no statistics were performed due the small size of the cohort [232], again similar in magnitude to the CF cohort. Maximum S2LJ force decreased by a median of 4.3%, and maximum power increased by a median of 4.7%, [232], again indicate improved muscle efficiency after WBVT which was not seen in the CF cohort, perhaps due to the higher frequency and use of additional weights in the Roth

et al [232] study. A third study in children with CF, 8-18 years, S2LJ power parameters did not significantly change after 4 weeks of side-alternating WBVT (20-22Hz, 1mm, 10-15 minutes, 3 times a week) [229], consistent with the results seen in the CF cohort.

3.4.4 Follow-up Period

In the follow-up period, maximum relative force and force SDS in the M1LJ decreased significantly from 2.7 – 2.5g, p=0.002 and from -2.2 to -2.7, p=0.002. These changes were similar in magnitude to the decreases seen in the observation period and provide further evidence to support a beneficial role of WBVT in interrupting the decline in muscle force produced during the L1LJ in the CF cohort. There were no significant changes in any of the S2LJ or CRT parameters during the follow-up period.

3.5 Exercise Tests

Table 3.10. Baseline Cardiopulmonary Exercise Test Parameters. Mean (SD).

CPET - Pre-Exercise Parameters	
Pre-exercise VO_2 (L/min)	0.3 (0.1)
Pre-exercise VO_2 (ml/kg/min)	7.1 (1.8)
Pre-exercise HR (bpm)	96.8 (17.3)
Pre-exercise HR (% Predicted)	46.7 (8.2)

Pre-exercise O_2 Pulse (ml/beat)	3.0 (1.1)
Pre-exercise VCO_2 (L/min)	0.3 (0.1)
Pre-exercise RQ	1.0 (0.2)
Pre-exercise VE (L/min)	10.3 (2.9)
Pre-exercise VE/VO_2	40.3 (10.9)
Pre-exercise VE/VCO_2	41.3 (7.9)
Pre-exercise Borg Score	0.6 (0.6)
Pre-exercise SBP (mmHg)	106.9 (8.3)
Pre-exercise DBP (mmHg)	66.8 (8.1)
Pre-exercise Oxygen Saturations (%)	97.5 (1.5)
CPET - Anaerobic Threshold (AT) Parameters	
AT VO_2 (L/min)	1.7 (0.7)
AT VO_2 (ml/kg/min)	39.4 (7.4)
AT VO_2 (% Predicted)	133.4 (36.6)
AT VO_2 (% Predicted VO_2)	95.9 (22.1)
AT VO_2 (% of Peak VO_2)	81.5 (10.7)
AT HR (bpm)	174.6 (13.9)
AT HR (% Predicted)	84.7 (6.9)

AT O_2 Pulse (ml/beat)	9.7 (3.8)
AT VCO_2 (L/min)	1.7 (0.7)
AT RQ	1.0 (0.0)
AT VE (L/min)	47.7 (20.0)
AT VE/VO_2	28.3 (2.3)
AT VE/VCO_2	28.8 (2.1)
CPET - Peak Exercise Parameters	
Peak VO_2 (L/min)	1.9 (0.8)
Peak VO_2 (ml/kg/min)	45.7 (7.3)
Peak VO_2 (% Predicted)	114.9 (22.5)
Peak HR (bpm)	181.6 (15.1)
Peak HR (% Predicted)	87.7 (8.1)
Peak O_2 Pulse (ml/beat)	10.4 (4.3)
Peak O_2 Pulse (% Predicted)	111.9 (26.4)
Peak VCO_2 (L/min)	1.9 (0.8)
Peak RQ	1.0 (0.1)
Peak VE (L/min)	55.2 (23.1)
Peak VE/VO_2	29.7 (3.3)

Peak VE/VCO_2	29.2 (2.0)
CPET Time (mins)	11.9 (1.9)
Peak Borg Score	7.0 (3.1)
Peak SBP (mmHg)	123.9 (16.7)
Peak DBP (mmHg)	68.3 (10.6)
Peak Oxygen Saturation (%)	95.9 (1.7)
CPET - Recovery Parameters	
Recovery VO_2 (L/min)	0.4 (0.1)
Recovery VO_2 (ml/kg/min)	8.5 (2.0)
Recovery HR (bpm)	108.5 (12.2)
Recovery HR (% Predicted)	2.5 (6.3)
Recovery O_2 Pulse (ml/beat)	53.3 (1.2)
Recovery VCO_2 (L/min)	0.4 (0.2)
Recovery RQ	1.1 (0.2)
Recovery VE (L/min)	13.4 (5.1)
Recovery VE/VO_2	40.0 (7.1)
Recovery VE/VCO_2	38.5 (5.0)
Recovery Borg Score	3.1 (2.2)

Recovery SBP (mmHg)	111.8 (12.2)
Recovery DBP (mmHg)	64.7 (11.1)
Recovery Oxygen Saturation (%)	96.9 (1.1)

Note: CPET, Cardiopulmonary Exercise Test; VO_2, oxygen uptake; HR, heart rate; O_2, oxygen; VCO_2, carbon dioxide production; RQ, respiratory quotient; VE, minute ventilation; VE/VO_2, ventilatory equivalent of oxygen; VE/VCO_2, ventilatory equivalent of carbon dioxide; SBP, systolic blood pressure; DBP, diastolic blood pressure; AT, anaerobic threshold.

3.5.1 Baseline

Baseline parameters for the CPET are presented in Table 3.10. All 16 participants had pre-exercise, peak and recovery parameters recorded however only 8 of the participants had a clear ventilatory AT identified. The pre-exercise VO_2 was elevated at 7.1ml/kg/min. The pre-exercise Borg Score of 0.6 indicated that the participants were experiencing minimal physical exertion prior to commencing the exercise test. Pre-exercise measurements of systolic and diastolic blood pressure and oxygen saturations were within normal limits. The anaerobic threshold occurred at 81.5% of the peak VO_2 and the VO_2 at which AT occurred was 133.4% of that predicted by the reference population, indicating good aerobic metabolism.

Peak exercise parameters demonstrated well preserved exercise capacity with a mean VO_2 of 45.7 ml/kg/min, 114.9% of predicted. Oxygen pulse at peak exercise was also normal with a peak value of 10.4 ml/beat, 111.9% of predicted. The ventilatory equivalents for oxygen and carbon dioxide were within normal limits. At peak exercise the mean heart rate was 87.7% of predicted, respiratory quotient 1.0 and Borg Score 7.0, all indicating that a peak test had been performed. Systolic and diastolic blood pressures were increased compared to pre-exercise values indicating increased physical effort, and oxygen saturations remained within normal limits. The recovery data indicated that all CPET parameters were returning towards pre-exercise measures.

Table 3.11. Cardiopulmonary exercise test parameters for the CF cohort at each visit, changes between visits and p values. *p<0.05.

		Pre-exercise HR (bpm)	**Pre-exercise HR % Predicted (%)**	**AT VO_2 (ml/kg/min)**
Visit 1	Value	96.8	46.7	36.5
	Δ in Observation	4.8	2.5	-5.3
	p	0.253	0.226	0.075
Visit 2	Value	101.6	49.2	31.2
	Δ with WBVT	-7.3	-3.4	-0.4
	p	0.086	0.095	0.900
Visit 3	Value	94.3	45.7	30.8
	Δ in Follow-up	-9.0	-4.2	-0.2
	p	0.039*	0.045*	0.954
Visit 4	Value	85.2	41.5	30.7

		AT VO_2 % Predicted VO_2 (%)	AT RQ	Peak VO_2 (ml/kg/min)
Visit 1	Value	89.2	1.0	45.7
	Δ in Observation	-8.8	0.0	-4.3
	p	0.223	0.019*	0.030*
Visit 2	Value	80.4	1.0	41.4
	Δ with WBVT	-4.9	0.0	0.2
	p	0.509	0.432	0.925
Visit 3	Value	75.5	1.0	41.6
	Δ in Follow-up	1.3	0.0	-1.3
	p	0.857	0.395	0.495
Visit 4	Value	76.8	1.0	40.3

		Peak VO_2 % Predicted (%)	Peak RQ	Peak VE/VO_2
Visit 1	Value	114.9	1.0	29.7
	Δ in Observation	-9.8	0.1	2.7
	p	0.035*	0.020*	0.001*
Visit 2	Value	105.0	1.1	32.4
	Δ with WBVT	0.3	-0.0	-0.6
	p	0.940	0.093	0.468
Visit 3	Value	105.4	1.0	31.8
	Δ in Follow-up	-3.8	0.0	0.2
	p	0.419	0.795	0.817
Visit 4	Value	101.6	1.0	32.0

		Peak VE/VCO_2	Recovery VE/VCO_2
Visit 1		29.2	38.5
	Δ in Observation	1.4	-0.1
	p	0.011*	0.916
Visit 2	Value	30.6	38.4
	Δ with WBVT	0.6	1.6
	p	0.236	0.204
Visit 3	Value	31.2	40.0
	Δ in Follow-up	-0.2	2.6
	p	0.758	0.047*
Visit 4	Value	32.0	42.6

Note: Δ, change; WBVT, whole body vibration training; VO_2, oxygen uptake; HR, heart rate; bpm, beats per minute; O_2, oxygen; VCO_2, carbon dioxide production; RQ, respiratory quotient; VE, minute ventilation; VE/VO_2, ventilator equivalent of oxygen; VE/VCO_2, ventilator equivalent of carbon dioxide; AT, anaerobic threshold.

3.5.2 Observation Period

During the observation period there were significant changes in CPET parameters at AT and peak-exercise, however there were no significant changes in pre-exercise or recovery parameters (Table 3.11 and Appendix E, Table E.1). The respiratory quotient at AT increased significantly, however the magnitude of the change was <0.1 points and is unlikely to be clinically relevant. There was a non-significant decrease in VO_2 at AT of 5.3ml/kg/min (15%), p=0.075, which while not statistically significant, would be considered clinically significant. This was associated with a non-significant decrease of the percent-predicted VO_2 at AT of 8.8%, p=0.223, which would also be considered clinically significant. The percent-predicted heart rate at which AT occurred did not change significantly, indicating that AT occurred at a similar cardiovascular effort. Together these finding indicate that a clinical deterioration in the VO_2 at AT occurred during the observation period, suggesting loss of aerobic fitness.

Consistent with the reduction in VO_2 seen at the AT, peak measures of VO_2 and its percent-predicted value decreased significantly, 45.7 – 41.4 ml/kg/min, p=0.030, and 114.9 – 105.0%, p=0.035. There were also significant increases in the respiratory quotient and ventilatory equivalents of oxygen and carbon dioxide at peak exercise: 1.0 – 1.1, p=0.020; 29.7 – 32.4, p=0.001; and 29.2 – 30.6, p=0.011 respectively. There was no significant change in the percent-predicted heart rate at peak exercise. These findings show that in the context of a similar cardiovascular effort, carbon dioxide production and the ventilatory response at peak exercise was significantly increased, while peak oxygen

uptake was significantly reduced, indicating a clinically significant reduction in aerobic capacity.

3.5.3 WBVT Period

During the WBVT period there were no significant changes in any CPET parameter. The significant reduction in peak VO_2, and clinically significant reduction in VO_2 at AT seen during the observation period was not seen after WBVT suggesting that WBVT may have slowed the rate of decline in aerobic capacity in young people with CF (Table 3.11 and Appendix E, Table E.1).

3.5.4 Follow-up Period

Significant changes in pre-exercise and recovery CPET parameters, but not AT or peak-exercise CPET parameters, were seen during the follow-up period (Table 3.11 and Appendix E, Table E.1). Pre-exercise heart rate and its percent-predicted value decreased significantly during the follow-up period from 94.3 – 85.2 beats/minute, p=0.039 and from 45.7 – 41.5% predicted, p=0.045. The recovery ventilatory equivalent for carbon dioxide increased significantly from 40.0 – 42.6, p=0.047. In contrast to the WBVT period, where peak VO_2 remained stable, there were no-significant reductions in peak VO_2 and its percent-predicted value in the follow-up period which were smaller in magnitude than the decreases seen during the observation period.

3.6 Revised Cystic Fibrosis Questionnaire (CFQ-R)

3.6.1 Baseline

Baseline parameters for the CFQ-R are presented in Table 3.12. The shared child and adult CFQ-R eating and treatment burden domains were below their corresponding normative means [714] indicating that the CF cohort had more eating disturbances and a greater treatment burden compared to the norm. All other domains were above their corresponding normative scores indicating better Quality of Life in these domains compared to the normative data [714]. When considering the adult only CFQ-R domains, the role and weight domains were lower than their normative means [714] indicating that the CF cohort found their disease to have a larger impact on their role functioning and ability to gain weight compared to the norm. Parent CFQ-R domains for eating and weight were lower than their normative means [714], consistent with that seen in the child CFQ-R domains, indicating greater impact on weight and eating problems in the CF cohort compared to the norm.

Table 3.12. Baseline CFQ-R Domain Scores. Mean (SD).

Child and Adult CFQ-R Domains (n=16)	
Physical	81.9 (18.9)
Emotion	80.5 (15.3)
Eating	81.3 (21.0)

Body	82.0 (16.7)
Treatment Burden	61.1 (23.0)
Respiratory	73.4 (16.8)
Digestion	79.2 (23.3)
Social	73.7 (14.7)
Adult Only CFQ-R Domains (n=7)	
Health Perceptions	69.9 (22.0)
Role	69.0 (24.9)
Weight	57.2 (31.7)
Parent CFQ-R Domains (n=9)	
Physical	92.6 (6.1)
Emotion	91.1 (11.0)
Vitality	74.1 (12.7)
School	80.3 (14.5)
Eating	70.4 (32.0)
Body Image	82.7 (23.7)
Treatment Burden	72.8 (26.1)
Health Perceptions	86.4 (10.8)

Respiratory	87.011 (9.191)
Digestion	74.1 (27.8)
Weight	51.9 (37.7)

3.6.2 Observation Period

Analysis data for CFQ-R parameters can be found in Appendix F, Table F.1. During the observation period, the child and adult digestion domain increased significantly from 79.2 – 89.6, p=0.017, indicating improved digestive symptoms. There was also a significant decrease in the parent reported respiratory domain from 87 – 76.5, p=0.046, indicating worsening of respiratory symptoms (Table 3.13 and Appendix F, Table F.1). One previous study has monitored quality of life during an observation period prior to a WBVT intervention in children with CF. Using the same questionnaire, there was no significant change in health-related quality of life during a 4 week observation period [229].

3.6.3 WBVT Period

After WBVT there were no significant changes in the child or adult domains, however parent domains for emotion and school increased significantly from 86.7 – 92.5, p=0.026 and from 71.6 – 90.7, p=0.043 (Table 3.13 and Appendix F, Table F.1).

3.6.4 Follow-up Period

During the follow-up period there were no significant changes in any child or adult domain scores, however there was a non-significant increase in the child and adult respiratory domain of 4.9 points, p=0.262, however this change is greater than the minimally clinically important different for this domain [714] and therefore indicates a clinically relevant improvement. The parent domains for emotion and respiratory increased significantly from 92.5 – 99.7, p=0.026 and from 74.3 – 87.9, p=0.037 (Table 3.13 and Appendix F, Table F.1)

3.7 Pulmonary Function Tests

3.7.1 Baseline

Baseline pulmonary function test are presented in Table 3.13. The percent predicted values for FEV_1, FVC and FEF_{25-75} from spirometry, the RV/TLC ratio from plethysmography and the lung clearance index all indicate the CF cohort had generally mild lung disease.

3.7.2 Study Periods

Analysis data for pulmonary function test parameters can be found in Appendix ?. During the observation period, the FEV1/FVC ratio and FEF_{25-75} percent predicted value decreased significantly from 79.2 – 76.6%, p=0.041 and from 73.5 – 64.6%, p=0.007

Table 3.13. Pulmonary function test parameters. Mean (SD).

Spirometry (n=13)	
FEV_1 (L)	2.4 (0.7)
FEV_1 (% Predicted)	84.4 (18.6)
FVC (L)	3.0 (0.9)
FVC (% Predicted)	92.3 (13.8)
FEV_1/FVC ratio (%)	79.2 (8.9)
FEF_{25-75}	2.3 (1.0)
FEF_{25-75} (% Predicted)	73.5 (28.5)
Plethysmography (n=12)	
RV/TLC (%)	27.5 (6.5)
Nitrogen Multiple-Breath Washout (n=16)	
Lung Clearance Index	7.8 (2.4)

Note: FEV1, forced expiratory volume in the first second; FVC, forced vital capacity; FEF25-75, forced expiratory flow over the middle half of the FVC; RV, residual volume; TLC, total lung capacity.

Table 3.14. Pulmonary function test parameters at each visit, changes between visits and p values. *p<0.05.

		FEV_1/FVC (%)	**FEF_{25-75} (% predicted)**
Visit 1	Value	79.2	73.5
	Δ in Observation	-2.6	-8.9
	p	0.041*	0.007*
Visit 2	Value	76.6	64.6
	Δ with WVBT	0.2	2.4
	p	0.809	0.443
Visit 3	Value	76.8	67.0
	Δ in Follow-up	-1.0	-0.9
	p	0.298	0.785
Visit 4	Value	75.8	66.2

Note: FEV1, forced expiratory volume in the first second; FVC, forced vital capacity; FEF25-75, forced expiratory flow over the middle half of the FVC.

(Table 3.14 and Appendix G, Table G.1), however there was no significant change in FEV_1 the RV/TLC ratio or lung clearance index, making the clinical significance of this difficult to interpret. After WBVT and during the follow-up period there were no

significant changes in any pulmonary function test parameters indicating that the CF cohort remained stable for a pulmonary function perspective for the duration of the study. These findings are consistent with a previous study in children with CF [229] which did not find any significant change in FEV_1, FVC or FEF_{25-75} after 1 month side-alternating WBVT of after 1 month follow-up and two studies in young adults with CF that found no significant changes in FEV1 after and 3 [233] and 6 [232] months side-alternating WBVT.

3.8 Adherence

Adherence was measured a counter in the vibration plate. Vibration plate counters were available for analysis in 15/16 participants (94%) and indicated adherence to treatment of 48%. Using the vibration plate counters 11/15 participants (73%) trained at least twice a week, 8/15 (53%) trained at least 3 times a week, 4/15 (27%) trained at least 4 times a week, and 2/15 (13%) trained at least 5 times a week. This level of adherence to vibration training is similar to the rate of adherence seen with other aspects of CF care (enzymes, airway clearance techniques and nebulisers) measured objectively in children with CF which was below 50% [719].

3.9 Summary of Findings

3.9.1 Baseline

The CF cohort presented with mild lung disease measured by spirometry, body plethysmography and nitrogen multiple-breath washout. Weight and BMI SDS were significantly reduced compared to the reference population however height SDS was preserved. The difficulty in maintaining weight and BMI was reflected in the CFQ-R child- and adult-reported eating domain, adult-reported weight domain, and parent-reported eating and weight domains, that were all lower than their corresponding normative means. Consistent with significantly reduced weight and BMI SDS, the total body lean tissue mass for height SDS measured by DXA and the muscle CSA SDS measured at the 66% tibial pQCT site were significantly reduced compared to the reference population, indicating that the lean tissue compartment was compromised in the CF cohort. A ramification of the significantly reduced lean tissue mass, was the significantly lower muscle force SDS produced during the M1LJ and S2LJ, however, peak oxygen uptake corrected for body weight was normal. The significant reductions in lean tissue mass and maximum forces produced during dynamic manoeuvres, compared to their healthy counterparts, would play a dominant role in the compromised bone health in both the trabecular and cortical bone compartments seen in the CF, cohort consistent with the mechanostat theory [35, 47, 72, 75]. Disturbances to the trabecular compartment were evidenced by significantly reduced SDS for vBMD of the lumbar vertebrae 1-4 and trunk BMD on the lumbar spine, and segmental analysis of total body DXA scans respectively, as well as the significant reduction in trabecular vBMD at the 4% tibial pQCT site. Compromised bone health in the cortical bone compartment was reflected in cortical bone mass, density, geometry and strength. Bone mass SDS were significantly reduced in the total body DXA scan and the cortical measurement at the 66% tibial pQCT

site. However, the SDS for BMC for LTM and BMC for muscle CSA were not significantly different to the reference population indicating that BMC was appropriately adapted for lean mass. Abnormal bone density was demonstrated by the significantly reduced arms BMD SDS, a non-weight-bearing site, and the significantly increased cortical vBMD at the 20% and 66% tibial pQCT weight-bearing sites. Sub-optimal geometric adaptations were evidenced by a significantly reduced DXA derived bone area for height SDS, periosteal circumference SDS at the 20% pQCT site and cortical CSA and cortical thickness SDS at the 66% pQCT site. Compromised bone strength was shown by the significantly reduced SDS for cortical measurements of polar CSMI and SSI at the 66% pQCT site. An uncoupling of normal bone turn-over in the CF cohort provided further support for the compromised bone health seen in the CF cohort, with 38% having the bone formation marker, osteocalcin below normal levels, and 44% having the bone resorption marker, urinary deoxypyridinoline: creatinine ratio, above normal levels, indicating reduced bone formation and increased bone resorption respectively.

3.9.2 Observation Period

The CF cohort followed their anthropometric trajectories, with no significant changes in SDS for height, weight or BMI demonstrated. The lean tissue compartment also remained unchanged, however fat CSA at the 66% pQCT site increased significantly, indicating that weight gains may have preferentially been in the fat and not lean tissue compartment. Pulmonary function tests demonstrated significant deterioration in the FEV_1/FVC ratio and FEF_{25-75} measurements and this was associated with significant reductions in the

parent-reported respiratory domain of the CFQ-R. Peak oxygen uptake and its percent-predicted value decreased significantly as did the relative maximum force and force SDS for the M1LJ, indicating a deterioration in muscle functional capacity in the setting of significantly reduced lean tissue mass. These deteriorations would have had further negative impacts on the functional muscle-bone unit in the CF cohort. To support this, the bone formation marker, osteocalcin, decreased significantly. The characteristics of the trabecular bone compartment remained effectively unchanged with no significant changes in SDS at the lumbar spine or 4% pQCT site. Cortical BMC SDS at the 20% and 66% pQCT sites increased, as did cortical density SDS at the 20% pQCT site. Cortical geometry demonstrated improvements measured by the SDS for cortical CSA, cortical thickness and periosteal circumference at the 20% pQCT site and for total and cortical CSA at the 66% site. Similarly, cortical strength SDS at the 20% and 66% pQCT sites increased.

3.9.3 WBVT Period

The CF cohort continued to follow their anthropometric trajectories, however the significant increase in fat CSA was not seen after WBVT, potentially indicating an improved distribution of weight gains to both the fat and lean tissue compartments. There was no further deterioration in pulmonary function or in the child-, adult- or parent-reported respiratory domain of the CFQ-R. The reduction in muscle functional capacity seen in the observation period was arrested with both peak oxygen uptake and force parameters in the M1LJ demonstrating non-significant increases after WBVT. To affirm beneficial effects of WBVT on the functional muscle bone-unit, bone turnover markers

remained stable and there were significant improvements in parameters of the trabecular bone compartment. Total bone mass and density at the 4% pQCT site increased significantly, a change not seen in the observation period and a beneficial outcome as trabecular density at the 4% pQCT site was significantly reduced at baseline. WBVT did not have the same effect on the cortical bone compartment. Bone mass in the cortical compartment showed some favourable changes with the total body DXA derived BMC to bone area SDS increasing significantly, however total and cortical bone BMC at the 20% and 66% pQCT sites showed similar changes to that seen in the observation period. Bone density in the cortical compartment remain largely unchanged with no changes seen at the 66% pQCT site, and similar changes to that seen during observation in total and cortical bone density at the 20% pQCT site. However, legs BMD increased 50% more than the increase seen in the observation period and the SDS showed a non-significant increase in contrast to a decrease seen in the observation period. Cortical bone geometry did not improve after WBVT. The DXA derived total bone area to height SDS decreased significantly however total and cortical bone CSA, cortical thickness and periosteal circumference had similar increases to those seen in the observation period. Similarly, total and cortical bone strength measures at the 20% and 66% pQCT sites demonstrated similar changes to those seen in the observation period. Improvements seen in the trabecular, but not the cortical bone compartment, is likely a reflection of the more metabolically active trabecular compartment, which would reflect changes more rapidly than the cortical bone compartment. Another consideration when interpreting the effect of WBVT on bone parameters is the adherence to the training protocol. Overall adherence was poor in the CF cohort, just shy of 50%, with only half the cohort training at least

three times a week. This amount of training appears to hinder the deterioration in muscle functional capacity seen in the CF cohort, and improve some bone parameters in the trabecular compartment, however this level of training stimulus was not adequate to elicit changes in the cortical bone compartment.

3.9.4 Follow-up Period

The CF cohort continued to follow their anthropometric trajectories, however there was a significant increase in muscle CSA and its corresponding SDS measured at the 66% pQCT site, indicating preferential distribution of weight gain to the lean tissue compartment. Pulmonary function and peak oxygen uptake remained stable with significant improvements in the parent-reported respiratory domain and clinically relevant improvements in the child- and adult-reported respiratory domain of the CFQ-R. Despite the significant increase in calf muscle CSA, the maximum relative force and force SDS for the M1LJ decreased significantly, once again impacting negatively on the functional muscle bone unit, with ramifications for the trabecular and cortical bone compartments. Gains in trabecular bone mass at the lumbar spine were smaller than those seen in the WBVT and observation periods there were no improvements in bone mass at the 4% pQCT site. Lumbar spine bone density also demonstrated smaller increases compared to the WBVT and observation periods and trunk BMD from segmental analysis of the total body DXA scan decreased significantly. In the cortical compartment, BMC for bone area decreased significantly and the significant improvements in total and cortical bone mass at the 20% and 66% pQCT sites were smaller in magnitude than those seen in the WBVT and observation periods. Cortical density was significantly reduced in

the arms BMD parameter, a non-weight bearing site, and the significant increase in legs BMD was smaller than that seen in the WBVT period and similar to that seen in the observation period. The increases in total and cortical bone vBMD at the 20% pQCT site were similar in magnitude to the WBVT period, however cortical vBMD at the 66% pQCT site increased significantly, perhaps indicating a reduced rate of bone turn-over, an unfavourable outcome as this parameter was significantly higher than the reference population at baseline. Cortical bone geometry also appeared to be negatively affected in the follow-up period. While total bone area to height SDS increased significantly, total and cortical bone CSA, cortical thickness and periosteal circumference had smaller increases compared to those seen in the WBVT and observation periods. Cortical bone strength measures at the 20% and 66% pQCT sites also demonstrated smaller increases compared to those seen in the WBVT and observation periods. Taken together, these findings suggest a subtle de-training effect in the follow-up period with significant reductions or smaller magnitude increases in trabecular and cortical bone parameters compared to the WBVT and observation periods.

4 MRCD Results

4.1 Participants

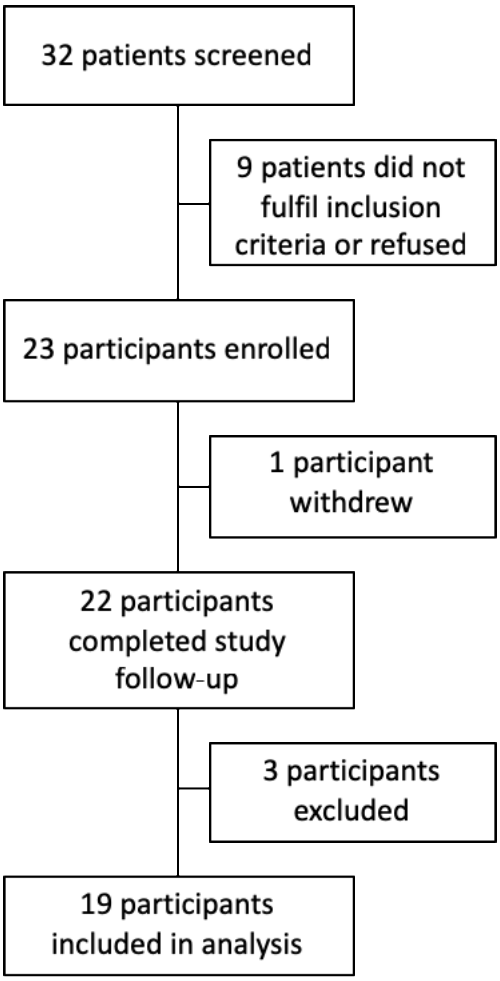

Figure 4.1: Flow diagram of Participant recruitment.

Twenty-three participants, 13 male, aged 5-77 years were recruited into the study (Table 4.1). Thirteen of these participants were recruited through the Children's Hospital at Westmead and the remainder from Royal North Shore Hospital. Four participants were later withdrawn: Participants 7 and 11 were found to have Congenital Myasthenia Gravis on whole exome sequencing; Participant 9 did not have a confirmed MRCD on muscle biopsy; and Participant 10 elected to withdraw from the study after his baseline visit to pursue alternative health care options to which WBVT training may have been detrimental.

Table 4.1. Participant Characteristics.

Patient	**Gender**	**Age**	**Diagnosis**
1	Male	17	Kearns-Sayre Syndrome
2	Female	16	MLASA
3	Male	24	Leigh Syndrome
4	Male	21	Leigh Syndrome
5	Female	8	Leigh Syndrome
6	Male	5	Leigh Syndrome
7	Male	6	WITHDRAWN - Congenital Myasthenia Gravis
8	Female	22	POLG
9	Female	77	WITHDRAWN - Unconfirmed MRCD

10	Male	43	WITHDRAWN - Pursued alternative treatment option
11	Male	8	WITHDRAWN - Congenital Myasthenia Gravis
12	Male	32	MELAS
13	Female	65	Mitochondrial Myopathy
14	Male	13	Kearns-Sayre Syndrome
15	Female	47	CPEO
16	Female	65	Mitochondrial Myopathy
17	Female	38	MELAS
18	Female	20	Complex I Deficiency
19	Male	11	MELAS
20	Female	62	MELAS
21	Male	55	Mitochondrial Myopathy
22	Male	31	MELAS
23	Male	11	Leigh Syndrome

Note: MLASA, mitochondrial myopathy, lactic acidosis and sideroblastic anaemia; POLG, DNA Polymerase Gamma; MELAS, Mitochondrial encephalomyopathy, lactic acidosis ad stroke like episodes; CPEO, Chronic progressive external ophthalmoplegia.

The remaining 19 participants, 10 male, with a mean (SD) age of 30.3 (20.1) years, presented with diverse genetic and phenotypic manifestations typical of mitochondrial respiratory chain disorders (Table 2.2) [278, 281, 283, 290, 295-297, 302, 305, 309, 321, 323-326, 336, 337, 347, 383, 431]. The bi-genomic regulation of the MRC and the ability of pathogenic mutations of both the mitochondrial and nuclear genomes to cause MRCD were represented in the cohort. Two participants had genetic mutations originating in the nuclear genome, one with childhood MLASA caused by a mutation in the nuclear encoded *YARS2* gene, a tRNA modifying gene important for mtDNA translation [296, 297, 355], and the other with adult onset mitochondrial disease due to a *POLG* mutation which interferes with mtDNA maintenance causing mtDNA depletion or multiple mtDNA deletions [296, 309, 370, 371]. The remaining six paediatric and eleven adult participants had genetic mutations originating in the mitochondrial genome.

The cohort of participants with MRCD in this study displayed diverse phenotypic heterogeneity and variability in the presentations in keeping with the literature [278, 283, 290, 295, 305, 326, 336, 337, 347, 383, 431]. There was a wide variation in age at presentation, spanning from the early childhood years to late in adult life. There was also a large spectrum in disease severity, with participants being oligosymptomatic with tissue specific involvement, while others had multisystemic manifestations causing chronic disability, significant morbidity and reduced quality of life. Some participants had symptoms that aligned with well-defined syndromes including LS, MELAS, PEO, KSS and MLASA, while others had largely non-specific disorders, these features also consistent with the literature [281-283, 295-298, 305, 327, 384, 432, 433]. As described

in the literature [296, 297, 305, 379, 434, 435], there were poor genotype-phenotype correlations within our cohort. The phenotypes consistent with MELAS in our cohort were caused by different mtDNA gene mutations, and MELAS caused by the same genetic mutation resulted in both child and adult onset disease. Similarly, the five participants in our cohort with LS due to the identical m.8993T>C mutation demonstrated significant variability in phenotypic severity. In keeping with the literature [281-283, 298, 305, 319, 321, 379, 383, 397], the participants in our cohort presented with symptoms in tissues highly reliant on OXPHOS including the brain, central and peripheral nervous systems, skeletal muscle, heart muscle, the retina and optic musculature, the cochlea, bone marrow, pancreas and gastro-intestinal system. The majority of participants in our cohort presented with features consistent with encephalomyopathy as described by Shapira et al in 1977 [398], including central nervous system sequelae such as stroke like episodes, migraines, seizures, psychomotor regression, ataxia, encephalopathy and cognitive impairment, as previously described [283, 379, 401], as well as skeletal muscle involvement, with common symptoms including muscle weakness, predominantly of the hip and shoulder girdle, and exercise intolerance, that are well recognised in the literature [403-407]. Exercise intolerance is considered to be a hallmark of MRCD [403, 405, 406, 410-412] and was universally manifested in our cohort.

Two-thirds of our cohort had MRCD involving multiple MRC complexes, three quarters of the adult participants and half of the paediatric participants, higher than that reported in the literature [295, 382, 391, 393, 395, 441]. These included participants with KSS and PEO caused by large-scale mtDNA deletions, mtDNA point mutations including those

causing MELAS and nDNA mutations in the *POLG* and *YARS2* genes as recognised in the literature [320, 352, 395, 441], [296, 297, 355]. The vast majority of participants with deficits in multiple MRC complexes presented with multisystem disease manifestations (Table 2.2) consistent with those reported in the literature for KSS [290, 321, 439, 465], PEO [321, 439], MELAS [281, 290, 298, 305, 321, 347, 455, 456, 459, 464], *POLG* gene mutations [296, 309, 440] and *YARS2* gene mutations [296, 297, 355]. The five participants with LS affecting the ATP synthase 6 subunit of Complex V of the MRC also presented with multisystemic disease manifestations which are in keeping with those reported in the literature [290, 296, 320, 347, 379, 445, 446, 450].

The cohort comprised of 7 paediatric and 12 adult participants. These cohorts were analysed separately due to the different biological process occurring in the immature musculoskeletal system during growth compared to the mature adult musculoskeletal system. Paediatric participants presented with more severe, progressive disease as is often reported in the literature [279, 321, 384, 385]. The majority of the paediatric MRCD cohort presented with mtDNA mutations, despite the literature suggesting that only 10-30% of paediatric presentations are due to mtDNA defects [290, 324, 382, 390, 393, 394]. This is likely due to the fact that the paediatric population of MRCD due to nDNA mutations have a disease onset much earlier than those with mtDNA mutations [328, 382, 390, 392, 395] and a more severe clinical course [279, 321, 384, 385] which would have precluded them from being enrolled in the study. As a result, the paediatric cohort in our study comprised the lesser-affected children with mtDNA mutations. The propensity toward nuclear gene mutations in the paediatric population accompanied by their earlier

onset and more severe clinical progression may also explain why we were unable to find enough eligible paediatric participants for the study. Half of the paediatric participants with mtDNA mutations presented with LS, not surprising as LS is the most commonly seen paediatric presentation in MRCD [320, 445]. All three of these paediatric participants had LS as a result of the point mutation m.8993T>C affecting the formation of the ATP Synthase 6 subunit of Complex V of the mitochondrial respiratory chain, one of the most common causes of maternally inherited LS [320, 434, 452, 453]. Two of the other paediatric participants with mtDNA mutations had KSS, both caused by large-scale deletions in mtDNA, the most common cause of KSS [439, 465]. The final paediatric participant with a mtDNA mutation had MELAS caused by the mtDNA point mutation m.3243A>G which is the most frequent pathogenic mtDNA point mutation associated with MELAS [281, 298, 321, 455-460] and the most common paediatric presentation caused by a mtDNA mutation [393].

The majority of adult participants also had mtDNA mutations, this pattern similar to that seen in the literature where mutations in mtDNA are the most common cause of adult onset mitochondrial disease [328, 386-388]. Eight of the eleven adults with mtDNA mutations were found in mitochondrial genes encoding tRNA consistent with the literature which has found that over 50% of all identified pathogenic mtDNA point mutations are located in the mitochondrial tRNA genes [282]. Furthermore, seven of these adults had mutations in the gene encoding the mitochondrial $tRNA^{Leu(UUR)}$, one of the most common sites for point mutations in tRNA genes [296]. Four of these adult participants presented with MELAS, caused by the m.3243A>G mutation in three, which

accounts for around 80% of individuals presenting with MELAS symptoms [281, 298, 321, 455-460], and by the less common m.3256C>T mutation in the fourth participant. The remaining four adult participants with mutations in mitochondrial genes encoding tRNA did not fulfil the criteria for well-defined syndromes. Three of these participants presented with largely non-specific mitochondrial myopathy, as is often seen in MRCD presentations [281, 282, 295-298, 327, 384], and the fourth had an isolated Complex I deficiency, which accounts for around a quarter of all MRCD presentations [290, 295]. Of the three remaining adult participants with mtDNA mutations, two had the point mutation m.8993T>C affecting the formation of the ATP Synthase 6 subunit of Complex V of the mitochondrial respiratory chain causing maternally inherited LS, which was the same point mutation seen in the three paediatric participants with LS. The last adult participant with a mtDNA mutation had Progressive External Ophthalmoplegia (PEO), most likely due to a single mtDNA deletion, a common cause of PEO in the literature [296, 305], although this was not genetically confirmed. Adult participants in our cohort presented with exercise intolerance and muscle weakness usually affecting the proximal muscles, seizures, migraines, cognitive impairment, sensorineural hearing loss, ophthalmologic problems including optic atrophy, ptosis, progressive external ophthalmoplegia and pigmentary retinopathy, cardiac arrhythmias, gastrointestinal symptoms. Three of the adult participants, 3, 4 and 18, were diagnosed during their paediatric years and had the most severe presentations of all the adult participants, supporting the greater disease severity and faster disease progression seen in paediatric diagnosed MRCD.

al dysmotility, diabetes and short stature, as previously reported in MRCD [305].

Within the cohort two distinct clinical cohorts could be identified: 5 participants, 4 male, aged 5-24 years with Leigh Syndrome; and 5 participants, 3 male, aged 11-62 years with MELAS. The Leigh Syndrome cohort consisted of paediatric and young adult participants all affected by the same mutation, m.8993T>C. This sub-cohort was analysed independently. The MELAS cohort was predominantly adult and comprised of different mutations, including the m.3251A>G mutation and the common m.3243A>G mutation. Due to the small number of participants in this cohort and the heterogeneous genetic mutations, this sub-cohort was not analysed independently.

Participants attended the hospital for four research visits. The observation period occurred between visits 1 and 2. The mean (SD) time elapsed between these visits was 4.3 (2.0) months. The WBVT period occurred between visits 2 and 4. The mean (SD) time between visits was 6.5 (0.6) months. The follow-up period occurred between visits 4 and 6. The mean (SD) time periods between these visits was 6.3 (0.9) months.

Table 4.2. Participant diagnoses, genetic mutations and clinical manifestations.

Patient	Diagnosis	Genome Affected	Mutation	Interrupted Function	Clinical Manifestations
1	KSS	mtDNA	mtDNA deletion		Sensorineural hearing loss requiring hearing aids, moderate - severe ptosis/ophthalmoplegia, dysphagia, intention tremor, muscle weakness (proximal > distal), exercise intolerance, community mobility in wheelchair
2	MLASA	nDNA	*YARS2*, homozygous for p.Phe52Leu	tRNA Tyr synthetase	Transfusion dependent sideroblastic anaemia, muscle weakness (proximal > distal), dysphagia requiring gastrostomy, community mobility in wheelchair, exercise intolerance
3	Leigh Syndrome	mtDNA	m.8993T>C	ATP Synthase subunit 6	Mild ptosis/ ophthalmoplegia, dysarthria, cerebellar ataxia, exercise intolerance, community mobility in wheelchair

4	Leigh Syndrome	mtDNA	m.8993T>C	ATP Synthase subunit 6	Proximal muscle weakness, bulbar dysfunction, dysarthria, mild tremor and cerebellar ataxia, exercise intolerance, community mobility in wheelchair
5	Leigh Syndrome	mtDNA	m.8993T>C	ATP Synthase subunit 6	Mild ataxia, dysarthria, action tremor, dysphagia, proximal muscle weakness, exercise intolerance, community mobility in wheelchair
6	Leigh Syndrome	mtDNA	m.8993T>C	ATP Synthase subunit 6	Mild ataxia and dysarthria, exercise intolerance
8	POLG	nDNA	*POLG*, with mutations of p.Pro163Ser, and p.Thr851Ala	mtDNA replication and stability	Seizures, peripheral neuropathy, cerebellar and sensory ataxia, mild ptosis/ ophthalmoplegia, GI dysmotility, insulin resistance, severe generalised sensorimotor polyneuropathy, exercise intolerance
12	MELAS	mtDNA	m.3256C>T	tRNA Leu (UUR)	Exercise intolerance

13	Mitochondrial Myopathy	mtDNA	m.3251A>G	tRNA Leu (UUR)	Sensorineural hearing loss, mild neuropathy, mild ptosis, impaired glucose tolerance, headaches, exercise intolerance, Sjorgens Syndrome
14	KSS	mtDNA	mtDNA deletion		Moderate-severe ptosis/ ophthalmoplegia/ retinitis pigmentosa, sensorineural deafness, mild dysdiadochokinesia, action tremor, ataxic gait, exercise intolerance
15	CPEO	mtDNA			Proximal muscle weakness, moderate to severe ptosis/ ophthalmoplegia, diplopia, exercise intolerance, depression, slow processing
16	Mitochondrial Myopathy	mtDNA	m.3251A>G	tRNA Leu (UUR)	Moderate ptosis/ ophthalmoplegia, optic neuropathy, sensorineural hearing loss, proximal muscle weakness, dysphagia, insulin resistance, respiratory muscle weakness and type 2 respiratory failure, depression,

					exercise intolerance
17	MELAS	mtDNA	m.3243A>G	tRNA Leu (UUR)	Mild ptosis, migraines, GI dysmotility, fibromyalgia, insulin resistance, exercise intolerance
18	Complex I Deficiency	mtDNA	tRNA-Leu mutation	tRNA Leu (UUR)	Ataxia, muscle weakness (proximal > distal), exercise intolerance
19	MELAS	mtDNA	m.3243A>G	tRNA Leu (UUR)	Headaches, seizures, stroke like episodes, dysarthria, ptosis, reduced visual acuity, reduced muscle strength, anxiety, exercise intolerance
20	MELAS	mtDNA	m.3243A>G	tRNA Leu (UUR)	Sensorineural hearing loss, migraines, proximal muscle weakness, GI dysmotility, exercise intolerance
21	Mitochondrial Myopathy	mtDNA	m.4269A>G	tRNA	Mild retinopathy/ ptosis/ ophthalmoplegia, sensorineural hearing loss, dysarthria, proximal muscle weakness, mild peripheral neuropathy, GI dysmotility, cognitive impairment, exercise intolerance

22	MELAS	mtDNA	m.3243A>G	tRNA Leu (UUR)	Sensorineural hearing loss, pigmentary retinopathy, seizures, stroke like episodes, exercise intolerance
23	Leigh Syndrome	mtDNA	m.8993T>C	ATP Synthase subunit 6	Ataxia, proximal muscle weakness, dysarthria, dysdiadochokinesia, exercise intolerance, wheelchair for community mobility

Note: KSS, Kearns-Sayre Syndrome; MLASA, mitochondrial myopathy, lactic acidosis and sideroblastic anaemia; POLG, DNA Polymerase Gamma; MELAS, Mitochondrial encephalomyopathy, lactic acidosis ad stroke like episodes; CPEO, Chronic progressive external ophthalmoplegia; mtDNA, mitochondrial DNA; nDNA, nuclear DNA; tRNA, transfer RNA; Tyr, tyrosine; Leu, leucine; ATP, adenosine triphosphate; GI, gastrointestinal.

4.2 Anthropometric Parameters

Analysis data for all anthropometric parameters can be found in Appendix H.

4.2.1 Paediatric Cohort

The baseline anthropometric data for the cohort are presented in Table 4.3 and Figure 4.1. The paediatric cohort, mean (SD) age of 12.1 (4.1) years, had a mean (SD height of 134.7 (18.7) cm and a mean (SD) height standard deviation score (SDS) of -2.1 (0.7) which was significantly lower than the reference population indicating growth retardation. Weight also indicated failure to thrive with mean (SD) 29.4 (8.5) kg and mean (SD) SDS -2.3 (1.8), which was also significantly lower than the reference population. BMI and BMI SDS were also low with a mean (SD) of 16.0 (2.5) kg/m^2 and -1.4 (2.1) respectively, however this was not significantly different to the reference population. These findings are consistent with the literature, which have found failure to thrive, growth retardation and short stature to be common manifestations in MRCD [305, 383].

During the observation period there was a significant increase in height from 134.7 to 136.4 cm (p=0.001), however there was no significant change in height SDS, indicating normal linear growth. Weight, weight SDS, BMI and BMI SDS did not change significantly in the observation period (Appendix H, Table H.2).

Height and weight increased significantly during the WBVT period, by 1.8cm, p=0.017 and 1.3kg, p=0.045, however their corresponding SDS were not altered significantly. There were no significant changes in BMI or BMI SDS indicating that the tracking of anthropometric measures was not altered during the WBVT period (Appendix H, Table H.2.).

Height increased significantly in the follow-up period from 138.2 to 139.8 cm (p=0.033), however there was no significant change in the height SDS. Weight, weight SDS, BMI and BMI SDS demonstrated no significant changes during the follow-up period indicating tracking of anthropometric variables (Appendix H, Table H.2.).

4.2.2 Adult Cohort

Baseline anthropometric data for the cohort are presented in Table 4.3. The adult cohort, mean (SD) age 40.9 (17.8) years, had normal nutritional status with a mean (SD) height, weight and BMI of 166.8 (9.8) cm, 64.3 (14.4) kg and 23.2 (5.0) kg/m^2 respectively. There was however a range of BMIs with two young adult females with low BMIs ($<18kg/m^2$) and two middle-aged females with high BMIs ($>30kg/m^2$).

There were no significant changes in the height, weight or BMI in the adult cohort during the observation, WBVT or follow-up periods (Appendix H, Table H.3).

4.2.3 Leigh Syndrome Cohort

Baseline anthropometric data for the cohort are presented in Table 4.3. The Leigh Syndrome cohort, mean (SD) age of 14.4 (8.1) years, had a mean (SD) height, weight and BMI of 144.8 (35.5) cm, 47.6 (31.0) kg and 20.2 (4.1) kg/m^2 respectively.

During the observation period, there were no significant changes in height, weight or BMI. Weight increased significantly by 1.7kg, p=0.019, after WBVT, consistent with the increase in weight seen in the paediatric cohort. There were no significant changes in height or BMI after WBVT or during the follow-up period. Weight significantly increased again in the follow-up period by 2.3kg, p=0.004, a change that was not seen in either the paediatric of adult cohorts (Appendix H, Table H.4).

Table 4.3. Baseline anthropometric data

Patient	**Gender**	**Age (years)**	**Height (cm)**	**Height SDS**	**Weight (kg)**	**Weight SDS**	**BMI (kg/m^2)**	**BMI SDS**
1	Male	17	160.8	-2.1	36.1	-4.7	13.96	-4.5
2	Female	16	151.6	-1.9	38.4	-2.8	16.71	-1.8
3	Male	24	188.5		92.6		26.06	
4	Male	21	173.5		64.6		21.46	
5	Female	8	119.0	-2.0	24.9	-0.6	17.58	0.7
6	Male	5	105.0	-1.9	17.0	-1.4	15.42	-0.1

8	Female	22	168.7		49.6		17.43	
12	Male	32	178.5		62.8		19.71	
13	Female	65	156.2		68.2		27.95	
14	Male	13	134.3	-3.5	25.7	-4.0	14.25	-3.0
15	Female	47	158.3		80.3		32.04	
16	Female	65	167.0		54.2		19.43	
17	Female	38	154.8		73.2		30.55	
18	Female	20	161.2		38.2		14.70	
19	Male	11	134.0	-1.6	24.3	-2.7	13.53	-2.5
20	Female	62	166.0		57.0		20.69	
21	Male	55	167.7		71.0		25.25	
22	Male	31	161.0		59.8		23.07	
23	Male	11	138.0	-1.4	39.1	0.2	20.53	1.2

Note: SDS, standard deviation score; BMI, body mass index.

4.3 Bone Parameters

4.3.1 DXA

4.3.1.1 Baseline

Baseline DXA parameters for the three cohorts are presented in Table 4.4. This includes: total body DXA scan parameters, segmental analysis of the trunk, arms and legs from the total body scans; and analysis of the lumbar spine (LS), including lumbar vertebrae 2-4; and analysis of the lumbar vertebrae 1-4 (L14).

Table 4.4. Baseline dual-energy x-ray absorptiometry parameters for scans of the total body and lumbar spine. Mean (SD).

Cohort	**Paediatric (n=7)**	**Adult (n=12)**	**Leigh Syndrome (n=5)**
Total Body DXA Parameters			
TBMC (g)	1022.1 (379.0)	2378.3 (358.5)	1562.5 (1063.2)
TBMC SDS	-0.8 (1.1)		
TBA (cm^2)	1199.0 (354.4)	2177.4 (246.0)	1603.1(893.5)
TBMD (g/cm^2)	0.839 (0.069)	1.090 (0.105)	0.916 (0.144)
TBMD SDS	-0.8 (1.4)	-0.4 (1.5)	-0.7 (1.5)

LTM (g)	20422.5 (5159.3)	38771.7 (8086.7)	29565.6 (17975.6)
Fat (%)	24.6 (11.8)	35.9 (11.9)	31.8 (9.3)
Fat (%) SDS	0.9 (1.5)		
LTM for Ht SDS	-1.5 (1.2)		
TBA for Ht SDS	-1.0 (1.1)		
TBMC for LTM SDS	0.9 (1.5)		
TBMC for TBA SDS	-0.3 (1.2)		
Parameters of Segmental Analysis of Total Body DXA Scans			
Trunk BMD (g/cm^2)	0.636 (0.072)	0.868 (0.108)	0.728 (0.179)
Trunk BMD SDS	-0.4 (0.4)		
Arms BMD (g/cm^2)	0.591 (0.073)	0.817 (0.106)	0.733 (0.185)
Arms BMD SDS	-0.7 (1.6)		
Legs BMC (g)	300.8 (147.8)	846.5 (149.0)	469.8 (354.9)
Legs BA (cm^2)	393.3 (135.8)	741.0 (75.8)	492.8 (284.5)
Legs BMD (g/cm^2)	0.735 (0.116)	1.139 (0.159)	0.856 (0.232)
Legs BMD SDS	-1.5 (1.5)		
Legs LTM (g)	6077.4 (1837.9)	12140.9 (260.9.7)	8884.3 (5849.7)
Legs Fat (%)	31.0 (12.3)	38.8 (11.4)	37.3 (6.78)

Lumbar Spine DXA Parameters			
LS BMC (g)	18.7 (8.6)	51.4 (9.2)	31.8 (25.5)
LS BMC SDS	-0.4 (0.6)		
LS BMD (g/cm^2)	0.672 (0.152)	1.123 (0.841)	0.785 (0.296)
LS BMD SDS	-0.9 (1.0)	-0.4 (1.4)	-1.1 (0.8)
L14 BMC (g)	23.8 (11.0)	64.3 (11.1)	39.2 (31.0)
L14 BA (cm^2)	34.6 (8.2)	58.6 (6.7)	44.8 (22.1)
L14 vBMD (g/cm3)	0.248 (0.046)	0.323 (0.067)	0.256 (0.055)
L14 vBMD SDS	-1.0 (1.1)		

Note: DXA, dual-energy x-ray absorptiometry; TBMC, total body bone mineral content; TBA, total body bone area; TBMD, total body bone mineral density; SDS, standard deviation score; LTM, lean tissue mass; Ht, height; BMD, bone mineral density; BMC, bone mineral content; BA, bone area; TF, tibia and fibula; LS, lumbar spine vertebrae 2-4; L14, lumbar spine vertebrae 1-4; vBMD, volumetric bone mineral density.

4.3.1.1.1 Paediatric Cohort

In addition to the baseline DXA parameters presented in Table 4.4, further analysis of DXA parameters in the paediatric cohort was obtained by investigating ratios between

total body DXA derived measures of BMC, BA, LTM, and height: LTM for height SDS; TBA for height SDS; TBMC for LTM SDS; and TBMC for TBA SDS.

The baseline SD scores for legs BMD, trunk BMD, LTM:Height, LS BMD and L14 BMD were significantly reduced compared to the reference population (Figure 4.1). Total body BMD SDS were < -1 in 5/7 (71%) and less < -2 in 1/7 (14%) of the paediatric cohort. At the lumbar spine, BMD SDS were < -1 in 3/7 (43%) and < -2 in 1/7 (14%). The mean total body fat percent was 24.6% with a SDS of 0.9 (Table 2.4), however this was not found to be significantly different to the reference population (Figure 4.1). Considering the paediatric cohort had a weight SDS significantly lower than the reference population, a much larger percentage of their weight is likely to be comprised of fat mass.

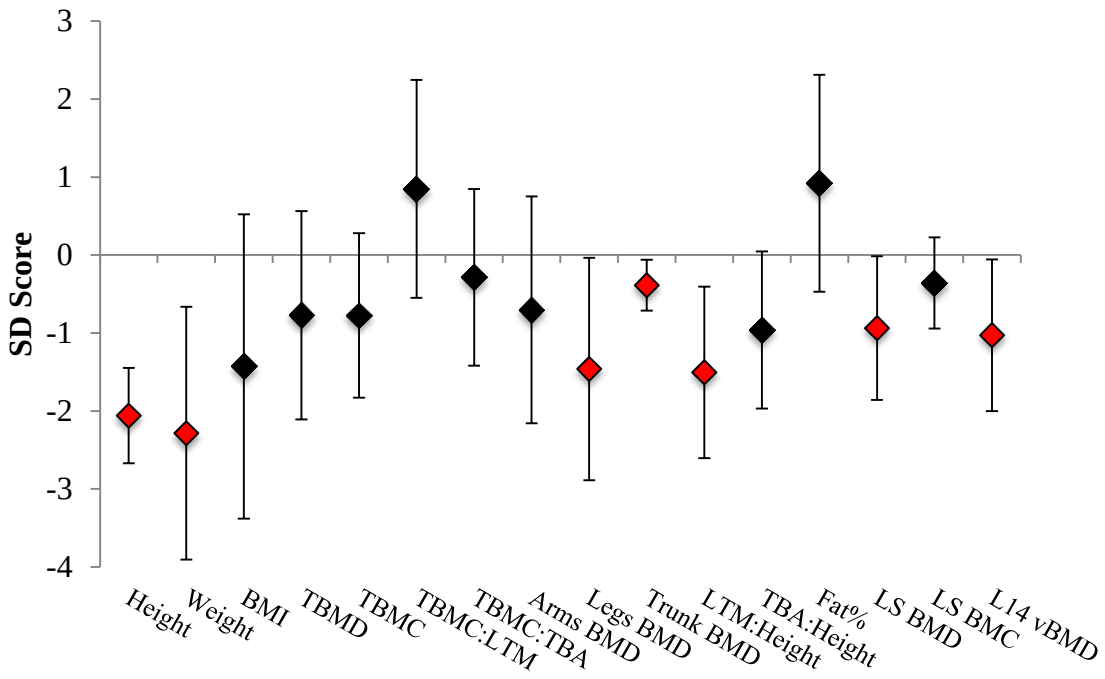

Figure 4.2. Standard deviation (SD) scores for anthropometric and DXA parameters in the paediatric cohort. Mean and 95% confidence intervals. Parameters shown in red were significantly different to the reference population on one-sample t-tests, $p<0.05$.

4.3.1.1.2 Adult Cohort

TBMD and LS BMD SDS were not significantly different to the reference population, -0.4, p=0.389 and -0.4, p=0.306 respectively. Total body BMD SDS were <-2 in 3/12 (25%) and lumbar spine BMD SDS were <-1 in 4/12 (33%) and <-2 in 1/12 (8%) of the adult cohort. Mean total body fat percent for the adult cohort was 35.9% (Table 4.4), the upper limit of the normal range for adult females but higher than that expected for adult males.

4.3.1.1.3 Leigh Syndrome Cohort

Consistent with the Leigh Syndrome cohort being a mixed adult and paediatric cohort all total body DXA scan parameters were lower than the adult cohort but higher than the paediatric cohort. However, the LS BMD SDS was lower than values seen in the other cohorts and significantly reduced compared to the reference population (p=0.035), with 4/5 (80%) having a SDS <-1. These results suggest this site may be more severely affected in Leigh Syndrome compared to other MRCD. Total body BMD SDS were <-1 in 2/5 (40%) and <-2 in 1/5 (20%) of the Leigh Syndrome cohort. Mean total body fat percent was 31.8% (Table 4.4), higher than that expected for adult males and children of either gender.

4.3.1.2 Observation Period

The observation period occurred between Visit 1 (Baseline) and Visit 2 (Start WBVT). Full analysis data for all DXA parameters for the observation period for each of the 3 cohorts can be found in Appendix A.

4.3.1.2.1 Paediatric Cohort

Analysis revealed significant changes in parameters of the total body DXA scan, segmental analysis of the total body DXA scan and the lumbar spine scan (Table 4.5 and

Appendix A, Table A.2). Total body LTM increased significantly from 20422.5 – 21258.9g (p=0.007) however the LTM for height SDS did not change significantly indicating this increase was associated with normal growth. Legs BA increased significantly, 393.3 – 406.5 cm^2 (p=0.008). Total body bone area (TBA) demonstrated a non-significant increase and TBA for height SDS did not change significantly, again indicating increases associated with normal growth. However, legs BMD SDS decreased significantly during the observation period falling from -1.5 to -1.7 (p=0.013), this decrease representing a quarter of a SDS. Lumbar spine scans revealed a significant increase in L14 BMC from 23.8g to 24.7g (p=0.039).

Table 4.5. DXA parameters for the paediatric MRCD cohort at each visit, changes between visits and p values. *p<0.05.

		TBMC (g)	TBMC SDS	TBA (cm^2)	TBMD (g/cm^2)	TBMD Ht SDS
Visit 1	Value	1022.120	-0.776	1199.002	0.839	-0.772
	Δ in Observation	25.488	-0.168	24.203	0.006	-0.087
	p	0.124	0.195	0.149	0.238	0.328
Visit 2	Value	1047.608	-0.944	1223.205	0.845	-0.859
	Δ with WBVT	46.778	0.019	37.931	0.012	0.026
	p	0.008*	0.883	0.029*	0.020*	0.771
Visit 3	Value	1094.368	-0.925	1261.136	0.858	-0.833
	Δ in Follow-up	15.264	-0.149	22.821	-0.002	-0.185
	p	0.347	0.250	0.172	0.671	0.048*
Visit 4	Value	1109.650	-1.074	1283.957	0.856	-1.018

		TLTM (g)	TLTM:Ht SDS	TBA:Ht SDS	TBMC:TLTM SDS	TBMC:TBA SDS
Visit 1	Value	20422.481	-1.504	-0.963	0.850	-0.286
	Δ in Observation	836.461	-0.006	-0.198	-0.248	0.017
	p	0.007*	0.959	0.299	0.191	0.869
Visit 2	Value	21258.941	-1.510	-1.160	0.602	-0.286
	Δ with WBVT	364.642	-0.155	0.031	0.266	0.079
	p	0.199	0.215	0.870	0.162	0.463
Visit 3	Value	21623.584	-1.665	-1.130	0.868	-0.190
	Δ in Follow-up	503.938	-0.062	-0.174	-0.112	-0.133
	p	0.082	0.612	0.360	0.549	0.220
Visit 4	Value	22127.521	-1.727	-1.304	0.756	-0.323

		Trunk BMD (g/cm^2)	Trunk BMD SDS	Legs BMC (g)	Legs BA (cm^2)	Legs BMD SDS
Visit 1	Value	0.636	-0.387	300.766	393.342	-1.461
	Δ in Observation	-0.002	-0.076	7.894	13.110	-0.250
	p	0.780	0.151	0.131	0.008*	0.013*
Visit 2	Value	0.634	-0.463	308.660	406.452	-1.711
	Δ with WBVT	0.018	0.077	11.778	12.737	-0.073
	p	0.008*	0.146	0.030*	0.009*	0.429
Visit 3	Value	0.652	-0.386	320.438	419.189	-1.784
	Δ in Follow-up	-0.006	-0.105	8.635	6.031	-0.039
	p	0.364	0.052	0.101	0.184	0.672
Visit 4	Value	0.646	-0.491	3.29.073	425.220	-1.823

		LS BMC (g)	LS BMC SDS	LSBMD (g/cm^2)	LS BMD SDS	L14 BMC (g)
Visit 1	Value	18.654	-0.358	0.672	-0.937	23.800
	Δ in Observation	0.579	-0.058	0.008	-0.045	0.883
	p	0.109	0.600	0.587	0.761	0.039*
Visit 2	Value	19.233	-0.416	0.679	-0.981	24.682
	Δ with WBVT	1.406	0.173	0.050	0.407	1.731
	p	0.001*	0.133	0.002*	0.012*	0.000*
Visit 3	Value	20.639	-0.244	0.729	-0.575	26.413
	Δ in Follow-up	-0.118	-0.221	-0.016	-0.278	-0.033
	p	0.735	0.059	0.272	0.071	0.935
Visit 4	Value	20.521	-0.464	0.713	-0.853	26.381

Note: Δ, change; TBMC, total bone mineral content; TBA, total bone area; TBMD, total bone mineral density; SDS, standard deviation score; TLTM, total body lean tissue mass; TFat, total body fat; BMD, bone mineral density; BMC, bone mineral content; BA, bone area; LTM, lean tissue mass; TF, tibia and fibula; LS, lumbar spine vertebrae 2-4; L14, lumbar spine vertebrae 1-4; vBMD, volumetric bone mineral density

4.3.1.2.2 Adult Cohort

On analysis, there were no significant changes in lumbar spine DXA scans, however there were significant decreases in parameters of the total body DXA scan and segmental analysis of the total body DXA scans (Table 4.6 and Appendix A, Table A.3). Total body LTM and legs LTM decreased significantly during the observation period: 38771.7 g to 38159.0 g (p=0.048); and 12141.0 g to 11889.1 g (p=0.033) respectively. Legs BMD decreased significantly from 1.139 g/cm^2 to 1.133 g/cm^2 (p=0.049), however falls within the CV for BMD measured by total body DXA scans reported in the adult literature [134], and that reported from our laboratory (Table 2.1),.

4.3.1.2.3 Leigh Syndrome Cohort

Investigation revealed significant changes in total body DXA scans and segmental analysis of total body DXA scans but not in lumbar spine scans (Table 4.7 and Appendix A, Table A.4). Total body BMD increased significantly during the observation period from 0.916 g/cm^2 to 0.925 g/cm^2 (p=0.025). Legs BMC increased significantly from 469.8 g to 480.7 g (p=0.022). The improvement in total body BMD was within the CV from our laboratory (Table 2.1), and of questionable clinical significance as the corresponding SDS did not change significantly. The increase in legs BMC was above the CV from our laboratory (Table 2.1) and in contrast to the significant decreases in legs BMD seen in both the paediatric and adult MRCD cohorts. This may reflect the small sample size or may indicate that in individuals with Leigh Syndrome, bone health is affected differently when compared to other MRCD.

Table 4.6. DXA parameters for the adult MRCD cohort at each visit, changes between visits and p values. *p<0.05.

		TLTM (g)	**Legs BMD (g/cm^2)**	**Legs LTM (g)**
Visit 1	Value	38771.665	1.139	12140.954
	Δ in Observation	-612.627	-0.006	-251.861
	p	**0.048***	**0.049***	0.033*
Visit 2	Value	38159.038	1.133	11889.093
	Δ with WBVT	-415.315	0.007	-188.452
	p	0.173	**0.036***	0.106
Visit 3	Value	37743.722	1.140	11700.640
	Δ in Follow-up	118.572	-0.008	6.224
	p	0.531	**0.019***	0.957
Visit 4	Value	37932.295	1.132	11706.864

Note: Δ, change; WBVT, whole body vibration training; TLTM, total body lean tissue mass; BMD, bone mineral density.

Table 4.7. DXA parameters for the Leigh Syndrome MRCD cohort at each visit, changes between visits and p values. *p<0.05.

		TBMD (g/cm^2)	**TBMD SDS**	**Legs BMC (g)**	**Legs BA (cm^2)**
Visit 1	Value	0.916	-0.702	469.760	492.843
	Δ in Observation	0.010	-0.003	10.984	9.518
	p	0.025*	0.976	0.022*	0.111
Visit 2	Value	0.925	-0.705	480.744	502.361
	Δ with WBVT	0.004	-0.123	15.056	14.367
	p	0.316	0.250	0.004*	0.023*
Visit 3	Value	0.929	-0.829	495.800	516.728
	Δ in Follow-up	0.004	-0.088	11.732	10.981
	p	0.263	0.406	0.016*	0.070
Visit 4	Value	0.934	-0.917	507.532	527.709

Note: Δ, change; WBVT, whole body vibration training; TBMD, total bone mineral density; SDS, standard deviation score; BMC, bone mineral content; BA, bone area.

4.3.1.3 Whole Body Vibration Training (WBVT) Period

The WBVT period occurred between Visit 2 (Start WBVT) and Visit 4 (End WBVT). Full analysis data for all DXA parameters for the WBVT period for each of the 3 cohorts can be found in Appendix A.

4.3.1.3.1 Paediatric Cohort

Total body DXA scans in the paediatric cohort showed significant increases in TBMC TBMD and TBA, 1047.6 g to 1094.4 g (p=0.008), 0.845 to 0.858 g/cm^2 (p=0.020) and 1223.2 to 1261.1cm^2 (p=0.029) respectively (Table 4.5). TBMC and TBMD SDS scores also increased but did not reach significance. Total body LTM did not increase significantly during the WBVT period as it did in the observation period and the magnitude of the increase was less than 50% of that seen during the observation period, however the LTM for height SDS did not change significantly, suggesting LTM acquisition was not significantly altered during WBVT (Appendix A, Table A.2).

On segmental analysis of the total body scan, legs BMC and legs BA increased significantly from 308.7 g to 320.4 g (p=0.030) and 406.5 to 419.2 cm^2 (p=0.009) respectively. Legs BMD SDS continued to decrease in the WBVT period however the decrease was not significant and was 70% less than the significant reduction in legs BMD SDS seen in the observation period. Legs LTM increased with WBVT however did not reach significance, p=0.059 (Table 4.5 and Appendix A, Table A.2).

Trunk BMD from the segmental analysis of the total body DXA scan increased significantly from 0.634 g/cm^2 to 0.652 g/cm^2 (p=0.008). The trunk BMD SDS score also increased with WBVT however this did not reach significance. Analysis of the lumbar spine scans demonstrated significant increases in both LS BMD and LS BMD SDS: 0.679 g/cm^2 to 0.729 g/cm^2 (p=0.002); and -1.0 to -0.6 (p=0.012). The mean absolute increase in the LS BMD SDS was close to half a SDS. LS BMC and L14 BMC also increased significantly from 19.2 to 20.6g (p=0.001) and from 24.7 to 25.4g (p<0.001) respectively (Table 2.5 and Appendix B, Table B.1).

4.3.1.3.2 Adult Cohort

There were no significant changes in the total body DXA scans (Appendix A, Table A.3). On segmental analysis of the total body DXA scan, legs BMD increased significantly from 1.133 g/cm^2 to 1.140 g/cm^2 (p=0.036). This result was in contrast to the significant decrease in legs BMD seen in the observation period and was slightly larger in value, a 0.007 g/cm^2 increase compared to a 0.006 g/cm^2 decrease. Legs LTM continued to decrease in the WBVT period with a mean reduction of 188.5 g however this was not significant and 25% less than the significant decrease of 251.9 g seen in the observation period. Similarly, the total body LTM continued to decline in the WBVT period however this decline was not significant and 32% lower than the significant decline observed in the observation period, 415.3 g compared to 612.6 g respectively (Table 4.6).

4.3.1.3.3 Leigh Syndrome Cohort

There were no significant changes in the total body DXA scans (Appendix A, Table A.4). Segmental analysis of the total body DXA scans demonstrated a significant increase in legs BMC and legs BA with WBVT: 480.7 to 495.8g (p=0.004), which was 50% greater than the significant increase seen in the observation period; and 502.4 to 516.7 cm^2 (p=0.023). The significant increases in total body BMD seen in the observation period did not continue during WBVT (Table 4.7). No significant changes in any parameters of the lumbar spine scans were seen (Appendix A, Table A.4).

4.3.1.4 Follow-up Period

The follow-up period occurred between Visit 3 (end WBVT) and Visit 4 (follow-up). Analysis data for all DXA parameters for the 3 sub-cohorts for the follow-up period can be found Appendix A.

4.3.1.4.1 Paediatric Cohort

Total body DXA scans demonstrated a significant decrease in TBMD SDS, -0.8 to -1.0 (p=0.048). This decrease was approximately twice the magnitude of the non-significant decrease seen in the observation period and in contrast to the significant increase seen with WBVT. TBMC acquisition also slowed in the follow-up period with a non-significant increase of 15.3g, one third of the magnitude of the significant increase seen in the WBVT period (Table 4.5).

On segmental analysis of the total body DXA scans, trunk BMD demonstrated a non-significant decrease which was equivalent to one third of the value gained during WBVT, and 3-fold greater than the decline seen in the observation period. Trunk BMD SDS decreased from -0.4 to -0.5, but did not reach significance (p=0.052). A similar trend was seen for LS BMD and LS BMD SDS which decreased from -0.6 to -0.9 (p=0.071). There was also a non-significant decrease in LS BMC and the LS BMC SDS decreased from -0.2 to -0.5 (p=0.059). The SDS decreases for LS BMD and LS BMC indicating a mean loss of over a quarter of a SDS in 6 months (Appendix A, Table A.2).

4.3.1.4.2 Adult Cohort

There were no significant changes in any parameters of the total body DXA scans (Appendix A, Table A.3). Although not significant, there was a mean increase in the total body LTM seen in the follow-up period which is in contrast to the mean decreases in total body LTM seen in the observation and WBVT periods. Segmental analysis of the total body DXA scans revealed a significant decrease in legs BMD, from 1.140 to 1.132 g/cm^2 (p=0.019) (Table 2.6). Similar to that seen in total body LTM, legs LTM also demonstrated non-significant mean increases in the follow-up period which was again in contrast to the mean decreases in these parameters seen in the observation and WBVT periods. The lumbar spine DXA scans found no significant changes in any parameters (Appendix A, Table A.3).

4.3.1.4.3 Leigh Syndrome Cohort

There were no significant changes in the total body DXA scans (Appendix A, Table A.4). Segmental analysis of the total body DXA scans demonstrated a significant increase in legs BMC from 495.8 to 507.5g (p=0.016) and non-significant increases in legs BA and legs LTM, p=0.079 and 0.061 respectively (Table 4.7 and Appendix A, Table A.4). The increases in legs BMC and legs BA were approximately two thirds of the increases seen with WBVT however the increase in legs LTM was approximately double that seen during the WBVT period. Analysis of the lumbar spine did not reveal any significant changes in any parameters (Appendix A, Table A.4).

4.3.2 Tibial pQCT

4.3.2.1 Baseline

The pQCT scans were not performed at Visit 1 (baseline) so baseline pQCT parameters were collected at Visit 2 (Start WBVT). Baseline pQCT parameters for the 3 cohorts are presented in Table 2.8.

Table 4.8. Baseline peripheral quantitative computed tomography parameters for the tibial 4%, 20% and 66% sites. Mean (SD).

	Paediatric (n=6)	**Adult (n=12)**	**Leigh Syndrome (n=5)**
4% Site Parameters			
Total BMC (mg/mm)	119.8 (25.4)	273.6 (53.6)	178.1 (94.6)
Total CSA (mm^2)	514.1 (181.8)	1076.8 (151.6)	654.8 (374.8)
Total vBMD (mg/cm^3)	246.800 (56.278)	257.550 (58.467)	278.580 (37.618)
Trabecular BMC (mg/mm)	32.5 (10.7)	94.4 (23.0)	48.3 (31.7)
Trabecular CSA (mm^2)	231.2 (81.8)	484.5 (68.2)	294.6 (168.6)
Trabecular vBMD (mg/cm^3)	146.120 (35.076)	197.250 (50.746)	163.380 (42.853)
Trabecular vBMD SDS	-2.8 (1.1)		
Periosteal Circumference (mm)	79.3 (14.4)	116.1 (8.1)	87.78(25.7)
20% Site Parameters			

Total BMC (mg/mm)	119.8 (19.0)	225.7 (34.8)	160.5 (62.4)
Total CSA (mm^2)	227.8 (71.7)	341.7 (46.2)	248.4 (106.7)
Total vBMD (mg/cm^3)	560.133 (149.659)	663.750 (82.917)	654.920 (59.221)
Cortical BMC (mg/mm)	100.4 (19.2)	207.0 (33.3)	144.7 (62.4)
Cortical BMC SDS	-0.3 (1.4)		
Cortical CSA (mm^2)	91.7 (16.1)	175.9 (27.8)	126.9 (49.3)
Cortical CSA SDS	-1.1 (1.3)		
Cortical vBMD (mg/cm^3)	1092.717 (26.841)	1176.358 (27.336)	1122.740 (57.253)
Cortical vBMD SDS	3.4 (1.4)		
Cortical Thickness (mm)	2.0 (0.5)	3.2 (0.5)	2.7 (0.5)
Cortical Thickness SDS	0.141 (2.302)		
Periosteal Circumference (mm)	52.9 (8.4)	65.4 (4.5)	54.8 (11.9)

Periosteal Circumference SDS	-1.5 (1.5)		
Cortical CSMI p (mm^4)	4688.5 (1963.0)	15026.9 (390.6)	8267.3 (7024.0)
Cortical SSI p (mm^3)	454.7 (140.8)	1182.1 (225.3)	725.3 (505.4)
Total CSMI p (mm^4)	6277.5 (2728.7)		
Total SSI p (mm^3)	501.3 (159.9)		
Total SSI p SDS	-0.9 (0.7)		
66% Site Parameters			
Total BMC (mg/mm)	149.9 (25.3)	341.7 (55.0)	212.8 (105.0)
Total CSA (mm^2)	293.2 (75.7)	582.3 (97.0)	389.2 (223.0)
Total CSA SDS	-0.8 (0.4)		
Total vBMD (mg/cm^3)	525.100 (82.078)	594.992 (96.813)	565.240 (67.949)
Cortical BMC (mg/mm)	120.8 (22.1)	289.8 (56.3)	173.3 (86.7)

Cortical BMC SDS	-2.0 (1.0)		
Cortical CSA (mm^2)	113.6 (19.9)	251.8 (48.3)	158.4 (70.2)
Cortical CSA SDS	-2.3 (0.8)		
Cortical CSA: Total CSA (%)	40.0 (7.8)	43.8 (8.1)	43.0 (6.5)
Cortical vBMD (mg/cm^3)	1063.083 (58.076)	1151.100 (24.954)	1072.260 (67.828)
Cortical vBMD SDS	2.0 (2.0)		
Cortical Thickness (mm)	2.3 (0.4)	3.6 (0.7)	2.7 (0.5)
Cortical Thickness SDS	-1.8 (1.4)		
Periosteal Circumference (mm)	57.6 (0.1)	82.5 (7.1)	64.9 (19.6)
Cortical CSMI p (mm^4)	8084.9 (3118.9)	37210.9 (11711.5)	18037.2 (17297.7)
Cortical CSMI p SDS	-2.2 (0.2)		
Cortical SSI p (mm^3)	617.6 (202.1)	2016.3 (456.1)	1086.9 (866.1)

Cortical SSI p SDS	-1.8 (0.5)		
Soft Tissue Total CSA (mm^2)	4801.2 (546.9)	8726.3 (1435.1)	6364.1 (2455.1)
Muscle CSA (mm^2)	2686.1 (513.3)	5225.4 (1017.4)	3784.6 (1886.7)
Muscle CSA SDS	-1.0 (0.6)		
Fat CSA (mm^2)	1815.3 (408.5)	2874.0 (1122.5)	2171.7 (540.0)
Fat CSA: Muscle CSA (%)	70.3 (21.6)	56.7 (22.6)	66.6 (26.1)
Muscle CSA: Soft Tissue Total CSA (%)	55.8 (7.3)	60.3 (8.8)	57.5 (8.8)
Cortical CSA: Muscle CSA (%)	4.3 (0.8)	4.9 (0.8)	4.3 (0.7)
Cortical BMC: Muscle CSA (mg/mm^2)	0.046 (0.009)	0.056 (0.009)	0.046 (0.007)
Cortical BMC: Muscle CSA SDS	-3.1 (8.1)		

Note: BMC, bone mineral content; CSA, cross sectional area; vBMD, volumetric bone mineral density; SDS, standard deviation score; CSMI, cross sectional moment of inertia; p, referring to the polar measurement; SSI, stress strain index.

4.3.2.1.1 Paediatric Cohort

Baseline pQCT parameters for 6 of the 7 paediatric participants are presented in Table 4.8 and Figure 4.2. At the 4% site, vBMD of the trabecular bone was abnormally low, with a SDS of -2.8, which was significantly reduced compared to the reference population and indicated that the trabecular compartment at the 4% site was osteoporotic in the paediatric cohort. All participants had a SDS <-1 and 2/6 (33%) had a SDS <-2.

Analysis of the 20% site found that the mean cortical bone SDS were within the normal range for BMC and the bone's geometric attributes including CSA, cortical thickness and periosteal circumference, and not significantly different to the reference population. The SDS for cortical vBMD was abnormally high at 3.4 and significantly higher than the reference population. The SDS was >1 in 2/6 (33%), >2 in 1/6 (17%) and <-1 in 1/6 (17%) of the paediatric cohort. Analysis of bone strength at the 20% tibial site found that the SDS for the polar measurement of SSI was significantly lower than the reference population.

At the 66% site, the SDS for cortical BMC, -2.0, was outside the normal range and significantly lower than the reference population. Bone geometry was also compromised with the SDS for cortical CSA (-2.3) and cortical thickness (-1.8) falling outside of the normal range and significantly lower than the reference population. Furthermore, the SDS for total bone CSA (-0.8) was also significantly reduced compared to the reference population. The SDS for cortical vBMD, 2.0, was abnormally high compared to the reference population but did not reach significance, p=0.055. The SDS was >1 in 5/6

(83%) and > 2 in 3/6 (50%) of the paediatric cohort. As a consequence of compromised geometry and elevated cortical vBMD, bone strength was impaired at the 66% site. The SDS for the polar CSMI and polar SSI were abnormally low at -2.2 and -1.8 respectively, and significantly lower than the reference population. Compositional analysis revealed that the muscle CSA SDS was within normal limits at -1.0 but was significantly lower than the reference population. The SDS for the cortical BMC: muscle CSA ratio was abnormally low at -3.1, but not significantly reduced compared to the reference population.

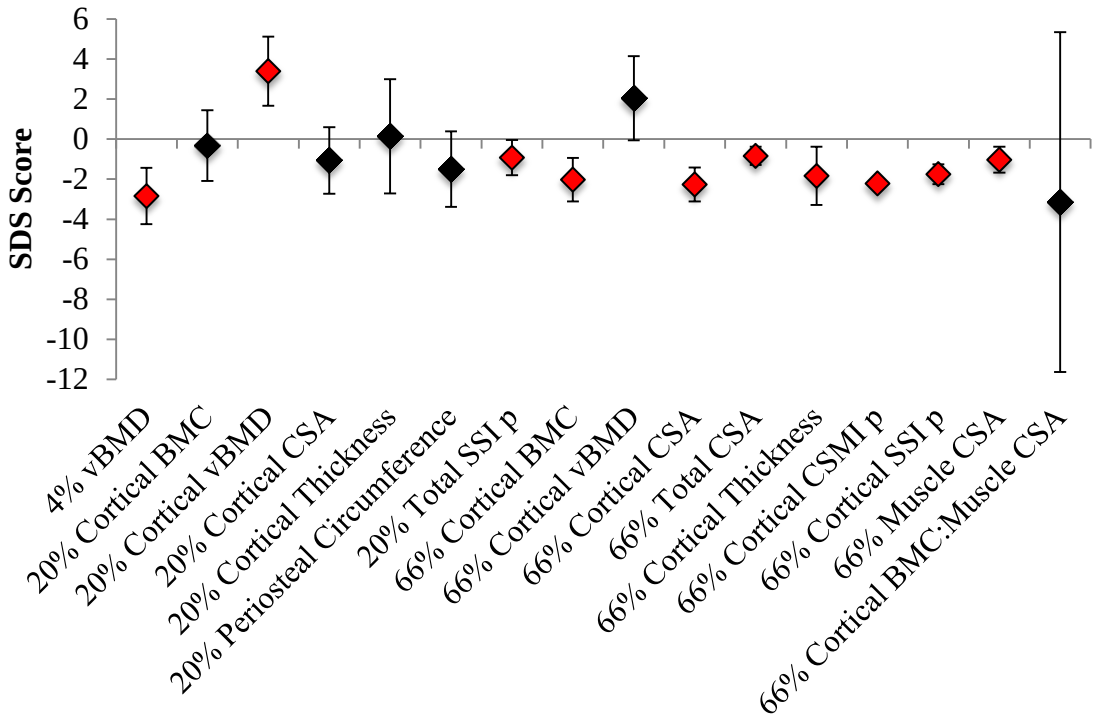

Figure 4.3. Standard deviation (SD) scores for peripheral quantitative computed tomography (pQCT) parameters in the paediatric cohort. Mean and 95% confidence interval. Parameters shown in red were significantly different to the reference population on one-sample t-tests, $p<0.05$.

4.3.2.1.2 Adult Cohort

Baseline pQCT parameters for the 12 adult participants are presented in Table 4.5. All 4% site parameters were greater in magnitude in the adult cohort compared to the paediatric cohort, consistent with bone modelling during childhood and adolescence. Comparison of the 4% site total vBMD of MRCD adults with control data from Dr Ferretti and colleagues found that the values for MRCD males but not females were significantly reduced compared to healthy controls (Figure 4.3).

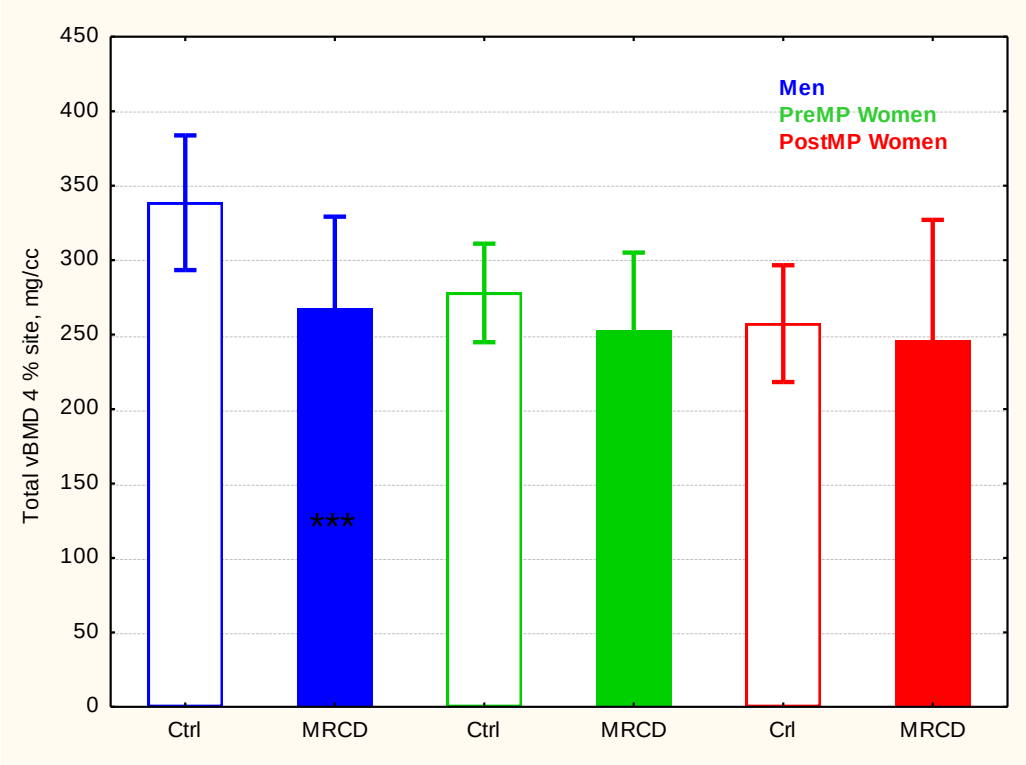

Figure 4.4. Total volumetric bone mineral density (vBMD) at the 4% tibial site for control and MRCD adult males and females. Mean and SD. PreMP, pre-menopausal; PostMP, post-menopausal. *** $p<0.005$. Graph prepared by Dr Ferretti and colleagues.

Analysis of the 20% site found that all pQCT parameters were greater in the adult cohort compared to the paediatric cohort, consistent with bone modelling during growth. When comparing the data from the adult cohort to that of the healthy control data from Dr Ferretti and colleagues for the 20% site, it was found that cortical BMC was significantly reduced in MRCD males but not females (Figure 4.4). Cortical bone geometry, including CSA, cortical thickness and periosteal circumference, were significantly smaller in both MRCD males and females compared to healthy controls (Figures 4.5 - 4.7). Cortical

vBMD was significantly increased in all MRCD participants (Figure 4.8). When exploring the correlation between cortical CSA and cortical BMC at the 20% site, a site that is not influenced by any allometric associations [126], the MRCD adult cohort had significantly higher cortical BMC for CSA compared to controls indicating a significantly greater vBMD (Figure 4.9).

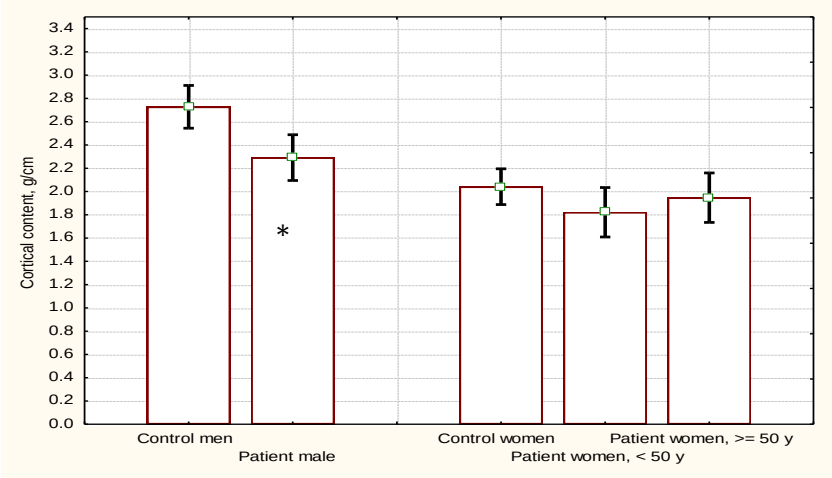

Figure 4.5. Cortical bone mineral content at the 20% tibial site for control and MRCD adult males and females. Mean and SD. **p<0.01. Graph prepared by Dr Ferretti and colleagues.

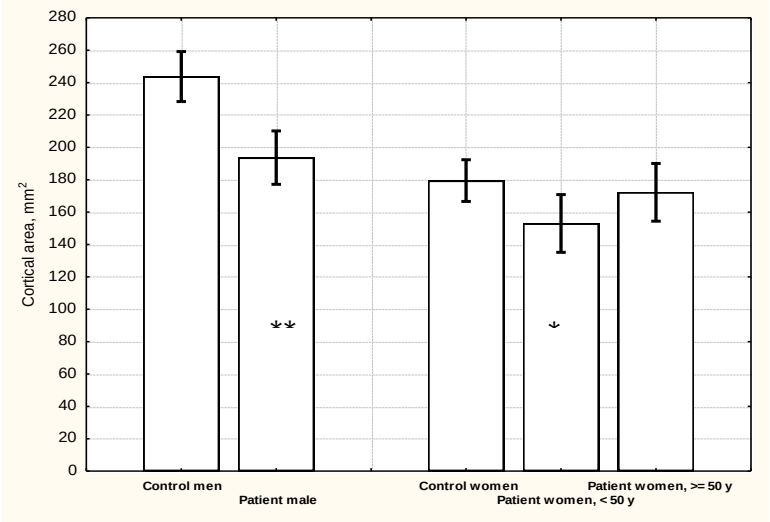

Figure 4.6. Cortical bone cross sectional area at the 20% tibial site for control and MRCD adult males and females. Mean and SD. **p<0.01, ***p<0.005. Graph prepared by Dr Ferretti and colleagues.

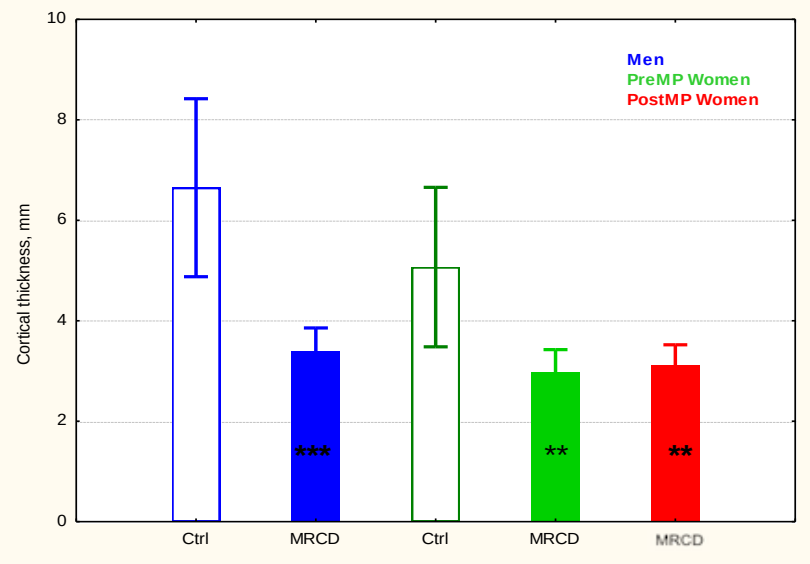

Figure 4.7. Cortical thickness at the 20% tibial site for control and MRCD adult males and females. Mean and SD. PreMP, pre-menopausal; PostMP, post-menopausal. *** p<0.005, **p<0.01. Graph prepared by Dr Ferretti and colleagues.

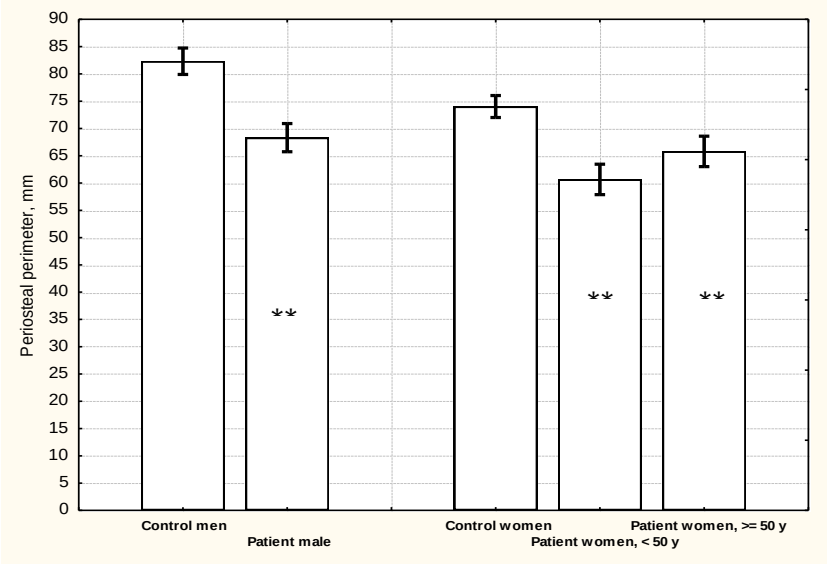

Figure 4.8. Periosteal circumference at the 20% tibial site for control and MRCD adult males and females. Mean and SD.*** p<0.005. Graph prepared by Dr Ferretti and colleagues.

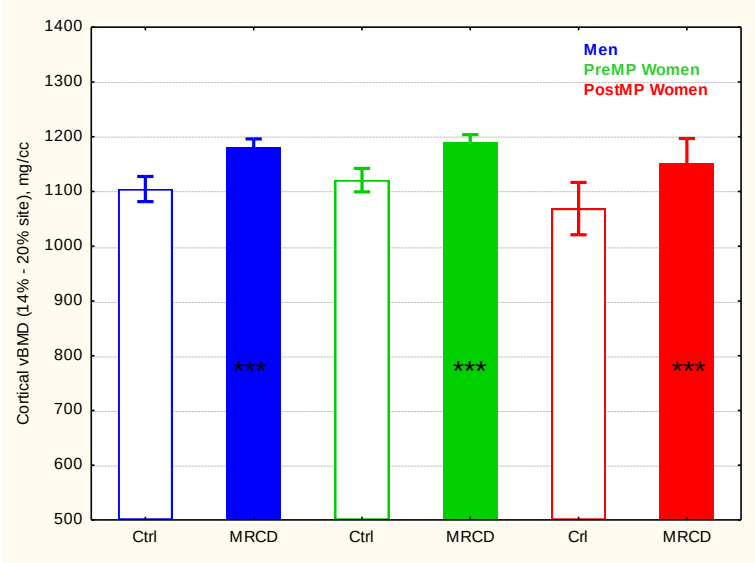

Figure 4.9. Cortical volumetric bone mineral density (vBMD) at the 14-20% tibial site for control and MRCD adult males and females. Mean and SD. PreMP, pre-menopausal; PostMP, post-menopausal. *** p<0.005. Graph prepared by Dr Ferretti and colleagues.

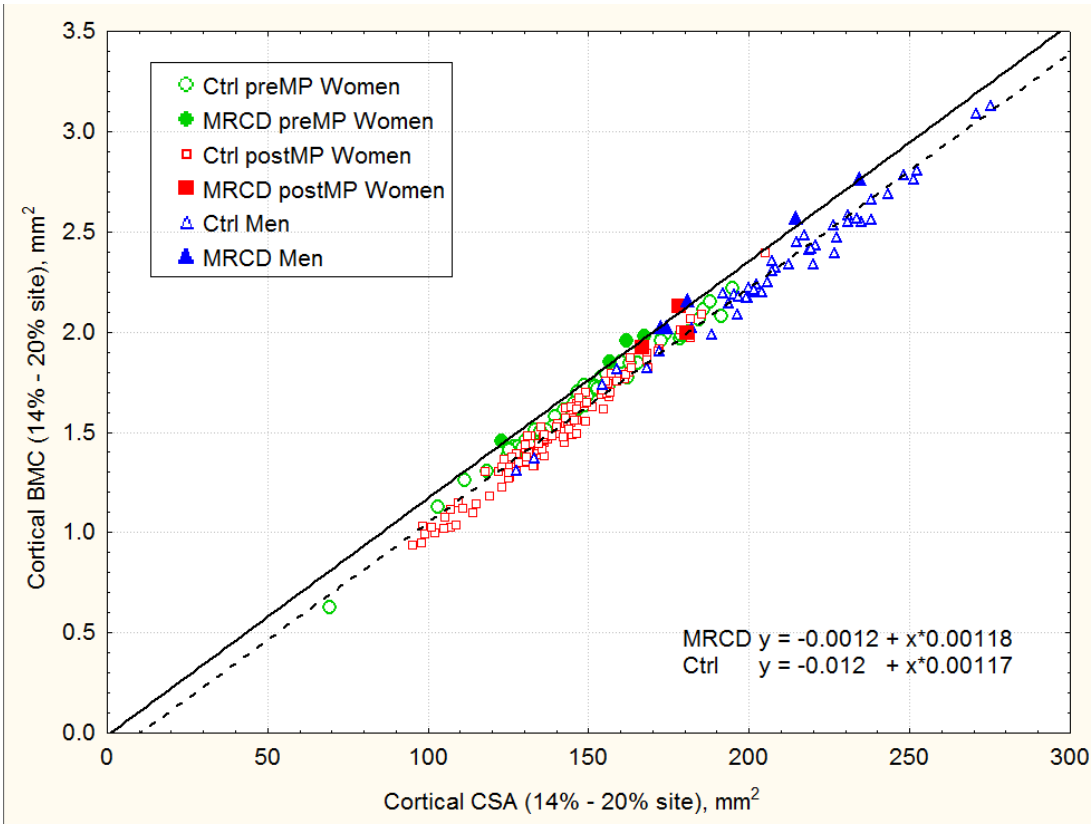

Figure 4.10. Cortical cross-sectional area (CSA) and cortical bone mineral content (BMC) at the 20% tibial site for control and MRCD adult males and females. Mean and SD. PreMP, pre-menopausal; PostMP, post-menopausal. Dashed line represents controls and solid line represents the MRCD cohort. ANCOVA analysis of the two equations indicated they were significantly different, $p<0.0001$. Graph prepared by Dr Ferretti and colleagues.

Similar to that seen in the paediatric MRCD cohort, smaller bone geometry and increased cortical vBMD caused reduced bone strength in MRCD adults. Polar CSMI at the 20% site was significantly reduced in MRCD men and pre-menopausal women compared to controls but not in post-menopausal women (Figure 4.10). CSMI were further investigated using distribution-mass and distribution-quality curves which also reflect the

functioning of an individual's mechanostat [118, 126]. In the reference population, the distribution mass curves did not differ according to gender or weight-bearing physical activity status, indicating a consistent physiological adaptation of the bone to the mechanical loads to which it is exposed [126], a response governed by the intact mechanostat. The distribution-mass curves at the 20% tibial pQCT site for the adult MRCD cohort demonstrate that this relationship was disrupted and there was a clear disadvantage, compared to reference population, in how well the mechanostat used the available cortical bone mass (Figure 4.11) and CSA (Figure 4.12) to optimise cross-sectional geometry and achieve the greatest CSMI. The equations representative of the MRCD cohort were shifted downwards but remained essentially parallel to the equations representative of the reference population, indicating the polar CSMI for the same cortical CSA and BMC in MRCD adults was significantly reduced compared to their healthy counterparts. When the MRCD data were plotted on the distribution-quality curve, which demonstrates the inverse relationship between cortical vBMD, or bone stiffness, and the ability to resist torsion forces [126] at the 20% pQCT site, the MRCD data plotted within the normal relationship for both males and females, albeit in the lower right hand corner, indicating that their abnormally high cortical vBMD values reduces the polar CSMI values and therefore the ability of their bone cortices to resist torsion forces (Figure 4.13). The data points do however follow the reference curves indicating that the mechanostat is directing the geometric distribution of cortical bone tissue in accordance with the stiffness of the diaphyseal bone and that the behaviour of the mechanostat is congruent with the reference population, albeit below the lower limits seen in the reference population. Bone strength at the 20% site was further investigated using the

buckling ratio, a reflection of the bone's ability to resist compressive stress. It is the ratio of the bone's outer radius and cortical thickness, a higher ratio indicating greater likelihood of failure in buckling. The ratio in the adult MRCD cohort was significantly increased compared to the reference population (Figure 4.14), likely due to their reduced cortical thickness compared to the reference population. Furthermore, the risk of fracture in the adult cohort will be significantly increased as cortical thickness decreases.

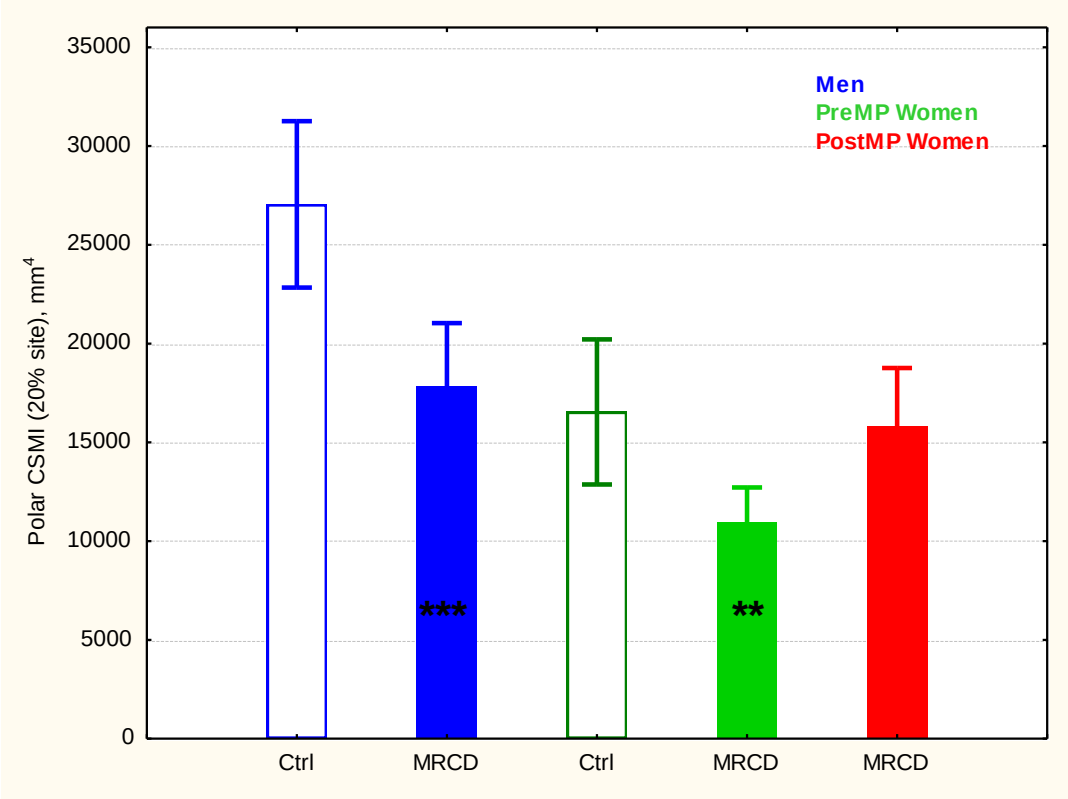

Figure 4.11. Polar CSMI at the 20% tibial site for control and MRCD adult males and females. Mean and SD. PreMP, pre-menopausal; PostMP, post-menopausal. *** $p<0.005$, ** $p<0.01$. Graph prepared by Dr Ferretti and colleagues.

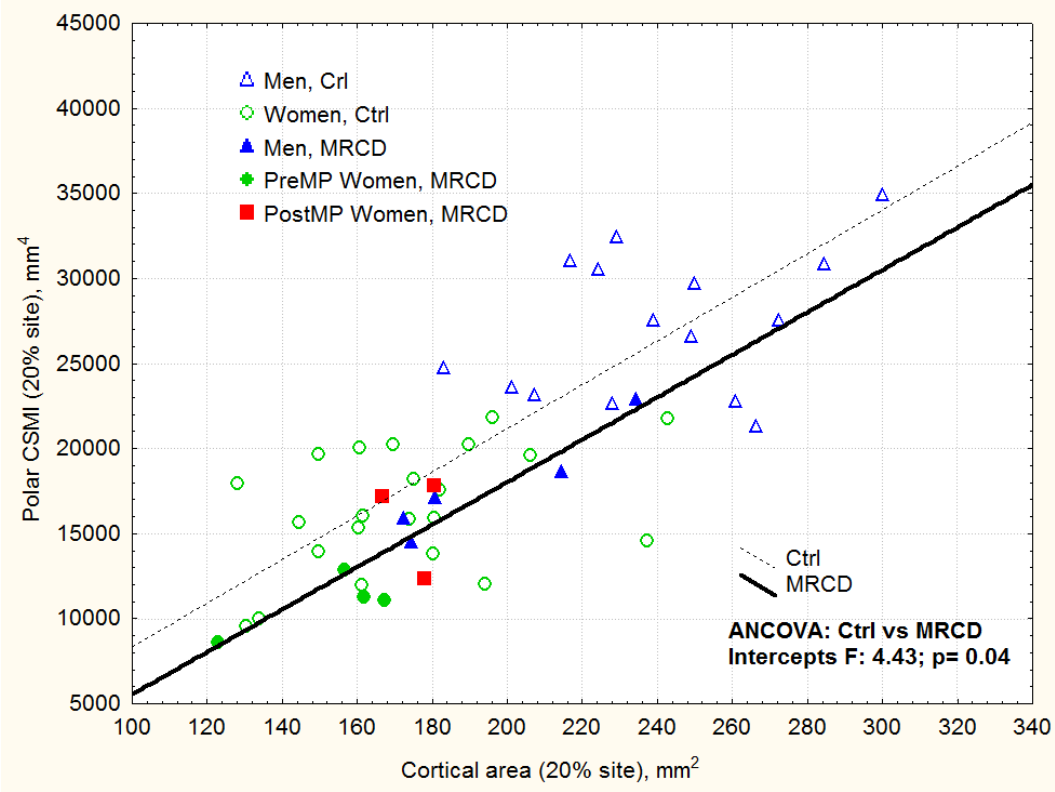

Figure 4.12. Distribution-mass curve: Cortical cross-sectional area and polar cross-sectional moment of inertia (CSMI) at the 20% tibial site for control and MRCD adult males and females. PreMP, pre-menopausal; PostMP, post-menopausal. ANCOVA analysis found the control and MRCD equations were significantly different, p=0.04. Graph prepared by Dr Ferretti and colleagues.

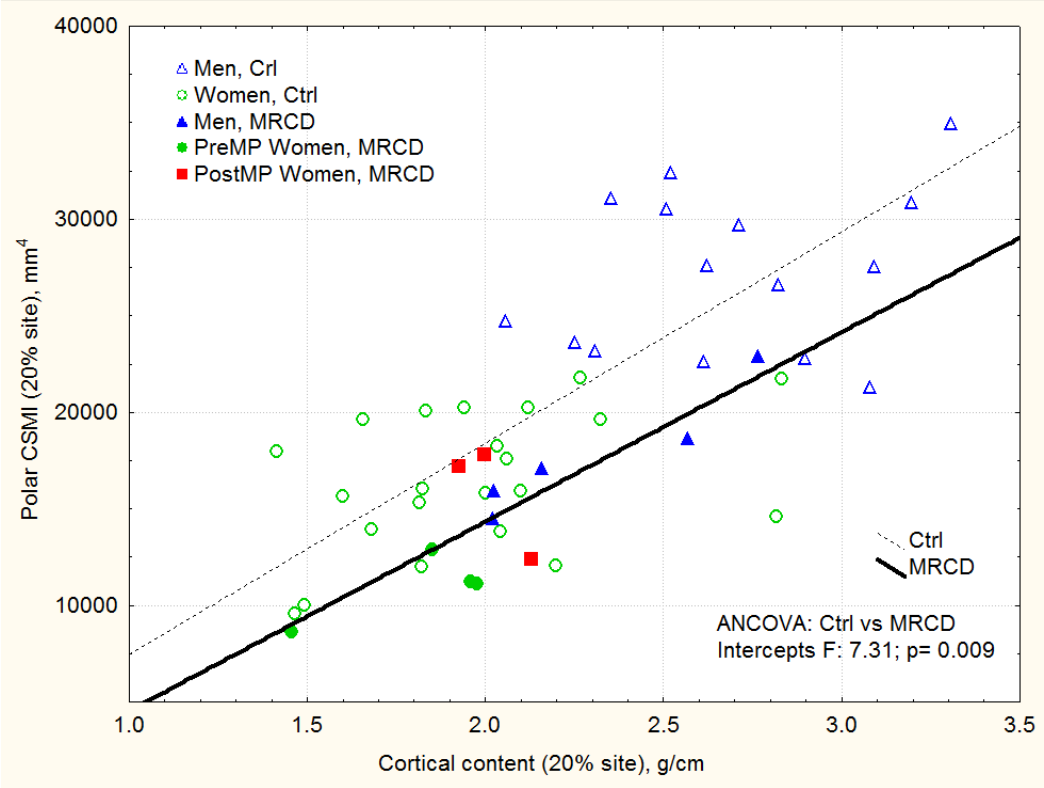

Figure 4.13. Distribution-mass curve: cortical bone mineral content and polar cross-sectional moment of inertia (CSMI) at the 20% tibial site for control and MRCD adult males and females. PreMP, pre-menopausal; PostMP, post-menopausal. ANCOVA analysis found the control and MRCD equations were significantly different, p=0.009. Graph prepared by Dr Ferretti and colleagues.

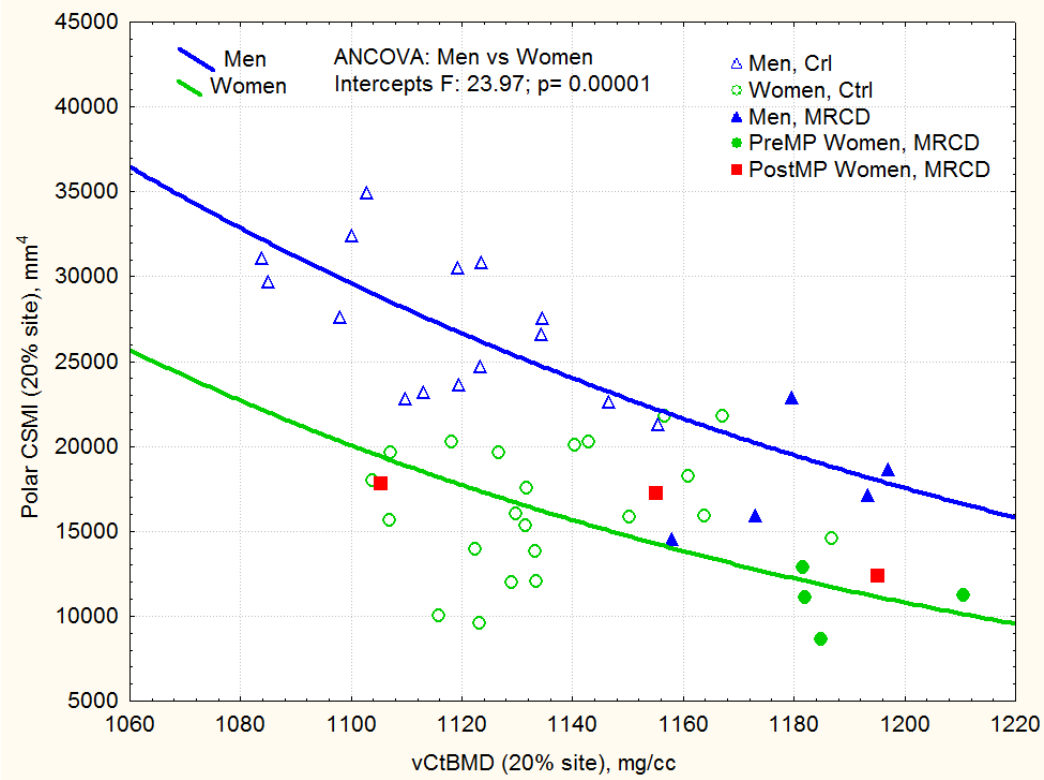

Figure 4.14. Distribution-quality curve: cortical volumetric bone mineral density (vCtBMD) and polar cross-sectional moment of inertia (CSMI) at the 20% tibial site for control and MRCD adult males and females. PreMP, pre-menopausal; PostMP, post-menopausal. Graph prepared by Dr Ferretti and colleagues.

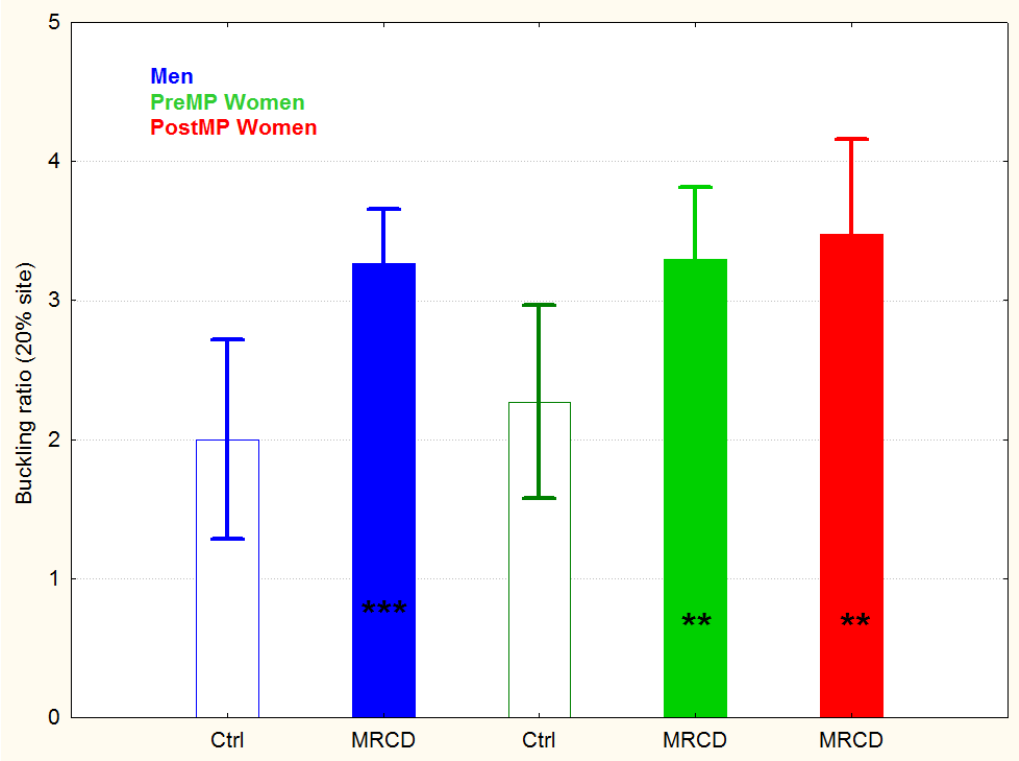

Figure 4.15. Buckling ratio at the 20% tibial site for control and MRCD adult males and females. Mean and SD. PreMP, pre-menopausal; PostMP, post-menopausal. *** $p<0.005$, ** $p<0.01$. Graph prepared by Dr Ferretti and colleagues.

At the 66% site, all total and cortical bone and composition parameters were greater in the adult cohort compared to the paediatric cohort, as would be expected for the mature adult musculoskeletal system compared to the evolving child and adolescent musculoskeletal system. The only exception was the ratio of fat CSA: muscle CSA, which was lower in the adult cohort. The adult cohort had a mean fat CSA of 28.74cm^2 (Table 4.5) which is between the mean values seen in healthy adult males, 18.2cm^2, and adult females 34.2cm^2 [720], however fat CSA as a percentage of muscle CSA had a mean value of 56.7% (Table 2.5), much higher than the 20% and 47% seen in the healthy

adults [720]. When comparing the adult MRCD cohort to the healthy control data for the 66% site provided by Dr Ferretti and colleagues, cortical CSA was significantly reduced in males but not females (Figure 4.15) and muscle CSA was significantly reduced in males and post-menopausal females but not pre-menopausal females (Figure 4.16). When analysing the correlation between muscle CSA and cortical CSA at the 66% site (Figure 4.17), the MRCD data plotted within 2 standard deviations of the mean of the reference population, indicating preserved muscle bone proportions and an intact anthropometric and biomechanical relationship between muscle and bone.

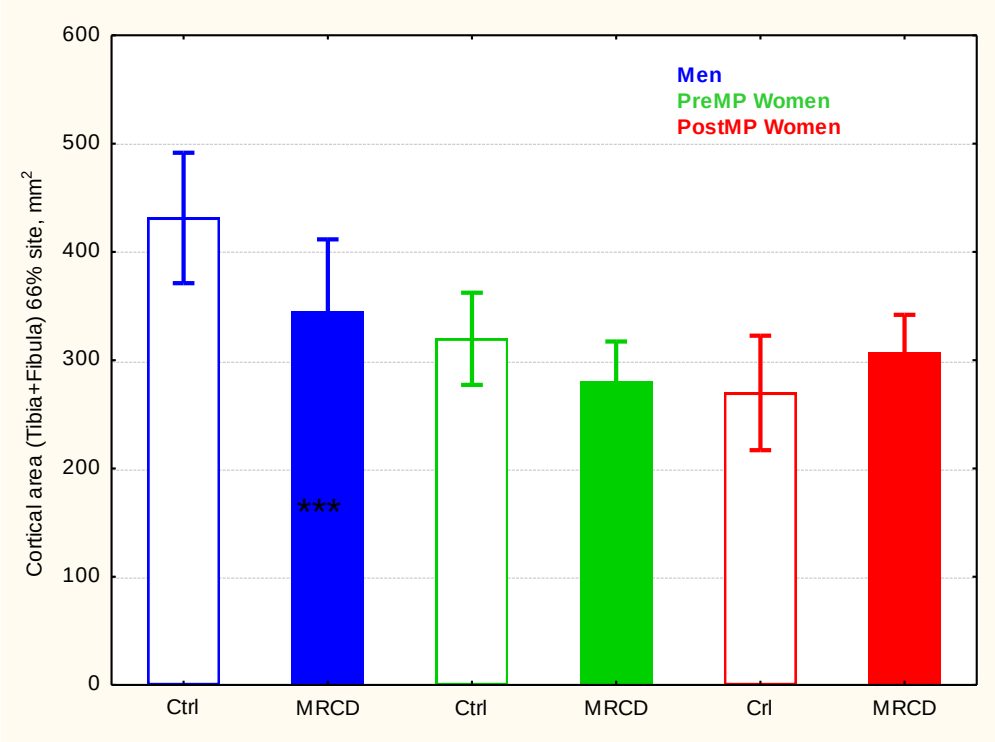

Figure 4.16. Cortical cross-sectional area at the 66% tibial site for control and MRCD adult males and females. Mean and SD. PreMP, pre-menopausal; PostMP, post-menopausal. ***p<0.005. Graph prepared by Dr Ferretti and colleagues.

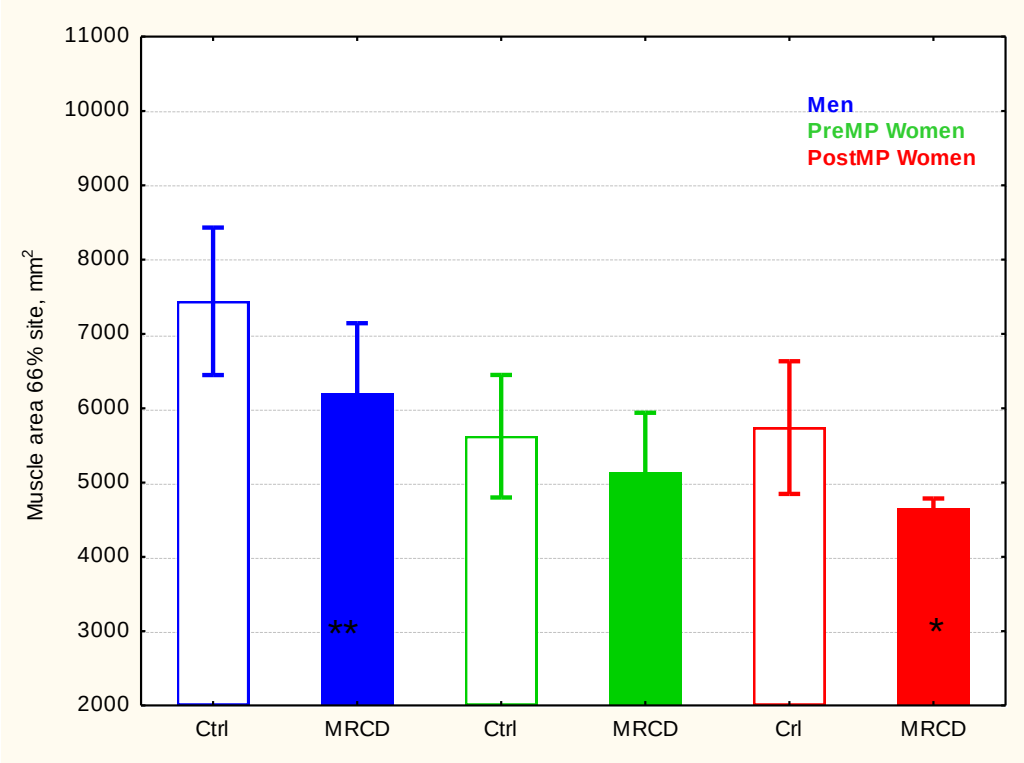

Figure 4.17. Muscle cross-sectional area at the 66% tibial site for control and MRCD adult males and females. Mean and SD. PreMP, pre-menopausal; PostMP, post-menopausal. **p<0.01, *p<0.05. Graph prepared by Dr Ferretti and colleagues.

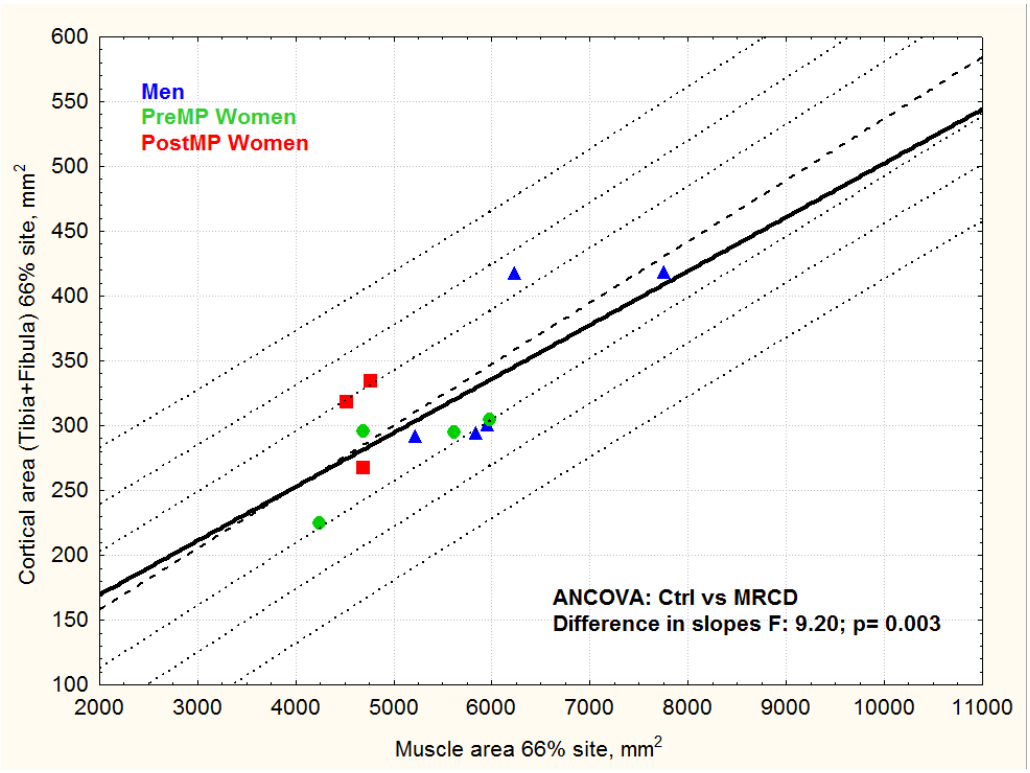

Figure 4.18. Muscle cross-sectional area and cortical cross-sectional area of the tibia and fibula at the 66% tibial site for control and MRCD adult males and females. Bands indicate 1, 2 and 3 standard deviation score units around either side of the regression line. PreMP, pre-menopausal; PostMP, post-menopausal. Graph prepared by Dr Ferretti and colleagues.

4.3.2.1.3 Leigh Syndrome Cohort

Baseline pQCT parameters for the 5 Leigh Syndrome participants are presented in Table 4.5. At all three tibial pQCT sites, all bone and composition parameters were larger than the paediatric but smaller than the adult sub-cohorts, indicating the mixed nature of the Leigh Syndrome Cohort, comprised of both paediatric and adult participants. One

exception was total bone vBMD at the 4% pQCT site, which was highest in the Leigh Syndrome cohort. As trabecular bone vBMD in the paediatric cohort and total bone vBMD in the adult cohort were significantly reduced compared to reference populations at this site, this result may indicate that bone deficits in the trabecular compartment may not be as severe in Leigh Syndrome compared to other MRCD.

4.3.2.2 Whole Body Vibration Training (WBVT) Period

Analysis data for all tibial pQCT parameters for the 3 sub-cohorts during the WBVT period, Visit 2 to Visit 3, can be found in Appendix B.

4.3.2.2.1 Paediatric Cohort

Analysis data for the 4%, 20% and 66% tibial pQCT sites for the paediatric cohort can be found in (Table 4.9 and Appendix B, Table B.2). After WBVT there was a significant increase in total bone BMC at the 4% site from 126.0-135.2 mg/mm, p=0.009. No other total bone or trabecular bone parameters at the 4% site changed significantly after WBVT.

Analysis of the 20% site demonstrated significant improvements in total and cortical bone parameters. Total bone BMC and vBMD increased significantly: 119.8 - 126.9 mg/mm, p<0.000; and 560.133 - 587.017 mg/cm^3, p=0.005 respectively. In the cortical compartment at the 20% site there were significant improvements in: BMC and BMC SDS from 100.4 - 109.2 mg/mm, p<0.000 and -0.3 - 0.4, p=0.001 respectively; CSA and

CSA SDS of 91.7 - 98.5 mm^2, p<0.000 and -1.1 to -0.5, p=0.002 respectively; vBMD from 1092.717 - 1106.150 mg/cm^3, p=0.049; and cortical thickness and cortical thickness SDS from 2.0 - 2.2 mm, p=0.001 and 0.1 - 0.7, p=0.008 respectively. Bone strength also increased significantly after WBVT. Polar CSMI and polar SSI of the cortical bone increased from 4688.5 - 4997.5 mm^4, p=0.017, and from 454.7 - 490.1 mm^3, p=0.002 respectively. Polar SSI of the total bone and its corresponding SDS also increased significantly from 501.3 - 532.8 mm^3, p=0.002, and -1.0 to -0.6, p=0.002 respectively. The improvement in the total bone polar SSI SDS representing nearly half a SDS.

WBVT also resulted in significant changes to the total, and cortical bone compartments as well as composition parameters at the 66% site. Total bone BMC increased from 149.9 - 156.7 mg/mm, p<0.000. Cortical bone BMC and CSA and their corresponding SD scores increased significantly from: 120.8 - 126.3 mg/mm, p=0.036; -2.0 to -1.7, p=0.029; 113.6 - 117.3 mm^2, p=0.020; and -2.3 to -2.1, p=0.021 respectively. The favourable geometric adaptation in cortical bone CSA after WBVT, resulted in improvements in bone strength parameters. The polar measurements of cortical CSMI and SSI as well as their corresponding SDS increased significantly from: 8084.9 - 8801.3 mm^4, p=0.021; -2.2 to -1.1, p=0.010; 617.6 - 662.7 mm^3, p<0.001; and -1.7 to -1.4, p<0.001 respectively. Muscle CSA, muscle CSA SDS and total soft tissue CSA also increased significantly during the WBVT period: 2686.1 - 2831.7 mm^2, p=0.014; -1.0 to -0.8, p=0.014; and 4801.2 - 5024.8 mm^2, p=0.017 respectively.

Table 4.9. pQCT parameters for the paediatric cohort MRCD cohort at each visit, changes between visits and p values. *p<0.05

		4% Total BMC (mg/mm)	**20% Total BMC (mg/mm)**	**20% Total vBMD (mg/ccm)**	**20% Cortical BMC (mg/mm)**	**20% Cortical BMC SDS**	**20% Cortical CSA (mm^2)**
Visit 2	Value	125.989	119.797	560.133	100.440	-0.321	91.707
	Δ with WBVT	9.238	7.083	26.883	8.718	0.692	6.827
	p	0.009*	0.000*	0.005*	0.000*	0.001*	0.000*
Visit 3	Value	135.227	126.880	587.017	109.158	0.371	98.533
	Δ in Follow-up	-1.348	2.388	0.967	1.993	0.119	1.840
	p	0.610	0.068	0.901	0.190	0.437	0.202
Visit 4	Value	133.878	129.268	587.983	111.152	0.490	100.373

		20% Cortical CSA SDS	20% Cortical vBMD (mg/ccm)	20% Cortical Thickness (mm)	20% Cortical Thickness SDS	20% Cortical CSMI p (mm^4)	20% Cortical SS1p (mm^3)
Visit 2	Value	-1.065	1092.717	2.039	0.141	4688.530	454.669
	Δ with WBVT	0.562	13.433	0.155	0.561	308.982	35.442
	p	0.002*	0.049*	0.001*	0.008*	0.017*	0.002*
Visit 3	Value	-0.504	1106.150	2.194	0.702	4997.512	490.111
	Δ in Follow-up	0.123	1.000	0.020	0.057	183.673	16.132
	p	0.363	0.871	0.567	0.728	0.118	0.081
Visit 4	Value	-0.381	1107.150	2.214	0.759	5181.185	506.243

		20% Total SS1p (mm^3)	20% Total SS1p SDS	66% Total BMC (mg/mm)	66% Total CSA (mm^2)	66% Total CSA SDS	66% Cortical BMC (mg/mm)
Visit 2	Value	501.269	-0.992	149.850	293.173	-0.839	120.813
	Δ with WBVT	31.561	0.319	6.840	15.307	0.259	5.528
	p	0.002*	0.002*	0.000*	0.133	0.161	0.036*
Visit 3	Value	532.830	-0.603	156.690	308.480	-0.580	126.342
	Δ in Follow-up	1.470	0.008	1.807	19.440	0.383	0.443
	p	0.851	0.911	0.172	0.065	0.049*	0.850
Visit 4	Value	534.3	-0.594	158.497	327.920	-0.198	126.785

		20% Total SS1p (mm^3)	20% Total SS1p SDS	66% Total BMC (mg/mm)	66% Total CSA (mm^2)	66% Total CSA SDS	66% Cortical BMC (mg/mm)
Visit 2	Value	501.269	-0.992	149.850	293.173	-0.839	120.813
	Δ with WBVT	31.561	0.319	6.840	15.307	0.259	5.528
	p	0.002*	0.002*	0.000*	0.133	0.161	0.036*
Visit 3	Value	532.830	-0.603	156.690	308.480	-0.580	126.342
	Δ in Follow-up	1.470	0.008	1.807	19.440	0.383	0.443
	p	0.851	0.911	0.172	0.065	0.049*	0.850
Visit 4	Value	534.3	-0.594	158.497	327.920	-0.198	126.785

		20% Total SS1p (mm^3)	20% Total SS1p SDS	66% Total BMC (mg/mm)	66% Total CSA (mm^2)	66% Total CSA SDS	66% Cortical BMC (mg/mm)
Visit 2	Value	501.269	-0.992	149.850	293.173	-0.839	120.813
	Δ with WBVT	31.561	0.319	6.840	15.307	0.259	5.528
	p	0.002*	0.002*	0.000*	0.133	0.161	0.036*
Visit 3	Value	532.830	-0.603	156.690	308.480	-0.580	126.342
	Δ in Follow-up	1.470	0.008	1.807	19.440	0.383	0.443
	p	0.851	0.911	0.172	0.065	0.049*	0.850
Visit 4	Value	534.3	-0.594	158.497	327.920	-0.198	126.785

		66% Cortical BMC SDS	66% Cortical CSA (mm^2)	66% Cortical CSA SDS	66% Periosteal Circumference (mm)	66% Cortical CSMI p (mm^4)	66% Cortical CSMI p SDS
Visit 2	Value	-2.023	113.600	-2.263	57.610	8084.868	-2.209
	Δ with WBVT	0.298	3.680	0.192	1.350	716.471	0.115
	p	0.029*	0.020*	0.021*	0.133	0.021*	0.010*
Visit 3	Value	-1.725	117.280	-2.072	58.960	8801.340	-2.094
	Δ in Follow-up	0.053	1.600	0.099	1.906	778.948	0.145
	p	0.662	0.256	0.190	0.044*	0.014*	0.003*
Visit 4	Value	-1.672	118.880	-1.973	60.866	9580.288	-2.209

		66% Cortical SSI p (mm^3)	66% Cortical SSI p SDS	66% Soft Tissue Total CSA (mm^2)	66% Muscle CSA (mm^2)	66% Muscle CSA SDS	66% Fat CSA (mm^2)
Visit 2	Value	617.598	-1.746	4801.227	2686.133	-1.029	1815.253
	Δ with WBVT	45.146	0.333	223.573	145.573	0.248	61.920
	p	0.000*	0.002*	0.017*	0.014*	0.015*	0.267
Visit 3	Value	662.744	-1.413	5024.800	2831.707	-0.781	1877.173
	Δ in Follow-up	25.420	0.168	149.760	2.187	0.041	129.120
	p	0.009*	0.060	0.084	0.965	0.636	0.034*
Visit 4	Value	688.164	-1.245	5174.560	2833.893	-0.739	2006.293

		66% Fat CSA:Muscle CSA (%)	66% Muscle CSA:Total CSA (%)
Visit 2	Value	70.277	55.820
	Δ with WBVT	-2.364	0.631
	p	0.292	0.329
Visit 3	Value	67.913	56.451
	Δ in Follow-up	4.939	-1.570
	p	0.043*	0.029*
Visit 4	Value	72.852	54.881

Note: Δ, change; 4%, referring to 4% tibial pQCT site; BMC, bone mineral content; CSA, cross-sectional area; vBMD, volumetric bone mineral density; 20%, referring to the 20% tibial pQCT site; CSMI, cross-section moment of inertia; p, referring to the polar measurement; SSI, stress strain index; 66%, referring to the 66% tibial pQCT site.

4.3.2.2.2 Adult Cohort

In the adult cohort there were no significant changes in any parameters for the total or trabecular bone compartments at the 4% site. Similarly, there were no significant changes in any total or cortical bone parameters at the 20% and 66% sites (Appendix B, Table B.3).

4.3.2.2.3 Leigh Syndrome Cohort

Analysis data can be found in Table 4.10 and Appendix B, Table B.4. During the WBVT period, there were no significant changes in any parameters at the 4% site. At the 20% site the Leigh Syndrome cohort demonstrated significant changes in the cortical bone compartment after WBVT. Cortical BMC and cortical thickness increased significantly: 144.8 - 149.6 mg/mm, p=0.027; and 2.66 - 2.75 mm, p=0.040 respectively. There was also a significant increase in the polar measurement of cortical SSI, 725.3 - 757.0 mm^3, p=0.029. Analysis of the 66% site revealed significant increases in total and cortical bone BMC of 212.8 - 218.1 mg/mm, p=0.035 and 173.3 - 178.6mg/mm, p=0.033, respectively. Cortical CSA also increased significantly from 158.4 - 161.4 mm^2, p=0.038. There were no other significant changes in any composition parameters.

Table 4.10. pQCT parameters for the Leigh Syndrome cohort at each visit, changes between visits and p values. *p<0.05.

		20% Cortical BMC (mg/mm)	20% Cortical Thickness (mm)	20% Cortical SS1p (mm^3)	66% Total BMC (mg/mm)
Visit 2	Value	144.767	2.657	725.271	212.832
	Δ with WBVT	4.894	0.088	31.765	5.236
	p	0.027*	0.040*	0.029*	0.035*
Visit 3	Value	149.570	2.745	757.036	218.068
	Δ in Follow-up	0.506	0.017	6.004	2.688
	p	0.786	0.643	0.628	0.230
Visit 4	Value	150.076	1125.100	763.040	220.756

		66% Cortical BMC (mg/mm)	66% Cortical CSA (mm^2)	66% Cortical CSMI p (mm^4)
Visit 2	Value	173.260	158.400	18037.238
	Δ with WBVT	5.298	2.976	323.269
	p	0.033*	0.038*	0.100
Visit 3	Value	178.558	161.376	18360.507
	Δ in Follow-up	2.054	2.240	405.134
	p	0.347	0.099	0.048*
Visit 4	Value	180.612	163.616	18765.640

Note: Δ, change; BMC, bone mineral content; CSA, cross-sectional area; vBMD, volumetric bone mineral density; 20%, referring to the 20% tibial pQCT site; CSMI, cross-section moment of inertia; p, referring to the polar measurement; SSI, stress strain index; 66%, referring to the 66% tibial pQCT site.

4.3.2.3 Follow-up Period

4.3.2.3.1 Paediatric Cohort

There were no significant changes in any parameters at the 4% site. All parameters demonstrated either a reduction in absolute value or the gains were blunted in comparison to gains seen during the WBVT period. At the 20% site, the significant improvements seen during the WBVT period were not maintained during the follow-up period and no significant changes occurred in any parameter. Analysis of the 66% site found continued significant changes in total bone, cortical bone and composition parameters. Total bone CSA showed a non-significant increase from 308.5 - 327.9 mm^2, p=0.065 however the corresponding SDS increased significantly from -0.6 to -0.2, p=0.049. Associated with this increase in total bone CSA the periosteal circumference also increased significantly from 59.0 - 60.9 mm, p=0.044. The ongoing improvements in the geometric attributes of the tibia at the 66% resulted in further significant improvements in bone strength parameters. Polar CSMI and its corresponding SDS increased significantly from: 778.9 - 9580.3 mm^4, p=0.014; and -2.1 to -1.9, p=0.003 respectively. Polar SSI increased significantly, 662.7 - 688.2 mm^3, p=0.009, however its corresponding SDS increase did not reach significance, -1.4 to -1.2, p=0.060. Fat CSA and the ratio of fat CSA: muscle CSA increased significantly in the follow-up period: 1877.2 - 2006.3 mm^2, p=0.034; and 67.9 - 72.8%, p=0.043 respectively. In contrast the ratio of muscle CSA: total soft tissue CSA decreased significantly from 56.5 - 54.9%, p=0.029 (Table 4.9 and Appendix B, Table B.2).

4.3.2.3.2 Adult Cohort

There were no significant changes in any parameters at the 4% or 20% tibial pQCT sites. At the 66% site there were significant decreases in cortical CSA, cortical thickness and the ratio of cortical CSA: total bone CSA: 252.7 - 251.0 mm^2, p=0.033; 3.6 - 3.5 mm, p=0.015; and 43.9 - 43.5%, p=0.019 respectively (Table 4.13 and Appendix B, Table B.3).

Table 4.11. pQCT parameters for the adult MRCD cohort at each visit, changes between visits and p values. *p<0.05.

		66% Cortical CSA (mm^2)	**66% Cortical CSA:Total CSA (%)**	**66% Cortical Thickness (mm)**
Visit 2	Value	251.773	43.808	3.556
	Δ with WBVT	0.947	0.105	0.008
	p	0.220	0.497	0.529
Visit 3	Value	252.720	43.913	3.564
	Δ in Follow-up	-1.707	-0.387	-0.031
	p	0.033*	0.019*	0.015*
Visit 4	Value	251.013	43.526	3.532

Note: Δ, change; 66%, referring to the 66% tibial pQCT site; CSA, cross-sectional area.

4.3.2.3.3 Leigh Syndrome Cohort

Analysis did not reveal significant changes in any parameters at the 4% or 20% tibial pQCT sites. At the 66% site, there was a significant increase in polar CSMI from 18360.5 - 18765.6 mm^3, p=0.048 (Appendix B, Table B.4).

4.3.3 Bone Turnover Markers and Serum Biochemistry

4.3.3.1 Baseline

Baseline parameters for serum biochemistry and bone turn-over markers are presented in Table 2.12. Serum alkaline phosphatase (ALP) was within normal reference ranges for all participants except for one paediatric participant, number 14, who had ALP levels below the normal reference range throughout the study. The paediatric and Leigh Syndrome cohorts had higher ALP levels than the adult cohort, consistent with bone modelling. Calcium and phosphate were within normal reference ranges in all participants and did not differ between cohorts. Thirteen of the 19 participants registered lactate levels above the normal reference range at least once during the study period, and 6 of these participants (participants 1, 2, 16, 18, 19 and 21) had consistently high lactate levels. Ten of the 13 patients with high lactate levels had at least one pyruvate reading above the normal reference range, with 3 of these participants (participants 18, 19 and 21) consistently registering high pyruvate levels. Mean lactate levels were above the normal reference range in the adult and paediatric cohorts indicating OXPHOS dysfunction, with

the adult cohort having the greatest mean lactate level. In contrast, the mean lactate in the Leigh Syndrome cohort was lower and within the normal reference range.

Osteocalcin levels were transiently higher than the normal reference range in 4 paediatric participants (participants 1, 2, 5 and 19) and transiently lower than the normal reference range in one paediatric participant (participant 5). All adult participants had at least one osteocalcin level lower than normal reference range. In three adult participants (participants 3, 4 and 8) the low levels were transient however the remaining 9 adult participants had consistently low osteocalcin levels throughout the study period. Mean osteocalcin levels were highest in the paediatric cohort and lowest in the adult cohort, again consistent with bone modelling in the paediatric cohort. Vitamin D levels were maintained above 50 pmol/L for the duration of the study in all participants except for participants 14 and 20 who had levels of 47 and 48 pmol/L respectively at visit 4 which occurred during the winter months. The urinary deoxypyridinoline:creatinine ratio was higher than the normal reference range at least once in 13 of the 19 participants (participants 2-6, 8, 12, 15-18, 20 and 21), and 8 of these participants had values higher than the normal range at least 3 of the 4 times they were tested. One paediatric participant (participant 17) had one urinary deoxypyridinoline:creatinine ratio that was below the normal reference range. The paediatric cohort had the highest urinary deoxypyridinoline:creatinine ratio, more than double that of the adult cohort that had the lowest urinary deoxypyridinoline:creatinine ratio, indicating higher bone turnover during the paediatric years reflecting bone modelling.

4.3.3.2 Study Periods

Analysis data for bone turnover markers and serum biochemistry in the three cohorts during the observation, WBVT and follow-up periods can be found in Appendix C.

4.3.3.2.1 Paediatric Cohort

During the observation period, serum vitamin D decreased significantly from 106.0 - 83.4 pmol/L, p=0.018 however this level was still well within the normal reference range, and above the minimum accepted value of 50pmol/L. Osteocalcin levels decreased significantly during the WBVT period from 6.9 - 4.0nmol/L, p=0.002, and phosphate decreased significantly during the follow-up period from 1.532 - 1.427mmol/L, p=0.042, however these remained within the normal reference ranges (Table 4.13). There were no statistically significant changes in any other bone turnover markers or serum biochemistry parameters in any of the study periods (Appendix C, Table C.2).

4.3.3.2.2 Adult Cohort

There were no statistically significant changes in bone turnover markers or serum biochemistry during the observation, WBVT or follow-up periods (Appendix C, Table C.3).

Table 4.12. Baseline Serum Biochemistry and Bone Turnover Marker Parameters. Mean (SD).

	Paediatric (n=7)	**Adult (n=12)**	**Leigh Syndrome (n=5)**
Alkaline Phosphatase (U/L)	155.3 (61.5)	74.9 (16.0)	155.8 (78.5)
Calcium (mmol/L)	2.4 (0.1)	2.4 (0.1)	2.4 (0.1)
Phosphate (mmol/L)	1.5 (0.1)	1.2 (0.2)	1.3 (0.2)
Lactate (mmol/L)	3.4 (2.5)	3.6 (4.9)	1.5 (0.9)
Osteocalcin (nmol/L)	6.4 (3.8)	1.6 (1.3)	4.6 (2.0)
Vitamin D (pmol/L)	106.0 (36.2)	78.9 (17 7)	93.0 (31.8)
Urinary Deoxypyridinoline: Creatine Ratio (nmol/mmol creatinine)	17.2 (11.0)	8.0 (2.9)	13.4 (5.8)

Table 4.13. Bone turn-over markers and serum biochemistry parameters for the paediatric cohort at each visit, changes between visits and p values. *p<0.05.

		Vitamin D (pmol/L)	**Osteocalcin (nmol/L)**	**Phosphate (mmol/L)**
Visit 1	Value	106.0	6.4	1.5
	Δ in Observation	-22.6	0.5	0.1
	p	0.018*	0.553	0.061
Visit 2	Value	83.4	6.9	1.5
	Δ with WBVT	-3.7	-2.8	-0.0
	p	0.670	0.002*	0.698
Visit 4	Value	79.7	4.0	1.5
	Δ in Follow-up	-8.0	-0.3	-0.1
	p	0.364	0.678	0.042*
Visit 6	Value	71.7	3.7	1.4

Note: Δ, change; WBVT, whole body vibration training.

4.3.3.2.3 Leigh Syndrome Cohort

During the observation period, phosphate increased significantly from 1.3 - 1.4mmol/L, p=0.006. There were no significant changes after WBVT, however, in the follow-up period, phosphate also decreased significantly 1.5 - 1.3mmlo/L, p<0.000 (Table 2.14).

Despite these changes, phosphate remained within the normal reference range throughout the study. There were on other statistically significant changes in bone turnover markers or serum biochemistry in any of the study periods (Appendix C, Table C.4).

Table 4.14. Bone turn-over markers and serum biochemistry parameters for the Leigh Syndrome cohort at each visit, changes between visits and p values. *p<0.05.

		Phosphate (mmol/L)
Visit 1	Value	1.3
	Δ in Observation	0.1
	p	0.006*
Visit 2	Value	1.4
	Δ with WBVT	0.0
	p	0.291
Visit 4	Value	1.5
	Δ in Follow-up	-0.2
	p	0.000*
Visit 6	Value	1.3

Note: Δ, change; WBVT, whole body vibration training.

4.4 Force Plate Parameters

4.4.1 Baseline

4.4.1.1 Paediatric Cohort

Baseline parameters for the M1LJ, S2LJ and CRT performed on the force plate are presented in Table 2.12 and Figure 2.18. The M1LJ gives information about the maximum force the leg muscles of an individual can produce, the S2LJ gives information about the maximum power the leg muscles of an individual can generate, and the CRT gives information about functional performance of the leg muscles in an everyday task. Only 2 paediatric participants were able to perform the M1LH and only 4 the S2LJ. This data has been presented to provide information about muscle function in the paediatric MRCD cohort in relation to the adult MRCD cohort and the reference population. No further analysis was performed on the data as the sample size was too small.

It is clear the ability of the leg muscles to produce force in the paediatric cohort was severely impaired as only 2 of 7 participants could perform the manoeuvre. The force SDS for the 2 participants able to perform the test was abnormal and significantly reduced compared to the reference population, -2.6, p=0.040 (Table 4.15 and Figure 4.18). Information from the S2LJ demonstrates that the ability of the leg muscles to generate power was also adversely affected in the paediatric cohort, impacting on muscle performance and efficiency. In the four paediatric participants able to perform the S2LJ, the SDS for EFI, FE and power were significantly lower than the reference population,

however the force SDS demonstrated a non-significant reduction: -5.3, p=0.001; -4.6, p=0.001; -4.7, p<0.000; and -1.6, p=0.119 respectively (Figure 2.18). These findings indicate inefficiency of muscle function and movement during the S2LJ. This dynamic manoeuvre demonstrates the inability of children with MRCD to use muscle force to generate power, superimposed on a reduced ability to produce normal muscle force. All S2LJ SDS parameters were lesser in value than the adult cohort indicating a greater functional impairment in this cohort of children with MRCD compared to adults with MRCD.

The findings from the M1LH and S2LJ help to inform the observations seen when investigating a functional movement, the CRT. All 7 paediatric participants were able to perform this test. The SDS for CRT speed, power and force were found to be abnormally low and significantly lower than the reference population at: -6.4, p=0.036; -2.5, p<0.000; and -2.2, p=0.003 respectively. The compromised ability of children with MRCD to generate muscle force and power culminated in grossly abnormal performance in the CRT as reflected in the speed SDS.

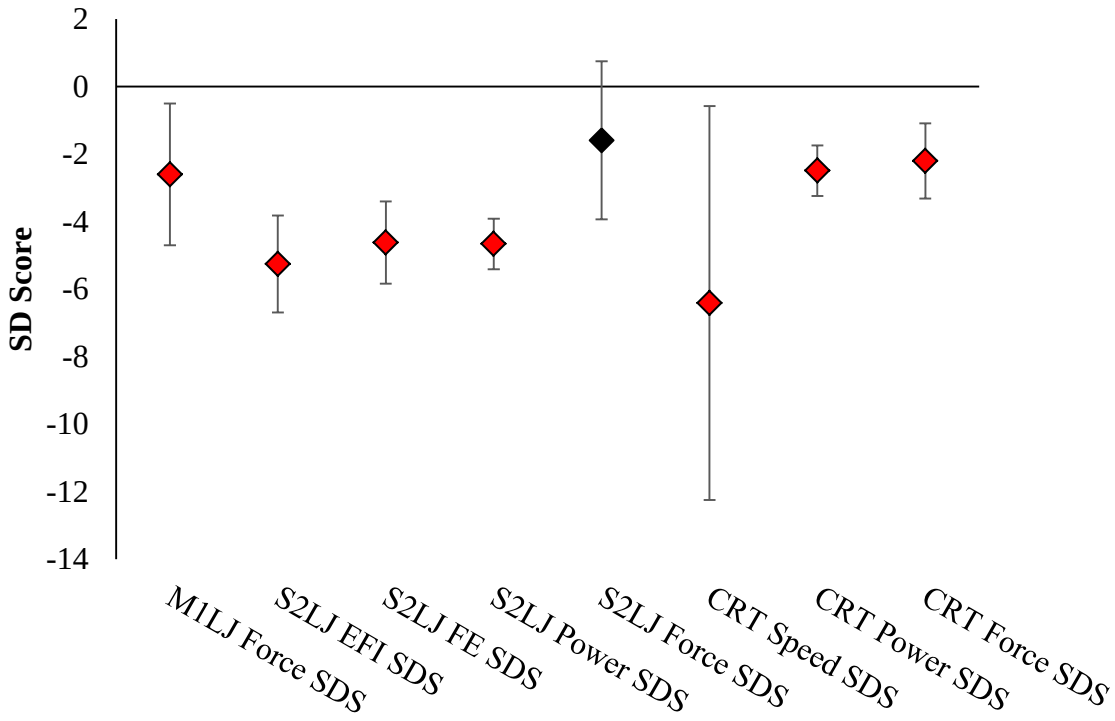

Figure 4.19. Standard deviation (SD) scores for force plate parameters in the paediatric cohort. Mean and 95% confidence interval. Parameters shown in red were significantly different to the reference population on one-sample t-tests, $p<0.05$.

4.4.1.2 Adult Cohort

Baseline force plate parameters are presented in Table 4.12 and Figure 4.19. All but one of the adult participants was able to perform the M1LJ. The force SDS was abnormal and significantly decreased compared to the reference population, -4.4, $p<0.001$ (Figure 4.19). This outcome is consistent with that seen in the paediatric MRCD cohort and indicates the leg muscles are unable to produce force normally in adults with MRCD. All of the adult participants were able to perform the S2LJ. The EFI, FE, power and force

SDS were all significantly lower than the reference population: -2.8, $p<0.000$; -3.4, $p<0.000$; -3.0, $p<0.000$; and -1.2, $p=0.002$ respectively (Figure 4.19), demonstrating similar impairments to those seen in the smaller paediatric cohort, confirming inadequacies of the muscle to produce force and generate power resulting in poor muscle efficiency in performing dynamic movements. All adult participants were also able to perform the CRT. The SDS for CRT speed, power and force were all significantly different to the reference population (Figure 4.19). The speed at which the CRT was significantly reduced, -3.4, $p<0.001$, however this was not as drastically reduced as the paediatric MRCD cohort. Power generated during the CRT was also significantly reduced compared to the reference population and more severely affected compared to the paediatric MRCD cohort, -2.7, $p<0.001$. The amount of force required to generate power during the CRT was significantly increased compared to the reference population, 2.9, $p=0.016$, indicating inefficiency in the ability of the leg muscles to perform the CRT. In contrast to the paediatric MRCD cohort, where force SDS indicated an inability of the leg muscles to produce adequate force during the CRT, the adult MRCD cohort was able to generate adequate force, however the amount of force required to perform this functional movement was significantly elevated compared to the reference population, a reflection of the poor efficiency of muscle work in adults with MRCD, as significantly more force was required to generate the power to perform the CRT. These results suggest that muscle function is more severely affected in the paediatric MRCD cohort compared to the adult MRCD cohort, reinforcing the more severe clinical presentations and disease progression often seen in children compared to adults with MRCD [279, 321, 384, 385].

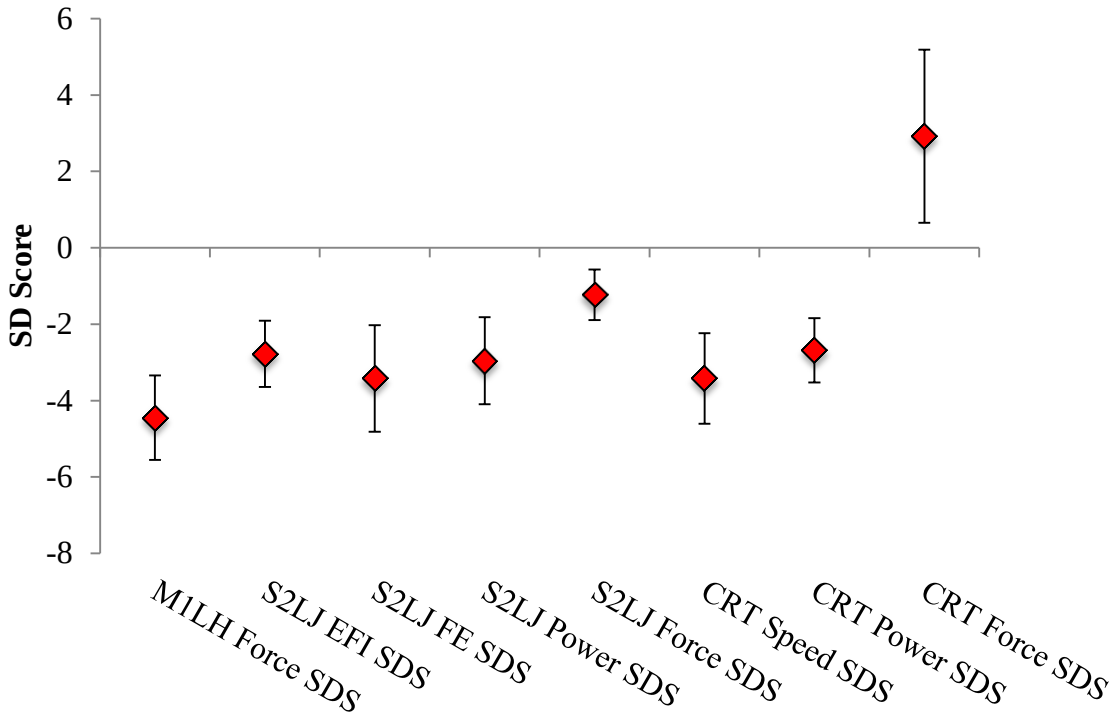

Figure 4.20. Standard deviation (SD) scores for force plate parameters in the adult cohort. Mean and 95% confidence interval. Parameters shown in red were significantly different to the reference population on one-sample t-tests, $p<0.05$.

Table 4.15. Baseline Force-plate parameters. Mean (SD).

Multiple One-Leg Jump (M1LJ) Parameters			
	Paediatric (n=2)	**Adult (n=11)**	**Leigh Syndrome (n=1)**
F max (kN)	5.9 (0.0)	11.8 (3.4)	10.4
F max rel (g)	2.5 (0.1)	2.0 (0.5)	1.7
Force SDS	-2.6 (0.2)	-4.4 (1.7)	-5.4
Single Two-Leg Jump (S2LJ) Parameters			
	Paediatric (n=4)	**Adult (n=12)**	**Leigh Syndrome (n=2)**
EFI (%)	40.9 (8.3)	53.8 (20.0)	33.8 (1.7)
EFI SDS	-5.3 (0.9)	-2.8 (1.4)	-4.8 (0.1)
FE (%)	53.0 (8.6)	65.8 (22.0)	47.8 (0.0)
FE SDS	-4.6 (0.8)	-3.4 (2.2)	-5.2 (0.0)
P max (kW)	0.6 (0.1)	1.5 (0.8)	1.6 (0.3)
P max rel (W/kg)	19.2 (3.9)	23.4 (11.4)	20.3 (1.0)
Power SDS	-4.7 (0.5)	-3.0 (1.8)	-5.3 (0.2)
F max (kN)	0.6 (0.1)	1.3 (0.3)	1.3 (0.2)
F max rel (N/kg)	19.2 (4.9)	20.0 (3.4)	17.3 (0.9)
Force SDS	-1.6 (1.5)	-1.2 (1.0)	-2.7 (0.3)
Chair Rise Test (CRT) Parameters			

	Paediatric (n=7)	Adult (n=12)	Leigh Syndrome (n=5)
Time per test (s)	2.5 (1.3)	2.8 (1.2)	2.7 (1.2)
V max (m/s)	0.7 (0.2)	0.7 (0.2)	0.6 (0.2)
Speed SDS	-6.4 (6.3)	-3.4 (1.9)	-4.1 (0.5)
P max (W)	234.1 (90.0)	473.5 (14.9)	326.4 (203.7)
P max rel (W/kg)	8.2 (2.4)	7.3 (3.1)	7.2 (2.1)
Ave P max rel (W/kg)	7.1 (2.4)	6.8 (2.8)	6.4 (1.9)
Power SDS	-2.5 (0.8)	-2.7 (1.3)	-2.6 (0.8)
F max (kN)	1.3 (0.2)	1.2 (0.1)	1.2 (0.2)
F max rel (N/kg)	49.5 (2.1)	19.7 (5.7)	39.9 (33.0)
Ave F max rel (g)	1.3 (0.1)	1.2 (0.1)	1.2 (0.1)
Force SDS	-2.2 (1.2)	2.9 (3.6)	-1.8 (1.4)

Note: SDS, standard deviation score; V max, maximum velocity; P max, maximum power; P max rel, maximum power in relation to body weight; F max, maximum force; F max rel, maximum relative force in relation to body weight; EFI, Esslinger Fitness Index; FE, Force Efficiency.

4.4.1.3 Leigh Syndrome Cohort

Baseline force-plate parameters are presented in Table 4.12 and Figure 4.20. Only 1 participant with Leigh Syndrome was able to perform the M1LH and only 2 the S2LJ. This data has been presented to provide information about muscle function in this cohort in relation to the paediatric and adult cohorts and the reference population. The one young adult able to perform the M1LH had a force SDS well below the reference population and lower than the mean of the adult cohort, suggesting that the muscle's ability to produce force may be more affected in Leigh Syndrome than other MRCD. The two participants able to perform the S2LJ had EFI, FE, power and force SDS significantly lower than the reference population: -4.8, $p=0.012$; -5.2, $p<0.000$; -5.3, $p=0.014$; and -2.7, $p=0.044$ respectively. The SDS for FE, power and force were lower than the paediatric and adult cohorts, again suggesting that muscle force, power and efficiency are more affected in Leigh Syndrome than other MRCD. All participants were able to perform the CRT. Speed, power and force SDS during the CRT were all lower than the reference population (Figure 4.20), the SDS for speed and power were significantly reduced compared to reference population: -4.1, $p<0.000$ and -2.6, $p=0.002$, however the force SDS did not reach significance, -1.8, $p=0.050$. As a mixed paediatric and adult cohort, the values for the CRT SDS were between those seen in the paediatric and adult cohorts. This finding does not support the theory that muscle force, power and co-ordination are more affected in Leigh Syndrome compared to other MRCD.

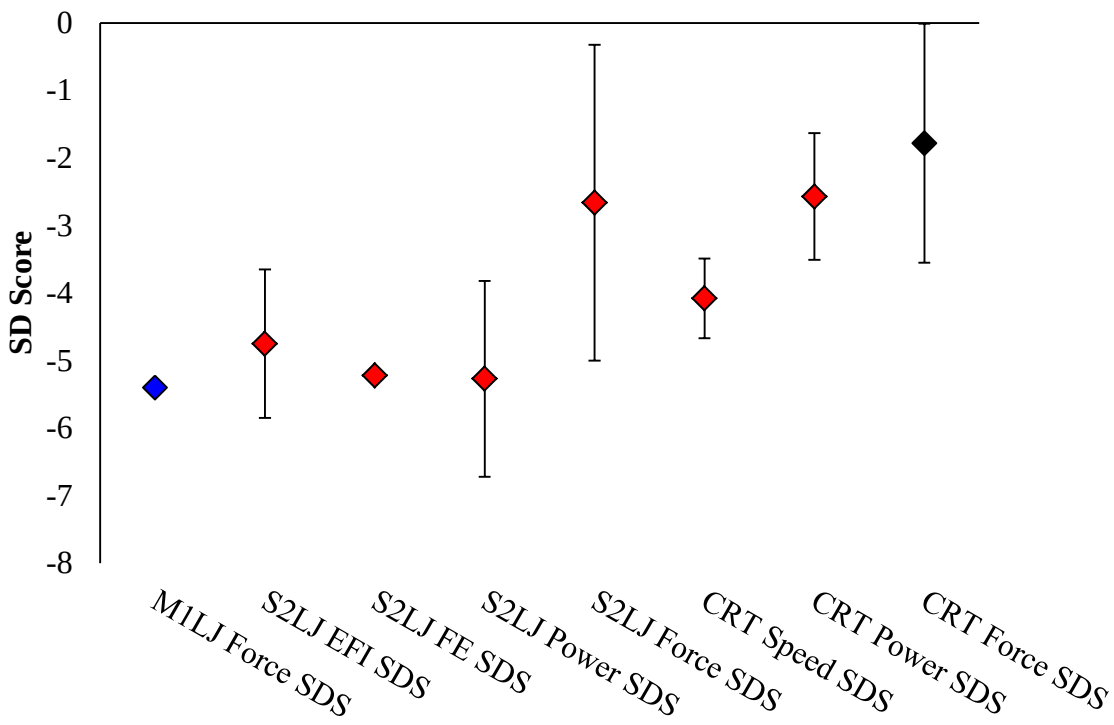

Figure 4.21. Standard deviation (SD) scores for force plate parameters in the Leigh Syndrome cohort. Mean and 95% confidence interval. Parameter in blue represent one participant. Parameters shown in red were significantly different to the reference population on one-sample t-tests, $p<0.05$.

4.4.2 Observation Period

Statistical analysis was performed on the M1LJ, S2LJ and CRT parameters for the adult cohort but only on the CRT parameters for the paediatric and Leigh Syndrome cohorts. There were no significant changes in any force plate parameters for the paediatric, adult or Leigh Syndrome cohorts during the observation period (Appendix D, Table D.2-D.4).

4.4.3 WBVT Period

4.4.3.1 Paediatric Cohort

Statistical analysis was only performed on the CRT parameters in the paediatric cohort. The speed at which the CRT was performed and the power required to perform the manoeuvre did not change significantly after WBVT (Appendix D, Table D.2). The force required to generate power however, decreased significantly (Table 2.13). Maximum force decreased from 1.3 – 1.2kN, p=0.037, and maximum relative force decreased from 47.6 – 41.3N/kg, p=0.019. The force SDS did not decrease significantly however it decreased by half a SDS from -2.4 to -2.9, p=0.160. These results may indicate that the force generating capacity of the muscle has worsened, however it may also indicate that the efficiency of movement may have improved after WBVT, as less force was required to generate similar power and speed.

Table 4.16. CRT parameters for the paediatric MRCD cohort at each visit, changes between visits and p values. *p<0.05.

		F max (kN)	**F max rel (N/kg)**	**Ave F max rel (g)**	**Force SDS**
Visit 1	Value	1.288	49.458	1.250	-2.201
	Δ in Observation	0.013	-1.878	-0.021	-0.196

	p	0.804	0.452	0.609	0.567
Visit 2	Value	1.302	47.580	1.230	-2.397
	Δ with WBVT	-0.119	-6.278	-0.054	-0.492
	p	0.037*	0.019*	0.196	0.160
Visit 4	Value	1.182	41.302	1.176	-2.889
	Δ in Follow-up	0.079	1.749	0.081	0.632
	p	0.153	0.482	0.058	0.076
Visit 4	Value	1.261	43.052	1.257	-2.257

Note: Δ, change; F max, maximum force; F max rel, maximum relative force; Ave F max rel, average maximum relative force; SDS, standard deviation score.

4.4.3.2 Adult Cohort

There were no significant changes in any M1LH or S2LJ parameters after WBVT (Appendix D, Table D.3). Analysis of the CRT parameters demonstrated a significant decrease in the time to perform each chair rise from 2.6 – 2.4s, p=0.030 (Table 2.14). However, there was no significant change in the corresponding speed SDS. Power and force parameters did not change significantly (Appendix D, Table D.2). These results likely indicate improved leg muscle agonist/antagonist co-ordination as the same force and power was required to perform each chair-rise in a faster time.

Table 4.17. CRT parameters for the adult sub-cohort at each visit, changes between visits and p values. *p<0.05.

		Time per test (s)	**P max (W)**	**P max rel (W/kg)**	**Power SDS**
Visit 1	Value	2.806	437.484	7.329	-2.684
	Δ in Observation	-0.162	24.854	0.411	0.226
	p	0.179	0.491	0.398	0.343
Visit 2	Value	2.644	498.338	7.741	-2.458
	Δ with WBVT	-0.269	-29.499	-0.203	-0.057
	p	0.030*	0.414	0.675	0.811
Visit 3	Value	2.375	468.839	7.537	-2.515
	Δ in Follow-up	-0.201	75.600	1.093	0.506
	p	0.099	0.042*	0.030*	0.038*
Visit 4	Value	2.174	544.440	8.630	-2.009

		F max (kN)	F max rel (N/kg)	Ave F max rel (g)	Force SDS
Visit 1	Value	1.205	19.652	1.205	2.924
	Δ in Observation	0.001	-0.103	-0.001	-0.025
	p	0.950	0.796	0.963	0.935
Visit 2	Value	1.206	19.549	1.204	2.898
	Δ with WBVT	0.034	0.696	0.032	0.346
	p	0.142	0.088	0.110	0.266
Visit 3	Value	1.240	20.245	1.236	3.244
	Δ in Follow-up	0.063	1.155	0.039	0.732
	p	0.009*	0.006*	0.052	0.022*
Visit 4	Value	1.303	21.400	1.275	3.976

Note: Δ, change; P max, maximum power; P max rel, maximum relative power; F max, maximum force; F max rel, maximum relative force; Ave F max rel, average maximum relative force; SDS, standard deviation score.

4.4.3.3 Leigh Syndrome Cohort

Statistical analysis was only performed on the CRT parameters. There were no significant changes in any CRT speed, power of force parameters after WBVT (Appendix D, Table D.4).

4.4.4 Follow-up Period

4.4.4.1 Paediatric Cohort

Analysis of the CRT parameters did not demonstrate any significant changes (Appendix D, Table D.2). In contrast to the WBVT period, there were non-significant increases in CRT average relative force and the force SDS: 1.2 – 1.3g, p=0.058; and -2.9 to -2.3, p-0.076, respectively, the latter over half a SDS which would be considered clinically relevant (Table 4.13). As the changes in CRT speed and power were not of the same magnitude as the changes seen in CRT force, these results may indicate worsening of muscle efficiency after withdrawal of WBVT as more force was required to achieve the same speed and power during the CRT.

4.4.4.2 Adult Cohort

During the follow-up period, there were no significant changes in any M1LJ or S2LJ parameters (Appendix D, Table D.3). Significant increases were seen in CRT power and

force parameters but not speed parameters. Maximum power, maximum relative power and the power SDS increased significantly: 468.8 – 544.4W, p=0.042; 7.5 – 8.6W/kg, p=0.030; and -2.5 to -2.0, p=0.038 respectively. Maximum force, maximum relative force and force SDS also increased significantly: 1.2 – 1.3kN, p=0.009; 20.2 – 21.4N/kg, p=0.006; and 3.2 – 4.0, p=0.022 respectively (Table 4.14). The further increase in the amount of force required to generate power to perform the CRT, coupled with the increased amount of power required to perform the test at the same speed, indicates worsening of muscle efficiency, an aspect which appears to have been hindered during the WBVT period.

4.4.4.3 Leigh Syndrome Cohort

Analysis of the CRT parameters revealed significant changes in CRT force parameters but not speed or power parameters (Appendix D, Table D.4). Maximum force, average relative force and force SDS increased significantly: 1.1 – 1.3kN, p=0.033; 1.1 – 1.2g, p=0.002; and -2.4 to -1.3, p=0.009 respectively (Table 2.15). These results would suggest that the ability of the Leigh Syndrome cohort to generate force improved during the follow-up period, however that lack of follow-on improvement in the power and speed parameters of the CRT, do not indicate improve muscle efficiency.

Table 4.18. CRT parameters for the Leigh Syndrome cohort at each visit, changes between visits and p values. *p<0.05.

		F max (kN)	Ave F max rel (g)	Force SDS
Visit 1	Value	1.217	1.182	-1.776
	Δ in Observation	-0.029	-0.036	-0.228
	p	0.617	0.373	0.536
Visit 2	Value	1.188	1.146	-2.004
	Δ with WBVT	-0.064	-0.047	-0.364
	p	0.275	0.245	0.329
Visit 3	Value	1.124	1.098	-2.369
	Δ in Follow-up	0.136	0.151	1.106
	p	0.033*	0.002*	0.009*
Visit 4	Value	1.260	1.249	-1.263

Note: Δ, change; F max, maximum force; Ave F max rel, average maximum relative force; SDS, standard deviation score.

4.5 Exercise Parameters

4.5.1 Six Minute Walk Test (6MWT)

4.5.1.1 Baseline

Baseline 6MWT parameters are presented in Table 4.19. Baseline, post and recovery measures of heart rate, systolic and diastolic blood pressure and oxygen saturations were all within normal boundaries indicating that the 6MWT was safely performed in this population.

4.5.1.1.1 Paediatric Cohort

Baseline 6MWT parameters for the 7 paediatric participants are presented in Table 4.19. The distance covered during the 6MWT, 319.2m, 52.2% of that predicted by the reference population, indicated moderate to severely impaired exercise capacity. The paediatric cohort also demonstrated the worst exercise capacity of the 3 cohorts. Borg scores and blood pressure parameters were increased at the post measurement compared to the baseline measurement and decreased toward baseline levels at the recovery measurement, however heart rate increased at the post measurement but did not show any decrease at the recovery measurement, indicating poor cardiovascular recovery after physical effort.

Table 4.19. Baseline 6MWT parameters. Mean (SD).

	Paediatric (n=7)	**Adult (n=12)**	**Leigh Syndrome (n=5)**
Six Minute Walk Test (6MWT) Parameters			
Distance (m)	319.2 (156.0)	413.0 (139.9)	341.6 (76.8)
Distance % Predicted (%)	52.2 (27.4)	71.3 (24.7)	56.8 (18.7)
Baseline HR (bpm)	94.0 (10.1)	86.0 (18.6)	83.7 (18.7)
Baseline Borg Score	0.6 (1.0)	1.2 (1.0)	0.4 (0.9)
Baseline SBP (mmHg)	104.6 (9.8)	126.1 (9.5)	114.3 (18.9)
Baseline DBP (mmHg)	66.6 (9.6)	83.2 (5.2)	77.8 (8.7)
Baseline SpO2 (%)	98.7 (1.8)	97.6 (1.7)	99.2 (1.8)
Post HR (bpm)	107.7 (9.1)	106.8 (20.2)	120.0 (2.0)
Post Borg Score	3.0 (1.4)	2.7 (1.1)	2.8 (1.3)

Post SBP (mmHg)	115.5 (13.9)	140.8 (14.1)	116.5 (17.7)
Post DBP (mmHg)	73.5 (12.9)	85.7 (11.5)	74.5 (13.4)
Post SpO2 (%)	98.6 (1.3)	97.7(1.4)	99.0 (1.4)
Recovery HR (bpm)	108.7 (9.7)	86.9 (19.6)	98.3 (16.3)
Recovery Borg Score	1.9 (1.3)	1.5 (1.1)	1.2 (0.8)
Recovery SBP (mmHg)	108.4 (11.5)	127.7 (11.2)	124.8 (21.8)
Recovery DBP (mmHg)	66.6 (10.9)	78.9 (7.2)	75.0 (16.9)
Recovery SpO2 (%)	98.3 (1.6)	97.2 (1.9)	98.6 (1.5)

Note: HR, heart rate; bpm, beats per minute; mmHg, millimeters of mercury; SBP, systolic blood pressure; DBP, diastolic blood pressure; SpO2, oxygen saturations.

4.5.1.1.2 Adult Cohort

Baseline 6MWT parameters for the 12 adult participants are presented in Table 4.19. The adult cohort covered the greatest distance in the 6MWT, compared to the other cohorts, and demonstrated a higher percent-predicted value of 71.3%, indicating moderately impaired exercise capacity. Baseline Borg scores were slightly higher than the other cohorts, however post and recovery scores were similar across all cohorts. Heart rate, Borg score and blood pressure parameters were all increased at the post measurement compared to the baseline measurement, indicating increased physical effort, and then dropped to near baseline levels at the recovery measurement.

4.5.1.1.3 Leigh Syndrome Cohort

Baseline 6MWT parameters for the 5 Leigh Syndrome participants are presented in Table 4.19. The Leigh Syndrome cohort covered a greater distance than the paediatric cohort but a shorter distance than the adult cohort. The percent-predicted value for the distance covered was also between the paediatric and adult cohorts and demonstrated a moderate to severely impaired exercise capacity. Heart rate, Borg score and blood pressure parameters increased between baseline and post measurements. At the recovery measurement, blood pressure had increased further and the recovery in heart rate was slow, indicating poor cardiovascular recovery, similar to that seen in the paediatric cohort.

4.5.1.2 Observation Period

Analysis data for all 6MWT parameters for the observation period can be found in Appendix E. Safety measures, including blood pressure and oxygen saturations, for each of the 3 cohorts, will not be discussed.

4.5.1.2.1 Paediatric Cohort

There were no significant changes in 6MWT distance or Borg scores at baseline, post or recovery measurements. However, the heart rate post the 6MWT increased by 26.8 bpm, p=0.037 indicating greater cardiovascular effort to achieve a similar 6MWT distance (Table 4.20 and Appendix E, Table E.2).

4.5.1.2.2 Adult Cohort

There were no significant changes in 6MWT distance, heart rate or Borg scores at baseline, post or recovery measurements in the adult cohort (Appendix E, Table E.3).

Table 4.20. Six-minute walk test parameters for the paediatric cohort at each visit, changes between visits and p values. *p<0.05.

		Baseline HR (bpm)	**Post HR (bpm)**	**Recovery Borg Score**
Visit 1	Value	96.040	106.7	1.8
	Δ in Observation	7.817	26.8	0.3
	p	0.149	0.037*	0.569
Visit 2	Value	103.857	133.429	2.1
	Δ with WBVT	-10.429	-27.9	-1.3
	p	0.011*	0.015*	0.018*
Visit 3	Value	93.429	105.571	0.9
	Δ in Follow-up	10.259	23.0	0.3
	p	0.017*	0.038*	0.569
Visit 4	Value	103.668	129	1.1

Note: Δ, change; WBVT, whole body vibration training; HR, heart rate; bpm, beats per minute.

4.5.1.2.3 Leigh Syndrome Cohort

There were significant decreases in the 6MWT distance and the percent-predicted value during the observation period: 341.6 - 274.9m, p=0.035; and 56.8 – 46.0%, p=0.023 respectively (Table 4.21). Significant decreases in 6MWT distance were not seen in the paediatric or adult MRCD cohorts during the observation period. These findings suggest that in comparison to other MRCD, Leigh Syndrome may exhibit a more accelerated decline in muscle endurance. There were no significant changes in heart rate or Borg scores at baseline, post or recovery measurements (Appendix E, Table E.4).

Table 4.21. 6MWT parameters for the Leigh Syndrome cohort at each visit, changes between visits and p values. *p<0.05.

		Distance (m)	Distance % Predicted (%)	Post Borg Score	Recovery Borg Score
Visit 1	Value	341.580	56.765	2.8	1.2
	Δ in Observation	-66.700	-10.794	-0.6	0.2
	p	0.035*	0.023*	0.068	0.584
Visit 2	Value	274.880	45.971	2.2	1.4
	Δ with WBVT	24.020	3.306	0.8	-1.0
	p	0.408	0.439	0.020*	0.016*
Visit 3	Value	298.900	49.278	3.0	0.4
	Δ in Follow-up	18.500	2.753	-0.6	0.4
	p	0.521	0.518	0.068	0.283
Visit 4	Value	317.400	52.031	2.4	0.8

Note: Δ, change; WBVT, whole body vibration training.

4.5.1.3 WBVT Period

Analysis data for all 6MWT parameters for the WBVT period can be found in Appendix E.

4.5.1.3.1 Paediatric Cohort

There were no significant changes in 6MWT distance after WBVT (Appendix E, Table E.2). Heart rate at baseline was significantly reduced by 10.5 bpm, p=0.011, after WBVT. There was also a significant reduction in heart rate post the 6MWT of 27.9 bpm, p=0.015, indicating less cardiovascular effort to achieve a similar 6MWT distance. To further support an improved response to exercise after WBVT, the recovery Borg score decreased significantly by 1.3 points, 0.018 (Table 4.20).

4.5.1.3.2 Adult Cohort

There were no significant changes in any of the 6MWT parameters after WBVT (Appendix E, Table E.3). The 6MWT distance did increase non-significantly by 19.2m (4.8%) compared to an 11.2m decrease during the observation period.

4.5.1.3.3 Leigh Syndrome Cohort

After WBVT there was a non-significant increase in 6MWT distance of 24.0m (8.7%) and a 3.3% increase in the percent-predicted value. This is in contrast to the significant decrease in 6MWT distance seen in the observation period. The post measurement for the Borg score was significantly increased by 0.8, p=0.020, indicating greater perceived physical effort required to perform the 6MWT. The recovery measurement of the Borg score was significantly decreased after WBVT by 1.0, p=0.016, indicating improved recovery, despite a greater effort to perform the test (Table 4.21). There were no significant changes in baseline, post or recovery measures of heart rate (Appendix E, Table E.4).

4.5.1.4 Follow-up Period

Analysis data for all 6MWT parameters for the Follow-up period can be found in Appendix E.

4.5.1.4.1 Paediatric Cohort

There were no significant changes in 6MWT distance or the baseline, post or recovery measurements for Borg score during the follow-up period (Appendix E, Table E.2). Baseline heart rate increased significantly by 10.3 bpm, p=0.017. Hear rate post the

6MWT also increased significantly by 23.0 bpm, p=0.038, indicating increased cardiovascular effort to cover a similar 6MWT distance.

4.5.1.4.2 Adult Cohort

There were no significant changes during the follow-up period for 6MWT distance or heart rate and Borg scores at baseline, post or recovery measurements (Appendix E, Table E.3).

4.5.1.4.3 Leigh Syndrome Cohort

After WBVT there were no significant changes in any of the 6MWT parameters (Appendix E, Table E.4). The 6MWT distance demonstrated a non-significant increase of 18.5m which was similar to the improvement seen during the WBVT period and in contrast to the significant reduction in 6MWT distance seen during the observation period (Appendix E, Table E.4).

4.5.2 Cardio-pulmonary Exercise Testing (CPET)

CPET data were available for 2 paediatric and 7 adult participants. The paediatric participants performed the exercise tests on a treadmill using the Bruce Treadmill Protocol, as did two of the young adult participants. The 5 remaining adult participants performed the exercise tests on a cycle ergometer using a 10-watt ramp protocol.

4.5.2.1 Baseline

Baseline CPET parameters are presented in Table 4.22. The limited paediatric data has presented to provide information about their response to exercise compared to the adult cohort and the reference population. The pre-exercise VO_2 was elevated in both the paediatric and adult cohorts at 7.6 and 4.5 ml/kg/min respectively, compared to that seen in the healthy adult reference population at rest of 3.5 ml/kg/min. The pre-exercise Borg Score of 1.0 and 2.8, in the paediatric and adult cohorts respectively, indicates that the participants were experiencing a mild level of physical exertion prior to commencing the exercise test. Pre-exercise measurements of systolic and diastolic blood pressure were within normal limits in both cohorts.

The anaerobic threshold occurred abnormally early in exercise in the adult cohort as demonstrated by a mean percent predicted VO_2 of 30.9% and percent predicted Watts of 23.9% which indicated severely impaired oxidative pathways. This was not as pronounced in the paediatric cohort, however the VO_2 at which AT occurred was 80.5% of that predicted by the reference population. This indicated mild-moderate impairment of the oxidative pathways in the paediatric cohort. The ventilatory equivalents for oxygen (VE/VO_2) and carbon dioxide (VE/VCO_2) were abnormally high at the anaerobic threshold in the adult cohort with mean values of 36.0 and 36.7 respectively, greater than their corresponding reference maximums of 28 and 32, indicating an exaggerated ventilatory response. These parameters were not elevated to the same degree in the

paediatric cohort. At AT, participants were already experiencing moderate to high muscular and cardiovascular effort. In the paediatric cohort, AT occurred at 48.3% of total exercise time, with a percent-predicted heart rate of 79.1% and VO_2 at 92.9% of its peak. In the adult cohort, AT occurred at 55.5% of total exercise time and 57.4% of peak Watts, when heart rate was at 72.2% predicted and VO_2 was at 70.6% of its peak.

Peak exercise parameters demonstrated moderate-severely reduced oxidative and exercise capacity with a mean VO_2 of 28.0 ml/kg/min, 61.0% of predicted in the paediatric cohort and a mean VO_2 of 15.4ml/kg/min, 45.0% of predicted, and mean power output of 63.2W, 45.2% of predicted, in the adult cohort. Oxygen pulse at peak exercise was also moderately-severely restricted with a peak value of 3.5ml/beat, 53.5% of predicted and 5.8ml/beat, 32.9% of predicted, in the paediatric and adult cohorts respectively. The ventilatory equivalent for oxygen was also high at peak exercise with a mean value of 48.0 in the paediatric cohort and 46.0 in the adult cohort. These findings at peak exercise, together with the findings at AT, reflect the inability of the exercising muscle to use the oxygen provided to it by the circulating blood. Consequently, the arterial-mixed venous oxygen difference does not progressively increase at it should, forcing a prematurely exaggerated response of the cardiovascular system to increase cardiac output in an attempt to increase VO_2. Concurrently, the working muscle is forced to use anaerobic pathways that produce lactic acid, the buffering of this increased lactic acid by bicarbonate, resulting in a rapid increase in VCO_2 causing exaggerated ventilatory responses. These exaggerated responses in the cardiovascular and respiratory systems, causing them to rapidly escalate toward their physiological maximums, culminated in

significantly reduced exercise capacity. At peak exercise the mean heart rate was 89.0 and 87.2% of predicted, respiratory quotient 1.4 and Borg Score 7.0 and 7.4 in the paediatric and adult cohorts respectively, all indicating that a peak test had been performed. Systolic and diastolic blood pressures were increased compared to pre-exercise values also indicating increased physical effort.

The recovery data were only available for the adult cohort and indicates that all CPET parameters were returning towards baseline levels except for the ventilatory equivalent for oxygen which remained high at 47.8, a likely reflection of elevated minute ventilation in response to the increased VCO_2.

Table 4.22. Baseline Cardiopulmonary Exercise Test Parameters. Mean (SD).

	Paediatric Cohort (n=2)	**Adult Cohort (n=7)**
CPET - Pre-Exercise Parameters		
Pre-exercise VO_2 (L/min)	0.2 (0.1)	0.2 (0.1)
Pre-exercise VO_2 (ml/kg/min)	7.6 (3.7)	4.5 (2.4)
Pre-exercise HR (bpm)	101.5 (3.5)	91.4 (21.5)
Pre-exercise HR (% Predicted)	49.0 (1.2)	52.6 (7.5)
Pre-exercise O_2 Pulse (ml/beat)	1.9 (0.8)	2.6 (1.1)
Pre-exercise VCO_2 (L/min)	0.2 (0.1)	0.2 (0.1)
Pre-exercise RQ	0.8 (0.1)	0.9 (0.1)
Pre-exercise VE (L/min)	7.6 (4.5)	9.9 (5.4)
Pre-exercise VE/VO_2	39.0 (5.8)	42.4 (11.2)
Pre-exercise VE/VCO_2	46.1 (3.0)	46.3 (13.4)
Pre-exercise Borg Score	1.0 (0.0)	2.8 (2.6)
Pre-exercise SBP (mmHg)	109.5 (0.7)	121.6 (10.4)
Pre-exercise DBP (mmHg)	73.0 (8.5)	84.0 (9.4)
CPET - Anaerobic Threshold (AT) Parameters		
AT VO_2 (L/min)	0.7 (0.1)	0.6 (0.3)

AT VO_2 (ml/kg/min)	26.1 (4.0)	10.9 (5.5)
AT VO_2 (% Predicted)	80.5 (14.8)	
AT VO_2 (% Predicted VO_2)	56.6 (1.6)	30.9 (11.1)
AT VO_2 (% of Peak VO_2)	92.9 (7.3)	70.6 (17.7)
AT HR (bpm)	164.0 (12.7)	125.0 (21.3)
AT HR (% Predicted)	79.1 (6.9)	72.2 (6.5)
AT O2 Pulse (ml/beat)	4.0 (0.5)	4.8 (2.4)
AT VCO_2 (L/min)	0.7 (0.1)	0.6 (0.3)
AT RQ	1.0 (0.0)	1.0 (0.0)
AT VE (L/min)	20.1 (0.1)	20.2 (8.8)
AT VE/VO_2	31.2 (6.1)	36.0 (13.4)
AT VE/VCO_2	30.9 (5.8)	36.7 (14.1)
AT (% of CPET Time)	48.3 (12.7)	55.51 (29.0)
AT Watts (W)		33.6 (19.2)
AT Watts (% Predicted)		23.9 (10.6)
AT Watts (W/kg)		0.6 (0.3)
AT Watts (% of Peak Watts)		57.4 (26.7)
CPET - Peak Exercise Parameters		

Peak VO_2 (L/min)	0.7 (0.1)	0.9 (0.5)
Peak VO_2 (% Predicted)	61.0 (2.9)	45.0 (16.3)
Peak VO_2 (ml/kg/min)	28.0 (2.1)	15.4 (7.3)
Peak HR (bpm)	184.5 (3.5)	150.0 (14.2)
Peak HR (% Predicted)	89.0 (2.6)	87.2 (7.0)
Peak O_2 Pulse (ml/beat)	3.5 (0.1)	5.8 (2.7)
Peak O_2 Pulse (% Predicted)	53.5 (1.5)	32.9 (13.0)
Peak VCO_2 (L/min)	0.9 (0.1)	1.1 (0.7)
Peak RQ	1.4 (0.2)	1.4 (0.2)
Peak VE (L/min)	30.1 (10.2)	38.0 (20.12)
Peak VE/VO_2	48.0 (15.6)	46.0 (12.3)
Peak VE/VCO_2	34.5 (6.4)	36.8 (14.1)
Peak Watts (W)		63.2 (32.2)
Peak Watts (% Predicted)		45.2 (19.1)
Peak Watts (W/kg)		1.1 (0.5)
CPET Time (mins)	5.9 (2.0)	5.4 (3.5)
Peak Borg Score	7.0 (2.8)	7.4 (2.5)
Peak SBP (mmHg)	124.5 (12.0)	169.4 (16.0)

Peak DBP (mmHg)	89.5 (2.1)	90.0 (9.7)
CPET - Recovery Parameters		
Recovery VO_2 (L/min)		0.4 (0.2)
Recovery VO_2 (ml/kg/min)		6.4 (3.9)
Recovery HR (bpm)		107.6 (23.5)
Recovery HR (% Predicted)		62.0 (9.2)
Recovery O_2 Pulse (ml/beat)		3.2 (1.6)
Recovery VCO_2 (L/min)		0.4 (0.3)
Recovery RQ		1.1 (0.1)
Recovery VE (L/min)		16.2 (8.7)
Recovery VE/VO_2		47.8 (8.2)
Recovery VE/VCO_2		47.0 (12.6)
Recovery Borg Score	3.0 (1.4)	2.8 (2.2)
Recovery SBP (mmHg)	115.5 (12.0)	137.8 (12.4)
Recovery DBP (mmHg)	84.0 (2.8)	82.6 (11.7)

Note: CPET, Cardiopulmonary Exercise Test; VO_2, oxygen uptake; HR, heart rate; O_2, oxygen; VCO_2, carbon dioxide production; RQ, respiratory quotient; VE, minute ventilation; VE/VO_2, ventilator equivalent of oxygen; VE/VCO_2, ventilator equivalent of

carbon dioxide; SBP, systolic blood pressure; DBP, diastolic blood pressure; AT, anaerobic threshold.

4.5.2.2 Observation Period

Analysis data for CPET parameters in the adult cohort for the observation period can be found in Appendix E. No longitudinal analysis was performed on the paediatric data.

4.5.2.2.1 Adult Cohort

Pre-exercise Borg score decreased significantly by 1.7 points, p=0.005 during the observation period. There were no other significant changes in pre-exercise, AT or peak exercise parameters. Heart rate at 5-minutes recovery decreased significantly by 12.9bpm, p=0.013. (Table 4.23 and Appendix E, Table E.5). These findings perhaps suggest that anxiety associated with the unfamiliarity of the testing environment and procedures, increased fatigue prior to the test on the first study visit, and heightened heart rate during recovery.

4.5.2.3 WBVT Period

Analysis data for CPET parameters can be found in Appendix E.

4.5.2.3.1 Adult Cohort

After WBVT, there were no statistically significant changes in any of the pre-exercise parameters. Similarly, at the anaerobic threshold, no parameters demonstrated statistically significant changes. Despite this, several anaerobic threshold parameters registered their highest values after WBVT. This included the percent-predicted values for VO_2 and power output as well as the oxygen pulse parameter. The peak exercise parameters did not reflect any significant changes during the WBVT period. However, peak VO_2 (ml/kg/min) increased 5% after WBVT, with 2 of the 7 participants improving their VO_2 by greater than 10% during the WBVT period. The ventilatory equivalent for oxygen at 5-minutes recovery increased significantly after WBVT, however there were no other significant changes in recovery parameters. (Table 4.23 and Appendix E, Table E.5).

4.5.2.4 Follow-up Period

Analysis data for CPET parameters can be found in Appendix E.

4.5.2.4.1 Adult Cohort

During the follow-up period, there were no significant changes in any of the pre-exercise, AT, peak exercise or recovery parameters. However, several anaerobic threshold parameters demonstrated non-significant decreases in the follow-up period. The VO_2 at AT decreased 0.2L/min (25%), p=0.076, and 2.5ml.kg/min (18%), p=0.068, corresponding to a 5.3% decrease in the percent-predicted value and a 5.7% decrease in

the AT VO_2 as a percentage of the peak VO_2. AT occurred 8.4% earlier when considering total exercise time. Oxygen pulse also decreased 1.1ml/beat (18%), p=0.076. The magnitude of these reductions would be considered clinically relevant. Taken together these results indicate that there was a clinical de-training effect on anaerobic threshold parameters during follow-up (Appendix E, Table E.5). A non-significant decrease in peak VO_2 of 1.5mlkg/min (8%), p=0.278 was also seen, with no change in power output at peak exercise and a non-significant reduction in exercise time of only 3%. These results also suggest a clinical de-training effect on peak oxygen uptake.

Table 4.23. CPET parameters for the adult MRCD cohort at each visit, changes between visits and p values. *p<0.05.

		Pre-exercise Borg Score	**AT VO_2 (L/min)**	**AT VO_2 % Predicted (%)**
Visit 1	Value	3.1	0.7	34.9
	Δ in Observation	-1.7	0.1	5.7
	p	0.005*	0.275	0.257
Visit 2	Value	1.4	0.8	40.5
	Δ with WBVT	-0.3	0.0	2.0
	p	0.533	0.814	0.630
Visit 3	Value	1.1	0.8	42.6
	Δ in Follow-up	-0.4	-0.2	-5.3
	p	0.440	0.076	0.246
Visit 4	Value	0.8	0.6	37.2

		AT VO_2 (ml/kg/min)	AT VO_2 % of Peak VO_2 (%)	AT O_2 Pulse (ml/beat)
Visit 1	Value	12.2	69.8	5.0
	Δ in Observation	1.7	5.4	0.9
	p	0.247	0.480	0.170
Visit 2	Value	13.8	75.2	5.9
	Δ with WBVT	-0.1	2.1	0.2
	p	0.929	0.760	0.755
Visit 3	Value	13.7	77.3	6.1
	Δ in Follow-up	-2.5	-5.7	-1.1
	p	0.068	0.424	0.076
Visit 4	Value	11.2	71.5	5.0

		AT Watts (% Predicted)	AT % Exercise Time (%)	Peak VO_2 (ml/kg/min)
Visit 1	Value	24.9	56.0	17.8
	Δ in Observation	5.5	-2.5	-0.3
	p	0.164	0.818	0.842
Visit 2	Value	30.4	53.5	17.5
	Δ with WBVT	0.2	-0.4	0.9
	p	0.944	0.964	0.519
Visit 3	Value	30.6	53.1	18.4
	Δ in Follow-up	-3.2	-8.4	-1.5
	p	0.365	0.410	0.278
Visit 4	Value	27.4	44.7	16.9

		Recovery HR (bpm)	Recovery HR % predicted (%)	Recovery VE/VO_2
Visit 1	Value	111.3	62.7	48.1
	Δ in Observation	-12.9	-7.3	-2.6
	p	0.013*	0.009*	0.376
Visit 2	Value	98.4	55.4	45.6
	Δ with WBVT	0.4	0.7	5.9
	p	0.915	0.730	0.029*
Visit 4	Value	98.9	56.2	51.4
	Δ in Follow-up	1.0	0.7	-0.1
	p	0.804	0.738	0.954
Visit 6	Value	99.9	56.9	51.3

Note: Δ, change; WBVT, whole body vibration training; VO_2, oxygen uptake; HR, heart rate; bpm, beats per minute; O_2, oxygen; VE, minute ventilation; VE/VO_2, ventilator equivalent of oxygen; AT, anaerobic threshold.

4.6 Disease Scale and Quality of Life Parameters

4.6.1 Baseline

4.6.1.1 Paediatric Cohort

The baseline Newcastle Paediatric Mitochondrial Disease Scale (NPMDS) Scores are presented in Table 4.24. Section 1, reflecting the patient and care-giver perspective of current function, demonstrated mild impact of disease on function. Section 2, reflecting system specific involvement, indicated minimal serious systemic manifestations of the disease. Section 3, reflecting the clinician's clinical assessment, indicated mild-moderate impact of the disease on current status. Section 4, reflecting patient and care-giver perspectives on Quality of Life, indicated mildly affected quality of life.

Table 4.24. Baseline Disease Scale and Quality of Life Scores for the paediatric and Leigh Syndrome cohorts. Mean (SD).

Newcastle Mitochondrial Paediatric Disease Scale Scores

	Paediatric Cohort (n=5)	**Leigh Syndrome Cohort (n=5)**
Section 1	4.7 (2.6)	4.0 (2.1)
Section 2	1.1 (1.1)	0.6 (0.9)
Section 3	10.1 (4.3)	9.6 (3.4)

Section 4	9.2 (3.1)	8.0 (2.7)

4.6.1.2 Adult Cohort

The baseline Newcastle Disease Adult Scale (NMDAS) and Quality of Life Scores are presented in Table 4.25. Section 1, reflecting the patient's perspective of current function, demonstrated minimal impact of disease on function, however they are aware of minor changes to what would be considered normal function. The patient's perception of their current function was affected to a similar extent in the paediatric and adult cohorts. Section 2, reflecting system specific involvement, indicated minimal serious systemic manifestations of the disease, similar to that seen in the paediatric cohort. Section 3, reflecting the clinician's clinical assessment, indicated mild impact of the disease on current status. The paediatric cohort was more adversely affected in this area compared to the adult cohort. Quality of Life, measured by the SF-12v2, was significantly reduced in the physical functioning, role physical, bodily pain and general health domains, but not in the vitality, social function, role emotional and mental health domains. The physical component score, but not the mental component score was also significantly reduced compared to the reference population (Figure 4.21). While different quality of life measurement shave been utilised, it would appear that the adult cohort had more severely affected Quality of Life compared to the paediatric cohort.

Table 4.25. Baseline Disease Scale and Quality of Life Scores for the adult cohort. Mean (SD).

	Adult Cohort (n=10)
Newcastle Mitochondrial Disease Adult Scale Scores	
Section 1	9.8 (6.5)
Section 2	5.0 (3.1)
Section 3	10.2 (6.3)
SF-12v2 Domain Standard Deviation Scores	
Physical Functioning	-1.5 (0.8)
Role Physical	-1.1 (0.9)
Bodily Pain	-1.0 (0.8)
General Health	-1.3 (1.2)
Vitality	-0.6 (1.1)
Social Function	-0.6 (1.2)
Role Emotional	-0.1 (0.6)
Mental Health	0.2 (0.7)
Physical Component Score	-1.7 (0.9)
Mental Component Score	0.3 (0.6)

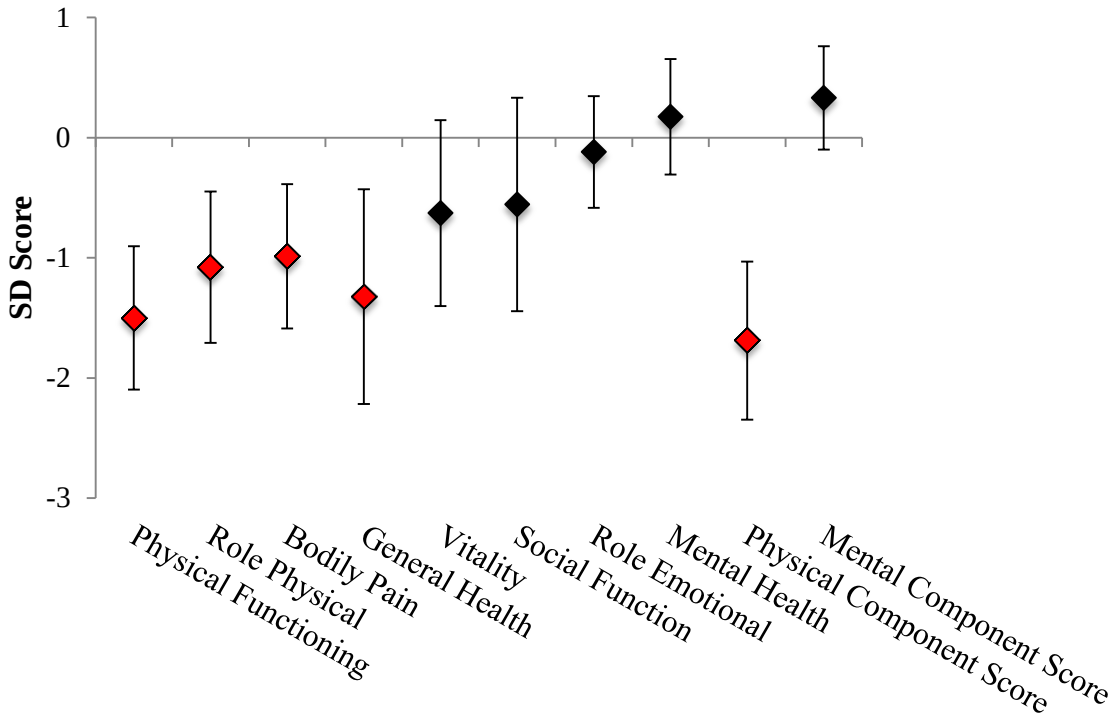

Figure 4.22. Standard deviation (SD) scores for Quality of Life Domains in the adult cohort. Mean and 95% confidence interval. Parameters shown in red were significantly different to the reference population on one-sample t-tests, $p<0.05$.

4.6.1.3 Leigh Syndrome Cohort

The baseline NPMDS Scores are presented in Table 4.24. Scores for each section were slightly lower than those seen in the paediatric cohort, indicating a higher current function, less system specific involvement, better clinical performance and more desirable Quality of Life, respectively. Similar to the paediatric cohort, the Leigh Syndrome cohort appears to be more affected on clinical assessment compared to the

adult cohort, however the adult cohort appears to have the lowest Quality of Life of the three cohorts.

4.6.2 Study Periods

All data analysis for disease scale and Quality of Life parameters for the three cohorts over the observation, WBVT and follow-up periods are presented in Appendix F, Table F2-4.

4.6.2.1 Paediatric Cohort

During the observation period, the NPMDS Section 1 score increased significantly by 1.9 points, $p<0.000$, indicating patient and care-giver perception of current function had worsened (Table 2.26, Appendix F.2). There was also a non-significant increase in the NPMDS Section 3 score of 1.3 points, $p=0.076$, indicating that the clinician's clinical assessment also demonstrated some deterioration. There were no significant changes in any of the NPMDS sections during the WBVT or follow-up periods. The progression of mitochondrial disease identified by an increase in the disease scores for current function and current clinical assessment during the observation period appears to have been interrupted by the WBVT intervention in the paediatric MRCD cohort and was not seen during the follow-up period, perhaps suggesting a latent effect of WBVT on mitochondrial disease progression measured by the NPMDS after cessation of the WBVT intervention in the paediatric MRCD cohort.

Table 4.26. Newcastle Paediatric Mitochondrial Disease Scale parameters for the paediatric cohort at each visit, changes between visits and p values. *p<0.05.

		Section 1	**Section 3**
Visit 1	Value	4.7	10.1
	Δ in Observation	1.9	1.3
	p	0.000*	0.077
Visit 2	Value	6.6	11.4
	Δ with WBVT	-0.3	-0.6
	p	0.484	0.415
Visit 3	Value	6.3	10.9
	Δ in Follow-up	0.0	1.0
	p	1.000	0.161
Visit 4	Value	6.3	11.9

Note: Δ, change; WBVT, whole body vibration training; NPMDS, Newcastle Paediatric Mitochondrial Disease Scale.

4.6.2.2 Adult Cohort

The NMDAS and SF12v2 scores did not change significantly during the observation period. After WBVT the NMDAS Section 3 score decreased significantly by 1.7 points, p=0.010, indicating improvement in the clinician's clinical assessment (Table 2.27, Appendix F, Table F.3). There was a non-significant decrease in the NMDAS Section 1 score of 1.8 points, p=0.083, indicating that the patients perceived their current function to have improved after WBVT. There were no significant changes in Quality of Life parameters, however a non-significant increase in the role physical domain, 0.5 SDS, p=0.074, was consistent with the significant improvements seen in the NMDAS Section 3, and encouraging as this domain was significantly reduced compared to the reference population at baseline. There were no significant changes in the NMDAS or SF-12v2 parameters during the follow-up period.

Table 4.27. Newcastle Mitochondrial Disease Adult Scale and Quality of Life parameters for the adult cohort at each visit, changes between visits and p values. *p<0.05.

		NMDAS Section 1	**NMDAS Section 3**	**Role Physical SDS (SF-12v2)**
Visit 1	Value	9.8	10.2	-1.1
	Δ in Observation	1.5	-0.5	-0.3
	p	0.145	0.423	0.275
Visit 2	Value	11.3	9.7	-1.4

	Δ with WBVT	-1.8	-1.7	0.5
	p	0.083	0.010*	0.074
Visit 3	Value	9.5	8.0	-0.9
	Δ in Follow-up	0.7	0.9	-0.2
	p	0.490	0.155	0.361
Visit 4	Value	10.2	8.9	-1.1

Note: Δ, change; WBVT, whole body vibration training; NMDAS, Newcastle Mitochondrial Disease Adult Scale.

4.6.2.3 Leigh Syndrome Cohort

There were no significant changes in the NPMDS section scores during the observation or WBVT periods. However, Section 3 of the NPMDS increased significantly by 1.8 points, p=0.008, during the follow-up period indicating deterioration on the clinician's clinical assessment.

Table 4.28. Newcastle Paediatric Mitochondrial Disease Scale parameters for the Leigh Syndrome cohort at each visit, changes between visits and p values. *p<0.05.

		NPMDS Section 3
Visit 1	Value	9.6
	Δ in Observation	0.4
	p	0.493
Visit 2	Value	10.0
	Δ with WBVT	-0.6
	p	0.310
Visit 3	Value	9.4
	Δ in Follow-up	1.8
	p	0.008*
Visit 4	Value	11.2

Note: Δ, change; WBVT, whole body vibration training; NPMDS, Newcastle Paediatric Mitochondrial Disease Scale.

4.7 Adherence

Adherence was measured a counter in the vibration plate. Vibration plate counters were available for analysis in 14/19 participants (74%) and indicated overall adherence to treatment of 63%. Using the vibration plate counters 12/14 participants (86%) trained at least twice a week, 11/14 (79%) trained at least 3 times a week, 9/14 (64%) trained at least 4 times a week, and 5/14 (36%) trained at least 5 times a week.

4.8 Summary of Findings

This is the first study investigating bone health in individuals with MRCD.

4.8.1 Paediatric Cohort

4.8.1.1 Baseline

The paediatric MRCD cohort presented with mild-moderate impact of their disease on current status as measured by the NPMDS. Height and weight SDS as well as the total body lean tissue mass for height SDS measured by DXA and the muscle CSA SDS measured at the 66% tibial pQCT site were significantly reduced compared to the reference population. The proportion of lean and fat mass also appears to be disrupted with the SDS for total body fat measured by DXA towards the upper end of normal and the ratio of fat CSA for muscle CSA at the 66% pQCT site elevated at 70.3%. These abnormalities in nutritional indices, including longitudinal growth, body weight, lean

tissue and the proportion of muscle and fat mass [3, 93, 94], are risk factors that negatively impact bone health, as they compromise achievement of peak bone mass, ultimately impacting long-term bone health [4, 55, 89].

The paediatric cohort also demonstrated impairments in muscle performance during functional activities and evidence of reduced habitual WBPA, further risk factors for the attainment of genetically determined peak bone mass and the maintenance of bone mass throughout life [4, 55]. The force SDS in the M1LJ and CRT and force efficiency SDS in the S2LJ were significantly reduced. Functional muscle performance was also significantly impaired with significantly increased time to perform the CRT, a 6MWT distance of 52% of predicted and peak oxygen uptake corrected for body weight of 61% predicted. The limitations in muscle performance, ability to perform functional tasks and aerobic capacity have ramifications on the paediatric MRCD cohort's ability to participate in regular WBPA, especially the types of activities that promote optimal bone adaptations. Such activities have a high strain magnitude, apply strain at a rapid rate, have fast acceleration, are multidirectional in nature and produce ground reaction forces in excess of those produced during normal daily activities [97, 98, 100]. These activities would be particularly difficult for the paediatric MRCD cohort as few were able to jump and hop. As WBPA has been shown to significantly increase BMC, BMD and bone strength in children and adolescents [97, 102, 103], with the benefits impacting on bone health in later life [91, 101, 108, 110-112], the inability to particpate in the appropriate WBPA and generate normal muscle forces, reducing the strain magnitude and frequency imparted on the underlying bone, will impact on their life-long bone health. This is

especially pertinent to the paediatric MRCD cohort as the foundations of long term bone health are established in the childhood and adolescent years which represents an optimal time for skeletal adaptation [50, 90, 97, 98, 101-103, 113].

Compromised bone health was evident in both the trabecular and cortical bone compartments. Trabecular bone density SDS were significantly reduced at the lumbar spine, the vBMD measurement for lumbar vertebrae 1-4 and at the 4% tibial pQCT site. Impairments were also seen in cortical bone mass, density, geometry and strength. Cortical bone mass SDS at the 66% pQCT site was significantly reduced. Compromised cortical density was evidenced by significantly reduced legs BMD SDS measured by DXA, but significantly increased cortical vBMD at the 20% and 66% pQCT sites. Sub-optimal geometric attributes were demonstrated by significantly reduced SDS for cortical CSA, cortical thickness and total bone CSA at the 66% pQCT site. Compromised bone strength was evident at both the 20% and 66% pQCT sites. A further risk factor for compromised bone health was the uncoupling of normal bone turn-over, with half of the cohort having an increased level of the bone resorption marker, urinary deoxypyridinoline: creatinine ratio.

In summary, the paediatric MRCD cohort presented with short, narrow diaphyses, reduced cortical bone mass and strength but increased cortical density. Paradoxically, bone density in the trabecular bone compartment was low. The mechanostat theory proposes a feedback system that regulates the stiffness of the diaphyseal bone through the regulation of modelling and remodelling processes by the osteocyte in response to strains

imposed on the bone from mechanical loading [35, 47, 72, 75]. A functional mechanostat requires intact mechanical and biological aspects and a disruption to either of these aspects can affect the bone's mass and geometric adaptation to mechanical loading [35]. The mechanical aspect of the mechanostat can be investigated by analysing the functional muscle-bone unit using total body DXA derived parameters [687] and pQCT derived parameters [78]. Using the algorithm by Hogler et al [687], BMC for height was at the lower end of the normal range, LTM for height was significantly reduced, however BMC was appropriately adapted for LTM, indicating low bone mass was secondary to reduced lean mass, a secondary bone defect. The significantly reduced LTM and inadequate muscle forces imparted on the skeleton during habitual mechanical loading, resulting in lower magnitude bone strains, would not activate osteocytes to elicit accrual of bone mass, periosteal apposition and geometric bone adaptations as seen in their healthy counterpart, and would cause bone losses in the trabecular bone compartment and on the endosteal surfaces of cortical bone [35, 56, 72]. Furthermore, in the setting of reduced WBPA, intra-cortical remodelling is reduced culminating in the accumulation of older bone which becomes hyper-mineralised, increasing the bone's material density [6]. This increased density, further reduces tissue strains generated in the bone from mechanical loading. Older bone also has reduced osteocyte density and empty lacunae, which impairs the ability of the osteocyte network to sense and communicate strains, adversely affecting their viability and function [12, 38, 39], again interfering with the normal modelling and remodelling processes of the bone.

Using the algorithm by Schoenau et al [78], muscle CSA for height at the 66% tibial pQCT site was significantly reduced and bone mass was not appropriately adapted for muscle CSA indicating a mixed bone defect, and disruptions to the mechanical and biological aspects of the mechanostat. To provide further evidence of a mixed bone defect, bone-muscle proportions were also disrupted when considering the ratio of cortical CSA to muscle CSA at the 66% pQCT site, which was reduced at 4.3%, 5% indicating normal adaptation of the muscle and bone compartments. The significantly elevated cortical vBMD also supports a mixed bone defect. Cortical vBMD is linearly correlated with the intrinsic stiffness of bone tissue [126], a material property [6], and is tightly controlled to remain within a narrow optimal range which varies minimally between individuals [47, 48, 126]. The elevated cortical vBMD indicates the cortical bone tissue is stiffer than normal. This increase in stiffness means that when the bone is subjected to mechanical challenges form increasing bone length during growth and increased muscle force from WBPA, bone deformation will be reduced in comparison to the same mechanical load being applied to a bone with normal cortical vBMD [721]. This reduction in deformation culminates in diminished generation of tissue strains within the cortical bone tissue, and if it falls below the optimal minimally effective strains (MES) threshold range, the strain is not perceived by the osteocytes to require alterations in bone mass and geometry, and so the osteocyte does not direct bone cells to increase bone mass or optimise bone modelling during growth. This ultimately culminates in narrow diaphyses and reduced cortical bone mass, these inadequacies adversely affecting bone strength which is dependent on both bone quantity (mass) [1], and bone quality, which includes the geometric attributes of the bone [47, 48] . Furthermore, persistent absence of

strains in the MES threshold range, results in the osteocyte perceiving bone in the cortical compartment to be superfluous, directing the removal of cortical bone mass by endosteal resorption in an attempt to thin the bone so it will bend more easily with the application of mechanical loading, with the resultant associated bone strains then falling within the desired MES threshold range [35, 56, 72], but adversely affecting cortical thickness. This same mechanism drives bone resorption in the trabecular bone compartment, resulting in reduced trabecular BMD. This paradoxically high cortical vBMD but low trabecular vBMD also described in osteogenesis imperfecta [721].

A disruption in the biological aspect of the mechanostat could involve a genetic alteration to components of the organic extra-cellular matrix or an abnormality in the hydroxyapatite crystal size, shape or speed of the mineralisation process, either of which could result in hyper-mineralisation of the bone matrix [722], altered MES thresholds [35, 56, 72, 76], the inability of the osteocyte to correctly sense and interpret bone strains, interruptions to osteocyte communication with osteoblasts and osteoclasts and their ability to direct modelling and remodelling processes [12, 14, 21, 22], or a problem in the function of osteoblasts and osteoclasts themselves [114, 723-725].

4.8.1.2 Observation Period

The paediatric MRCD cohort reported significant reductions in patient and care-giver perceptions of current function as measured by the NPMDS. They followed their anthropometric trajectories, with no significant changes in SDS for height, weight or BMI

demonstrated nor were there significant changes in body composition SDS parameters. There were no changes in muscle functional performance measured by the CRT and 6MWT. In the trabecular bone compartment, bone mass of lumbar spine increased significantly (3.7%), which was above the reported CV at our laboratory (Table 2.1). however there were no changes in bone density. In the cortical compartment there were no changes bone mass however the SDS for legs BMD decreased significantly. This decline in leg BMD can be explained by the significant increases in leg bone area, which was not matched by increases in BMC of similar magnitude. It would appear that the rate of bone mineral accrual did not match the rate of bone area expansion, and this is a likely consequence of reduced habitual weight-bearing physical activity and progressive decline in muscle strength and function associated with MRCD in childhood [403-407, 410-412]. This mechanism is consistent with the mechanostat theory [47, 75] where bones adapt their structure, including bone mass, to withstand mechanical loads placed upon them from muscle contractions [73]. These findings are consistent with those previously documented, where impaired muscle activity or reduced weight-bearing physical activity has been linked to compromised bone mass accrual during skeletal growth [35, 56, 72]. Bone turnover markers remained stable indicating an ongoing state of increased bone resorption. In the paediatric MRCD cohort, bone density in the lower limbs was adversely affected during the observation period.

4.8.1.3 WBVT Period

Patient, care-giver and clinician assessment found the impact of disease to have remained stable as measured by the NPMDS. The paediatric MRCD cohort continued to follow their anthropometric trajectories, however there was a significant increase in soft tissue CSA at the 66% pQCT site which was accompanied by a significant increase in the muscle CSA, indicating a preferential distribution of weight gain into the lean tissue compartment. There was a significant reduction in the amount of force required to perform the CRT, indicating improved muscle efficiency, however performance in the 6MWT was unchanged. There were also significant improvements in parameters of both the trabecular and cortical bone compartments. Bone mass in the trabecular compartment increased significantly at the lumbar spine and 4% pQCT site and bone density at the lumbar spine after WBVT. In the cortical bone compartment, bone mass increased significantly on total body DXA and at the 20% and 66% pQCT sites. Cortical bone density measured by DXA and at the 20% pQCT site also demonstrated significant increases after WBVT. The significant reduction in leg BMD seen in the observation period was also attenuated. There were also significant increases in parameters of cortical bone geometry and strength at both the 20% and 66% pQCT sites. There was no significant change in the bone resorption marker, however osteocalcin, a bone formation marker, significantly decreased, but remained within its reference range. These favourable improvements in trabecular bone mass and density, cortical bone mass, density, geometry and strength, appear to be a result of the WBVT as they were not seen during the observation period. Adherence to the WBVT protocol in the paediatric MRCD

cohort was 62%. This amount of WBVT appears to be successful in improving trabecular and cortical bone health and muscle CSA in the calf of children with MRCD.

4.8.1.4 Follow-up period

Patient, care-giver and clinician assessment found the impact of disease to have remained stable as measured by the NPMDS. The paediatric MRCD cohort continued to follow their anthropometric trajectories, however there was a significant increase in fat CSA at the 66% pQCT site which was accompanied by a significant increase in the ratio of fat to muscle CSA and in the ratio of muscle CSA to total soft tissue CSA, indicating a preferential distribution of weight gain into the fat compartment. There was a significant increase in the amount of force required to perform the CRT, indicating worsening muscle efficiency, and performance in the 6MWT remained unchanged. Bone mass and density in the trabecular bone compartment demonstrated non-significant reductions. Cortical bone mass remained stable however the total body bone density decreased significantly. Cortical bone geometric attributes and strength demonstrated significant increases at the 66% pQCT site. There were no significant changes in bone turnover markers. These findings suggest a de-training effect in the follow-up period with particular effects in the trabecular bone compartment and soft tissue composition of the calf.

4.8.2 Adult Cohort

4.8.2.1 Baseline

The adult MRCD cohort presented with mild impact of their disease on current status as measured by the NMDAS. The mean BMI of the cohort was within the normal range however the muscle CSA measured at the 66% tibial pQCT site was significantly reduced compared to the reference population in males and post-menopausal females, indicating that the lean tissue compartment was compromised. Furthermore, total body fat percent for the adult MRCD cohort was 35.9%, the upper limit of normal for adult females but higher than that expected for adult males, and at the 66% pQCT site, fat CSA was 28.74cm^2, between the mean values seen in healthy adult males, 18.2cm^2, and females 34.2cm^2 [720], however fat CSA as a percentage of muscle CSA had a mean value of 56.7%), much higher than the 20% and 47% seen in their healthy counterpart [720]. The reduced lean mass and increased proportion of fat to muscle are important factors in the maintenance of bone health and these abnormalities may cause accelerated bone loss and the early development of osteoporosis [3, 95].

Consistent with the paediatric MRCD cohort, the adult MRCD cohort had impairments in muscle performance during functional activities and reduced habitual WBPA, further risk factors the maintenance of bone health [4, 55]. Force SDS in the M1LJ, S2LJ and CRT and force efficiency SDS in the S2LJ were significantly reduced. Functional muscle performance was also impaired with significantly increased time to perform the CRT, a

6MWT distance of 71% of predicted and peak oxygen uptake corrected for body weight and peak power output of 45% predicted. The inadequacies in lean mass, functional muscle performance and habitual WBPA were reflected in significantly reduced physical function related domains of the SF12v2. These limitations in muscle performance and ability to participate in regular WBPA, that is osteogenic or osteo-protective in nature [97, 98, 100], would have negative ramifications on the maintenance of bone health in adulthood.

Similar to the paediatric MRCD cohort, compromised bone health was seen in both the trabecular and cortical bone compartments. In the trabecular compartment, bone density at the 4% tibial pQCT site was significantly reduced compared to the reference population in males. Impairments were also seen in cortical bone mass, density, geometry and strength. Cortical bone mass at the 20% pQCT site was significantly reduced in males. Compromised cortical density was evidenced by significantly increased cortical vBMD at the 20%. Cortical bone geometric attributes were also significantly reduced at the 20% and 66% pQCT sites and compromised bone strength was evident at the 20% site. A further risk factor for compromised bone health was seen by the uncoupling of normal bone turnover, with the majority of the adult cohort having a decreased level of the bone formation marker, osteocalcin and an increased level of the bone resorption marker, urinary deoxypyridinoline: creatinine ratio, indicating that bone remodelling in the adult MRCD cohort favoured bone resorption and accelerated loss of bone, a suboptimal climate for the maintenance and preservation of long term bone health. In

adult medicine, bone turnover markers have been used to predict the presence and development of osteoporosis [726, 727] and risk of fracture [727, 728].

In summary, the adult MRCD cohort presented with a remarkably similar bone phenotype to the paediatric MRCD cohort, with narrow diaphyses, reduced cortical bone mass and strength, increased cortical density and reduced trabecular density. Total BMC for muscle CSA was not significantly different to the reference population, indicating that BMC was appropriately adapted to muscle CSA, suggesting the low bone mass phenotype seen in the adult cohort was secondary to reduced lean mass, a disruption to the mechanical aspect of the mechanostat. The preservation of the bone-muscle proportions when considering the ratio of cortical CSA to muscle CSA at the 66% pQCT site, also supports a secondary bone defect. The disruption to the mechanical aspect of the mechanostat was impacting on the magnitude and frequency of bone tissue strains generated, as well as the viability and function of the osteocytes [12, 38, 39] in their ability to sense and respond to bone strains, and altering the bone tissue environment by reducing intra-cortical remodelling resulting in older, more dense bone [6] and directing bone losses in the trabecular bone compartment and on the endosteal surfaces of cortical bone [35, 56, 72].

The significantly elevated cortical vBMD, cortical BMC for CSA ratio and distribution-mass curves at the 20% provide evidence for a mixed bone defect and an additional disruption to the biological aspect of the mechanostat. This is further supported by the findings in the distribution-quality curve at the 20% pQCT site that demonstrates reduced

cortical CSMI was a direct consequence of the elevated cortical density as the normal relationship of the mechanostat was preserved. The significantly reduced bone CSA in the MRCD adult cohort also supports an interruption to the biological aspect of the mechanostat. Any of the biological disruptions causing increased cortical density, described for the paediatric MRCD cohort, are likely to have been present since birth and could explain the small bone size seen in the adult MRCD cohort, as bone mass accrual, periosteal apposition and geometric adaptations would have been hindered during growth impacting on the size and integrity of the mature adult bone. This is an interesting finding, as only three of the twelve adult participants reported symptoms in their paediatric years, and despite the remaining nine adult participants describing symptom onset and diagnosis in their adult years, there appears to have been a disruption to normal bone modelling during the paediatric years.

4.8.2.2 Observation Period

Patient and clinician assessment found the impact of disease to have remained stable as measured by the NMDAS. There were no significant changes in anthropometric variables however total body LTM and legs LTM measured by DXA decreased significantly, a clinically concerning outcome due to the already reduced baseline LTM in this cohort seen at the 66% pQCT measured by muscle CSA. The loss of lean tissue mass was greater in the legs compared to the total body indicating that this area of the body is most sensitive to disease progression in MRCD. This is likely a consequence of the progressive decline in muscle strength, function, weight bearing physical activity and exercise

tolerance that is reported in MRCD [403-407, 410-412]. There were no changes in muscle force parameters or functional measures and physical domains of the SF12v2 remained stable. There were no changes to bone mass or density in the trabecular compartment or to bone mass or geometry in the cortical compartment, however legs BMD decreased significantly, likely associated with the significant decrease in legs LTM. According to the mechanostat theory [47, 75], bones adapt their structure, to withstand mechanical loads placed upon them from muscle contractions [73], to resist mechanical failure [74]. The reduction in LTM during the observation period would have likely reduced the magnitude of the habitual forces imposed on the underlying bones resulting in the reduced bone density seen in the adult MRCD cohort. Bone turnover markers did not change significantly, consistent with one previous study [729], and continued to reflect un-coupled bone turnover, with remodelling of the adult skeleton continuing to favour bone resorption and accelerated bone loss, consistent with the significant loss of BMD in the legs. In the adult MRCD cohort, lean tissue mass and bone density in the lower limbs demonstrated deterioration in the observation period.

4.8.2.3 WBVT Period

After WBVT, patient and clinician assessment found the impact of disease to have improved as measured by the NMDAS. There were no significant changes in anthropometric variables however the significant decline in total body LTM and legs LTM seen during the observation period was hindered. There were no significant changes in muscle force parameters however chair rise test speed increased significantly and there

was a non-significant increase on the 6MWT distance of 5%. A similar magnitude non-significant increase was also seen in peak VO_2 corrected for body weight. The role physical domain of the SF12v2 also demonstrated a non-significant increase of half a SDS. The trabecular bone compartment did not demonstrate any significant changes after WBVT however in the cortical compartment, legs bone density measured by DXA increased significantly. No changes in bone mass, geometry or strength were seen in the cortical bone compartment. Bone turnover markers remained unchanged, in a state of un-coupled bone turnover. Adherence to the WBVT protocol in the adult MRCD cohort was 64%. This amount of WBVT appears to have a beneficial effect of WBVT on physical function, lean tissue mass and bone density of the legs, counteracting deficits seen during the observation period.

4.8.2.4 Follow-up Period

Patient and clinician assessment found the impact of disease to have remained stable as measured by the NMDAS. There were no significant changes in anthropometric variables or lean mass. Maximum force and power in the CRT increased significantly, however 6MWT distance did not change significantly. There was a non-significant reduction in peak VO_2 corrected for body weight of 8% and several parameters at the anaerobic threshold also demonstrated clinically relevant reductions, indicating a deterioration in oxidative capacity. Physical domains of the SF12v2 remained stable. There we no significant changes in the trabecular bone compartment or in bone mass or strength in the cortical compartment. However, cortical density and geometry demonstrated significant reductions. Bone turnover markers did not change significantly with the persistence of

uncoupled bone turnover favouring bone resorption. When considering all outcomes, there appears to have been a detraining effect in the follow-up period with particular effects on muscular performance and the cortical bone compartment.

4.8.3 Leigh Syndrome Cohort

4.8.3.1 Baseline

The Leigh Syndrome cohort presented with mild impact of their disease on current status as measured by the NPMDS. The mean BMI of the cohort was at the lower end of the normal range. The distribution of soft tissue in the lean and fat compartments was unable to be analysed due to the mixed paediatric and adult nature of the cohort. Muscle force SDS were significantly reduced in the M1LJ and S2LJ, dynamic manoeuvres, however the force SDS for the CRT, a functional test, was significantly elevated, indicating adversely affected force production and muscle efficiency. Functional muscle performance was also impaired with a significantly increased time to perform the CRT and a 6MWT distance of 57% of predicted. The only bone parameters available to assess the cohort compared to a reference population were the total body and lumbar spine BMD SDS. The total body SDS was not significantly different to the reference population but the lumbar spine SDS was significantly reduced and lower than both the paediatric and adult cohorts, suggesting that the trabecular bone compartment may be more affected in Leigh Syndrome compared to other MRCD. Bone turnover markers indicated an increased level of the bone resorption marker, urinary deoxypyridinoline: creatinine ratio. Deficits in muscle force production and functional muscle performance

as well as the uncoupled bone turnover favouring bone resorption in the Leigh Syndrome would have contributed to the compromised bone health seen in the trabecular bone compartment.

4.8.3.2 Observation Period

Patient, care-giver and clinician assessment found the impact of disease to have remained stable as measured by the NPMDS. There were no significant changes in anthropometric variables or in muscle force parameters. However, the 6MWT distance and its percent of predicted decreased significantly indicating worsening of functional exercise capacity. There were no changes to bone mass or density in the trabecular compartment measured by DXA. In the cortical bone compartment, legs BMC and total body BMD increased significantly. Bone turnover markers did not change significantly, continuing to reflect increased bone resorption. Functional exercise capacity demonstrated the most prominent changes during the observation period.

4.8.3.3 WBVT Period

After WBVT, the impact of the disease on current function remained stable according to patient, care-giver and clinician assessment measured by the NPMDS. Weight increased significantly, a change that was not seen in the paediatric or adult cohorts. Muscle force parameters measured by the CRT did not change, however there was a non-significant 9% increase in the 6MWT distance, which contrasts to the significant decline seen in the

observation period. There were no changes to bone mass or density in the trabecular compartment measured by DXA or pQCT. In the cortical bone compartment, bone mass increased in the legs DXA measurement and at the 20% and 66% pQCT sites. There were no significant changes in bone density, however geometric attributes demonstrated significant increases in the legs DXA measurement and at the 20% and 66% pQCT sites. Bone strength increased significantly at the 20% pQCT site. Bone turnover markers did not change significantly, with an uncoupling of bone turnover favouring bone resorption persisting. Adherence to the WBVT protocol in the Leigh Syndrome cohort was 61%. This amount of WBVT appears to have been beneficial in slowing the decline in functional exercise capacity and in promoting changes in bone mass, geometry and strength in the cortical bone compartment.

4.8.3.4 Follow-up Period

Clinician assessment of current status measured by the NPMDS increased significantly during follow-up indicating worsening of clinical manifestations. Significant increases in weight were again seen, in contrast to that seen in the paediatric and adult cohorts. Muscle force parameters in the CRT increased significantly indicating further progression of abnormal muscle efficiency. There was not a significant change in the 6MWT distance. Similar to the observation and WBVT periods, there were no significant changes in bone mass or density in the trabecular bone compartment. In the cortical bone compartment, legs BMC measured by DXA increased significantly as did bone strength at the 66% pQCT site. There were no significant changes in bone turnover markers with

bone resorption measured by the urinary deoxypyridinoline: creatinine ratio remaining higher than the reference range. There does not appear to be a de-training effect after removal of the WBVT stimulus in the Leigh Syndrome cohort.

5 Discussion

The discussion regarding the effect of WBVT on chronic disease will be presented from the paediatric perspective, considering the CF cohort and the paediatric MRCD cohort, followed by the adult perspective, considering the adult MRCD cohort. Finally, the findings for the Leigh Syndrome cohort, a genetically identical, mixed adult and paediatric MRCD cohort will be presented. This approach is consistent with the existing literature in WBVT which predominantly reports paediatric or adult cohorts, and allows the investigation of the effects of WBVT on the immature growing skeleton as well as the mature adult skeleton, which may respond differently to WBVT.

5.1 Paediatric Perspective

5.1.1 Effect of WBVT on Bone and Body Composition Parameters

5.1.1.1 WBVT Period

5.1.1.1.1 Trabecular Bone Compartment

The trabecular bone compartment was investigated using lumbar spine DXA scans and pQCT at the 4% tibial site.

5.1.1.1.1.1 Bone Mass

The CF cohort and paediatric MRCD cohort demonstrated significant improvements in bone mass in the trabecular compartment measured by lumbar spine DXA and pQCT at the 4% site after WBVT. In the CF cohort, lumbar spine BMC increased significantly by 1.7g (5.4%), as did the BMC of lumbar vertebrae 1-4 by 2.1g (5.2%) In the paediatric MRCD cohort, lumbar spine BMC increased significantly by 1.4g (7.3%), as did the BMC of lumbar vertebrae 1-4 by 1.7g (7.0%). These increases in both cohorts were well above the reported CV at our laboratory (Table 2.1). The lumbar spine BMC SDS did not show a significant change after WBVT in either cohort indicating that these changes were likely consistent with normal growth. In the CF cohort, the improvements seen after WBVT were slightly smaller than the improvements seen during the observation period, suggesting no effect of WBVT on bone mass in the trabecular compartment. Previous studies have also suggested a negligible effect of WBVT on trabecular bone mass. In a study of adolescent females, mean age 17 years, with low bone density and a history of fracture, randomised to home-based low-magnitude vertical WBVT (30Hz, 0.3g, 10 minutes a day) or control, lumbar spine BMC significantly increased after WBVT by 2.1g (2%), however these improvements were not significantly different to their control group [207]. In another study of osteopoenic adolescent girls with idiopathic scoliosis, mean age 18 years, randomised to home-based low-magnitude vertical WBVT (32-37Hz, 0.3g, 20 minutes, 5 times a week for 12 months) or control, lumbar spine BMC increased by 1.2g (3.5%), p=0.05, after WBVT, however this was not significantly difference to the control group [206]. The smaller improvement in lumbar spine BMC seen in these studies, compared to the CF and MRCD cohorts, may be due to the use of low-magnitude

WBVT in comparison to the higher magnitude vibration used in the CF cohort. Another study of 19 children, 5-16 years of age, with cerebral palsy and other neurological conditions, using school-based low-magnitude vertical WBVT (40-42Hz, 0.2mm, 5 -15 minutes, at least twice a week for 6 months) improved lumbar spine BMC by 1.2g (7%). This improvement, however, was not found to be significantly different to their baseline measures [730].

In the paediatric MRCD cohort, as the observation and WBVT periods cannot be directly compared due to the varying lengths of the two periods, 4.3 months compared to 6.5 months respectively, the rates of increase in DXA parameters were calculated accounting for the disparity in the length of the two research periods. The rate of increase in lumbar spine BMC and the BMC of lumbar vertebrae 1-4 was 63%, 32% higher than the rate of increase seen during the observation period respectively. These improvements in bone mass support a beneficial effect of WBVT in the paediatric MRCD cohort, which has some support in the existing literature. Healthy children and children with Down Syndrome, 12-18 years of age, significantly increased lumbar spine (L1-4) BMC in both the Down Syndrome and healthy groups by 2.5g (6.6%), and by 3.4g (8.0%) after vertical WBVT (25-30Hz, 2mm, 5-10 minutes, 3 times a week, for 20 weeks) [731]. The percent changes in L1-4 BMC were similar in value to the increases seen in the paediatric MRCD cohort, despite the higher vibration frequency used. In another study of adolescents and young adults, mean age 16 years, with cerebral palsy GMFCS levels II and III, side-alternating WBVT (20Hz, 9 minutes a day, 4 times a week for 20 weeks) significantly

increased lumbar spine BMC by 2.7g (5.1%) [732], similar in magnitude to the results seen in both the CF and paediatric MRCD cohorts with a similar training stimulus.

Significant improvements in bone mass were also seen at the 4% pQCT site in the CF and paediatric MRCD cohorts after WBVT. In the CF Cohort, total bone BMC increased significantly by 7.5mg/mm (3.3%), much larger than the non-significant increase seen during the observation period in the CF cohort. Total bone BMC increased by 9.2mmg/mm (7.3%) in the paediatric MRCD cohort. Neither cohort demonstrated a significant improvement in trabecular BMC at the 4% tibial pQCT site which is calculated on the inner 45% of the 4% site cross-section. These results indicate that at the 4% pQCT site, WBVT appears to have improved bone mass from the outer perimeter inwards rather than the central area of the cross-section outwards. There are no reports in the existing literature of the effects of WBVT on bone mass at the 4% pQCT site.

5.1.1.1.1.1.1 Summary

Bone mass in the trabecular bone compartment, measured at different sites using two- and three-dimensional imaging techniques demonstrated significant increases after WBVT in the CF and paediatric MRCD cohorts. In the CF cohort, increases seen in the lumbar spine were very similar to those seen during the observation period and appears to be consistent with normal growth, however the increases seen at the 4% pQCT site were larger than those seen during the observation period in the CF cohort suggesting a benefit of WBVT on trabecular bone mass. The paediatric MRCD cohort demonstrated increases at the lumbar spine of greater magnitude compared to those seen during the observation

period as well as a significant increase at the 4% pQCT site, supporting a beneficial effect of WBVT on trabecular bone mass. Despite the lack of consensus, in the limited existing literature, on the effect of WBVT on trabecular bone mass in children, the increases seen in bone mass in the trabecular bone compartment, particularly at the 4% tibial pQCT site, will contribute beneficially towards improving trabecular BMD which was significantly reduced compared to the reference population prior to the commencement of WBVT at this site in both cohorts.

5.1.1.1.1.2 Bone Density

During the WBVT period, there were significant increases in trabecular bone density in the CF and paediatric MRCD cohorts. In the CF cohort, lumbar spine BMD increased significantly by 0.023g/cm^2 (2.6%), and trunk BMD from the segmental analysis of the total body DXA scan increased significantly by 0.018g/cm^2 (2.3%), similar in magnitude to the improvements seen during the observation period and above the CVs of our laboratory. The lumbar spine BMD SDS did not change significantly after WBVT, suggesting these changes in BMD were associated with normal growth, and suggest no effect of WBVT on trabecular bone density. Other studies have found similar outcomes. In a study of children with cerebral palsy and other neurological conditions, low-magnitude vertical vibration increased lumbar spine BMD by 0.016g/cm^2 (3%), however this improvement was not statistically different to the baseline value in this cohort and there was no corresponding change in the lumbar spine BMD SDS [730]. In children with Osteogenesis Imperfecta (OI), with a mean age 9 years, using side-alternating WBVT with a tilt-table (15-20Hz, 9 minutes twice a day for 6 months) as part of a 12-month

intensive physiotherapy program, lumbar spine BMD increased significantly by 0.05g/cm^2 (12%), however there was no change in the corresponding SDS [203]. These improvements in lumbar spine BMD were much larger than those seen in the CF cohort, perhaps due to the longer daily training, 18 minutes compared to 9 minutes, or the concurrent intensive physiotherapy program. In another study of adolescent females, home-based low-magnitude vertical WBVT improved lumbar spine BMD by 0.02g/cm^2 (2.3%), however this improvement was not significantly different to the control group [207]. This study also measured spinal volumetric BMD by quantitative computed tomography and found a 2.1% increase in this measure after WBVT, however, did not hold its significance when compared to the control group [207]. Two other studies investigating the effect of WBVT on spinal volumetric BMD using quantitative computed tomography. Six months low-magnitude vertical WBVT (90Hz, 0.3g, 10 minutes, 5 times a week) during a standing program in children with disabling conditions and motor impairment, with a mean age 9 years, found a signifincat increase of 5.5% in spinal vBMD, however this was not signifncatly different to the untreated group [197]. A second study in children with cerebral palsy, with a mean age 9 years, investigating the same intervention (low-magnitude vertical WBVT 30Hz, 0.3g, 10 minutes a day) using a randomised cross-over design, did not find any improvements in spine vBMD over time or compared to the control group [199]. Two previous studies have investigated the effect of WBVT on lumbar spine vertebrae 1-4 BMAD. The first, a shorter duration study of side-alternating WBVT (16-24Hz, 4mm, 2-6 minutes, 2-3 times a week for 3 months) in boys with Duchenne Muscular Dystrophy (DMD), mean age 7 years, found BMAD of the lumbar vertebrae 1-4 and its corresponding SDS did not change significantly after

WBVT [204], consistent with the results seen in the CF and paediatric MRCD cohorts. In contrast, adolescents with Down Syndrome and age-matched healthy controls, had significant improvements in lumbar vertebra 1-4 BMAD of 0.023g/cm^2 (2.9%) in those with Down Syndrome and 0.028g/cm^2 (3.5%) in the healthy controls after 20 weeks of vertical WBVT [731].

In the paediatric MRCD cohort, lumbar spine BMD increased 0.050g/cm^2 (7.4%), and the lumbar spine BMD SDS also increased significantly by 0.4. There was also a significant increase in trunk BMD of 0.018g/cm^2 (2.8%), however the corresponding SDS did not change significantly. The rate of increase in lumbar spine BMD was over 300% greater than the rate of increase seen in the observation period and the significant increase in trunk BMD was in contrast to a reduction in trunk BMD seen during the observation period. The increase in lumbar spine BMD and trunk BMD supports a beneficial role of WBVT in this cohort of children with MRCD, as the lumbar spine BMD SDS as well as the trunk BMD SDS were significantly reduced compared to the reference population prior to the commencement of WBVT. These increases in lumbar spine BMD seen in the paediatric MRCD cohort are consistent with the findings of previous studies investigating the effects of WBVT in other paediatric cohorts. Lumbar spine BMD increased significantly by 0.013g/cm^2 in children with cerebral palsy, 6-12 years of age, randomised to side-alternating WBVT with a tilt-table (12-18Hz, 2-4mm, 9 minutes, 5 times a week for 6 months) in addition to conventional physiotherapy, however this improvement was not significantly greater than the improvement seen in those randomised to conventional physiotherapy alone [198]. The magnitude of this

improvement was less than that seen in the paediatric MRCD cohort, and may be due to any of the following difference between the MRCD study and the Ruck et al [198] study: daily compared to 5 times a week WBVT; the vibration frequency of 20Hz compared to 18Hz; the use of shoes in the Ruck et al [198] study which dampens the transmission of the vibration stimulus to the legs; foot placement further away from the central fulcrum in the MRCD cohort resulting in greater amplitude displacement and therefore a greater vibration stimulus; and the use of a tilt table in the Ruck et al [198] study which reduces the amount of weight perpendicular to the vibration platform, again reducing the magnitude of the vibration stimulus delivered to the legs [198]. Another study in children with CP, utilising WBVT in addition to a conventional exercise training, found that lumbar spine BMD increased 0.16g/cm^2 after a similar intervention period [201]. The larger results seen in this study compared to the paediatric MRCD cohort may be due to additional benefit of the conventional exercise training in addition to the WBVT. In adolescents and young adults with cerebral palsy, 20 weeks of side-alternating WBVT significantly increased lumbar spine BMD by 0.014g/cm^2 (1.3%) [732], smaller than the improvements seen in the paediatric MRCD cohort despite a similar training protocol. However, the paediatric MRCD cohort had a younger mean age, which may have allowed greater improvements in the trabecular bone compartment in response to WBVT.

WBVT also had a significant effect on total vBMD measured by pQCT at the 4% site in the CF cohort, which increased by 6.056mg/cm^3 (2.0%), larger than the reported CV reported in the current literature [61], and no significant changes in these measures were seen during the observation period, suggesting the WBVT is having a beneficial effect on

vBMD in the trabecular bone compartment. Similar to the finding for BMC at the 4% pQCT site, there were no significant changes in trabecular vBMD, which measures the inner 45% of the pQCT cross-section, indicating that improvements in vBMD are occurring from the outer perimeter inwards. However, this increase in total vBMD will contribute beneficially towards improving trabecular bone density which was significantly reduced compared to the reference population at baseline at the lumbar spine and 4% pQCT site. In the paediatric MRCD cohort there was no effect of WBVT on total or trabecular vBMD at the 4% pQCT site. The results seen in the CF and paediatric MRCD cohorts for trabecular vBMD at the 4% pQCT site were consistent with a previous study in 6 ambulant boys with DMD, 7 years of age, where there was no significant change in trabecular vBMD at the 4% pQCT site after 3 months of side-alternating WBVT (16-24Hz, 4mm, 2-6 minutes, 2-3 times a week for 3 months) [214]. Two other studies investigated the effects of 6 months low-magnitude vertical WBVT during a standing program on proximal tibial trabecular vBMD measured by quantitative computed tomography. The first study involving children and adolescents, with disabling conditions, found a significant increase in proximal tibial trabecular vBMD of 6.27mg/ml (6.3%) in those using low-magnitude WBVT compared to a decrease in the same measure in the untreated group [197]. The second study in children with cerebral palsy, found no significant changes in proximal tibial trabecular vBMD with the addition of low-magnitude WBVT to a daily standing program using a randomised cross-over design [199]. The difference in results between these studies may be due to the higher vibration frequency used by Ward et al [197], 90Hz, compared to 30Hz in the Wren et al [199] study. Another study in adolescent girls, with idiopathic scoliosis, did not find an effect

of 12 months home-based low-magnitude vertical WBVT on distal tibial vBMD measured by high-resolution pQCT over time or compared to controls [206].

5.1.1.1.1.2.1 Summary

The greatest improvements in bone density in the trabecular bone compartment after WBVT occurred at the 4% distal tibial pQCT site in the CF cohort. The effects of WBVT may have been more pronounced at the 4% distal pQCT site compared to the lumbar spine, due to the proximity of this trabecular bone site to the vibration platform, which would have received the greatest vibration stimulus, before the acceleration was dissipated as it travelled caudally towards the lumbar spine. In contrast, the greatest improvements occurred in the lumbar spine in the paediatric MRCD cohort. The effects of WBVT may have been more pronounced in the lumbar spine compared to the 4% distal pQCT site, due to the side-alternating nature of the vibration stimulus which produces rotational movements around the pelvis and lumbar spine [154], the rapid repeated concentric and eccentric muscle contractions [159, 160] in this area having a more profound effect on the trabecular bone in the spine than the effect of the vibration stimulus on the distal tibia.

There are conflicting results in the current literature as to whether WBVT has a beneficial effect on bone density in the trabecular bone compartment measured by both two-dimension and three-dimensional techniques. This is likely a consequence of the variation in analysis sites and analysis methods described, the variable WBVT intervention protocols employed and the different patient groups investigated, whose underlying

conditions may cause bone to behave differently in response to WBVT. Larger studies, investigating different paediatric cohorts with factors compromising the attainment or maintenance of optimal bone health are required, and need to utilise similar analysis techniques to elucidate the effect of WBVT on the trabecular bone compartment centrally in the spine and peripherally in the metaphyseal regions of long bones.

5.1.1.1.2 Cortical Bone Compartment

The cortical bone compartment was investigated using total body DXA scans, segmental analysis of these scans, and the 20% and 66% sites of the tibial pQCT scans.

5.1.1.1.2.1 Bone Mass

Bone mass increased in the cortical bone compartment after WBVT in the CF cohort on analysis of total body DXA scans and pQCT scans at the 20% and 66% sites. Total body BMC increased significantly by 78.2g (4.6%), above the reported CV for this measure from our laboratory (Table 2.1), however this increase was smaller than the increase seen during the observation period and the corresponding SDS did not change significantly, suggesting normal increase with growth. The total body BMC for total body bone area SDS increased significantly during the WBVT period by 0.1, indicating that bone mass was being accrued at a greater rate than bone area expansion. Similar changes were seen in the paediatric MRCD cohort after WBVT. Total body BMC increased significantly by 46.8g (4.5%), however the corresponding SDS did not change significantly. The rate of increase in total body BMC during the WBVT period was 23% greater than the rate of

increase in BMC seen during the observation period and occurred in the context of equivalent increases in height in the two time periods, so cannot be accounted for by skeletal growth alone, supporting a beneficial effect of WBVT in the paediatric MRCD cohort. Furthermore, while not statistically significant, total body BMC SDS and the total BMC to total LTM SDS increased during the WBVT period, in contrast to reductions in these parameters seen during the observation period, and total body BMC to total body bone area SDS showed larger improvements during the WBVT period than during the observation period.

The improvements in total body BMC seen in the CF and paediatric MRCD cohort after side-alternating WBVT are similar to those reported in existing studies investigating the effects of side-alternating WBVT in other paediatric populations. In a study of children with bilateral spastic cerebral palsy, GMFCS I-V, with a mean age 9.8 years, using side-alternating WBVT with a tilt-table (5-25Hz, up to 9mm, 9 minutes twice a day for 6 months) as part of an intensive physiotherapy program (including resistance training, treadmill walking and hydrotherapy), total body BMC significantly increased 5.7% after 6 months of WBVT [200]. A similarly designed study in children with OI reported significant improvements in total body BMC of 63.1g (21%), but no significant change in the corresponding SDS, after side-alternating WBVT as part of a 12 month intensive physiotherapy program [203]. The larger increases seen in these studies compared to the CF cohort, likely attributable to the physiotherapy intervention which was concurrently performed, the higher amplitude used or longer training times. Another study using side-alternating WBVT in adolescents and young adults with cerebral palsy, found a

significant improvement in total body BMC after WBVT of 48g (2.3%) [732]. This improvement was slightly smaller than the one seen in the CF and paediatric MRCD cohorts, despite a very similar training protocol. In another study, 6 months vertical WBVT in children with cerebral palsy and other neurological conditions, significantly increased total body BMC by 33.5g (7%), a slightly larger percentage increase than that seen in the CF and paediatric MRCD cohorts, perhaps a reflection of the different vibration stimulus employed, vertical compared to side-alternating. Another study investigating the effect of side-alternating WBVT in children with DMD, found that 3 months of WBVT increased total body BMC by 47g (6.6%) [204], a slightly greater percentage increase compared to that seen in the CF and paediatric MRCD cohorts, perhaps due to the younger mean age in the Soderpalm et al [204] study. In a study of adolescent females, with a mean age 17 years, with low bone density and a history of fracture, randomised to low-magnitude vertical WBVT (30Hz, 0.3g, 10 minutes a day for 12 months) or control, total body BMC significantly increased by 53.5g (3.5%) however this was not significantly different to the control group [207]. Another study utilising vertical WBVT in adolescents with Down Syndrome and age-matched healthy controls, 12-18 years, demonstrated significant improvements in total body BMC of 51.7g (3.7%) in the Down syndrome group and 145.8g (8.0%) in the control group after 20 weeks of WBVT (25-30 Hz, 5-10 minutes, 3 times a week) [731]. The improvements seen in the Down Syndrome cohort were similar in magnitude to those seen in the CF cohort but smaller than the improvements seen in the control group, an indication that healthy adolescents with no musculoskeletal compromise may have a larger benefit from WBVT.

Improvements in bone mass were also seen when segmental analyses of the total body DXA scans were performed. In the CF cohort there was a statistically significant increases in legs BMC, of 35.1g (5.5%), above the reported CV from our laboratory, but similar in magnitude to the increases seen in these measures during the observation period, indicating that WBVT is unlikely to have influenced bone mass accrual.

In the paediatric MRCD cohort, legs BMC increased significantly by 11.8g (3.8%), however, the rate of increase in legs BMC during the WBVT period was similar to the rate of increase seen during the observation period again indicating no effect of WBVT on bone mass accrual. These findings were similar to those seen in the existing literature. Lower limb BMC significantly improved by 13g (2%) in adolescents and young adults with cerebral palsy, GMFCS II and III, with a mean age 16 years, after 20 weeks of side-alternating WBVT (20Hz, 406mm, 9 minutes, four times a week) [732], slightly smaller than the increases seen in the CF and paediatric MRCD cohorts despite the similar vibration training protocol. Another study using vertical WBVT (25-30Hz, 5-10 minutes, 3 times a week) in adolescents with Down Syndrome and age-matched healthy controls, 12-18 years, found that 20 weeks of WBVT, significantly increased lower limb BMC in those with Down Syndrome by 26.7g (6.0%) and in the healthy controls by 46.7g (6.9%) [731]. The gains in both these cohorts were greater than the gains seen in the CF and paediatric MRCD cohorts and may be a reflection of the WBVT stimulus used, vertical compared to side-alternating, or alternatively due to the adolescent age group studied by Matute-Llorente et al [731] where gains in BMC are likely to have been enhanced by the adolescent growth spurt [733]. One previous study, investigated the effect of WBVT on

femoral BMC and it did not find significant improvements in BMC of the femur after side-alternating WBVT in adolescents and young adults with cerebral palsy [732].

At both the 20% 66% tibial pQCT sites, significant increases in BMC were seen after WBVT in the CF cohort. Total bone and cortical BMC at the 20% pQCT site increased significantly by 8.0mg/mm (4.0%) and 7.3mg/mm (4.3%) respectively. The SDS for cortical BMC also increased significantly by 0.3. At the 66% pQCT site significant increases in total and cortical BMC of 7.3mg/mm (3.8%) and 7.6 mg/mm (3.5%), and the cortical BMC SDS increased significantly by 0.3. These improvements in BMC parameters at the 20 and 66% pQCT site were similar in magnitude to those seen during the observation period and are most likely associated with growth and not a direct consequence of the WBVT. In contrast the paediatric MRCD cohort demonstrated increases that would support a beneficial effect of WBVT. The largest increase in BMC in the paediatric MRCD cohort was seen in cortical bone at both the 20% and 66% pQCT sites. At the 20% site BMC significantly increased 8.7 mg/mm (8.7%) and the corresponding SDS increased 0.7, and at the 66% site BMC significantly increased 5.5mg/mm (4.6%) with a 0.3 standard deviation increase. Total bone BMC also increased significantly at the both pQCT sites. At the 20% site BMC increased by 7.1mg/mm (5.9%) and at the 66% site BMC increased by 6.8mg/mm (4.6%). There is no pQCT data for the observation period in the paediatric MRCD cohort so comparison between the two periods cannot be performed for parameters at the 20% and 66% pQCT sites.

All increases in BMC were larger in the 20% site cross-section than the 66% site cross-section in both cohorts. While the forces imparted to the underlying bone from the effects of the WBVT on the overlying muscle bulk should be greatest at the 66% site where the calf muscle has a larger cross-section and thus greater bulk and the capacity to generate larger forces to the underlying bone, greater increase in BMC was seen at the 20% site. This may be explained by the slightly closer proximity of the 20% site to the vibration platform in comparison to the 66% site. Due to this closer proximity greater force and acceleration occurs in the muscles at the 20% site compared to the 66% site, which are dissipated as the vibration stimulus travels in a cephalad direction [174]. However, the large disparity in muscle bulk between the two sites and the small distance between the two sites in the same long bone, suggests that the larger increases seen at the 20% tibial pQCT site in comparison to the 66% site may not be predominantly due to the effect of the vibration stimulus acting through the muscle, but rather a direct action of the vibration stimulus on the underlying bone. This mechanism has previously been described, where the WBVT acts directly on the bone by inducing mechanical deformation of the bone causing perturbations in canalicular fluid flow and stimulation of the osteocyte and the WNT/β-catenin pathway [196].

The existing literature does not report on the effects of WBVT on cortical bone mass in the peripheral skeleton measured by pQCT. While there is little evidence to support a beneficial role of WBVT for cortical bone mass in the CF cohort, the paediatric cohort with MRCD consistently demonstrated significant increases in cortical bone mass in the total body DXA scans, segmental analysis of these scans as well as at both the 20% and

66% pQCT sites after WBVT, which would support a beneficial role of WBVT in improving cortical bone mass. The significant increases in cortical bone mass are of particular importance for the bone health of children with MRCD as their cortical BMC SDS at the 66% pQCT site was significantly reduced compared the reference population prior to the commencement of WBVT and their cortical BMC SDS at the 20% tibial pQCT site as well as their total body BMC SDS were also below zero, though not significantly different to the reference population.

5.1.1.1.2.2 Bone Density

WBVT had a beneficial effect on the bone density in the cortical bone compartment when measured by both two- and three-dimensional analysis techniques. In the CF cohort total body BMD measured by DXA increased significantly by 0.020g/cm^2 (2.0%), larger than the CV for our laboratory (Table 2.1), but similar in magnitude to the increase seen during the observation period and not large enough to elicit a corresponding significant increase in the total body BMD SDS. In the paediatric MRCD cohort, total body BMD measured by DXA increased significantly by 0.012g/cm^2 (1.0%), and rate of increase was 34% larger than the rate of increase seen during the observation period, however the magnitude of this increase was within the CV for our laboratory (Table 2.1), and while statistically significant, cannot conclusively be attributed to WBVT. While there was no corresponding significant increase in the total body BMD SDS during the WBVT period, this parameter did increase during the WBVT period, in contrast to a reduction during the observation period.

The increases in total body BMD in the CF and paediatric MRCD cohorts was similar to that reported in existing studies investigating the effects of side-alternating WBVT in other paediatric cohorts. In a study of children with bilateral spastic cerebral palsy, GMFCS I-V, mean age 9.8 years, using side-alternating WBVT with a tilt-table, (5-25Hz, up to 9mm, 9 minutes twice a day) as part of an intensive physiotherapy program, total body BMD significantly increased by 2.3% after 6 months of WBVT [200]. This increase was as slightly larger in magnitude than the increases seen in the CF and paediatric MRCD cohorts however their intervention also involved resistance training and treadmill walking [200], which would have contributed to the BMD improvements. A similarly designed study in children with Osteogenesis Imperfecta, with a mean age 9 years, reported significant improvements in total body BMD of 0.01g/cm^2 (2%) after side-alternating WBVT with a tilt-table (15-20Hz, up to 9mm, 9 minutes twice a day for 6 months) as part of an intensive 12 month physiotherapy program [203]. There was no significant improvement in the total body BMD SDS [203]. These findings are similar to those seen in the CF and paediatric MRCD cohorts however they were measured after 12 months, indicating a faster improvement in the CF and MRCD cohorts as it occurred in half the time. Another study using side-alternating WBVT (20Hz, 4-6mm, 9 minutes, 4 times a week) in adolescents and young adults with cerebral palsy, GMFCS II and III, with a mean age 16 years, found a significant increase in total body BMD of 0.008g/cm^2 (0.8%) after 20 weeks of training [732]. The smaller increase in BMD in the Gusso et al [732] study compared to the CF and paediatric MRCD cohorts, despite the similar training protocol, is likely due to the younger mean age of the CF and paediatric MRCD cohort and greater capacity for optimisation of bone density in the short intervention

period. In a study of children with cerebral palsy and other neurological conditions, with a mean age 12.5 years, 6 months vertical WBVT (40-42Hz, 0.2mm, 2-15 minutes, twice a week for 6 months) significantly improved total body less head BMD by 0.014g.cm^2 (2%) [730], similar in magnitude to the improvement seen in the CF and paediatric MRCD cohorts. Furthermore, this same study did not find any improvement in the total body BMD SDS score after 6 months of WBVT [730]. Another study investigating the effect of WBVT in children with Duchenne Muscular Dystrophy, mean age 7 years, found that 3 months of side-alternating WBVT (16-24Hz, 4mm, 2-6 minutes, 2-3 times a week) did not result in any significant improvement in total body BMD [204] perhaps due to the lower training volume and shorter intervention time compared to the CF and paediatric MRCD cohorts. In a study of adolescent females with low bone density and a history of fracture, with a mean age 17 years, home-based, low-magnitude vertical WBVT (30Hz, 0.3g, 10 minutes a day for 12 months) increased total body BMD by 0.01g/cm^2 however this increase was not significantly different to baseline or the control group, a likely consequence of the absence of limitations in the muscle function, mobility or participation in weight-bearing physical activity of the cohort [207]. Vertical WBVT (25-30Hz, 5-10 minutes, 3 times a week for 20 weeks), in adolescents with Down Syndrome and age-matched healthy controls found significant improvements in total body BMD in the healthy controls of 0.037g/cm^2 (3.7%), however there was no significant change in total body BMD in the Down Syndrome group [731]. The improvements in total body BMD in the control group likely reflect the greater capacity of able-bodied adolescents without musculoskeletal impairments to improve their BMD with WBVT during adolescence.

In the CF cohort, bone density significantly improved during the WBVT period when the segmental analyses of the total body DXA scans were analysed. Arms BMD increased significantly by 0.009g/cm^2 (1.3%), however there was no significant change in the corresponding SDS and this improvement fell within the CV reported by our laboratory (Table 2.1). Legs BMD increased significantly after WBVT by 0.031g/cm^2 (3.0%), demonstrating a larger improvement compared to the observation period, indicating that while only small in magnitude, these improvements in BMD in the lower limbs may be associated with WBVT.

Several existing studies support a beneficial effect of WBVT on the lower limbs in paediatric populations. A study in adolescents and young adults with cerebral palsy, GMFCS II and III, with a mean age 16 years, which demonstrated significant improvements in BMD of the legs by 0.023g/cm^2 (2.2%) after 20 weeks of side-alternating WBVT (20 Hz, 4-6mm, 9 minutes, 4 times a week) [732]. Another study in children with cerebral palsy found a significant improvement in femur BMD of 0.27g/cm^2, following WBVT in addition to conventional exercise compared to exercise alone [201]. This improvement was much larger than the improvement seen in the current study any may be a reflection of the additional benefit of the conventional exercise training in conjunction with WBVT. The benefit of vertical WBVT (25-30Hz, 5-10 minutes, 3 times a week) in improving legs BMD was also demonstrated in adolescents with Down Syndrome and age-matched healthy controls after 20 weeks of WBVT, with legs BMD significantly increasing in the Down Syndrome and healthy control groups by

0.052g/cm^2 (2.7%) and 0.064g/cm^2 (3.1%) respectively [731], similar in magnitude to that seen in the CF cohort. Arms BMD also increased significantly in the healthy control by 0.029g/cm^2 (2.4%), larger than the increase seen in the CF cohort, however there was no significant improvement in arms BMD in the Down Syndrome group [731]. A study investigating the effect of home-based low-magnitude, vertical WBVT (32-37Hz, 0.3g, 20 minutes, 5 times a week, for 12 months) in adolescent girls (mean age 18 years) with idiopathic scoliosis, femoral neck BMD improved significantly by 0.015g/cm^2 (2.2%) on those randomised to the WBVT and this was significantly greater than the control group [206].

In children with MRCD, bone density on segmental analyses of the total body DXA did not change significantly after WBVT. However, the significant reduction in leg BMD SDS seen during the observation period did not continue during the WBVT period and the rate of decline in this parameter was hindered. These results are encouraging and support a benefit of WBVT for improving bone health in children with MRCD through the maintenance of BMD. The lack of significant improvements in lower limb BMD in the paediatric MRCD cohort compared to the existing literature [731, 732], despite a similar training protocol [732] suggests the differences in effects are likely due to the underlying disease processes, or may be explained by the different WBVT stimulus used, vertical [731] compared to side-alternating respectively. Ruck et al [198] did not find any significant changes in femur BMD after 6 months of side-alternating WBVT with a tilt-table (12-18Hz, 2-4mm, 9 minutes a day, 5 times a week for 6 months) and conventional physiotherapy, compared to conventional physiotherapy alone [198].

Volumetric BMD increased significantly at the 20% but not at the 66% pQCT site in both the CF and paediatric MRCD cohorts. In the CF cohort, the vBMD of the total and cortical sectors at the 20% site increased by 9.8g/cm^3 (1.6%), and 7.1g/cm^3 (0.6%) respectively, and the SDS for cortical vBMD increased significantly by 0.2. These increase in vBMD of the cortical sector were above the CV reported in the literature [117] (Table 2.2), however the improvements in all these parameters were similar to those seen during the observation period and are unlikely to be attributable to the WBVT intervention. In the paediatric MRCD cohort, the vBMD of the total and cortical sectors at the 20% site increased by 26.9mg/cm^3 (4.8%), and 13.4mg/cm^3 (1.2%), however there was no change in the cortical vBMD SDS. Despite the lack of significant increases in vBMD at the 66% pQCT site, there was a non-significant increase in the cortical vBMD SDS of 0.60 after WBVT, a clinically relevant improvement. There is no pQCT data for the observation period in the paediatric MRCD cohort so comparison between the two periods cannot be made.

Previous studies have not found significant improvements in bone density measured by three-dimensional techniques after WBVT. A study investigating the effect of side-alternating WBVT (20Hz, 4-6mm, 9 minutes, 4 times a week) on vBMD at the 20% pQCT site in adolescents and young adults with cerebral palsy, GMFCS II and III, with a mean age 16 years, and they did not find a significant increase after 20 weeks of WBVT [732] despite a similar training protocol, a difference which may be related to the younger age of the CF and paediatric MRCD cohorts compared to the cerebral palsy

cohort. A study investigating the effect of 6 months low-magnitude vertical WBVT (90Hz, 0.3g, 10 minutes, 5 times a week) during a standing program in children with disabling conditions and motor impairment, with a mean age 9 years, did not find a significant improvement in cortical vBMD measured between 25-50% of proximal tibial length using quantitative computed tomography in those randomised to the WBVT or placebo groups [197]. Another study using side-alternating WBVT (16-24Hz, 4mm, 2-6 minutes, 2-3 times a week) in boys with Duchenne Muscular Dystrophy, mean age 7 years, did not find significant changes in cortical vBMD at the 66% pQCT site after 3 months of training [214]. The difference on the current outcomes compared to these studies may be explained by the difference in the vibration platforms used [197], the different analysis site [197] and shorter intervention period [214].

5.1.1.1.2.3 Bone Area and Geometry

In the cortical bone compartment, WBVT in the CF and paediatric MRCD cohorts was associated with significant increases in bone area measured by DXA and the long bone's geometric attributes measured by pQCT at the 20% and 66% pQCT sites. Total body bone area significantly increased by 45.1cm^2 (2.8%), however this improvement was less than that seen during the observation period, and the total bone area for height SDS decreased significantly during the WBVT by 0.2. Segmental analysis of the total body DXA scans also revealed significant improvements in legs bone area of 16.5cm^2 (2.8%) after WBVT, slightly smaller in magnitude compared to the change seen during the observation period. The results in the CF cohort suggest that WBVT did not appear to be beneficial in improving bone area measured by DXA.

In the paediatric MRCD cohort, total body bone area significantly increased 37.9cm^2 (3.1%). The rate of increase in total bone area was 5% greater in the WBVT period than the rate of increase seen during the observation period and occurred in the context of equivalent increases in height in the two study periods so cannot be attributed to growth alone. Furthermore, while there was no significant increase in the total bone area to height SDS during the WBVT period, this parameter did increase, which was in contrast to a reduction in this parameter during the observation period. Segmental analysis of the total body DXA scans revealed significant improvements in leg bone area after WBVT of 12.7cm^2 (3.1%). In contrast to the total body DXA scan where the rate of expansion of total bone area was greater during the WBVT period, the rate of expansion of the legs bone area was greater during the observation period. The results in the paediatric MRCD cohort suggest that WBVT may be beneficial in improving bone area measured by DXA. There are no published data on the effect of WBVT on bone area measured by DXA.

The effect of WBVT on bone geometry in the cortical bone compartment was further investigated with pQCT scans of the 20% and 66% pQCT sites which allows a three-dimensional analysis of the bone's geometric attributes. WBVT was associated with significant increases in CSA and cortical thickness at both the 20% and 66% pQCT sites. At the 20% tibial pQCT site, CSA of the total and cortical bone sectors increased significantly after WBVT in the CF cohort by 7.4mm^2 (2.3%) and 5.6mm^2 (3.6%), and the total and cortical bone CSA SDS increased significantly by 0.3 and 0.2 respectively. These increases were larger than the reported CV in the existing literature (Table 2.2) and

the changes in the total and cortical bone CSA and their corresponding SDS changed with similar magnitude to the observation period. Cortical thickness and periosteal circumference and their corresponding SD scores also increased significantly after WBVT at the 20% pQCT site in the CF cohort by 0.1mm (2.7%) and 0.2, and 0.8mm (1.2%) and 0.2, the magnitude of all these improvements being similar to those seen during the observation period. Total bone CSA at the 66% pQCT site increased significantly by 19.0mm^2 (3.9%) and 0.3 SDS, cortical bone CSA increased significantly by 6.8mm^2 (3.1%) and by 0.2 in the corresponding SDS, and periosteal circumference increased significantly by 1.5mm (1.9%), these changes also similar in magnitude to the changes seen during the observation period. There were no significant changes in cortical thickness at the 66% pQCT site.

In the paediatric MRCD cohort, there were no significant changes in total bone CSA at the 20% or 66% pQCT sites however, cortical CSA at the 20% site increased significantly by 6.8mm^2 (1.1%) and the corresponding SDS increased significantly by 0.6. The percentage improvement in cortical CSA may fall within the reported CV for this measure [117, 148], however the corresponding significant increase in the SDS of over half a standard deviation confirms the clinical relevance of this improvement. Cortical CSA at the 66% tibial pQCT site increased significantly by 3.7mm^2 (3.2%) with a significant increase of 0.2 in the associated SDS. The percentage improvement in cortical CSA was greater than the CV reported in the literature [61]. Cortical thickness also increased significantly after WBVT at the 20% pQCT site by 0.2mm (7.6%) with an associated significant increase in the SDS of 0.6. There were no significant changes in

periosteal circumferences seen after WBVT at either the 20% of 66% pQCT sites. While there is no pQCT data for the observation period in the paediatric MRCD cohort to make comparisons, it appears that in contrast to the CF cohort where WBVT did not seem to have a beneficial effect on bone geometry, WBVT appears to have been beneficial in the paediatric MRCD cohort, especially in the cortical bone sector.

Few studies have investigated the effect of WBVT on the geometric attributes of the cortical bone compartment in children using three-dimensional analyses. The study by Ward et al [197] using low-magnitude vertical WBVT (90Hz, 0.3g, 10 minutes 5 times a week for 6 months) during a standing program in children with disabling conditions and motor impairment, with a mean age 9 years, did not find a significant improvement in cortical CSA, cortical thickness or periosteal circumference measured between 25-50% of proximal tibial length using QCT over time or between those randomised to the WBVT or placebo groups [197]. In another study investigating the effects of low-magnitude vertical WBVT (30Hz, 0.3g, 10 minutes a day for 6 months) with a randomised cross-over study in children with cerebral palsy GMFCS I-IV, with a mean age 9 years, QCT of the tibial mid-shaft found an 8.5% increase in cortical CSA after WBVT which was significantly greater than the control group [199]. This improvement was much larger than that seen in the CF cohort. The disparity in the results reported in these studies compared to the current study, is likely a reflection of the different type of WBVT used, low magnitude and high frequency, generating a much lower acceleration compared to the WBVT used in the current study, the different analysis site and technique used, 25-50% of tibial length measured from the proximal end using QCT

compared to pQCT measured from the distal end in the current study, or the different paediatric populations investigated [197, 199].

Consistent with DXA findings in the paediatric MRCD cohort, results from pQCT at the 20% and 66% distal tibial sites in this cohort support a beneficial effect of WBVT in improving bone geometry in paediatric patients with MRCD. The significant improvements seen in the paediatric MRCD cohort are of particular benefit to their bone health, as the SD scores for total bone CSA and cortical CSA at the 66% tibial pQCT site were significantly reduced compared to the reference population prior to the commencement of WBVT, and the SD score for cortical CSA at the 20% tibial pQCT site was reduced at -1.07 but not significantly different to the reference population (Table 2.5 and Figure 2.18). The small magnitude of the significant improvements in bone geometry seen in the current study, along with the paucity of data in the in the existing body of research and the conflicting results in that body of evidence, indicate that larger studies, with a longer WBVT intervention period are required to fully understand the effects of WBVT on bone geometry in children.

5.1.1.1.2.4 Bone Strength

Surrogate measures for bone strength, including cross-sectional moment of inertia (CSMI), section modulus and stress-strain index (SSI), increased significantly in the cortical bone compartment at both the 20% and 66% tibial pQCT sites after WBVT in the CF and paediatric MRCD cohorts. In the CF cohort, the polar measurement of cortical CSMI at the 20% site increased significantly by 649.7mm^4 (5.4%), with similar results

seen for the polar measurement of cortical SSI of 43.5mm^3 (4.7%). Total bone polar CSMI also increased significantly after WBVT by 807.9mm^4 (5.3%), as did total bone polar SSI and its corresponding SDS by 46.2mm^3 (4.6%) and 0.3 respectively. The CV reported in the current literature for polar SSI at the 20% tibial pQCT site is less than 5% [148], so caution should be used when interpreting clinical relevance of these results. At the 66% site, the polar measurement of cortical CSMI and its corresponding SDS increased significantly by 1967.0mm^4 (7.0%) and 0.2, as did the polar measurement for cortical SSI and its corresponding SDS by 77.7mm^3 (5.2%) and 0.3. The increases in these 20% and 66% site parameters were similar in magnitude to the increases seen in the observation period, indicating that WBVT may not have had a beneficial effect on bone strength in the CF cohort.

In the paediatric MRCD cohort, cortical polar CSMI increased significantly at the 20% by 309.0mm^4 (6.6%) and at the 66% site, by 716.4mm^4 (8.9%) with a significant improvement in its corresponding SDS score of 0.1. Cortical polar SSI significantly increased at the 20% site by 35.4mm^3 (7.8%) and at the 66% site by 45.1mm^3 (7.3%) with the SDS of the latter parameter also increasing significantly by 0.3. Significant improvements were also seen in the total bone polar SSI and the corresponding SDS score at the 20% site of 31.6mm^3 (6.3%) and 0.3 respectively. These findings were larger than the CV reported for polar SSI in the literature [148]. There was no pQCT data for the observation period in the paediatric MRCD cohort to make comparisons, however all increases were larger in magnitude than those seen in the CF cohort, and were in keeping with the significant improvements in CSA and cortical thickness seen in the paediatric

MRCD cohort which reflect an enhancement of the geometric attributes of the cortical bone cross-section at these two analysis sites. These favourable geometric adaptations, along with the significant increases in bone mass, played a focal role in the significant improvements in bone strength seen in the cortical bone compartment after WBVT.

Three previous studies have investigated the effect of WBVT on cortical bone strength in children. The study by Ward et al 2004 [197] did not find a significant improvement in polar CSMI measured between 25-50% of proximal tibial length using QCT after WBVT [197]. Gusso et al 2016 [732] did not find any significant improvements in polar SSI at the 20% tibial pQCT site in adolescents and young adults with cerebral palsy [732] despite a very similar training protocol to that used in the CF and MRCD cohorts, however the significant increases seen may be due the younger mean age of participants and a greater ability of the developing skeleton to respond to the WBVT. The study by Wren et al 2010 [199] found significant improvements in the polar measurement of CSMI in the tibial mid-shaft, which increased ~17% after WBVT, significantly larger than the control group [199] and also the increases seen in the CF and MRCD cohorts. The differences between the results reported in the current literature and the CF and MRCD cohorts are likely a consequence of different study populations, vibration platforms used, analysis sites and techniques performed [197, 199].

5.1.1.1.2.5 Body composition

WBVT in the CF cohort was also associated with significant increases in lean tissue mass. Total body LTM measured by DXA increased significantly by 1026.3g (3.1%),

above the CV reported by our laboratory (Table 2.1), however there was no significant change in the LTM for height SDS indicating that the improvement seen during the WBVT was not likely to be attributed to the WBVT but rather normal growth. Segmental analysis of the total body DXA scans found significant improvements in legs LTM of 283.0g (2.7%) after WBVT. These increases in LTM were smaller increase compared to that seen during the observation period, suggesting WBVT did not have a beneficial effect on LTM in the CF cohort. Two previous studies investigating the effect of WBVT on LTM measured by total body DXA scans found similar responses to those seen in the CF cohort. In a study of children with cerebral palsy using side-alternating WBVT as part of an intensive physiotherapy program, total body LTM measured by DXA significantly increased 3.1% after 6 months of WBVT [200], similar in magnitude to the increase seen in the CF cohort, despite the addition of resistance training and treadmill walking during the WBVT intervention, which would have contributed to the improvement seen in LTM [200]. Another study found 6 months of side-alternating WBVT in adolescents and young adults with cerebral palsy significantly increased LTM of the total body and legs by 2.1% and 2.2% respectively [732], slightly smaller than the increases seen in the CF cohort.

The paediatric MRCD cohort did not reveal any significant increases in total body LTM, the LTM to height SDS or LTM of the legs. This area however did have the greatest non-significant improvement in LTM during the WBVT period compared to the observation and follow-up periods. Three previous studies also found no effect of WBVT on LTM measured by DXA. In a study of children with cerebral palsy and other neurological

conditions, 6 months of vertical WBVT did not significantly increase total body LTM [730]. In adolescents with Down Syndrome, 20 weeks of vertical WBVT did not significantly increase total body LTM or LTM of the lower limbs [227]. The use of vertical WBVT in adolescent females with low bone density and a history of fracture, did not significantly increase total body LTM measured by DXA after 12 months of WBVT [207].

Analysis of the soft tissue compartment at the 66% pQCT site did not show any significant changes after WBVT in the CF cohort, however the increase in fat CSA seen during the observation period was ablated, a potentially beneficial effect of WBVT. These findings are consistent with two previous studies. pQCT analysis of muscle CSA at the 66% tibial site in boys with Duchenne Muscular Dystrophy did not find a significant increase in muscle CSA after 3 months of side-alternating WBVT [214], and the study by Ward et al [197] using low magnitude, high frequency WBVT during a standing program in children with disabling conditions and motor impairment did not find a significant improvement in muscle CSA measured between 25-50% of proximal tibial length using QCT [197].

When the 66% pQCT site was analysed in the paediatric MRCD cohort, significant increases were found after WBVT in total soft tissue CSA, muscle CSA and the muscle CSA SDS. These parameters increased significantly by 223.6mm^2 (4.7%), 145.6mm^2 (5.4%) and 0.2 respectively. One previous study supports these findings. After 20 weeks of side-alternating WBVT in adolescents and young adults with cerebral palsy, muscle

CSA at the 50% tibial pQCT site increased significantly by 3.8% [732], a result similar to that seen in the paediatric MRCD cohort. The improvements in LTM may only have been significant at the 66% pQCT site in the paediatric MRCD cohort as this analysis technique can measure true muscle CSA [145], a more accurate measure than DXA scanning techniques allow.

The current study, with some support from the existing literature, would suggest a positive benefit of WBVT for increasing LTM in the paediatric cohorts, especially the paediatric MRCD cohort. The significant improvements in muscle CSA is of particular importance to the paediatric MRCD cohort as their muscle CSA SDS was significantly reduced compared to the reference population prior to the commencement of WBVT, and likely played an important role in the significantly reduced bone mass seen at the 66% pQCT site, as well as the compromised geometric attributes (cortical CSA, total CSA and cortical thickness), and reduced bone strength (polar SSI and polar CSMI) at the 20% and 66% pQCT sites also seen prior to commencement of WBVT (Table 4.5 and Figure 4.18). The significant improvements in muscle CSA after WBVT may have been focal in the significant improvements in bone mass, geometry and strength seen after WBVT. The close interaction between LTM and bone mineral content is known as the functional muscle-bone unit [78], and strong correlations between total body BMC and total body LTM have been shown in studies using DXA [82-84] and between BMC and muscle cross-sectional area in studies using pQCT [61, 78, 85]. The functional muscle-bone unit is also involved in the changes in long bone geometry observed during growth, whereby the geometry of the bone is adapted to the mechanical loads habitually applied to it to

maintain its strength [1, 51, 64]. As previously suggested, the effect of WBVT on the leg muscles, generating rapid muscular contractions and increasing muscle tone [159, 160], along with the subsequent transmission of muscle forces generated to the underlying bone [196], cannot be recreated through the normal movements of children with MRCD due to their compromised muscle strength and function and impaired ability to participate in normal locomotion and weight-bearing physical activities [403-407, 410-412]. Therefore, we can propose that WBVT has a beneficial effect on LTM, the accrual of bone mass and promotes favourable geometric adaptations of long bones in children with MRCD during growth directly through its actions on muscles and the underlying bone, which in turn precipitated improvements in cortical bone strength parameters. In conclusion, WBVT appears to have significantly improved many aspects of the cortical bone compartment, including those significantly compromised compared to their peers prior to commencement of WBVT, culminating in generally improved bone health in children with MRCD.

5.1.1.1.2.6 Bone Biochemistry and Turnover Markers

After WBVT, there were no significant changes in bone turnover markers in the CF cohort. Bone biochemistry, demonstrated significant decreases in calcium and magnesium however they remained within their corresponding normal reference ranges. There was a significant decrease in osteocalcin in the paediatric MRCD cohort, which is of questionable clinical significance as improvements in bone health were seen during the WBVT period. These results were consistent with those of a paediatric study investigating the effect of WBVT on a dynamic platform with a mean of 22 minutes a

week at 40-42Hz, in patients with severe motor disabilities including cerebral palsy [730]. After 6 months of training, there were no significant differences in calcium, phosphate, parathyroid hormone, vitamin D, osteocalcin, PINP, bone specific alkaline phosphatase or CTX [730].

The WBVT period was associated with several favourable adaptations in the trabecular bone compartment for bone mass and density and also in the cortical bone compartment for bone mass, density, area and geometry, and strength in the paediatric MRCD cohort. The magnitude and diversity of the response to WBVT seen in these bone parameters measured by DXA and pQCT were much larger than those seen in the observation period however there were not any changes in the bone turnover markers to reflect this. This may be explained by the fact that the bone turnover markers used have wide reference ranges and large inter-individual variation, resulting in no discernible difference between the two study periods despite the difference in bone response. Alternatively, the bone turnover markers used in the paediatric MRCD cohort may not have been sensitive enough to identify a difference with WBVT. Markers of bone formation including bone specific alkaline phosphatase [734] and PINP [735] are considered to be more robust measures than osteocalcin and alkaline phosphatase due to confounding influences on these measures, including the sample instability and susceptibility to processing and storage practices for osteocalcin as well as it being non-homogenously distributed in the circulation and having high individual variability [736], and the influences from gut, liver, kidney and brain when alkaline phosphatase is used [736]. Bone specific alkaline phosphatase, an osteoblast ectoenzyme that helps to regulate bone mineralisation, more

specifically assesses osteoblast function compared to alkaline phosphatase, and the production of bone specific alkaline phosphatase has been found to positively correlate with bone formation measured by histomorphometry [736, 737]. PINP is released during the synthesis of type I collagen from procollagen, reflecting a different aspect of osteoblast activity and bone formation compared to osteocalcin, a protein synthesised by osteoblasts that assists in the regulation of bone mineralisation, and bone specific alkaline phosphatase, an osteoblast enzyme which also assists in the regulation of bone mineralisation [736-738]. Age-specific reference ranges are available for the analysis of BAP [727, 738, 739] and PINP [735, 738] in paediatric patients. Serum-derived markers of bone resorption are also preferred over urinary-derived measures as there is less variability [727, 736, 740-742] due to difficulties with serial measurements [738, 743, 744], susceptibility to UV exposure [736], the effect of changing muscle mass and age when expressed relative to creatinine [727, 740, 745] and large variations between individuals [741, 746] that are associated with urinary-derived measures. Age and gender-specific references ranges for CTX are available in the paediatric population [727, 738]

5.1.2 Follow-up Period

5.1.2.1 Trabecular bone compartment

5.1.2.1.1 Bone Mass and Density

During the follow-up period, the CF cohort demonstrated significant increases in BMC of the lumbar spine and lumbar vertebra 1-4 of 1.4g (4.3%) and 1.7g (4.1%), both smaller in magnitude than the increases seen during the WBVT period. There were no significant changes in the corresponding SDS of these two parameters however the trunk BMD SDS for segmental analysis of the total body DXA scan decreased significantly by 0.1. The paediatric MRCD cohort did not demonstrate any significant changes in trabecular bone parameters measured by DXA at the lumbar spine. However, in contrast to the significant increases seen after WBVT and relative stability of these parameters in the observation period, the lumbar spine BMC SDS and BMD SDS decreased by 0.2 and 0.3 of a standard deviation respectively, and these decreases neared significance, p=0.059 and p=0.071 respectively. There were no significant changes in bone mass or density at the 4% pQCT site in either cohort. This finding was similar to that seen in the observation period for the CF cohort but in contrast to the significant increases in total BMC seen after WBVT in both cohorts and the significant increase in total vBMD in the CF cohort.

Two previous studies have investigated the effects of WBVT after completion of training. In children with cerebral palsy, 6 months after completing a 6 month side-alternating WBVT intervention, lumbar spine BMC, BMD and the BMD SDS had not signifincatly changed from their post WBVT values [730]. In 6 boys with Duchenne Muscular Dystrophy, followed for nine months after completion of their 3 months side-alternating WBVT intervention, total body and lumbar spine DXA scans and distal tibial pQCT at the 4% and 66% sites were performed 3 and 9 months after completion of WBVT. Lumbar spine BMD and its associated SDS did not change significantly during follow-

up. Trabecular vBMD measured at the 4% tibial pQCT site demonstrated progressive non-significant reductions at 3 and 9 month follow-up after a non-significant increase seen with WBVT [214]. The results of the Soderpalm et al 2013 study [214], as well as the results in the paediatric MRCD cohort, suggest a benefical effect of WBVT in maintaining or potentially increasing trabecular bone mass and density. While there is no pQCT data for the observation period to help deduce whether the effects seen during the WBVT period in the paediatric MRCD cohort are a direct response of the WBVT, the follow-up data supports a beneficial role of WBVT for improving trabecular bone mass and density in children with MRCD. The significant improvements seen in DXA and pQCT measures of trabecular bone mass and density at multiple sites during the WBVT period, including DXA analysis of the lumbar spine, and pQCT analysis of the 4% distal pQCT site, were not reproduced during the follow-up period of similar duration. In fact, during the follow-up period, all DXA and pQCT measures of the trabecular bone compartment demonstrated mean decreases, and while non-significant, contrast with the significant increases in bone mass and density seen across both analysis modalities and at multiple analysis sites during the WBVT period. These differences between the WBVT and follow-up periods support a benefical effect of side-alternating WBVT in improving trabecular bone mass and density in the paedaitric MRCD cohort. These beneficial effects are particularly pertinent to this cohort of children as their baseline data demonstrate that the health of the trabecular bone compartment is compromised with trunk BMD, lumbar spine BMD and lumbar spine vertebrae 1-4 vBMD standard deviation scores all significantly reduced compared to the reference population (Table 4.4 and Figure 4.1), as well as the mean trabecular vBMD SDS at the 4% tibial pQCT site (Table 4.5 and Figure

4.18). Despite the short six-month intervention period used, considering a primary bone outcome, significant improvements in trabecular bone health were demonstrated. A longer study with greater durations of all study periods, that are equal in length to allow direct comparison between study periods, as well as the use of pQCT analysis for the duration of the study in a larger cohort of children with MRCD would help to further define the benefit of WBVT as well as any detraining effects after completion of WBVT on the trabecular bone compartment.

5.1.2.2 Cortical bone compartment

5.1.2.2.1 Bone Mass

In the cortical bone compartment, there were significant changes in bone mass in the CF cohort measured by DXA and pQCT during the follow-up period. Total body BMC increased significantly by 88.2g (4.9%), similar in magnitude to the increase seen in the WBVT period. There was no significant change in the total body BMC SDS however the total body BMC for total body bone area SDS decreased significantly by 0.3, which is in contrast to the significant improvement in this parameter seen in the WBVT period. Legs BMC, also increased significantly during the follow-up period by 24.6g (3.7%), smaller than those seen during the WBVT period. Total and cortical BMC at the 20% tibial pQCT site increased significantly during the follow-up period by 6.6mg/mm (3.2%) and 6.2mg/mm (3.5%), and the cortical BMC SDS increased significantly by 0.3. At the 66% tibial pQCT site, total BMC and cortical BMC increased significantly during the follow-up period by 8.3mg/mm (2.8%) and 7.7mg/mm (3.1%). The cortical BMC SDS increased

significantly by 0.2. These significant increases at the 20% and 66% pQCT sites were smaller than the increases seen during the WBVT period. These significant changes were consistent with Kilebrant et al [730] who found a significant increase in total body BMC of 20g in the 6 months following completion of WBVT in children with cerebral palsy and other neurological conditions [730], this change being smaller in magnitude compared to the increase seen in the CF cohort.

In the paediatric MRCD cohort, there were no significant changes in bone mass measured by DXA or pQCT during the follow-up period. This contrasts to the significant improvements in total body and legs BMC seen after WBVT as well as the significant improvement in total and cortical bone sectors on analysis at the 20% and 66% pQCT sites. Similarly, Soderpalm et al [214] did not find significant improvements in total body BMC in the follow-up period.

5.1.2.2.2 Bone Density

The CF cohort did not demonstrate any significant changes in bone mineral density measured by total body DXA during the follow-up period, however segmental analysis of these scans demonstrated significant improvments in legs BMD of 0.016g/cm2 (1.5%), smaller in magnitude than the improvements seen in the WBVT period. In the paediatric MRCD cohort, there was a significant decrease in the total body BMD SDS during the follow-up period of 0.2 standard deviations, opposite to the significant increase in total body BMD documented during the WBVT period. Furthermore, the significant decrease in leg BMD SDS seen in the observation period, was not seen in the follow-up period,

instead the decline in this parameter continued to be hindered in the follow-up period . There is no conistenncy in the current literature regarding changes in BMD measured by DXA after cmpletion of a WBVT intervention. In children with cerebral palsy and other neurological conditions, total body BMD and its associated SDS did not significnatly change 6 month after completing a WBVT intervention [730], however there was a significant increase in total body BMD at 9 months follow-up in boys with DMD [214].

At the 20% pQCT site, total and cortical vBMD increased significantly during the follow-up period in the CF cohort by 7.4g/cm^3 (1.2%) and 9.3g/cm^3 (0.8%), and the cortical vBMD SDS increased significantly by 0.2. These improvements were similar in magnitude to those seen during the WBVT period, however they fall within the reported CV for this measure (Table 2.2). At the 66% distal tibial pQCT site, total bone vBMD did not change significantly during the follow-up period in the CF cohort, however cortical bone vBMD and its corresponding SDS increased significantly by 9.543mg/cm^3 (0.9%) and 0.3. This change falls within the CV reported in the literature for cortical vBMD at the 66% tibial pQCT site (Table 2.2). While the clinical relevance of this change is questionable, a further increase in the already elevated cortical vBMD at the 66% site would indicate a likely reduction in the rate of intra-cortical bone turnover, an unfavourable change as this would result in the accumulation of older bone that less effectively responds to bone strains resulting in sub-optimal geometric adaptations. There were no significant changes in BMD measured by pQCT at the 20% or 66% sites during the follow-up period in the paediatric MRCD cohort. These results are in contrast to the significant improvements in vBMD seen in the total bone and cortical bone sectors at the

20% pQCT site during the WBVT period. No significant changes in cortical vBMD measured at the 66% pQCT site were found at 3 or 9 months follow-up in boys with DMD [214].

5.1.2.2.3 Bone Area and Geometry

In the CF cohort, bone area measured by total body DXA and the segmental analysis of those scans demonstrated significant improvements in bone area during the follow-up period. Total bone area increased by 77.6cm^2 (4.5%), legs bone area by 17.8cm^2 (2.9%), and the total bone area for height SDS increased significantly by 0.2, all larger in magnitude than the changes seen during the WBVT period, indicating favourable bone geometric adaptations during the follow-up period. Cortical bone geometry at the 20% and 66% tibial pQCT sites demonstrated enhancements during the follow-up period. At the 20% pQCT site, total CSA, cortical CSA and the cortical CSA SDS increased significantly during the WBVT period by 6.2mm^2 (1.9%), 4.2mm^2 (2.6%) and 0.2 respectively. Cortical thickness and periosteal circumference as well as their corresponding SDS also significantly improved during the WBVT period by 0.1mm (1.9%) and 0.1, and by 0.6mm (1.0%) and 0.2 respectively. Increases in these parameters were smaller than the increases seen in during the WBVT period, and close to the CVs reported for these measures in the current literature (Table 2.2). At the 66% tibial pQCT site, cortical CSA and its corresponding SDS increased significantly by 4.8mm^2 (2.1%) and 0.2, however these improvements were smaller than those seen during the WBVT period.

In the paediatric MRCD cohort there were no significnat changes in cortical bone geometry on DXA or at the 20% pQCT site during the follow-up period. Total bone CSA SDS at the 66% pQCT site increased significantly by 0.4 and this was accompanied by a significant increase in the periosteal circumference at the 66% pQCT site of 1.9mm (3.2%). These significant increases occurred in different parameters to the significant increases seen during the WBVT period, which occurred in cortical CSA and its associated SDS at the 66% pQCT site. It appears that periosteal apposition may have been more active in the follow-up period, culminating in the significnant increases in total bone CSA and periosteal circumference seen at the 66% tibial pQCT site, two parameters associated with long bone strength [1, 51, 58, 64].

5.1.2.2.4 Bone Strength

During the follow-up period, bone strength in the cortical bone compartment increased significantly in the CF cohort. At the 20% pQCT site, cortical polar CSMI and polar SSI increased significantly by 457.2mm^4 (3.6%) and 44.5mm^3 (4.6%) as did total polar CSMI and polar SSI by 519.4mm^4 (3.3%) and 41.0mm^3 (3.9%) All these improvements were smaller than those seen during the WBVT period. At the 66% pQCT site, significant increases were seen in the polar measurements for cortical CSMI and SSI and their corresponding SDS of 1201.6mm^4 (4.0%) and 0.2 and by 67.5mm^3 (4.3%) and 0.3, these changes smaller than those seen during the WBVT period.

In the paediatric MRCD cohort there were no significant changes in total or cortical bone strength parameters at the 20% tibial pQCT site. At the 66% pQCT site, cortical polar CSMI significantly increased by 778.9mm^4 (8.9%) with its associated SDS significantly

increasing by 0.1, similar increases to those seen during the WBVT period. Cortical polar SSI also increased significantly by 25.4mm^3 (3.8%), a smaller improvement than that seen during the WBVT period which did not elicit a change in the polar SSI SDS, as seen in the WBVT period. No previous studies have investigated bone strength indicators after completion of a WBVT intervention.

5.1.2.2.5 Body Composition

During the follow-up period, the CF cohort demonstrated significant alterations in their body composition. Total body and legs LTM increased significantly by 1110.1g (3.3%), 381.9g (3.5%) similar in magnitude to the changes seen during the WBVT period. There was also a significant increase in muscle CSA and its corresponding SDS measured at the 66% tibial pQCT site, of 186.8mm^2 (4.2%) and 0.2. this finding only reported in the follow-up period and beneficial for the CF cohort as muscle CSA at the 66% site was significantly reduced at baseline. This is also more favourable than the increase in fat CSA at the 66% site seen during the observation period, perhaps indicating preferential enhancement of the lean tissue compartment after the WBVT intervention.

The paediatric MRCD cohort demonstrated significant alterations in their body composition. Fat CSA measured at the 66% pQCT site significantly increased 129.1mm^2 (6.9%) and was associated with a significant increase in the ratio of fat CSA to muscle CSA, of 4.9%. These increases in fat CSA also had an adverse effect on the muscle CSA to total soft tissue CSA ratio which significantly decreased by 1.6%. These finding are similar to those found in children with cerebral palsy and other neurological conditions

who demonstrated no changes in total body lean tissue mass but significantly increased their total body fat mass, measured by total body DXA, 6 months after completion of a WBVT intervention [730].

These alterations in body composition are concerning as the fat % SDS measured by DXA was elevated in the paediatric MRCD cohort with a value of 0.92, prior to commencement of WBVT. Furthermore, the increases in fat CSA seen on analysis of the 66% tibial pQCT site appears to be impacting on the proportion of muscle at the 66% tibial pQCT site. Reductions in the proportion of muscle CSA are particularly alarming for the paediatric MRCD cohort as their muscle CSA SDS at the 66% tibial pQCT site was significantly reduced compared to the reference population at baseline (Figure 4.5 and Table 4.18) and this reduction in muscle CSA was postulated to be closely involved in compromising other aspect of bone health in the cortical bone compartment of the paediatric MRCD cohort as discussed above. The significant increases in fat CSA as well as the significant reduction in the proportion of muscle CSA in the cross-section could negatively impact on the bone health of the paediatric MRCD cohort.

5.1.2.2.6 Bone Biochemistry and Turnover Markers

During the follow-up period there were no significant changes in bone turnover markers in either cohort. The CF cohort demonstrated a small but significant reduction in calcium and the paediatric MRCD cohort a reduction in phosphorus, however these measures remained well within their reference ranges. Despite ongoing significant increases in the geometry and strength of the cortical bone compartment measured by tibial pQCT, the

bone turnover markers remained unchanged. It may be that these markers were not sensitive enough to reflect such changes or that more specific, stable and sensitive markers including the bone formation markers, bone specific alkaline phosphatase and PINP, and the bone resorption marker, CTX, are required to elucidate the ongoing effects after completion of WBVT in the paediatric patients with MRCD.

5.1.3 Effect of WBVT on Force Plate Parameters

M1LJ and S2LJ analysis was only performed in the CF cohort due to the limited number of participants in the paediatric MRCD cohort able to perform these manoeuvres. CRT parameters were analysed in both cohorts.

5.1.3.1 WBVT Period

After WBVT, maximum force in the M1LJ increased in the CF cohort by 0.5kN (4.6%), p=0.050, however there were no corresponding changes in relative force or the force SDS and this change fell within the CV reported in the literature (Table 2.5), indicating the change is likely physiological. This finding is however, in contrast to the significant reduction in relative force and the force SDS seen during the observation period, suggesting the WBVT had a beneficial effect on preserving the force producing capability of the lower limb muscles during the M1LJ. Three previous studies have investigated the effects of WBVT on force generating capacity in paediatric cohorts. In children with OI, mean age 9 years, participating in a paired randomised controlled trial of side-alternating WBVT (24Hz, 4-6mm, 9 minutes twice a day, with exercises

including squats and weight shifts for 20 weeks) or control, there was no significant change in M1LJ maximum relative force after WBVT or compared to the control group [747]. Two other studies using a tilt-table to provide WBVT have also shown improvements in maximum force after WBVT. Significant improvements in maximum relative force, corresponding the increased ground reaction force required to keep the patient upright as the angle of the tilt table was increased, were seen in children with OI types III and IV, and mobility impairments, 5-15 years, after side-alternating WBVT (15-25Hz, 1-2mm, 9 minutes twice a day) [257] and in children with bilateral spastic cerebral palsy, GMFCS classification I-V, with a mean age 9.8 years, after side-alternating WBVT 9 minutes twice a day for 6 months as part of an intensive physiotherapy program including resistance training hydrotherapy and treadmill walking [748].

During the WBVT period, the CF cohort did not demonstrate any significant changes in efficiency or power parameters during the S2LJ, however, maximum force increased significantly by 0.1kN (10%), well above the reported CV (Table 2.5). There were no significant changes in relative force or the force SDS, likely indicating increases associated with normal musculoskeletal growth and development. However, considering the SDS for this parameter was significantly reduced at baseline and significant increases in force during the S2LJ were not seen in the observation or follow-up periods, this may indicate a beneficial effect of WBVT on preserving normal increases in force production in the CF cohort. This would be consistent with the beneficial effects of WBVT in preserving force production in the M1LJ.

Previous studies have investigated the effects of WBVT on S2LJ performance in paediatric cohorts in good health and with underlying disease. Similar to the result seen in the CF cohort, S2LJ power parameters did not significantly change after 4 weeks of side-alternating WBVT (20-22Hz, 1mm, 10-15 minutes, 3 times a week) in children with CF [229], after 20 weeks of side-alternating WBVT (20 Hz, 4-6mm, 9 minutes, 4 times a week) in children with cerebral palsy, mean age 16.2 years [732], or after side-alternating WBVT (24Hz, 4-6mm, 9 minutes twice a day, with exercises including squats and weight shifts for 20 weeks) in children with OI, mean age 9 years, and matched controls [747]. In two studies of elite adolescent female basketball players, jump height parameters, which are closely correlated to power parameters, significantly increased by 2.6% after vertical WBVT (35Hz, 4mm, 3-6 minutes with exercises including squats and heel raises, twice a week for 14 weeks) in addition to normal aerobic, resistance and basketball training [749] and by 2.9cm (10%) after vertical WBVT (25-35Hz, 4mm, 5 minutes with exercises including squats and lunges for 15 weeks) prior to routine resistance training [750]. In the former study, increases were not significantly different to the those randomised to the control group (normal training without WBVT) [749] perhaps a reflection of an inadequate training stimulus as only 6 minutes of WBVT was performed twice a week, or that the elite status of the participants left little room for improvement. The latter study demonstrated an improvement that was larger in magnitude and significantly greater than those randomised to control (resistance training only) [750]. One previous study found no significant changes in maximum relative force after side-alternating WBVT, or compared to the control group, in children with OI [747], consistent with the results of the CF cohort.

The CF cohort demonstrated significant changes in CRT speed and force parameters after WBVT. Time per test decreased significantly by 0.1s (9.1%), and the corresponding SDS increased significantly by 0.5, indicating improve muscle efficiency and co-ordination after WBVT, however was similar in magnitude to improvements in CRT performance seen in the observation period. CRT time also decreased significantly by 1.5s (18%) after 20 weeks of side-alternating WBVT (20 Hz, 4-6mm, 9 minutes, 4 times a week) In 40 children with cerebral palsy, GMFCS II and III, mean age 16.2 years [732], a larger improvement than that seen in the CF cohort using a similar vibration training protocol, however CRT performance at baseline was much better in the CF cohort compared to the cerebral palsy cohort so they may not have had the same capacity to improve. Alternatively, Gusso et al [732] used a higher amplitude, 6mm, compared to 4 mm in the CF cohort, which may have impacted on the outcome and this should be considered in future studies. However, an RCT of side-alternating WBVT (24Hz, 4-6mm, 9 minutes twice a day, with exercises including squats and weight shifts for 20 weeks) or no intervention, in 20 children with Osteogenesis Imperfecta (OI) types I-IV and controls paired for gender and pubertal status, mean age 9 years, found no significant improvements in time per test [747].

There were no significant changes in CRT power parameters in the CF or paediatric MRCD cohorts, similar to that reported in the existing literature in children with cerebral palsy [732] and children with OI [747]. Maximum force during the rising phase of the CRT increased significantly by 0.1kN (6.7%) in the CF cohort, however relative force

and force SDS did not change significantly, indicating increases associated with normal musculoskeletal growth and development, consistent with the increased maximum force seen during the S2LJ in the CF cohort. The paediatric MRCD cohort demonstrated significant decreases in maximum force and maximum relative force of 0.1kN (7.7%) and 6.3N/kg (13.2%) respectively, larger than the reported CV for maximum force (Table 2.5) and a non-significant decrease in force SDS of 0.5, a clinically relevant improvement. The significant reduction in muscle force without significant changes in power or speed parameters indicates improved muscle efficiency as less force is required to generate a similar power and speed [718]. No previous studies have reported on CRT force parameters.

5.1.3.2 Follow-up Period

After 6 months follow-up, the CF cohort demonstrated significant reductions in relative force and force SDS of 0.2g (7.4%) and 0.5. These reductions were larger than the reductions seen in the observation period and indicate that WBVT was effective in preserving and perhaps improving the force producing capacity of the lower limb muscles during M1LJ in the CF cohort. This is especially pertinent as the M1LJ force SDS was significantly reduced at baseline and further deterioration would have negative ramifications on long term bone health. No previous studies have investigated the effects of WBVT removal on muscle force parameters.

The CF cohort did not demonstrate any significant changes in any of the efficiency, power or force parameters measured by the S2LJ during the follow-up period. One

previous study investigated the effects of cessation of WBVT in a cohort of paediatric patients with CF. Four weeks after the withdrawal of side-alternating WBVT (20-22Hz, 1mm, 10-15 minutes, 3 times a week for 4 weeks), there were no significant changes in power parameters during the S2LJ [229].

During the follow-up period, there were no significant changes in speed, power or force parameters during the CRT in the CF cohort. These finding indicating that the significant improvements in muscle efficiency and co-ordination gained during the WBVT were not lost in the first 6 months after cessation of the WBVT intervention. The paediatric MRCD cohort also did not demonstrate any significant changes in CRT force, power or speed parameters in the follow-up period, however, average relative force and the force SDS demonstrated non-significant increases of 0.1g (8.3%), p=0.058 and 0.6, p=0.076 respectively. As this increase occurred in the absence of other significant changes in power and speed parameters, this indicates a worsening of muscle function, as a significantly larger amount of force was required to maintain the same muscle power and velocity. No previous studies have evaluated the effect of WBVT cessation on CRT performance.

5.1.4 Effect Of WBVT on Exercise Capacity

Exercise capacity in the CF cohort was investigated using formal cardio-pulmonary exercise testing (CPET) with the Bruce Treadmill Protocol. In the paediatric MRCD cohort, exercise capacity was measured using the six-minute walk test (6MWT) and

CPET. There is a complete dataset for the six-minute walk test, however, only data for two paediatric participants were available for CPET and thus statistical analysis was not performed due to the small size of the sample.

5.1.4.1 WBVT Period

After WBVT there were no significant changes in any CPET parameters at baseline, AT, peak exercise or recovery in the CF cohort. This is contrast to the significant declines in peak VO_2 and its percent-predicted value and a non-significant, but clinically relevant decline in VO_2 at AT in the observation period. Similar to the pattern seen in maximum relative force and force SDS in the M1LJ, WBVT appears to have been beneficial in hindering the decline in peak VO_2 and in preserving the submaximal VO_2 kinetics at AT. One previous study has investigated the effects of WBVT on peak VO_2 using an incremental treadmill test in children with CF and did not find any significant changes after WBVT [229], consistent with the findings in the CF cohort.

The paediatric MRCD cohort did not demonstrate any significant changes in the 6MWT distance after WBVT. Post-6MWT heart rate decreased significantly by 27.9 beats/minute (20.9%), suggesting an improved cardiovascular response to the 6MWT, especially as 6MWT distance had not significantly changed. It was also in contrast to a significant increase in this parameter in the observation period of 25.1%. The Borg Score, measured after 5 minutes of seated recovery, was also significantly reduced after WBVT

by 1.3 points, also indicating an improved aerobic efficiency, as recovery after this endurance test was attained at a more rapid rate.

The effect of WBVT on walking performance has been investigated in the existing literature in paediatric cohorts with underlying medical conditions including CP, DMD, SMA, OI and SB. Consistent with the paediatric MRCD cohort, two previous studies have found no effect of WBVT on 6MWT distance in children with DMD after progressive side-alternating WBVT (10-24Hz, 4mm, 9 minutes twice a day, 5 times a week for 8 weeks) [228] and in in four boys with DMD and two boys with Becker Muscular Dystrophy after 6 months of low-magnitude vertical WBVT (30-90Hz, 0.4g, 10 minutes daily) [751]. In contrast, 6MWT distance was found to be significantly improved by 44m (11%) in GMFCS II and by 40m (35%) in GMFCS III, after 20 weeks of side-alternating WBVT (20 Hz, 4-6mm, 9 minutes, 4 times a week) [732]. The larger improvements seen in this study compared to the paediatric MRCD cohort may be due to the higher peak vibration amplitude used during training, 6mm compared to 4mm. 6MWT distance also increased significantly by 31.5m (8%) in children with SMA, mean age 10 years, after progressive side-alternating WBVT (10-24Hz, 4mm, 9 minutes twice a day, 5 times a week for 8 weeks) [228]. The higher peak amplitude, 24Hz compared to 20Hz and the longer daily training duration, 18 minutes compared to 9 minutes, may have contributed to the improvements in 6MWT performance seen in the SMA group that were not seen in the paediatric MRCD cohort. In children with OI, mean age 9 years, participating in an intensive functional physical therapy program (including resistance trailing, treadmill walking and neurodevelopmental therapy) for 12 months in addition to

side-alternating WBVT with a tilt-table (15-20Hz, 9 minutes twice a day, for 6 months), the one-minute walk test increased significantly by 43% after 6 months of WBVT [203]. Children with cerebral palsy, 6-12 years of age, receiving twice weekly physical therapy sessions, were randomised to progressive side-alternating WBVT with a tilt-table (12-18Hz, 2-4mm, 5 times a week for 6 months) or control [198]. Walking speed in the 10m walk test significantly increased by 38% in the WBVT group which was significantly larger than the control group [198]. Side-alternating WBVT (using individualised frequency and amplitude setting, 9 minutes twice a day, 5 days a week for 6 months) in addition to an intensive physical therapy program (including resistance training, treadmill walking and hydrotherapy), resulted in a 39% increase in self-selected 6m gait speed, in children with spina bifida, mean age 8.7 years [258]. Another study in children with bilateral spastic cerebral palsy, mean age 10 years, participating in a conventional physical therapy program (including massage, stretching and balance training), found a significant improvement in common gait pattern speed measured by 3D gait analysis of 30% in those randomised to progressive side-alternating WBVT (5-25Hz, 2-6mm, 18 minutes, with dynamic squats, 3 times a week for 8 weeks), which was significantly larger than the control group [226]. A systematic review and meta-analysis investigating the effects of WBVT in children with cerebral palsy found that compared to control, WBVT significantly increased gait speed by 34% [202].

5.1.4.2 Follow-up Period

After 6 months follow-up, there were no significant changes in CPET parameters at AT or peak exercise in the CF cohort, similar to that seen one moth post withdrawal of

WBVT in a study of children with CF [229]. The significant reductions in peak VO_2 seen in the CF cohort during the observation period did not continue in the follow-up period, perhaps due to a latent effect of the WBVT.

There were no significant changes in the 6MWT distance during the follow-up period in the paediatric MRCD cohort. The maintenance of 6MWT distance during the follow-up period in the paediatric MRCD cohort is consistent with the current literature which has not shown any significant changes in walking test performance during a follow-up period after cessation of WBVT. In 8 ambulant children with spinal muscular atrophy, mean age 10 years, and 14 ambulant children with Duchenne Muscular Dystrophy, mean age 9 years, there was a non-significant decrease in 6MWT distance of 2% 4 weeks after ceasing progressive side-alternating WBVT (10-24Hz, 4mm, 9 minutes twice a day, 5 times a week for 8 weeks) [228]. Six months after completing low-magnitude vertical WBVT (30-90Hz, 0.4g, 10 minutes daily, for 6 months), in four boys with Duchenne Muscular Dystrophy and two boys with Becker Muscular Dystrophy, 6-22 years of age, there was a non-significant increase in 6MWT distance of 0.8m (0.2%) [751]. In children with OI, mean age 9 years, there was no change in one-minute walk distance 6 months after completing side-alternating WBVT with a tilt-table (15-20Hz, 9 minutes twice a day, for 6 months), however participants continued to receive an intensive functional physical therapy program (including resistance training, treadmill walking and neurodevelopmental therapy) during the 6 month follow-up after cessation of the WBVT intervention [203]. Self-selected 6m gait speed in children with spina bifida, mean age 8.7 years, did not change significantly, 6 month after completing side-alternating WBVT

(using individualised frequency and amplitude setting, 9 minutes twice a day, 5 days a week for 6 months) in addition to an intensive physical therapy program [258].

The post-6MWT heart rate increased significantly by 23.0 beats/minute (21.8%) indicating a deterioration in the cardiovascular response to the endurance test, especially considering this increase in heart rate occurred without a significant increase in 6MWT performance, and may indicate worsening OXPHOS capacity.

5.1.5 Effect of WBVT on Quality of Life

Quality of life in the CF cohort was measured using a disease-specific questionnaire, the CFQ-R. In the paediatric MRCD cohort, Quality of Life was measured using section IV of the NPMDS.

5.1.5.1 WBVT Period

After WBVT, the CF cohort did not demonstrate any significant changes in child- and adult-reported domains, however the parent-reported emotion and school domains increased significantly, indicating better quality of life in emotional regulation and school attendance and participation. Importantly, participants did not perceive the home-based WBVT intervention as an increased treatment burden, as there were no significant changes in this domain after WBVT. This is especially important as the treatment burden domain was below the normative mean at baseline. There were no significant changes in quality of life in the paediatric MRCD cohort. Two previous studies have investigated the

effects of WBVT on quality of life in paediatric cohorts. In children with cystic fibrosis, with a mean age of 11.7 years, participating in a randomised cross-over trial of side-alternating WBVT (20-22Hz, 1mm, 10-15 minutes, 3 times a week for 4 weeks), health-related quality of life measured by the CFQ-R did not change significantly after WBVT [229]. In children with cerebral palsy, GMFCS II and III, mean age 16.2 years, quality of life measured by the Cerebral Palsy Quality of Life Questionnaire did not find any significant changes in patient-reported health-related quality of life, however caregivers reported significant improvements in school well-being, general well-being and participation, perceived pain and impact of disability and global quality of life scores, after 20 weeks of side-alternating WBVT (20 Hz, 4-6mm, 9 minutes, 4 times a week) [732], sharing some similarities with the results in the CF cohort.

5.1.5.2 Follow-up Period

After 6 months follow-up, there were no significant changes in child- or adult-reported domains however parent-reported emotion and respiratory domains increased significantly, indicating improvements in emotions and respiratory symptoms. There was also a non-statistically significant, but clinically relevant improvement in the child- and adult-reported respiratory domain. The paediatric MRCD cohort did not demonstrate any significant changes in quality of life during the follow-up period. No previous studies have investigated health-related quality of life after cessation of a WBVT intervention.

5.2 Adult Perspective

5.2.1 Effect of WBVT on Bone and Body Composition Parameters

5.2.1.1 WBVT Period

5.2.1.1.1 Trabecular Bone Compartment

The trabecular bone compartment was investigated using lumbar spine DXA scans and pQCT at the 4% tibial site.

5.2.1.1.1.1 Bone Mass

In the adult MRCD cohort, 6 months WBVT did not have a significant impact on lumbar spine bone mass or density. These results are consistent with systematic reviews and meta-analyses in postmenopausal women [214] and in the older adult population [216, 217], which found no treatment effects of WBVT on lumbar spine BMD when compared to a control group performing no intervention or an active exercise group. However, a more recent systematic review and meta-analysis in post-menopausal women found a significant treatment effect of WBVT on lumbar spine BMD when compared to control, sham and active exercise interventions, however this was only preserved when low-magnitude WBVT (<1g) was used [752]. The use of high magnitude WBVT in the adult MRCD cohort may explain why we did not find significant improvements in lumbar

spine BMD. However, the four low magnitude studies [211, 753-755] in the meta-analysis [752] utilised WBVT for 12-18 months, compared to 6-12 months in the high magnitude studies [209, 210, 221, 756], the longer intervention period likely contributing to the difference in outcomes.

Five studies using side-alternating vibration platforms in post-menopausal women have reported lumbar spine BMD outcomes [209, 210, 215, 754, 757]. Gusi et al [209] and Liphardt et al [215] did not find any improvements in lumbar spine BMD after 8 months WBVT (12Hz, 3mm, 3-6 minutes, 3 times a week) and 12 months WBVT (20Hz, 3-4mm, 10 minutes, 2-3 times a week) respectively, these findings consistent with those seen in the adult MRCD cohort. The results of one study suggested a bone-sparing effect of 8 months WBVT (12Hz, 2mm, 6 minutes, twice a week) with no significant effect on lumbar spine BMD, BMC or area seen after WBVT compared to significant reductions in the control group [757]. The combination of dynamic exercises including squats and heel raises, with 12 months WBVT (12Hz, 12mm, 10 minutes, 3 times a week) significantly increased lumbar spine BMD 0.007g/cm^2 (0.7%), significantly larger than the control group [754]. Iwamoto et al [210] found a significant increase in lumbar spine BMD in post-menopausal women after 12 months of WBVT at 20Hz, however the participants in the were also receiving alendronate, and this may have contributed to the positive response in lumbar spine BMD seen.

Several studies using vertical vibration platforms have reported lumbar spine BMD outcomes. Four studies using low-magnitude vertical vibration in post-menopausal

women and community dwelling elderly for 12-24 months [211, 753, 758, 759] and four using high-magnitude vertical vibration in post-menopausal women, older adults and healthy, young adults for 3-8 months [208, 221, 760, 761] did not find a beneficial treatment effect of WBVT for lumbar spine BMD and BMC, which is consistent with the results of the adult MRCD cohort. Two studies demonstrated a potential bone preservation effect of vertical WBVT on the lumbar spine in post-menopausal women when compared to control groups after 8 months low-magnitude WBVT (30Hz, 0.3g, 15 minutes, twice a week) [757] and 6 months of high-magnitude WBVT (35-40Hz, 1.5mm, 7-12 minutes, 3 times a week) [762]. Three studies in post-menopausal women found 6-18 months of vertical WBVT to have a significant improvement on lumbar spine BMD [208, 754-756].

The existing literature suggests high-magnitude vibration [210, 754-756, 762] with amplitudes up to 12mm [754], frequency $\geq$25Hz [754-757, 762, 763], $\geq$12 months in duration [210, 754, 755, 763], progressive nature of the vibration training with dynamic exercises [754, 755], and the combination of WBVT with a conventional exercise intervention [755], may contribute to the improvements in lumbar spine bone mass and density after WBVT. Larger improvements in lumbar spine bone mass and density may have been found in the adult MRCD cohort if the duration of the study was prolonged, ideally to at least 12 months, and if the WBVT protocol used a frequency $\geq$ 25Hz. Progressing the intensity of the vibration training by increasing frequency, amplitude or adding in some dynamic exercises may also improve lumbar spine bone mass and density

outcomes. The effects of high and low-magnitude vibration training should also be investigated in adults with MRCD.

In the adult MRCD cohort, there were no significant changes in bone mass and density parameters measured at the 4% tibial pQCT site after WBVT. These results are consistent with the existing literature. In healthy, young adults, 8 months of vertical WBVT (25-45Hz, 2mm, 2-8g, 2-4 minutes, 3 times a week) with dynamic exercises, showed no changes in distal tibial trabecular vBMD measured by pQCT [208]. Side-alternating WBVT (12-28Hz, 2-6 minutes, twice a week) for 6 months in post-menopausal women did not change trabecular vBMD at the 4% pQCT site compared to controls [212]. In another study of post-menopausal women, randomised to 12 months side alternating WBVT (20Hz, 2-4mm, 10 minutes, 2-3 times a week) or control, there was no significant differences in total or trabecular bone vBMD at the distal tibia measured by high resolution pQCT [215]. In another study using high resolution pQCT of the distal tibia, 12 months of home-based low-magnitude vertical vibration in post-menopausal women found no significant changes in total and trabecular vBMD [759]. Trabecular vBMD measured by QCT of the proximal femur did not significantly differ between low-magnitude vertical WBVT compared to placebo after 2 years in community-dwelling elderly [758]. Another study in older adults found no effect of 11 weeks of vertical WBVT (40Hz, 2mm, 7.5 minutes, 3 times a week) on total and trabecular BMC and vBMD measured at the 4% tibial pQCT site [761].

The results reflected in the available literature as well as those found in the adult MRCD cohort, investigating the effect of WBVT on trabecular bone mass and density in the lower limbs, suggest that WBVT may have limited efficacy in improving bone health in trabecular bone. However, all the studies investigating distal tibial vBMD were only up to 12 months in duration. It may be that a longer duration intervention period is required in adults, especially when bone health is already compromised, to precipitate changes in the trabecular bone compartment. Intervention periods of at least 18 months, along with progressive, high intensity WBVT are likely required to elicit a beneficial response in trabecular bone in the adult MRCD cohort.

5.2.1.1.2 Cortical Bone Compartment

5.2.1.1.2.1 Bone Mass

After WBVT, no significant changes were seen in bone mass when analysing the total body DXA scans or segmental analysis of those scans in the adult MRCD cohort. These results are consistent with previous studies. In post-menopausal women, no significant differences in total body BMC were found after 8 months side-alternating WBVT (12Hz, 2mm, 6 minutes, twice a week) [757]. Similar results were found in older adults, after 11 weeks vertical WBVT (40Hz, 2mm, 7.5 minutes, 3 times a week) with static squats [761].

There were no significant changes in bone mass parameters measured at the 20% or 66% distal tibial pQCT sites in the adult MRCD cohort during the WBVT period. These findings are consistent with one study in the existing literature investigating the effects of 11 weeks vertical WBVT (40Hz, 2mm, 7.5 minutes, 3 times a week), compared to controls, in older adults [761]. Total BMC and cortical BMC at the 38% tibial pQCT site did not change significantly after WBVT or between groups however there was a significant reduction in total BMC at the 38% tibial pQCT site in the control group [761]. These results suggest there may be a bone sparing effect of WBVT at the 38% tibial pQCT site in older adults.

5.2.1.1.2.2 Bone Density

In the adult MRCD cohort, WBVT did not have an effect on total body bone density. These finding are consistent with the existing literature in post-menopausal women using side -alternating [757], low-magnitude [757] and vertical [221, 760], WBVT and in older adults using vertical WBVT [761]. Segmental analysis of the total body DXA scans in the adult MRCD cohort found a significant increase in legs BMD of 0.007g/cm^2 or 0.6% after WBVT. This value is within the CV reported in the adult literature, 0.7% [134], and that reported from our laboratory, 1.55% (Table 2.1), and may not be of any clinical relevance. The effect of WBVT on legs BMD in the adult cohort cannot be directly compared to the literature as previous studies in the adult population used total hip and femoral neck DXA scans, however we can make some comparisons. The magnitude of the change in legs BMD in the adult MRCD cohort was smaller than the changes recorded in recent systematic review and meta-analyses in post-menopausal women

where hip BMD significantly increased after WBVT with a standardised mean difference of 0.015g/cm^2 [214]. However it was similar in magnitude to that seen in a meta-analysis in older adults where WBVT was found to have a significant treatment effect on femoral neck BMD with a mean difference of 0.04g/cm^2 [216]. Another meta-analysis in post-menopausal women did not find a significant treatment effect of WBVT on femoral neck BMD with an intervention period of at least 6 months [752] and no significant treatment effect of WBVT on total hip BMD was found in another meta-analysis in older adults [217]. It is obvious that WBVT affects different areas of the skeleton differently, even when analysis is performed in areas of close proximity, for example the total hip and femoral neck areas. While we cannot directly compare the results of proximal hip analysis to that of the segmental analysis of the leg BMD in the adult MRCD cohort, improvements in the BMD of the cortical bone in the lower limbs of adults with MRCD may be augmented with a longer intervention period and frequencies greater than 25Hz [763].

When considering side-alternating vibration platforms, 8 months WBVT (12 Hz, 3mm, 2-6minutes, 3 times a week), significantly improved femoral neck BMD in post-menopausal women compared to a walking program [209]. However the same positive response in femoral neck BMD in post-menopausal women was not found in three more recent studies using side-alternating WBVT for 8-12 months [215, 754, 757]. Studies using vertical vibration platforms in post-menopausal women also report conflicting results for the effect of WBVT on hip BMD. One study demonstrated a significant increase in total hip BMD of 0.008g/cm^2 (0.93%) after 6 months of WBVT [221], similar

in magnitude to the increase in legs BMD seen in the adult cohort despite the addition of dynamic exercises during the vibration stimulus and the different anatomical site analysed. Other studies investigating the effects of vertical vibration platforms however, did not show any beneficial effect of WBVT on hip BMD in post-menopausal women [211, 754, 755, 759, 760, 764]. Studies using vertical vibration platforms in older adults also did not find a beneficial effect of WBVT on hip BMD [758, 761, 765].

In the adult MRCD cohort, 6 months of WBVT did not have any significant effect on bone density measured at the 20% or 66% tibial pQCT sites. These results are similar to those seen in healthy, young adults, which showed no change in the cortical vBMD of the tibial mid-shaft compared to control after 8 months of progressive vertical WBVT [208]. In another study using side-alternating WBVT in post-menopausal women, 6 months of progressive WBVT (12-28Hz, 3-6 minutes with increasing amplitude, twice a week), cortical vBMD at the 38% tibial site did not change significantly in the WBVT group compared to a significant decrease in the control group [212], these results indicating a potential bone sparing effect WBVT. In contrast, 11 weeks of vertical WBVT (40Hz, 2mm, 7.5 minutes, 3 times a week) in older adults resulted in a significant reduction in total vBMD and cortical vBMD at the 38% tibial pQCT site, which was significantly greater than the decline seen in the control group for total vBMD [761]. The decline in vBMD seen in the latter study may be due to the shorter intervention period, older age of participants or the non-progressive nature of the WBVT protocol.

Side-alternating WBVT [208, 209] appears to be more successful in improving cortical bone density in the lower limbs compared to vertical WBVT, which may be explained by the rapid alternating displacements at the level where the hip meets the pelvis generated during side-alternating WBVT, the resulting muscle action imparting larger site specific forces to the underlying bone in the hip and lower limb region during side-alternating vibration compared to vertical vibration. High-magnitude vibration training appears to be more beneficial than low-magnitude vibration training, which did not elicit any beneficial effects on cortical bone density in any of the studies reviewed [211, 753, 758, 759], an observation confirmed in a recent meta-analysis in post-menopausal women [752], and likely a result of a reduced mechanical stimulus imparted on the hip and lower limb region compared to high-magnitude vertical and side-alternating WBVT platforms. Vertical vibration appears to have been more beneficial in improving cortical bone density when frequencies $\geq$30Hz were employed [221, 765], an aspect of vibration training protocols which was found to be particularly osteogenic for hip BMD in a recent meta-analysis in post-menopausal women [763]. Future studies investigating the effects of WBVT in adults with MRCD should involve a larger sample size, exploring the effects of different vibration frequencies, as well as the use of proximal hip DXA scans, including total hip and femoral neck scans, to elucidate the efficacy of WBVT on cortical bone in the lower limbs.

5.2.1.1.2.3 Bone Area and Geometry

There were no significant changes in any parameter of bone area or geometry in either of the analysis techniques used in the adult MRCD cohort. These results are consistent with

the findings of Russo et al [212] who did find any changes in bone area at the 38% tibial site in post-menopausal women compared to controls after 6 months of progressive side-alternating WBVT (12-28Hz, 3-6 minutes with increasing amplitude, twice a week) [212], and Torvinen et al [208] who found no improvements in bone area measured at the tibial mid-shaft after 8 months of progressive vertical WBVT (25-45Hz, 2mm, 2-8g, 2-4 minutes, 3 times a week) with dynamic exercises in healthy young adults [208]. Total and cortical bone area at the 38% tibial pQCT site did not change significantly after 11 weeks of vertical WBVT (40Hz, 2mm, 7.5 minutes, 3 times a week) in older adults, however total bone area decreased significantly in the control group [761], suggesting a potential bone-sparing effect of WBVT.

5.2.1.1.2.4 Bone Strength

In the adult MRCD cohort there were no significant changes in any parameter of bone strength measured at the 20% or 66% tibial pQCT site after WBVT. Two previous studies investigating the effect of vertical WBVT on bone strength in the tibia were consistent with the findings in the adult MRCD cohort. Progressive vertical WBVT (25-45Hz, 2mm, 2-8g, 2-4 minutes, 3 times a week) with dynamic exercises in healthy young adults found no improvement in bone strength of the tibial mid-shaft after 8 months [208]. In older adults, 11 weeks of vertical WBVT (40Hz, 2mm, 7.5 minutes, 3 times a week) polar SSI at the 38% tibial pQCT site [761].

Similar to findings in the trabecular bone compartment, 6 months WBVT in adults with MRCD did not demonstrate any significant improvements in bone mass, density,

geometry or strength in the cortical bone compartment. Analogous to the conclusions drawn for the trabecular compartment, it is likely studies with a longer WBVT period using progressive protocols may elucidate whether WBVT in adults is beneficial in improving bone health in the cortical bone compartment. Furthermore, larger studies investigating different vibration platforms, vertical and side-alternating, as well as different vibration frequencies are required to determine optimal training protocols for enhancing cortical bone parameters.

5.2.1.1.2.5 Body Composition

During the WBVT period, body composition was measured using total body DXA scans, segmental analysis of these scans and the measurement of muscle and fat CSA at the 66% distal tibial pQCT site. In the adult cohort, WBVT did not have an effect on total body lean tissue mass or fat mass. This finding is consistent with the Verscheuren et al [221] study which found no change in total body lean tissue mass in post-menopausal women, after 6 months of progressive vertical WBVT (35-40Hz, 1.7-2.5mm) with dynamic exercises compared to controls [221], however, a significant decreased in fat mass of 2.3% was found [221]. Similar results were found by von Stengel et al [766] in post-menopausal women, where 18 months of conventional training (including aerobic, resistance and balance exercises) and progressive vertical WBVT (25-35Hz, 1.7-2.0mm, with dynamic exercises including squats and heel raises, twice a week) did not find a significant change in LTM over time or compared to the control group, however total body fat decreased significantly by 2.2% after WBVT and significantly more than control [766]. In older adults, 11 weeks of vertical WBVT (40Hz, 2mm, 7.5 minutes with static

squats, 3 times a week) did not significantly change total body lean tissue mass over time or compared to controls [767]. Fat free mass, measured by underwater weighing, increased significantly after progressive vertical WBVT (35-45Hz, 2.5-5mm, 13-20 minutes with static and dynamic exercises) in untrained young adult females but was not significantly different to the control group [768]. There was no change in body fat over time or between groups [768].

There were no significant changes in body composition parameters on segmental analysis of the total body DXA scans, however, WBVT appears to have reduced the rate of decline and in some cases reversed the significant decline of LTM that was seen in the total body and legs during the observation period (Table B.2). The study by Gomez-Cabello et al [767], in older adults, did not find significantly altered LTM in the legs after 11 weeks of vertical WBVT (40Hz, 2mm, 7.5 minutes with static squats, 3 times a week) [767]. Verschueren et al 2011 [765] did not find a significant change in upper leg muscle mass measured by multi-slice CT after 6 months progressive vertical WBVT (30-40Hz, 1-12 minutes, 1.6-2.2g, with dynamic exercises, 3 times a week) in elderly institutionalised females [765]. Using a similar method, Bogaerts et al [769] found a significant increase in muscle mass (3.4%) measured by CT of the upper thigh after 12 months of progressive vertical WBVT (35-40Hz, 5mm, 2-15 mins with dynamic exercises) which was significantly greater than control group, in older men, [769]. Machado et al [770] found significant increases in the muscle CSA of the vastis medialis and biceps femoris measured by CT, which were significantly greater than control, after 10 weeks of progressive vertical WBVT (20-40Hz, 2-4mm, 1.5-8 minutes, 3-5x/week

with dynamic exercises) in community-dwelling older females. Quadriceps muscle CSA of the upper third of the thigh, measured by MRI, did not change significantly after 8 months vertical WBVT (20Hz, 2mm, 3.2g, 3-3.5 minutes, twice a week) in elderly women, however, those randomised to the control group demonstrated significant reductions in quadriceps muscle CSA, suggesting that WBVT may have a muscle-preserving effect [771].

Body composition parameters at the 66% tibial pQCT site in adults with MRCD remained stable during the WBVT period. There are no reports in the literature describing the effect of WBVT on muscle and fat CSA at the 66% distal pQCT site in adults. In a study investigating the effects of 13 weeks of vertical WBVT (12Hz, 1mm, 3 times a week) in older adults, mid-calf muscle CSA measured by pQCT did not change significantly with WBVT [244].

The previous literature investigating the effects of WBVT on body composition in adults has been focused on vertical vibration platforms. It would appear from the available literature that improvements in LTM occur when progressive vertical vibration protocols are utilised with frequencies $\geq$40Hz, amplitudes of 4-5mm, with dynamic exercises for at least 10 minutes duration [768-770]. Future larger studies in adults with MRCD with higher training stimulus that are progressive in nature, including amplitude and frequency, with the addition of static or dynamic exercises on the platform may improve outcomes for body composition, including lean tissue mass and fat mass, in adults with MRCD.

5.2.1.1.2.6 Bone Biochemistry and Turn-over Markers

There were no significant changes in blood biochemistry or bone turnover markers in the adult MRCD cohort during the WBVT period indicating that WBVT did not interrupt the accelerated bone loss seen in this cohort due to bone remodelling favouring bone resorption. Despite the absence of change in bone turnover markers and the climate favouring bone resorption during the WBVT period, bone parameters did not continue to decline significantly as seen during the observation period and there was a significant improvement in leg BMD, which was of borderline clinical significance. This may be explained by the small size of the cohort and the inherent variability of the bone turnover markers used.

Elmantaser et al [729] found that 8 weeks of side-alternating WBVT (18-22Hz, 9 minutes, 3 times a week) in healthy young men, significantly decreased the serum bone resorption marker CTX, however there was no change in the other bone resorption marker TRACP5b, or the serum bone formation markers BAP and osteocalcin [729]. In the same study, men randomised to low magnitude vertical WBVT 32-37Hz, 10-20 minutes, did not demonstrate any significant changes in markers of bone resorption or formation after WBVT [729]. Despite the similarities in the side-alternating training protocol used in the MRCD cohort, the favourable reduction in resorptive activity seen in healthy young men [729] was not seen in the adult MRCD cohort, however this may be due to the different markers of osteoclast activity that were used. The urinary deoxypyridinoline:creatine ratio used in the MRCD cohort is not as preferable as the

serum measure of CTX utilised in the Elmantaser et al 2012 study [729]. Urinary derived measures of bone resorption have several limitations including large variations between individuals, the influence of circadian variation [741, 746], difficulty with serial measurements [738, 743, 744], susceptibility to UV exposure [736] and the effect of changing muscle mass and age when expressed relative to creatinine [727, 740, 745]. Consequently, serum derived measurements of bone resorption, such as CTX, are preferred [727, 740-742].

Two other studies investigating vertical WBVT in post-menopausal women found WBVT to have a beneficial effect on bone resorption. Low-magnitude vertical WBVT (12Hz, 0.3g, 10 mins 3 times a week) had significant reductions in the urinary N-terminal telopeptide (Ntx)/creatine ratio, however serum-derived BAP (bone alkaline phosphatase) did not change significantly [772]. Progressive vertical WBVT (35-40Hz, 1.5mm, 7-12 minutes, 3 times a week) for 6 months, found a significant reduction in urinary hydroxyproline (29%) compared to a non-significant increase in the control group (55%) [762].

In another study investigating the effects of side-alternating (30Hz, 3mm, 3.6g), vertical (30Hz, 1.3mm, 1.5g), or sham WBVT in older adults, a 12 week progressive program with 3 training sessions a week, 1-6 minutes, found significant increases in the bone formation marker aminoterminal propeptide of type I collagen (P1NP) in the side-alternating WBVT group (35%) and vertical WBVT group (26%) which were significantly greater than sham, however the bone resorption marker CTX did not change

significantly over time or between groups [222]. The findings in this study with regard to bone resorption support the findings in the adult MRCD cohort despite the different markers used, however the significant increase in the bone formation marker P1NP is in contrast to the results seen in the adult MRCD cohort, where different markers of bone formation, osteocalcin and alkaline phosphatase, did not significantly improve after WBVT. The differing results may be explained by the different bone formation markers used. In comparison to the bone formation markers osteocalcin and alkaline phosphatase, utilised in the adult MRCD cohort, which are released at different stages of differentiation and proliferation of the osteoblast [773], PINP estimates the rate of synthesis of type I collagen, the most prolific collagen in bone [735]. PINP may be a more sensitive measure of bone formation compared to osteocalcin, which is more labile with marked diurnal variation and susceptibility to processing and storage practices [734, 739], and alkaline phosphatase, which lacks specificity to bone due to influences from other organs including the gut, liver, kidney and brain [734, 736]. Corrie et al [222] also speculated that their study may have found a significant increase in bone formation due to the shorter intervention period compared to most of the existing literature, 12 weeks compared to at least 6 months. They proposed that bone formation may be increased during the initial period of exposure to the WBVT stimulus, but that this increase would only be transient, subsiding once adaptations in the skeleton had occurred, hence explaining why longer duration studies did not see a similar increase in bone formation markers [222].

Several studies in the existing literature did not find a beneficial effect of WBVT on bone turnover markers using side-alternating [212], vertical [208, 220, 221, 760], and low-magnitude [211, 758] WBVT in healthy adults [208], post-menopausal women [211, 212, 221, 760], older adults [758], and post-stroke [220], consistent with the findings in the adult MRCD cohort.

Alterations to bone resorption markers [729, 762, 772] appears to occur more regularly than changes in bone formation markers [222] indicating that WBVT may be more successful in reducing the rate of bone resorption than stimulating bone formation in adult populations. The studies significantly improving bone resorption markers affected a variety of markers, CTX [729], urinary NTX/Creatinine ratio [772], and urinary hydroxypyroline [762], and utilised different vibration training protocols, progressive side-alternating [729], progressive vertical [762], and low-magnitude [772] of varying durations, 8 weeks [729], 6 months [762] and 8 months [772] in young adults [729] and post-menopausal women [762, 772]. Only one study found significant improvements in in bone formation markers, P1NP, after 12 weeks of progressive side-alternating vibration in older adults [222]. It is difficult to elucidate the most effective training protocol to elicit changes in bone formation and resorption markers. Side-alternating WBVT [222, 729] has been successful in reducing bone resorption markers and increasing bone formation markers, whereas vertical vibration [762, 772] has only been successful in reducing bone resorption markers. Identifying changes in bone formation markers may be time-sensitive, with one study speculating that changes in bone turnover

occur in the first few months of the training stimulus with a transient change that subside once adaptations in bone have occurred [222].

Serum derived measurements of bone resorption, such as CTX, that is released during collagen breakdown [736, 737], are preferred [727, 740-742] due to limitations in urinary derived measures that increase variability [727, 736, 738, 740, 741, 743-746]. Bone-specific alkaline phosphatase (BAP), which eliminates confounding influences from other organs including the gut, liver, kidney, and brain when alkaline phosphatase is used [734, 736] and has been found to positively correlate with bone formation measured by histomorphometry [736, 737], should also be considered. P1NP, which reflects the rate of synthesis of type I collagen [735], a different aspect of bone formation to that measured by osteocalcin, a protein synthesised by osteoblasts and alkaline phosphatase, an osteoblast ectoenzyme, both of which are involved in the regulation of bone mineralisation [736-738, 773] is likely to be a more sensitive measure of bone formation compared to osteocalcin [734, 739] and alkaline phosphatase [734, 736] and has been utilised in studies of post-menopausal women to assess osteoporosis and assess the effects of anti-resorptive agents [734]. Both BAP and PINP are considered to be more stable than osteocalcin which exhibits sample instability and susceptibility to processing and storage practices as well as it being non-homogenously distributed in the circulation and having high individual variability [736]. The international Osteoporosis Foundation recommends the use of the bone formation marker PINP and the bone resorption marker, CTX, for monitoring osteoporosis in the adult population [774]. The incorporation of these more specific, sensitive and stable, serum derived bone turnover markers in future

larger and longer-term studies are required to determine whether WBVT has the capacity to alter bone turnover markers and improve bone health in adults with MRCD.

5.2.1.2 Follow-up Period

5.2.1.2.1 Trabecular Bone Compartment

5.2.1.2.1.1 Bone Mass and Density

During the follow-up period, there were no significant changes in bone mass parameters measured at the lumbar spine or 4% distal tibial pQCT site in the adult MRCD cohort. Cheung et al [775] followed-up post-menopausal women, 12 months after completing an 18 month randomised controlled trial comparing low-magnitude WBVT (35Hz, 0.3g, 5 times a week) to control. Lumbar spine BMD remained stable during the follow-up period and was not significantly different to those randomised to the control group [775], their finding consistent with those in the adult MRCD cohort. High-resolution pQCT of the distal tibia did not find a significant change in total or trabecular vBMD in the 8 month follow up period in post-menopausal women, randomised to control or side-alternating WBVT (20Hz, 3-4mm, 10 minutes, 2-3 times a week) for 12 months [215]. This result supports the finding at the 4% distal pQCT site in the adult MRCD cohort.

5.2.1.2.2 Cortical Bone Compartment

5.2.1.2.2.1 Bone Mass and Density

In the adult MRCD cohort there were no significant changes in bone mass or density measured by total body DXA scans or at the 20% and 66% pQCT sites during the follow-up period. However, on segmental analysis of the total body DXA scans, legs BMD decreased significantly by 0.008g/cm^2 or 0.7%, just within the reported CV in the adult literature of 0.7% [134]. The magnitude of this significant decrease was larger than the decrease seen in the observation period and the significant increase seen in the WBVT period. It could be postulated that the reduction in legs BMD seen in the follow-up period may be a result of the withdrawal of the vibration stimulus, which may have improved legs BMD through its actions on the leg muscles and the subsequent transfer of forces to the underlying bone during the WBVT period. The forces generated during WBVT cannot be recreated through the normal movements of adults with MRCD during the follow-up period, despite preserved muscle mass seen. Alternatively, the reduced leg BMD may be due to the withdrawal of the actions of the vibration stimulus directly on the bone where it is postulated to cause mechanical deformation of the underlying bone resulting in perturbations in canalicular fluid flow and stimulation of the osteocyte and the WNT/β-catenin pathway [196] resulting in the preservation or augmentation of BMD as was seen in the WBVT period.

One previous study has followed-up post-menopausal women, 12 months after completing an 18 month randomised controlled trial comparing low-magnitude WBVT, (35Hz, 0.3g, 5 times a week) to control [775]. Total hip BMD remained stable during the

follow-up period and was not significantly different to those randomised to the control group [775]. The different anatomic sites measured in the Cheung et al 2016 study [775], total hip, in contrast to segmental analysis of the legs from the total body DXA scan in the MRCD cohort needs to be considered. The larger surface area covered by segmental analysis of the legs from the total body DXA scan may have been more sensitive in identifying changes in the entire lower limb BMD than the small region of interest investigated when analysing the total hip BMD and may account for differences in outcomes. Nevertheless, the adult MRCD cohort may be more susceptible to bone loss after withdrawal of the vibration training compared to post-menopausal females as a significant reduction in legs BMD was found after just 6 months of follow-up in contrast to the non-significant change in post-menopausal women after 12 months follow-up.

5.2.1.2.2.2 Bone Area, Geometry and Strength

The adult MRCD cohort did not display any significant changes in bone area measured by total body DXA scans or the segmental analysis of those scans during the follow-up period. However, there were significant reductions in cortical CSA and cortical thickness measured at the 66% pQCT site of 1.7mm^2 or 0.7% and 0.0mm or 0.9% respectively. There was also a significant reduction in the ratio of cortical CSA to total CSA of 0.4%. The reported CVs for cortical CSA in adults is less than 1% [61], so the changes in cortical CSA and cortical thickness may well be clinically relevant, suggesting endosteal resorption. Considering the cohort of adults with MRCD demonstrated significantly reduced cortical CSA at the 66% site prior to commencing WBVT (Figure 4.16), further reductions in cortical CSA will have negative ramifications on bone health in this cohort.

The distribution-mass relationship in the adult MRCD cohort is disrupted with a given cortical CSA associated with a lower polar CSMI compared to the reference population indicating compromised bone strength (Figure 2.11). Although it did not reach significance there was an associated decrease in the polar CSMI at the 66% pQCT site with this significant decrease in cortical CSA during the follow-up period. If the cortical CSA continues to decline polar CSMI will follow and bone strength will be further compromised in this cohort. Furthermore, the significant reductions in cortical thickness and the cortical CSA to total CSA ratio are likely to increase the buckling ratio at the 66% site causing the bone to be at a greater risk of fracture further compromising bone health in adults with MRCD. As bone and muscle parameters measured by pQCT in the adult MRCD cohort remained stable during the WBVT period, we might speculate that the vibration stimulus had a positive effect on the underlying bone, especially at the 66% tibial pQCT site where the calf muscle mass is at its greatest. The activation of the muscle at this site during WBVT and the associated forces imparted on the underlying bone may have prevented the contraction of cortical CSA and cortical thickness, by endosteal resorption, seen in the follow-up period when the vibration stimulus was withdrawn.

5.2.1.2.2.3 Body Composition

There were no significant changes in any body composition parameters measured using total body DXA scans, or at the 66% distal tibial pQCT site during the follow-up period in adults with MRCD. One previous study followed-up older men, one year after completing a 12 month trial of progressive vertical WBVT (35-40Hz, 5mm, 2-15 mins

with dynamic exercises) and found muscle mass, measured by CT of the upper thigh, decreased significantly (2.6%) [776]. The maintenance of lean tissue mass in the adult MRCD cohort during the follow-up period contrasts to the significant reductions in total body and legs LTM and seen during the observation period, which was also shorter in duration compared to the follow-up period. It may be that WBVT has had a muscle-sparing effect on adults with MRCD that has persisted 6 months post-intervention. The preservation of LTM during the follow-up period did not prevent the loss of legs BMD in the follow-up period as was seen during the WBVT period. These results suggest that the beneficial neuromuscular adaptations likely gained during WBVT are no longer having a beneficial impact on the underlying bone through muscle contractile activity or that the bone was directly stimulated during WBVT, which is postulated to cause mechanical deformation of the underlying bone resulting in perturbations in canalicular fluid flow and stimulation of the osteocyte and the WNT/β-catenin pathway [196].

5.2.1.2.2.4 Bone Biochemistry and Turnover Markers

In the MRCD cohort there were no significant changes in bone turnover markers in the follow-up period indicating continued accelerated bone loss as osteocalcin levels remained below the reference range and the urinary deoxypyridinoline:creatinine ratio above the reference range. These findings support the adverse consequences of removal of the WBVT stimulus on the cortical bone compartment evidenced by significant reductions in legs BMD and cortical CSA and thickness measured at the 66% pQCT site. One study has investigated bone turnover markers 4 weeks after cessation of a side-alternating WBVT intervention in adults and found no significant changes in the bone

formation markers, BAP or osteocalcin, or the bone resorption marker TRACP5b, however CTX, another bone resorption marker, increased significantly [729]. The significant increase in CTX seen after one month of removing the WBVT stimulus is consistent with the adverse effects seen in the cortical bone compartment in the MRCD cohort after removal of the WBVT stimulus. As previously discussed, serum-derived CTX is preferred over urinary-derived measures of bone resorption and may have been more sensitive in identifying any changes in bone resorption after withdrawal of the WBVT in the adult MRCD cohort.

5.2.2 Effect of WBVT on Force Plate Parameters

Only 11 of the 12 participants in the adult MRCD cohort were able to perform the multiple one-leg hop manoeuvre and many of the remaining participants found this outcome measure difficult to perform.

5.2.2.1 WBVT Period

5.2.2.1.1 Multiple One-leg Jump (M1LJ)

The adult MRCD cohort did not exhibit any significant changes in force parameters during the M1LJ after WBVT. Three previous studies have investigated the effects of WBVT on force production during the M1LJ, two in young adults with CF and one in osteopoenic post-menopausal women. In 10 CF adults, 22-27 years, 3 months of

progressive home-based side-alternating WBVT (20-25Hz, 0.6mm, 18 minutes, 5 times a week), found a non-significant increase in force of 72N (~5%) [233]. In the second study of young adults with CF, 21-41 years, six months of home-based side-alternating WBVT (12Hz, 6 minutes with trunk bends, rotations and extensions, 5 times a week, as well as 26Hz, 6 minutes, with the progressive addition of weights up to 9kg, 3 times a week), increased M1LJ force by a median of 6.7%, however no statistics were performed due the small size of the cohort [232]. In osteopoenic post-menopausal women, 9 months of progressive side-alternating WBVT (22-24Hz, 2-4mm, 4 minutes with squat exercises, twice a week) in addition to a conventional aerobic and resistance training program, significantly increased peak force and peak relative force ~4% after WBVT [777]. Each of these studies saw improvements in force parameters during the M1LJ of between 4-7% after WBVT, much larger than the improvements seen in the adult MRCD cohort. The lack of improvements seen in the adult MRCD cohort may have been due to a sub-optimal WBVT protocol. All three studies used a higher frequency than that used in the adult MRCD cohort, and a progressive protocol [232, 233, 777]. The addition of weights [232] or the performance of squat exercise [777] during WBVT may have also improved the effectiveness of the WBVT in improving M1LJ performance.

The M1LJ measures the maximum forces acting through the tibia, making it an attractive outcome measure to use in WBVT studies, as it can be correlated directly to bone health measurements taken of the tibia using pQCT [698, 699]. The lack of improvement in force parameters during the M1LJ may explain the lack of improvements seen in pQCT parameters in the adult MRCD cohort as muscle forces are essential for improving and

maintaining the underlying bone health. The complexity of the movement pattern required to perform the M1LJ with correct technique, made it a difficult outcome measure for the adult MRCD cohort. Many participants were unable to perform the hops on the ball of the foot, or complete 10 consecutive hops, and most required support to maintain balance during the manoeuvre. This, coupled with the severely impaired muscle function demonstrated by the adult MRCD cohort at baseline, indicate that it may not have been the best measurement to assess peak forces and gauge the efficacy of WBVT, perhaps explaining why no changes in force production during the were seen. A study in healthy, older adults, used an isokinetic dynamometer to assess ankle plantar-flexor force after 8 weeks of progressive side-alternating WBVT (26Hz, 5-8mm, 4-8 minutes, with static and dynamic exercises) and found isokinetic ankle plantar-flexion strength increased significantly (18.5%) [778]. Force measurements of the ankle plantar-flexor using an isokinetic motorised dynamometer should be considered for future studies.

Furthermore, maximal force measurements could be assessed by measuring knee extension strength isometrically or isokinetically using a motorised dynamometer or leg press dynamometer. Meta-analyses have investigated the effects of WBVT on knee extension strength measured in this way. In healthy adolescents and adults, 12-78 years, chronic WBVT, compared to the identical training condition without WBVT significantly increased maximum knee extensor strength [300, 301, 307, 314, 197, 299, 259, 315, 203, 262] with a standardised mean difference of 0.76 [224]. A meta-analysis in older adults, mean age 68 years, found WBVT [213, 770, 779, 780] significantly increased isometric knee extension strength, compared to a control intervention, with a standardised mean

difference of 2.15 [216]. However, dynamic knee extension strength [213, 778, 781, 782] was not significantly improved after WBVT compared to a control intervention or exercise intervention [216]. In contrast, another meta-analysis in older adults, 57-82 years of age, found that chronic WBVT [213, 240, 778], significantly improved dynamic knee extension strength compared a control intervention, with a standardised mean difference of 0.63 [217], however no effect of WBVT was found when compared to an exercise intervention [217], the differences in these meta-analyses due to the studies included and the analysis methods used. The latter meta-analysis also found that chronic WBVT [213, 783] had no significant effect on isometric knee extension strength, measured using a motorised isometric dynamometer, when compared to a control intervention or an exercise intervention [217], however, isometric leg extension strength, measured using a leg press dynamometer [766, 770], significantly increased after WBVT, compared to a control intervention ,with a standardised mean difference of 0.57 [217]. These results indicated that the method of assessing lower limb muscle strength is important and can affect the interpretation of the benefits following WBVT.

A purer measure of knee extension strength, using isometric and/or isokinetic motorised dynamometry or leg press dynamometry should be considered in future studies. In fact isometric and isokinetic motorised dynamometry have been used to evaluate the effectiveness of WBVT on knee extension muscle strength in adult cohorts with underlying medical conditions involving movement disorders, including cerebral palsy [235], Pompe Disease [254, 784], multiple sclerosis [252, 265, 785], stroke [786], as well as post-menopausal women [213, 215, 239, 240, 787] and older adults [765, 769, 775,

776, 778, 781-783, 788-792] who often display age-related impairments in neuromuscular function and strength. Many of these studies using this method to assess knee extension strength demonstrated beneficial effects after side-alternating [254, 778, 782, 784, 787, 791, 792] and vertical [213, 239, 769, 775, 781, 783, 788] WBVT. The type of exercise performed during WBVT (isometric or dynamic) should be taken into account when deciding which measurement of knee extension strength (isometric or isokinetic) will be utilised as an outcome measure in future studies, with static exercises likely to result in better isometric muscle strength, and dynamic exercise, isokinetic muscle strength [216].

Another aspect to consider, when trying to elucidate why we did not find any strength benefits after WBVT in the adult MRCD cohort, is the WBVT protocol used. Optimising the protocol in future studies may provide an adequate stimulus to elicit the muscle morphological or neurological changes required for improvements force production to be seen. Two meta-analyses investigated the effect of vibration frequency and amplitude on knee extension muscle strength [224, 793]. They both found WBVT to be more beneficial in improving muscle strength when frequencies were over 30Hz [224, 272], and as high as 50Hz were used [793]. These findings indicate that the frequency in the protocol employed by the MRCD cohort was likely inadequate to elicit changes in neuromuscular performance. Frequency is an important aspect of training as it activates the tonic vibration reflex, which in turn enhances motor unit synchronisation [794], with higher frequencies believed to more closely mimic the rate of motor unit discharge during more forceful muscle contractions [223]. The meta-analyses were not as consistent in

their results for the effect of amplitude on muscle strength, the smaller meta-analysis, which derived data from 7 randomised controlled trials, finding an amplitude of up to 4mm was most beneficial [224], however the larger meta-analysis, deriving data from 18 randomised controlled trials, finding that amplitudes of 8-10mm [793] were most beneficial. This latter meta-analysis also found that amplitude was the vibration training parameter most highly correlated with strength improvements [793]. As the amplitude increases, larger electromyographic activity is elicited [795], which in turn correlates with muscle activation, ultimately impacting on the magnitude of the training stimulus delivered. It would appear that the frequency and amplitude used in the adult MRCD cohort did not deliver an adequate training stimulus to induce the muscle morphological and neuromuscular changes required to improve muscle strength after WBVT. One meta-analyses investigated the effect WBVT volume during each training session and found that 12-15 minutes of WBVT was the most beneficial volume to improve knee extensor strength [793]. The delivery of WBVT in the adult MRCD cohort, 3 bouts of 3 minutes, interspersed with a minimum 3 minute rest period, may also have impacted on the lack of improvement in muscle strength after WBVT, as training intervals of 30-90 seconds, interspersed with 60 second rest periods have been suggested to be most beneficial, likely because they reflect the work/rest intervals of conventional resistance training programs [793]. One meta-analysis found that performing dynamic as well as static exercises during the WBVT improved the beneficial effect of WBVT on muscle strength [793]. The lack of dynamic exercise during WBVT in the adult MRCD cohort may explain why no changes in force generated during the single-two leg jump were seen in the adult MRCD cohort, as the single two-leg jump is a measure of dynamic function. The

incorporation of dynamic exercises into the WBVT protocol likely improves muscle strength outcomes as dynamic exercises demand greater muscle force to be generated which increases motor unit recruitment resulting in improved synchronisation and co-ordination of motor units after WBVT [793]. The majority of studies in this same meta-analysis were progressive in nature and included squat and other exercises [793]. The authors [793] also discuss the potential benefits of including additional weights to WBVT as this results in greater acceleration [796]. The incorporation of progressive protocols that include dynamic as well as static exercise are likely necessary to optimise benefits from WBVT, according to overload principle which is required for favourable muscle morphological and neuromuscular adaptations to occur [223]. These aspects of WBVT were not incorporated in the protocol used by the adult MRCD cohort.

5.2.2.1.2 Single Two-leg Jump (S2LJ)

There were no significant changes in any of the S2LJ power, force, or efficiency parameters during the WBVT period in the adult MRCD cohort. Previous studies in side-alternating WBVT have demonstrated largely beneficial effects of WBVT on S2LJ performance. Only studies investigating the effects of chronic WBV, at least 8 weeks in duration, have been discussed to align more closely with the 6-month intervention period in the adult MRCD cohort. In many studies, jump height was used as a surrogate measure for muscle power [223].

When considering side-alternating WBVT, one RCT in healthy, trained young adults, mean age 20 years, randomised to 11 weeks of progressive side-alternating WBVT (30Hz, 8 mm, 5-8 minutes with exercises) did not demonstrated any significant changes in jump height [238]. While consistent with the results in the adult MRCD cohort, the capacity of this cohort of healthy young adults to improve their functional muscle performance after WBVT may be limited due to their baseline high level of function, in contrast to the adult MRCD cohort, who demonstrated significantly impaired muscle function at baseline. The remaining studies into the effects of side-alternating WBVT on S2LJ performance have demonstrated beneficial effects in cohorts with underlying medical conditions, or age-related decline in muscle function, causing impairments in muscle function, in keeping with the impaired muscle function seen in the adult MRCD cohort. After 3 months of home-based side-alternating WBVT 20-25Hz, 0.6mm, 18 minutes, 5 time a week, 10 young adults with CF, 24-27 years, maximum S2LJ force decreased significantly by 122N (9%), without a significant change in maximum power [233] indicating improved efficiency of muscle function as less force was required to generate an equivalent amount of power [718]. Similar improvements in muscle efficiency were seen in another study of 8 young adults with CF, 21-41 years, after 6 months of home-based side-alternating WBVT (12Hz for 6 minutes with trunk bends, rotations and extensions, 5 times a week, and 26Hz for 6 minutes with additional weights up to 9kg, 3 times a week) with a reduction in maximum S2LJ force by a median of 4.3%, and increase in maximum power by a median of 4.7% [232]. Muscle efficiency during the S2LJ was also improved after 4 weeks side-alternating WBVT (20Hz, 20mm, combined with exercises for muscle co-ordination, 4 times a week) in middle-aged adults

with pulmonary artery hypertension, whose maximum power significantly increased by 4.4%, and maximum force significantly decreased by 2.7% [797]. Furthermore, in a case-study of a 34 year old female with late-onset Pompe Disease, 15 weeks of progressive side-alternating WBVT (5-20Hz, 4mm, 2-4 minutes, 3 times a week) significantly increased maximum S2LJ power by 64% [254]. Pompe Disease sufferers exhibit progressive decline in muscle strength and impaired mobility, similar to that seen in MRCD, however the large improvement seen in power generation during the S2LJ in this case study is much greater than that seen in the MRCD cohort, despite similar features of the training protocol.

Several RCTs in post-menopausal women have found significant improvement in S2LJ performance after side-alternating WBVT: 8 months side-alternating WBVT (12.6Hz, 6 mm, 3-6 minutes, 3 times a week) with a 10 minute stationary bike warm-up significantly increased S2LJ height 1.57cm (13%) [240]; 6 months of progressive side-alternating WBVT (12-28Hz, 3-6 minutes, twice a week) significantly increased maximum S2LJ power by 8.4W (5%) without significant changes in the maximum force [212], indicating improved muscle efficiency; progressive side-alternating WBVT (22-24Hz, 2-4mm, 4 minutes, with static and dynamic squats, twice a week) in addition to conventional aerobic and resistance training for 9 months, increased maximum S2LJ power and the Esslinger Fitness Index significantly after WBVT by 0.6W/kg (2%) and 4% respectively, however, Jump Efficiency Index remained stable in contrast to a significant decline in this parameter seen in those randomised to the balance group [777], this study suggesting WBVT may provide an additional benefit for muscle efficiency over conventional

aerobic, resistance and balance exercise in isolation, as this aspect of jump performance was maintained over the 9 months intervention in the WBVT group but not in the balance group. A RCT older adults, found 6 months progressive side-alternating WBVT (30Hz, 3.9mm, 6-18 minutes, with static and dynamic squat exercises, twice a week) significantly increased jump height by 18.6% [789].

Individuals with underlying medical conditions, post-menopausal women and older adults with disease- or age-related reductions in muscle neuromuscular performance were amenable to improvements after WBVT (often delivered and assessed with the same devices used in the adult MRCD cohort), however similar improvements were not seen in the adult MRCD cohort, despite compromised neuromuscular function at baseline. The lack of improvement seen in the adult MRCD cohort may be due to differences in the vibration training protocols used. The use of a progressive protocol [212, 777, 789], higher vibration frequencies (22-28Hz) [212, 232, 233, 777, 789], higher vibration amplitude [240] [797], the longer duration of each training session [233], the addition of squat exercises during the vibration stimulus [232, 777, 789, 797], or the addition of weights during WBVT [232] may improve the efficacy of the side-alternating WBVT protocol in improving S2LJ performance and should be considered in future studies investigating the effects of WBVT in adults with MRCD.

The majority of studies investigating the effects of WBVT on S2LJ performance have used vertical vibration platforms. A meta-analysis in healthy adults 17-80 years of age, including 15 randomised control trials [158, 169, 175, 208, 238, 239, 769, 798-805],

showed that compared to a control intervention, WBVT significantly increased jump height with a standardised mean difference of 0.77, which was increased to 0.96 when considering non-athletes [223]. This result is consistent with another meta-analysis in older adults, 57-82 years of age, that found chronic vertical WBVT significantly improved jump height with a standardised mean difference of 0.51 [217] when compared to a control intervention [239, 769]. Another meta-analysis investigated the effects of chronic WBVT with exercises (including squats, lunges and heel raises) performed during WBVT compared to the identical training condition without WBVT [169, 212, 237, 766, 806-808], on muscle power in healthy people 11.8-77.5 years of age and found that jump height was significantly increased after WBVT, with a standardised mean difference of 0.87 [224]. The authors suggest that the improvements in jump height after WBVT are likely due to the effects of WBVT on the stretch reflex, which is believed to be an important aspect in enabling increases in muscle stiffness, which underlies the effectiveness of the stretch-shortening cycle in generating force to precipitate the jump [224]. One of the theories behind the effectiveness of WBVT is that it increases the excitability of the stretch reflex via Ia afferents [809].

When WBVT was compared to an exercise intervention [169, 239, 240, 769], including conventional aerobic and resistance training and walking programs, in healthy adults, the meta-analysis found that WBVT had a significantly greater effect on jump height, with a standardised mean difference of 0.63 [223]. This indicates that chronic vertical WBVT is at least as effective in improving S2LJ power as conventional exercise interventions, with these benefits achieved in a shorter time, 15-30 minutes of WBVT compared to up to 90

minutes [769] of conventional training, and with less physical effort, unweighted standing exercises during WBVT, compared to aerobic and resistance training. However another meta-analysis in older adults, 57-82 years of age, found that when compared to an exercise intervention [239, 240, 769], no significant effect of WBVT on jump height was found [217], likely a reflection of the fact that younger participants, less than 60 years [169, 237, 806-808], performed better after WBVT, compared to older participants [212, 766] as reported by Osawa et al [224].

Three studies have investigated the effects of WBVT on single two-leg jump performance after both side-alternating and vertical WBVT and no differences were found between vertical and side-alternating vibration platforms in their effectiveness at improving S2LJ performance in healthy young adult males after 8 weeks of training [729], in post-menopausal women, after 12 months of training [754] or in older adults after 12 weeks of training [222].

Published meta-analyses [217, 223, 224, 272] consistently report a beneficial effect of WBVT S2LJ power parameters healthy adults. The lack of significant improvements in power parameters after WBVT in the adult MRCD cohort are disappointing especially considering the baseline muscle function of the adult MRCD cohort was significantly impaired and it is believed that WBVT may improve muscle function to a larger extent in those with poorer baseline muscle function [223, 272, 810]. This may indicate that the S2LJ test is not the most appropriate test to investigate power in the adult MRCD cohort or that the WBVT protocol used in the adult MRCD cohort was not optimised. As only

healthy individuals were included in the meta-analysis, it is difficult to elucidate whether similar improvements would be expected in cohorts with underlying medical conditions. The few studies that have investigated the effects of WBVT on S2LJ performance in cohorts with underlying medical conditions, have all used side-alternating vibration platforms and have all shown improvements in after WBVT [232, 233, 254, 797], so it would not be unreasonable to expect to see improvements in S2LJ performance in the adult MRCD cohort after side-alternating WBVT.

The measurement of muscle power in the adult MRCD cohort, using the S2LJ, may not have been the most appropriate method to investigate the effects of WBVT on muscle power. The meta-analysis by Marin et al 2010 [272], which included measures of muscle power from a motorised isokinetic dynamometer [169, 781, 798, 801, 802, 811] as well as jump performance, demonstrated the largest standardised mean difference, 0.99 [272], compared to 0.77 [223] and 0.87 [224] for the effect of WBVT on muscle power as well as a larger effect in untrained participants 1.52 [272] compared to 0.96 [223] and those over 50 years of age, 2.24 [272], compared to 0.6 [224] and 0.51 [217]. These results indicate that larger improvements were found in muscle power using methods other than jump performance, especially in untrained individuals and those older in age who have age-related deficits in neuromuscular function, two aspects consistent with the adult MRCD cohort. Future studies should consider using a motorised isokinetic dynamometer to measure the effects of WBVT on muscle power. This method has been used successfully in more recent studies investigating the effects of WBVT in older adults

[222] and stroke patients [220], two cohorts with compromised muscle function and mobility, features congruent with the adult MRCD cohort.

It would appear that the WBVT protocol used in the adult MRCD cohort did not provide sufficient stimulus for the muscle morphological or neurological changes required for improvements in S2LJ performance to occur, and could be optimised in future studies. Three of the meta-analyses investigated the effect of vibration frequency and amplitude on single two-leg jump performance [223, 224, 272]. They all found WBVT to be more beneficial on jump performance when frequencies were over 30Hz [223, 224, 272], higher than the 20Hz used in the adult MRCD cohort. The effect of amplitude on jump performance was not as consistent, however at least 3mm [223] and as high as 8-10mm [272] were found to be the most beneficial. There also appeared to be a beneficial interaction between frequency and amplitude on jump performance, higher amplitudes (>3mm) combined with higher frequencies (>30Hz) the most beneficial combination [223]. While these recommendations are based predominantly on vertical WBVT studies, more recent chronic side-alternating WBVT studies, including studies in those with an underlying medical condition, that have shown beneficial effects on jump performance have used frequencies between 22-28Hz [212, 232, 233, 777, 789] and amplitudes of at least 6mm [240, 797]. Two of the meta-analyses investigated the effect of the volume of WBVT performed during each training session [223, 272]. One study found that 6-10 minutes of WBVT was the most beneficial volume to improve jump performance [272], and the other found that over 10 minutes was more beneficial [223]. The way the WBVT was delivered in the adult MRCD cohort may also have impacted on the lack of

improvement in S2LJ performance after WBVT. The training protocol employed delivered the WBVT in three bouts of 3-minutes training, with at least 3 minutes rest between bouts. It has been suggested that shorter training intervals, up to 60s in duration, will likely to limit neuromuscular fatigue [272], which may have been a factor for the adult MRCD cohort who trained for 3-minute durations. One meta-analysis found that WBVT over 12 weeks in duration was more beneficial to jump performance [223]. The MRCD cohort trained for 6-months, however longer intervention periods of 12 -18 months may need to be considered to elicit improvements in muscle function during the S2LJ. The beneficial effects of WBVT were also improved with progressive WBVT protocols (manipulating frequency, amplitude and volume), when dynamic exercises were performed during WBVT, and when exercises other than squats (including lunges and heel raises) were performed during WBVT [272]. These aspects of the WBVT protocol ensure the overload principle is met which likely optimises neuromuscular adaptations for improving power after WBVT [272] and were not used in the adult MRCD cohort.

5.2.2.1.3 Chair-rise Test (CRT)

After WBVT, the adult MRCD cohort significantly decreased CRT time by 0.2s (7.7%), well above the CV reported in the literature [691, 692], however the corresponding SDS did not change significantly. There were no significant changes in force or power parameters. Three previous studies have investigated the effects of WBVT on the CRT using a force platform generating parameters of force, power, velocity and time per test.

In young adults with CF, 24-27 years, 3 months of home-based side-alternating WBVT (20-25Hz, 0.6mm, 18 minutes, 5 times a week) significantly reduced the time per test by 1.05 seconds (15%) and significantly increased mean velocity by 0.13m/s (21%), maximum force and relative force by 438N and 7N/kg (41%) and maximum power and relative power by 135W and 2.38W/kg (19%) [233]. Similar results were found in institutionalised elderly, mean age 85 years, randomised to 8 weeks of progressive vertical WBVT (30-35Hz, 4mm, 5-10 minutes, with exercises, 3x/week) or control [812]. The number of chair-rises performed in 30 seconds significantly increased by 36% and this was significantly greater than the control group, however the maximum velocity achieved during the chair-rise test did not change significantly over time or between groups [812]. There was a significant increase in maximum power of 12%, significantly greater than the control group [812]. These increases in force and power parameters were not seen in the adult MRCD cohort. The improvement in the time to complete the CRT was larger than adult MRCD cohort, perhaps a reflection of the longer duration of each training session in the Rietschel et al study [233] or the addition of exercises during WBVT in the Alvarez-Barbosa et al study [812]. Conventional aerobic and resistance training with progressive side-alternating WBVT (22-24Hz, 2-4mm, 4 minutes, with static and dynamic squats, twice a week) or a balance program including proprioceptive and balance training for 9 months, in post-menopausal women with osteopenia or osteoporosis, significantly decreased the time to perform the CRT by 0.11 seconds (8%), and significantly increased the relative force by 0.02g (1.5%) in the WBVT group, however no between-group differences were found [777]. There were no significant

changes in relative power parameters [777]. Similar magnitude changes in time per test were seen in the adult MRCD cohort, despite a much less intensive training regime.

The remaining literature has investigated the effect of WBVT on CRT time parameters. There have been several studies investigating the effect of WBVT on the time to complete the CRT in older adults, which is used as a surrogate measure of functional muscle strength of the lower limbs. A systematic review and meta-analysis found that WBVT had a significant treatment effect on the time to complete the CRT or the number of CRT completed within a defined time, with a standardised mean difference of 0.72 when compared to control [778, 813, 814] interventions [217].

Previous studies investigating the effect of side-alternating WBVT have in general, shown beneficial treatment effects in reducing the time to perform the CRT in post-menopausal women [240, 757], middle aged adults [797], older adults [792, 815, 816]. Studies reporting greater improvements in CRT time compared to the adult MRCD cohort, performed exercises during the WBVT intervention [797, 816] or utilised a higher frequency of 24-26Hz [815, 816]. Progressive side-alternating vibration interventions in older adult populations [778, 791, 814, 817-819] have reported larger improvements in chair-rise test performance than those seen in the adult MRCD cohort. These studies all used higher frequencies, up to 30Hz, and some utilised larger amplitudes of 6-14mm [778, 791, 817]. The existing literature in vertical WBVT in patients with Multiple Sclerosis [820], post-menopausal women [757] and older adults [222, 244, 771, 820] demonstrate conflicting results on the effects of vertical WBVT on CRT time, however

the studies demonstrating a beneficial effect of WBVT [757, 771, 820, 821] were generally larger in magnitude compared to the adult MRCD cohort, perhaps due to their longer duration [757, 771], higher frequency [820], the addition of squat exercise during WBVT [820], or concurrent participation in a multi-disciplinary rehabilitation program [820]. In general, progressive vertical WBVT protocols were beneficial in improving CRT performance in older adults [813, 822]. The improvements in chair-rise time seen in these studies were greater in magnitude compared to the adult MRCD cohort, perhaps a consequence of the different vibration stimulus used, the progressive nature of the vibration protocol, the higher maximum frequency (25-40Hz) or addition of exercises [813, 822] during the WBVT.

5.2.2.1.3.1 Chair-rise Summary

There is limited literature exploring the effects of WBVT on power, force and velocity parameters during the chair-rise test as few studies have used a force platform during the manoeuvre, the vast majority instead performed as field tests with a chair and timing device. Despite the paucity of data and variety of cohorts investigated [233, 777, 812], it appears that both side-alternating [233] and vertical platforms [812] are able to significantly increase the power generated during the CRT. This parameter remained unchanged in the adult MRCD cohort after 6 months of WBVT. Force [233, 777] and velocity [233] parameters only demonstrated significant changes after side-alternating, but not vertical [812] WBVT. This observation should be considered with caution however due to the small amount of literature available. Both the side-alternating and

vertical WBVT protocols that were successful in improving power parameters in the CRT used higher frequencies than those utilised in the adult MRCD cohort, 25Hz [233] and 35Hz [812] respectively, compared to 20Hz. The side-alternating WBVT protocol with beneficial effects on power and velocity also used a smaller amplitude 0.6mm and longer training duration, 18 minutes [233], compared to 4mm and 9 minutes in the adult MRCD study, two other important aspects that should be considered when designing WBVT protocols for future studies.

There is an abundance of literature investigating the effect of WBVT on the time to perform the CRT and the number of chair-rises performed in a defined time period. The vast majority of these studies have been performed on older adults. Both progressive [778, 791, 814, 817-819] and non-progressive [240, 757, 792, 797, 815, 816] side-alternating and vertical [757, 771, 813, 820-823] WBVT protocols, as well as low-magnitude vertical WBVT [757], have shown beneficial effects on chair-rise time, side-alternating protocols more consistently demonstrating beneficial effects compared to vertical protocols. The majority of the existing literature has demonstrated that WBVT improves CRT time when participants act as their own controls [233, 792, 797] or in comparison to a control group [757, 771, 778, 812-815, 817-819, 823], however some studies, all using vertical WBVT protocols, did not find an effect of WBVT over control [222, 244, 781, 821].

There may be evidence in the existing literature to suggest that progressive WBVT protocols [777, 778, 791, 812-814, 817-819, 822, 823], using side-alternating or vertical

vibration platforms, result in larger magnitude gains in chair-rise time in shorter time periods, compared to non-progressive WBVT protocols [233, 240, 757, 771, 792, 797, 815, 816, 820, 821], and should be considered when designing WBVT protocols for future studies in adults with MRCD. Progressive WBVT protocols in the literature generally used higher frequencies, 25-40Hz, than that used in the adult MRCD study, and an amplitude of at least 3mm. Two studies investigated the effect of the number of training session performed each week on CRT performance [813, 823]. Both studies found that a minimum of 2 training sessions a week were required to elicit improvements in CRT performance, with improvements increasing with the number of sessions performed [813, 823].

5.2.2.2 Follow-up Period

5.2.2.2.1 Multiple One-leg Jump

In the follow-up period, there were no significant changes in muscle force parameters during the M1LJ in the adult MRCD cohort. No previous studies have investigated the effects of WBVT on multiple one-leg hop performance after withdrawal of the WBVT stimulus.

5.2.2.2.2 Single Two-leg Jump

During the follow-up period, there were no significant changes in any S2LJ force, power or efficiency parameters in the adult MRCD cohort. These results are consistent with a study of healthy young adults that found no significant changes in S2LJ maximum force or power 4 weeks after completing 8 weeks of side-alternating WBVT (18-22Hz, 9 minutes, 3 times a week) or low-magnitude vertical WBVT (32-37Hz, 10-20 minutes, 3 times a week) [729]. In another group of young adults, jump height decreased significantly by 5% 5 weeks after completing 13 weeks of vertical WBVT (35Hz, 2mm, 9 minutes, twice a week) with progressive resistance training for the lower limbs and trunk [824].

In older males, jump height decreased significantly by 6.1% 12 months after completing a 12 month progressive vertical WBVT (35-40Hz, 5mm, 2-15 minutes with static and dynamic exercises, 3 times a week), but remained significantly greater than baseline performance [776]. Those in fitness group which combined aerobic, resistance and balance training for 90 minutes, and control group demonstrated larger reductions in jump height that were not significantly different to their baseline performance [776]. This suggest a slower decline in the S2LJ benefits seen after WBVT compared to a conventional fitness program.

The persistence of benefits in S2LJ performance after ceasing a WBVT intervention, or lack thereof, requires further investigation. It is likely the length of the intervention period, the intensity of the WBVT protocol as well as the baseline level of muscle

function, age of participants, and the presence of any underlying medical conditions will influence the outcome.

5.2.2.2.3 Chair-rise Test

During the follow-up period, the adult MRCD cohort demonstrated significant increases in CRT force and power parameters. Maximum force increased by 0.1kN (8.3%), maximum relative force increased by 1.2N/kg (5.9%) and the SDS increased by 0.7. Maximum power increased by 75.6W (16.1%), maximum relative force by 1.1W.kg (14.7%) and the SDS increased by 0.5. The significant increases in both force and power parameters with any significant improvement in CRT speed parameters indicates worsening of muscle efficiency.

Two previous studies in older adults measured CRT after a follow-up period once WBVT had ceased. Neither of these studies performed the CRT on a force plate and thus did not report on velocity, power or force parameters during the chair-rise test however they did investigate time parameters. Six months after a 6 week progressive vertical WBVT with exercises, CRT time decreased significantly by 13.25s (44%) institutionalised elderly [822]. The ongoing significant improvement in CRT performance in contrast to the lack of improvement seen in the adult MRCD cohort and may be due to bi-weekly group exercise sessions attended by participants during the follow-up period [822]. In community-dwelling older adults, the number of chair-rises completed in 30 seconds decreased significantly during a 3-week follow-up period, in those randomised to progressive WBVT with exercises twice a week for eight weeks, however those

randomised to four times a week did not demonstrate significant differences to their post-training results [823]. The results of this study indicate that a training threshold may need to be attained for maintenance of muscle function improvements during the chair-rise test after cessation of WBVT. Both studies also included exercises during their WBVT intervention, an aspect that was not incorporated into WBVT for the adult MRCD cohort, and which may enhance or extend the benefits of WBVT on muscle function during the CRT.

5.2.3 Effect of WBVT on Exercise Capacity

5.2.3.1 WBVT Period

5.2.3.1.1 Six-minute Walk Test (6MWT)

The pre-exercise Borg Score was significantly reduced by 0.4 of a point, which may indicate a minor reduction in general fatigue in the adult MRCD cohort after 6 months of WBVT, however the clinical relevance of this small change is questionable. After WBVT period there were no significant changes in 6MWT distance, however there was an improvement of 19.2m (4.8%). This change was slightly larger than the smallest meaningful change in 6MWT distance of 20m found in a large cohort of community-dwelling older adults, sub-acute stroke survivors and older adults with mobility impairments [825]. In a smaller cohort of individuals with Alzheimer Disease, affected by dementia, mean age 80 years, the minimal detectable change was found to be 33.5m

[826], and another small study of individuals with Parkinson Disease, mean age 71 years, found the minimal detectable change for the 6MWT distance to be 82m [827], the change seen in the adult MRCD cohort smaller than both these cohorts sharing impairments seen in adults with MRCD, dementia and movement disorders respectively. Multi-center drug trials in other metabolic conditions including mucopolysaccharidosis have demonstrated improvement in 6MWT distance of 38m (11%) [828] and 30m (8%) [829] after 26 and 52 weeks respectively. In another multi-center drug trial, individuals with Pompe Disease increased 6MWT distance by 28m (9%) after 78 weeks [830]. The 22m (6%) increase in 6MWT distance after WBVT in the adult MRCD cohort is similar in magnitude to the 30m (8%) [829] and the 28m (9%) [830] improvement in 6MWT distances seen after drug trials in other metabolic conditions after much longer intervention periods, and indicates that while not statistically significant, the improvements seen in the adult MRCD cohort may be clinically relevant, suggesting a beneficial effect of WBVT on functional capacity.

The effects of side-alternating and vertical WBVT on 6MWT distance have been investigated previously. The previous literature investigating the effects of side-alternating WBVT has shown beneficial effects of WBVT on 6MWT distance in: case studies of late-onset Pompe Disease [254, 784]; post lung transplant [831]; middle-aged adults with Multiple Sclerosis [785]; middle-aged adults with pulmonary artery hypertension [797]; and older adults with COPD [791, 816]. These studies reported improvements in 6MWT distance of similar or greater magnitude than that seen in the adult MRCD cohort. Those reporting larger increases in 6MWT distance were also

participating in a concurrent rehabilitation program [784, 816, 831], used higher frequencies 26-40Hz [785, 791, 816], or larger amplitudes of 6-20mm [791, 797, 816], used progressive protocols [785, 791, 797], performed exercises during the WBVT [785, 797, 816], used a training frequency of at least 3 times a week [254, 784, 785, 797, 816, 831], for at least 12 weeks duration [254, 784, 785, 791]. These aspect of WBVT should be considered in future studies to optimise the improvement in 6MWT distance after side-alternating WBVT.

Previous literature investigating the effect of vertical WBVT on 6MWT performance have shown varied results. Beneficial effects of WBVT on 6MWT distance have been seen in: middle aged adults with Multiple Sclerosis [820]; stroke survivors [268, 832]; older adults [244, 821, 833]; and older adults with COPD [790, 834], but not in young adults with cerebral palsy [235]. No effect of WBVT was found on the two-minute walk test distance in middle-aged adults with Multiple Sclerosis [252], however older adults demonstrated significant improvement in the shuttle walk test after WBVT [332]. Those studies demosntrating improvements of greater magnitude in the 6MWT, to those seen in the adult MRCD cohort were concurrently participating in a rehabiitation program [820]; used higher frequencies of 25-40Hz [268, 790, 820, 821, 832-834]; used progressive protocols [268, 834]; performed squat exercise during the WBVT [790, 820, 821, 832, 833]; or performed WBVT for 12-15 minutes each session [268, 790, 832], at least 3 times a week. [788, 790, 820, 821, 834]. These aspects of the WBVT protocol should be considered in future studies using vertical platforms.

5.2.3.1.2 Cardiopulmonary Exercise Test

During the WBVT period there were no significant changes in any anaerobic threshold, or peak exercise parameters during CPET in the adult MRCD cohort, however there was a non-significant increase in VO_2 of 5%, similar in magnitude to the increase in 6MWT distance after WBVT. These results are in contrast to two published studies that have investigated the effects of WBVT on CPET. In middle-aged adults with pulmonary hypertension, oxygen uptake at AT and peak exercise significantly increased by 6% and 7% respectively, after 4 weeks of side-alternating WBVT (20Hz, 20mm, 4 times a week with exercises for muscle co-ordination) [797]. Even larger results were seen in community-dwelling older adults, mean age 67 years, randomised to progressive vertical WBVT (30-40Hz, 2.5-5mm, 2-15 minutes with exercises including squats, lunges and heel raises, 3 times a week for 12 months), significantly increased peak oxygen uptake by 18% after WBVT [783]. Both these studies included exercise as part of their vibration protocols, the former also utilised a larger vibration amplitude compared to the adult MRCD study and the latter a higher vibration frequency, longer training duration and longer intervention period, all aspects of their respective protocols which may have contributed to larger improvements in oxygen uptake after WBVT.

5.2.3.1.3 Summary

The outcome measures used to determine exercise capacity may not have shown any significant changes after WBVT as the vibration protocol did not reach a high enough

intensity to elicit meaningful cardiovascular work. To improve cardiovascular fitness and endurance, as measured by the 6MWT and CPET, the prescribed training should provide a workload that is 40-50% of an individual's peak VO_2 and/or 60% of their age-predicted maximum heart rate [835]. While these parameters were not directly measured in the adult MRCD cohort during WBVT, previous studies have found minimal cardiovascular response to WBVT, even when squatting exercises were added during the WBVT stimulus [154, 185, 836]. The addition of extra weight during the squatting exercise and increasing frequency and amplitude [154, 185] still did not elicit an adequate cardiovascular response, the fatigue experienced by the participants driven by the neuromuscular system and not the cardiovascular system [185].

Future studies may consider adding an additional measure of functional mobility that can assess maximal gait speed which reflects the ability to adapt to changing environmental demands and obstacles [837]. The 10m walk test is most commonly used however lengths can vary between 6-12m. Participants are asked to walk at their comfortable or fastest walking speeds and the time taken to cover the defined distance is recorded. The 10m walk test has been used extensively as an outcome measure, with established reliability and validity, in neuromuscular disease including Multiple Sclerosis [838], children with neuromuscular diseases [839], older adults [825], Parkinson Disease [827], stroke [840-842] and normative data exists [841, 843]. The 10m walk test has also been used as an outcome measure investigating the effectiveness of WBVT interventions in individuals with multiple sclerosis [265, 820, 844], Parkinson Disease [263] and older

adults [778, 821, 845-848] and demonstrated improved performance after vertical WBVT [820] and side-alternating WBVT [263, 844-847] interventions.

5.2.3.2 Follow-up Period

5.2.3.2.1 Six-minute Walk test

There were no significant changes in 6MWT distance, heart rate or Borg Score parameters during the follow-up period in the adult MRCD cohort. Similar results were seen in chronic stroke survivors, where there was no significant change in 6MWT distance one month after an eight week progressive vertical WBVT intervention (20-30Hz, 0.4-0.6mm, 9-15 minutes with exercises including squats, lunges and heel raises, 3 times a week) [268]. After completing 24 months of side-alternating WBVT (8-18Hz, 4mm, 6 minutes, 3 times a week), a 72 year old female with late-onset Pompe Disease, reduced her 6MWT distance by 42m (9%) at one month follow-up [784]. The more rapid decline in 6MWT distance demonstrated in this case-study, compared the adult MRCD cohort, may be a reflection of the lower frequency, training volume or frequency of training sessions each week used in comparison to the adult MRCD vibration protocol, the effect of which may not have been as effectively sustained.

5.2.3.2.2 Cardiopulmonary Exercise Test

During the follow-up period, there were no significant changes in any of the AT or peak exercise parameters. However, several anaerobic threshold parameters, including VO_2

and oxygen pulse, demonstrated non-significant, but clinically relevant decreases of 18% in the follow-up period, indicating that the anaerobic threshold occurred earlier in exercise, a reflection of worsening aerobic efficiency as the transition to anaerobic metabolism during exercise began earlier. There was also a non-significant, but clinically relevant decrease in peak VO_2 of 8%, indicating that there was a clinical de-training effect on anaerobic threshold parameters and peak VO_2. These findings would suggest that WBVT preserved aerobic capacity in the adult MRCD cohort and in particular, aerobic efficiency at submaximal exercise. No previous studies have investigated CPET outcomes after cessation of WBVT intervention.

5.2.4 Effect of WBVT on the Mitochondrial Disease Scale and Quality of Life

5.2.4.1 WBVT Period

After WBVT the NMDAS Section 3 score decreased significantly by 1.7 points, indicating improvement in the clinician's assessment of clinical function. There was also a non-significant decrease in the NMDAS Section 1 score of 1.8 points, indicating that the patients also perceived their current function to have improved after WBVT and a non-significant increase in the role physical domain of the SF12v2 of 0.5 SDS. These results suggest that while there was minimal improvement in function measured by the M1LJ, S2LJ and CRT or endurance measured by the 6MWT and CPET, the adult MRCD cohort felt improvements in function, including activities of daily living, gait stability and exercise tolerance, that were not reflected in the chosen outcome measures. This finding

supports the consideration of additional and/or alternative outcome measures for functional mobility and lower limb strength as discussed above.

The effect of WBVT interventions on disease-specific scales have been investigated in Parkinson Disease using the Unified Parkinson Disease Rating Scale. After 3 weeks of side-alternating WBVT (25Hz, 7-14mm, 15 minutes, twice a day, 5 times a week) as part of a multi-component inpatient rehabilitation program, the total score improved significantly [263]. After 5 weeks of side-alternating WBVT (6Hz, 13mm, 5 minutes, 2-3 times a week), there were significant improvements in the total score and motor section scores however these improvements were similar to the placebo group [264].

Two previous studies in older adults have used the SF-12 to investigate the effects of WBVT on quality of life. Significant improvements in the SF-12 physical component score were found after progressive side-alternating WBVT (6-26Hz, 1-3mm, 4-5 minutes, 3-5 times a week for 8 weeks) [819] and after progressive side-alternating WBVT (15-30Hz, 2-8mm, 5 minutes, 3 times a week for 8 weeks) in addition to an exercise program including strength, balance and functional mobility training [845] in older adults. Neither study found significant changes in the mental component score [819, 845] consistent with that seen in the adult MRCD cohort. Both these studies demonstrated findings consistent with the adult MRCD cohort for the mental component scores, however they found a beneficial effect of WBVT on the physical component score which was not seen in the adult MRCD cohort. These findings in contrast to the adult MRCD cohort where not improvements in the physical component score were seen, perhaps a reflection of the

progressive nature of the vibration protocols [819, 845], the higher frequency [819, 845] and amplitude [845] used in these studies or the concurrent participation in an exercise intervention [845].

The SF-36, a longer version of the SF-12, has been used more extensively to investigate the effect of WBVT on quality of life and demonstrated conflicting results in the literature. No effect of WBVT on the SF-36 was found in older adults [222, 753, 823] and post-menopausal females [849] using side-alternating, [222] vertical [222, 823, 849] and low-magnitude [753] WBVT. In contrast, several studies have found a beneficial effect of WBVT on quality of life. Improvements in quality of life measured by the SF-36 were found in middle-aged adults with pulmonary artery hypertension [797], middle-aged adults post lung transplant [831], middle-aged adults with Multiple Sclerosis [844] post-menopausal women [850], older adults [813, 851, 852] using side-alternating [797, 831, 844] or vertical [813, 850-852] WBVT.

Other quality of life measures have also demonstrated conflict in the literature about the effect of WBVT on quality of life. Significant increases in quality of life were found after vertical WBVT in older adults measured by the EuroQol-5D [812]; after vertical WBVT in older males with COPD measured by the St George Questionnaire [834] after side-alternating WBVT in older adults with COPD measured by the Chronic Respiratory Questionnaire [816]. However, no change in Quality of Life was seen after side-alternating WBVT in middle-aged females with fibromyalgia measured by the Fibromyalgia Impact Questionnaire or 15D [853]; after side-alternating WBVT in older adults with COPD measured by the St George Questionnaire [791]; after vertical WBVT

in middle-aged adults with Multiple Sclerosis measured by the Multiple Sclerosis Impact Scale [265]; after side-alternating WBVT in older adults with Parkinson's Disease measured by the Parkinson Disease Questionnaire [264].

Studies demonstrating the most beneficial effects on quality of life used vibration protocols with different training parameters to those used in the adult MRCD cohort, which could be considered in training protocols for future studies. Vibration training frequencies of 25-35Hz [812, 813, 834, 844, 850-852] and amplitudes of 6-20mm [797, 844, 852], combined with exercises including squats, lunges and heel raises [797, 812, 850], for 10-20 minutes [812, 831, 851] should be incorporated into future protocols to improve quality of life. Vibration training alongside balance training [850] or physical training [852] programs may also be beneficial.

5.2.4.2 Follow-up Period

There were no significant changes in the NMDAS or SF12v2 during the follow-up period in the adult MRCD cohort. One previous study in Parkinson's Disease has investigated the effect of WBVT cessation on a disease-specific scale and found similar results to those seen in the adult MRCD cohort. Four weeks after completion of side-alternating WBVT (25Hz, 7-14mm, 15 minutes, twice a day, 5 times a week for 3 weeks) as part of a multi-component inpatient rehabilitation program, there were no significant changes in the Unified Parkinson Disease Rating Scale score in older adults [263].

One previous study has investigated the effect of cessation of a WBVT intervention on the SF-12. At 6 months follow-up, the physical component score significantly decreased in older adults, [845] in contrast to no change seen in the adult MRCD cohort, however, consistent with the MRCD cohort, the metal component score did not change significantly [845]. Three previous studies have investigated the effects of WBVT on quality of life, using the SF-36, after the WBVT intervention has been ceased. There were no changes in the SF-36 in post-menopausal women 12 months after completing low-magnitude WBVT [775], in community-dwelling older adults 3 weeks after completing progressive vertical WBVT [823], or in middle-aged adults with Multiple Sclerosis two weeks after completing progressive side-alternating WBVT [775].

5.3 Leigh Syndrome

5.3.1 Effect of WBVT on Bone and Body Composition Parameters

5.3.1.1 WBVT Period

After WBVT, the Leigh Syndrome cohort did not have any significant changes in parameters of the trabecular bone compartment. This was consistent with the findings in the adult MRCD cohort but in contrast to the paediatric MRCD cohort that demonstrated increases in bone mass in the lumbar spine and at the 4% pQCT site.

In the cortical bone compartment the Leigh Syndrome cohort demonstrated significant changes in bone mass, area, geometry and strength. Bone mass parameters from segmental analysis of total body DXA scans as well as parameters from the 20% and 66% distal tibial pQCT sites increased significantly after WBVT training. Legs BMC increased significantly by 15.1g (3.1%), larger than the reported CV at our laboratory (Table 2.1), however the rate of increase slower than that seen during the observation period and smaller than increases seen in the paediatric MRCD cohort, reflecting the combination of paediatric and adult participants in the cohort. Cortical BMC at the 20% pQCT site increased significantly by 3.4% and total and cortical BMC at the 66% pQCT site increased significantly by 2.5% and 3.1% respectively. Cortical polar SSI at the 20% pQCT site increased significantly by 4.4%. All pQCT parameters were smaller in magnitude compared to the significant improvements seen in the corresponding parameters in the paediatric cohort, indicating that these changes are likely a reflection of responses in the paediatric participants in the Leigh Syndrome cohort. There were no significant changes in markers of bone turnover, consistent with both the paediatric and adult cohorts.

5.3.1.2 Follow-up Period

The Leigh Syndrome cohort did not demonstrate any significant change in the trabecular bone compartment during the follow-up period, consistent with the findings of both the paediatric and adult MRCD cohorts, however there were significant changes in the cortical bone compartment. Legs BMC increased significantly by 2.4% an improvement that was not seen when the adult and paediatric cohorts were analysed separately,

however the rate of increase in this parameter was slower than that seen in both the observation and WBVT periods, and may indicate that WBVT is beneficial in maintaining bone mass accrual in the legs of individuals with Leigh Syndrome. Bone strength measured by the polar cortical CSMI at the 66% pQCT site increased significantly by 2.2%, a quarter of the significant increase seen in the same parameter in the paediatric cohort, indicating that the Leigh Syndrome cohort may not have been able to maintain improvements in bone strength after WBVT as well as children with other MRCD. There were no significant changes in bone turnover markers consistent with the findings of the adult MRCD cohort. A much larger cohort of participants with genetically identical Leigh Syndrome is needed to confirm any differences in bone health and bone responses to WBVT between Leigh Syndrome and other MRCD.

5.3.2 Effect of WBVT on Force Plate Parameters

5.3.2.1 WBVT Period

CRT speed, force and power parameters did not change significantly after WBVT in the Leigh Syndrome cohort. In comparison to the paediatric MRCD cohort, this indicated maintenance of muscle efficiency during the CRT, however in comparison to the adult MRCD cohort, there did not appear to be improved leg muscle agonist/antagonist co-activation.

5.3.2.2 Follow-up Period

CRT force parameters increased significantly in the Leigh Syndrome cohort during the follow-up period. Maximum force by 0.2kN (18.2%), average relative force by 0.1g (9.1%) and the force SDS by 1.1. These findings were consistent with adult MRCD cohort as well as non-significant changes in the same parameters in the paediatric cohort, and indicate a de-training effect and worsening of muscle efficiency during a functional task.

5.3.3 Effect of WBVT on Exercise Capacity

5.3.3.1.1 Six-minute Walk Test (6MWT)

5.3.3.2 WBVT Period

In comparison the significant decrease in 6MWT distance and percent-predicted values seen during the observation period, there was a non-significant increase in 6MWT distance of 24.0m (8.7%) and a 3.3% increase in the percent-predicted value after WBVT. This increase in 6MWT distance was larger than the 4.8% increase seen in the adult MRCD cohort and in contrast to a reduction in 6MWT distance seen in the paediatric MRCD cohort. There was also a significant reduction in the recovery Borg Score, indicating less perceived effort and improved recovery, this change also seen in the paediatric MRCD cohort.

5.3.3.3 Follow-up Period

There was no significant change in 6MWT distance during the follow-up period, which was also seen in the paediatric and adult cohorts. This is particularly beneficial for the Leigh Syndrome cohort as significant reductions were seen during the observation period, suggesting a latent beneficial effect of WBVT in maintaining sub-maximal muscular endurance.

5.3.4 Effect of WBVT on the Mitochondrial Disease Scale and Quality of Life

5.3.4.1 WBVT Period

The Leigh Syndrome cohort did not demonstrate any changes in the NPMDS disease scales or Quality of Life measure after WBVT, consistent with that seen in the paediatric MRCD cohort.

5.3.4.2 Follow-up Period

During the follow-up period, Section 3 of the NPMDS increased significantly, indicating deterioration on the clinician's clinical assessment. The paediatric and adult MRCD cohorts did not demonstrate a similar decline in function, consistent with a detraining

effect after removal of the WBVT stimulus which was also postulated for reductions in CRT performance during the follow-up period in the Leigh Syndrome cohort.

6 Conclusions, Limitations and Future Directions

The primary aim of this thesis was to determine whether a six-month home-based side-alternating WBVT intervention influences bone mass, density, geometry and strength in children with CF or MRCD. Secondary aims included determining the influences of the WBVT intervention on bone turnover markers, muscle mass and function, exercise capacity and Quality of Life. The results presented in this thesis have shown that WBVT was well tolerated, with contrasting effects on children with different chronic illnesses and on children and adults with same chronic illness. WBVT in the CF cohort produced increases in trabecular bone mass and density, that were not seen during the observation or follow-up periods whereas WBVT in the paediatric MRCD cohort elicited significant increases in both the trabecular and cortical bone compartments with the greatest effects on cortical bone mass, geometry and strength. In contrast, WBVT in the adult MRCD cohort had very little impact on the trabecular or cortical bone compartments, likely a reflection of the lower metabolic activity of the adult skeleton compared to the immature skeleton of the growing child. When considering the Leigh Syndrome cohort, WBVT had no effect on the trabecular bone compartment however cortical bone mass, geometry and strength were favourably influenced. There was minimal influence of the WBVT intervention on bone turnover markers in any of the cohorts. WBVT augmented muscle CSA in the paediatric MRCD cohort and appeared to hinder the rate of decline in LTM in the adult MRCD cohort but did not have an impact on muscle mass in the CF or Leigh Syndrome cohorts. WBVT appeared to inhibit the deterioration in muscle force and exercise capacity in the CF and Leigh syndrome cohorts, provide some subtle benefits to

muscle function and exercise capacity in the adult MRCD cohort, but did not appear to influence muscle function or functional exercise capacity in the paediatric MRCD cohort. WBVT did not influence Quality of Life in any of the three MRCD cohorts however, the parent-reported school and emotion domains of the CFQ-R improved significantly in the CF cohort. This data is the first to investigate the effects of WBVT in people with MRCD and is the first to investigate the effects of WBVT on bone health in children with CF, adding to a small body of adult literature in this area.

When analysing the baseline DXA and pQCT scans in the MRCD cohorts, a compromised bone phenotype was identified, which could not be completely attributed to a secondary bone defect. The underlying mechanism contributing to the mixed nature of this bone defect cannot be elucidated with non-invasive measures. Verification of the possible mechanisms that may be contributing to the MRCD bone phenotype requires further investigation with the use of invasive techniques including but not limited to bone biopsies, static and dynamic histomorphometry, quantitative backscattered electron imaging, mechanical testing of bone stiffness and toughness, tissue mineralisation, intra-cortical remodelling rate, osteoblast and osteoclast number and bone turnover rate [6, 80, 114, 132, 722, 723, 854]. These findings will be instructive in better managing bone health in people with MRCD.

When comparing outcomes in the CF and MRCD cohorts to the existing literature, it became clear that the WBVT protocol used during the intervention period was likely sub-optimal, and did not provide sufficient stimulus for the muscle morphological or

neurological changes required for improvements in muscle function and bone mass, as better improvements were seen in other cohorts with more intensive WBVT protocols. It is likely that WBVT protocols will need to be tailored specifically for the desired outcome (bone density or muscle function) and the musculoskeletal site where the outcome is desired (lumbar spine or total body). Recommendations from the existing literature have been summarised at the end of each section, however it is important to have a progressive WBVT protocol to meet the requirements of the overload principle. Future studies should consider progressing vibration training protocols by increasing frequency (25-40Hz), amplitude (6-10mm), training time (12-15 minutes) or adding exercises (static/dynamic lunges, squats, heel raises, weight shifts) and/or the addition of weights during the vibration-training stimulus. The addition of exercises or using WBVT interspersed with short intervals of aerobic training may improve aerobic capacity. Shorter training intervals of 30-90 seconds, interspersed with 60-second rest periods, will limit neuromuscular fatigue, compared to the 3 minutes intervals used in the CF and MRCD cohorts, and may be more beneficial. Training should be performed at least 3 times a week. The effects of vertical as well as side-alternating WBVT platforms should also be investigated. While interventions of at least 12 weeks may be effective in eliciting changes in muscle mass and function, it is likely intervention periods of at least 9-18 months are required to elicit changes in bone parameters, especially in adult populations. The de-training effect in muscle and bone parameters seen in the CF cohort, adult MRCD and Leigh Syndrome cohort during the follow-up period also indicates that future studies designed to incorporate intermittent training periods (6 months WBVT, 3 months off, 6

months WBVT, 3 months off) may also be beneficial in preserving muscle and bone mass.

The choice of outcome measures should also be reconsidered in future studies. DXA scans should include the use of proximal hip DXA scans, including total hip and femoral neck scans, to elucidate the efficacy of WBVT on cortical bone in the lower limbs, particularly in adult cohorts as a lot of literature exists in this area. pQCT analysis of the tibia should consider using more commonly analysed sights including the 14% and 38% sites instead of the 20% site. More specific, sensitive and stable, serum derived bone turnover markers should be used. The bone formation marker PINP should be used instead of osteocalcin and alkaline phosphatase and the bone resorption marker, CTX, should be used instead of urinary deoxypyridinoline. Maximum force and power could be investigated using isometric and/or isokinetic motorised knee extension dynamometry or leg press dynamometry. The 10m walk test should be considered as an additional measure of functional mobility that can assess maximal gait speed. A measure of habitual physical activity should also be considered as moderate-vigorous activity is important for bone modelling during growth and remodelling over the lifespan.

This study had several limitations, including the small sample size, lack of sample-size calculations for secondary measures, the large age-range, short intervention period of 6 months WBVT and lack of direct supervision of daily training routine. Future studies should aim for randomised controlled trials, stratifying for gender and pubertal stage in paediatric cohorts and by menopausal state in adult females. Intervention periods of at

least 12 months comparing vertical and side-alternating vibration platforms using progressive or cycling training protocols with more widely used densitometry measures, more sensitive bone turnover markers and more easily performed measures of muscle force and power. Telemedicine options could be utilised to support adherence to home-based training protocols. Despite these limitations, the data presented in this thesis demonstrates a potential therapeutic role for WBVT in a subset of chronic illnesses affecting musculoskeletal function. This intervention is safe but more work is required to determine its optimal efficacy.

7 Appendices

Appendix A: DXA Parameters

Table A.1 CF Cohort
Table A.2 Paediatric MRCD Cohort
Table A.3 Adult MRCD Cohort
Table A.4 Leigh Syndrome Cohort

Table A.1. DXA parameters for the CF cohort at each visit with changes between visits and associated p values from linear mixed models analysis. *p<0.05.

		TBMC (g)	TBMC SDS	TBA (cm^2)	TBMD (g/cm^2)	TBMD SDS	TLTM (g)
Visit 1	Value	1611.8	-0.7	1615.6	0.974	-0.5	31407.1
	Δ in Observation	101.7	-0.0	73.6	0.018	0.0	1246.7
	p	0.000*	0.855	0.000*	0.000*	0.820	0.000*
Visit 2	Value	1713.6	-0.7	1689.2	0.992	-0.5	32653.7
	Δ with WBVT	78.2	-0.1	45.1	0.020	0.0	1026.3
	p	0.000*	0.144	0.000*	0.000*	0.501	0.001*
Visit 3	Value	1791.8	-0.8	1734. 3	1.011	-0.4	33680.0
	Δ in Follow-up	88.2	0.0	77.6	0.007	-0.1	1110.1
	p	0.000*	0.600	0.000*	0.059	0.078	0.001*
Visit 4	Value	1880.0	-0.748	1811.9	1.018	-0.5	34790.1

		TFat (%)	TFat % SDS	TLTM:Ht SDS	TBA:Ht SDS	TBMC:TLTM SDS	TBMC:TBA SDS
Visit 1	Value	18.6	-0.2	-0.7	-0.9	-0.2	0.0
	Δ in Observation	1.0	0.1	-0.1	0.0	0.1	-0.1
	p	0.106	0.181	0.172	0.730	0.294	0.205
Visit 2	Value	19.6	-0.1	-0.8	-0.9	-0.1	-0.0
	Δ with WBVT	0.3	0.0	-0.1	-0.2	0.1	0.1
	p	0.592	0.745	0.103	0.034*	0.661	0.027*
Visit 3	Value	20.0	-0.0	-0.9	-1.0	-0.0	0.1
	Δ in Follow-up	0.7	0.1	-0.0	0.2	0.1	-0.3
	p	0.308	0.426	0.611	0.024*	0.236	0.000*
Visit 4	Value	20.7	0.1	-1.0	-0.9	0.1	-0.2

		Legs BMD (g/cm^2)	Legs BMD SDS	Legs LTM (g)	Legs Fat (%)	LS BMC (g)	LS BMC SDS
Visit 1	Value	0.998	-0.3	10114.2	24.1	29.5	-0.23
	Δ in Observation	0.024	-0.1	515.3	0.4	2.2	-0.0
	p	0.000*	0.414	0.000*	0.488	0.000*	0.727
Visit 2	Value	1.021	-0.4	10629.5	24.5	31.7	-0.3
	Δ with WBVT	0.031	0.0	283.0	0.5	1.7	-0.1
	p	0.000*	0.448	0.034*	0.445	0.000*	0.342
Visit 3	Value	1.051	-0.3	10912.6	25.0	33.4	-0.4
	Δ in Follow-up	0.016	-0.1	381.908	0.4	1.4	-0.1
	p	0.012*	0.410	0.007*	0.578	0.003*	0.341
Visit 4	Value	1.068	-0.4	11294.5	25.3	34.9	-0.4

		LSBMD (g/cm^2)	LS BMD SDS	L14 BMC (g)	L14 BA (cm^2)	L14 vBMD (g/cm3)	L14 vBMD SDS
Visit 1	Value	0.863	-0.4	36.9	42.5	0.291	-0.9
	Δ in Observation	0.027	-0.1	2.9	1.8	0.001	-0.1
	p	0.004*	0.256	0.000*	0.000*	0.865	0.446
Visit 2	Value	0.891	-0.5	39.8	44.3	0.292	-1.0
	Δ with WBVT	0.023	-0.1	2.1	1.4	0.003	-0.0
	p	0.015*	0.470	0.000*	0.001*	0.553	0.807
Visit 3	Value	0.913	-0.6	41.9	45.7	0.295	-1.0
	Δ in Follow-up	0.006	-0.1	1.7	1.5	0.001	-0.1
	p	0.533	0.090	0.004*	0.001*	0.870	0.592
Visit 4	Value	0.919	-0.7	43.6	47.2	0.296	-1.1

Note: Δ, change; TBMC, total bone mineral content; TBA, total bone area; TBMD, total bone mineral density; SDS, standard deviation score; TLTM, total body lean tissue mass; TFat, total body fat; BMD, bone mineral density; BMC, bone mineral content; BA, bone area; LTM, lean tissue mass; TF, tibia and fibula; LS, lumbar spine vertebrae 2-4; L14, lumbar spine vertebrae 1-4; vBMD, volumetric bone mineral density; R, region of distal femur scan.

Table A.2. DXA parameters for the paediatric MRCD cohort at each visit with changes between visits and associated p values from linear mixed models analysis. *p<0.05.

		TBMC (g)	TBMC SDS	TBA (cm^2)	TBMD (g/cm^2)	TBMD Ht Z	TLTM (g)
Visit 1	Value	1022.1	-0.8	1199.0	0.839	-0.8	20422.5
	Δ in Observation	25.5	-0z	24.2	0.006	-0.1	836.5
	p	0.124	0.195	0.149	0.238	0.328	0.007*
Visit 2	Value	1047.6	-0.9	1223.2	0.845	-0.9	21258.9
	Δ with WBVT	46.8	0.0	37.9	0.012	0.0	364.6
	p	0.008*	0.883	0.029*	0.020*	0.771	0.199
Visit 3	Value	1094.4	-0.9	1261.1	0.858	-0.8	21623.6
	Δ in Follow-up	15.3	-0.1	22.8	-0.002	-0.2	503.9
	p	0.347	0.250	0.172	0.671	0.048*	0.082
Visit 4	Value	1109.7	-1.1	1284.0	0.856	-1.0	22127.5

		TFat (%)	TFat % SDS	TLTM:Ht SDS	TBA:Ht SDS	TBMC:TLTM SDS	TBMC:TBA SDS
Visit 1	Value	24.6	0.9	-1.5	-1.0	0.9	-0.3
	Δ in Observation	0.1	0.0	-0.0	-0.2	-0.2	0.0
	p	0.850	0.903	0.959	0.299	0.191	0.869
Visit 2	Value	24.7	0.9	-1.5	-1.1	0.6	-0.3
	Δ with WBVT	1.5	0.2	-0.2	0.0	0.3	0.1
	p	0.067	0.077	0.215	0.870	0.162	0.463
Visit 3	Value	26.2	1.1	-1.7	-1.1	0.9	-0.2
	Δ in Follow-up	0.0	-0.0	-0.1	-0.2	-0.1	-0.1
	p	0.955	0.909	0.612	0.360	0.549	0.220
Visit 4	Value	26.3	1.1	-1.7	-1.3	0.8	-0.3

		Trunk BMD (g/cm^2)	Trunk BMD SDS	Arms BMD (g/cm^2)	Arms BMD SDS	Legs BMC (g)	Legs BA (cm^2)
Visit 1	Value	0.636	-0.4	0.591	-0.7	300.8	393.3
	Δ in Observation	-0.002	-0.1	0.004	-0.1	7.9	13.1
	p	0.780	0.151	0.401	0.204	0.131	0.008*
Visit 2	Value	0.634	-0.5	0.595	-0.8	308.7	406.5
	Δ with WBVT	0.018	0.1	0.009	0.0	11.8	12.7
	p	0.008*	0.146	0.067	0.893	0.030*	0.009*
Visit 3	Value	0.652	-0.4	0.604	-0.8	320.4	419.2
	Δ in Follow-up	-0.006	-0.1	0.001	-0.1	8.6	6.0
	p	0.364	0.052	0.812	0.113	0.101	0.184
Visit 4	Value	0.646	-0.5	0.605	-1.0	329.1	425.2

		Legs BMD (g/cm^2)	Legs BMD SDS	Legs LTM (g)	Legs Fat (%)	LS BMC (g)	LS BMC SDS
Visit 1	Value	0.735	-1.5	6077.4	31.0	18.7	-0.4
	Δ in Observation	-0.001	-0.3	113.1	0.6	0.6	-0.1
	p	0.830	0.013*	0.169	0.389	0.109	0.600
Visit 2	Value	0.734	-1.7	6210.5	31.7	19.2	-0.4
	Δ with WBVT	0.009	-0.1	187.4	1.1	1.4	0.2
	p	0.168	0.429	0.059	0.147	0.001*	0.133
Visit 3	Value	0.743	-1.8	6397.92	32.8	20.6	-0.2
	Δ in Follow-up	0.013	-0.0	156.7	0.0	-0.1	-0.2
	p	0.077	0.672	0.108	0.948	0.735	0.059
Visit 4	Value	0.756	-1.8	6554.7	32.9	20.5	-0.5

		LSBMD (g/cm^2)	LS BMD SDS	L14 BMC (g)	L14 BA (cm^2)	L14 vBMD (g/cm3)	L14 vBMD SDS
Visit 1	Value	0.672	-0.9	23.8	34.6	0.248	-1.0
	Δ in Observation	0.008	-0.0	0.9	1.0	0.003	-0.0
	p	0.587	0.761	0.039*	0.052	0.816	0.973
Visit 2	Value	0.679	-1.0	24.7	35.6	0.251	-1.0
	Δ with WBVT	0.050	0.4	1.7	0.0	0.001	-0.0
	p	0.002*	0.012*	0.000*	0.956	0.961	0.875
Visit 3	Value	0.729	-0.6	26.4	35.6	0.252	-1.1
	Δ in Follow-up	-0.016	-0.3	-0.0	0.7	0.000	-0.1
	p	0.272	0.071	0.935	0.172	0.968	0.863
Visit 4	Value	0.713	-0.9	26.4	36.3	0.252	-1.1

Note: Δ, change; TBMC, total bone mineral content; TBA, total bone area; TBMD, total bone mineral density; SDS, standard deviation score; TLTM, total body lean tissue mass; TFat, total body fat; BMD, bone mineral density; BMC, bone mineral content; BA, bone area; LTM, lean tissue mass; TF, tibia and fibula; LS, lumbar spine vertebrae 2-4; L14, lumbar spine vertebrae 1-4; vBMD, volumetric bone mineral density; R, region of distal femur scan.

Table A.3. DXA parameters for the adult MRCD cohort at each visit with changes between visits and associated p values from linear mixed models analysis. *p<0.05.

		TBMC (g)	TBA (cm^2)	TBMD (g/cm^2)	TBMD SDS	TLTM (g)	TFat %
Visit 1	Value	2378.3	2177.4	1.090	-0.4	38771.7	35.9
	Δ in Observation	21.1	23.8	-0.003	-0.0	-612.6	0.6
	p	0.260	0.174	0.296	0.817	0.048*	0.251
Visit 2	Value	2399.4	2201.2	1.087	-0.4	38159.0	36.5
	Δ with WBVT	6.0	10.4	-0.002	-0.0	-415.3	0.8
	p	0.747	0.548	0.660	0.772	0.173	0.156
Visit 3	Value	2405.4	2211.6	1.085	-0.4	37743.7	37.3
	Δ in Follow-up	-21.0	-20.0	-0.000	-0.0	118.6	-0.7
	p	0.264	0.251	0.859	0.544	0.531	0.179
Visit 4	Value	2384.4	2191.5	1.085	-0.461	37932.3	36.6

		Trunk BMD (g/cm^2)	Arms BMD (g/cm^2)	Legs BMC (g)	Legs BA (cm^2)	Legs BMD (g/cm^2)	Legs LTM (g)
Visit 1	Value	0.868	0.817	846.5	741.0	1.139	12141.0
	Δ in Observation	-0.005	-0.003	-6.6	-2.5	-0.006	-251.89
	p	0.380	0.490	0.100	0.460	0.049*	0.033*
Visit 2	Value	0.863	0.814	839.9	738.5	1.133	11889.1
	Δ with WBVT	-0.003	0.003	1.5	-2.6	0.007	-188.5
	p	0.551	0.482	0.704	0.436	0.036*	0.106
Visit 3	Value	0.860	0.817	841.4	735.9	1.140	11700.6
	Δ in Follow-up	0.000	-0.004	-4.2	1.5	-0.008	6.2
	p	0.939	0.335	0.290	0.661	0.019*	0.957
Visit 4	Value	0.860	0.813	837.2	737.4	1.132	11706.9

		Legs Fat (%)	LSBMC (g)	LSBMD (g/cm^2)	LS SDS	L14BMC (g)	L14 BA (cm^2)	L14 vBMD (g/cm3)
Visit 1	Value	38.8	51.4	1.123	-0.4	64.3	58.6	0.323
	Δ in Observation	0.5	0.1	0.004	0.1	0.1	-0.2	0.007
	p	0.274	0.794	0.694	0.467	0.898	0.646	0.326
Visit 2	Value	39.3	51.6	1.126	-0.4	64.4	58.8	0.330
	Δ with WBVT	0.5	-0.6	-0.010	-0.1	-0.3	0.2	-0.011
	p	0.293	0.245	0.246	0.276	0.571	0.617	0.125
Visit 3	Value	39.8	51.0	1.116	-0.5	64.1	59.0	0.319
	Δ in Follow-up	-0.6	0.4	0.003	0.1	0.5	0.3	0.009
	p	0.237	0.397	0.732	0.461	0.359	0.441	0.235
Visit 4	Value	39.3	51.4	1.119	-0.4	64.6	59.3	0.327

Note: Δ, change; TBMC, total bone mineral content; TBA, total bone area; TBMD, total bone mineral density; SDS, standard deviation score; TLTM, total body lean tissue mass; TFat, total body fat; BMD, bone mineral density; BMC, bone mineral content; BA, bone area; LTM, lean tissue mass; TF, tibia and fibula; LS, lumbar spine vertebrae 2-4; L14, lumbar spine vertebrae 1-4; vBMD, volumetric bone mineral density; R, region of distal femur scan.

Table A.4. DXA parameters for the Leigh Syndrome MRCD cohort at each visit with changes between visits and associated p values from linear mixed models analysis. *p<0.05.

		TBMC (g)	TBA (cm^2)	TBMD (g/cm^2)	TBMD SDS	TLTM (g)	TFat (%)
Visit 1	Value	1562.5	1603.124	0.916	-0.7	29565.6	31.8
	Δ in Observation	48.8	33.9	0.010	-0.0	-26.6	0.5
	p	0.227	0.348	0.025*	0.976	0.966	0.606
Visit 2	Value	1611.3	1637.0	0.925	-0.7	29538.9	32.24
	Δ with WBVT	28.9	32.8	0.004	-0.1	731.3	1.2
	p	0.465	0.363	0.316	0.250	0.252	0.211
Visit 3	Value	1640.3	1669.9	0.929	-0.8	30270.2	33.5
	Δ in Follow-up	14.3	13.6	0.004	-0.1	943.6	0.3
	p	0.716	0.702	0.263	0.406	0.146	0.730
Visit 4	Value	1654	1683.5	0.934	-0.9	31213.8	33.8

		Arms BMD (g/cm^2)	Trunk BMD (g/cm^2)	Legs BMC (g)	Legs BA (cm^2)	Legs BMD (g/cm^2)	Legs LTM (g)
Visit 1	Value	0.733	0.728	469.8	492.8	0.856	8884.3
	Δ in Observation	0.001	0.004	11.0	9.5	0.006	-188.8
	p	0.885	0.665	0.022*	0.111	0.438	0.312
Visit 2	Value	0.734	0.732	480.7	502.4	0.862	8695.5
	Δ with WBVT	0.008	0.007	15.1	14.4	0.012	182.2
	p	0.301	0.424	0.004*	0.023*	0.108	0.3
Visit 3	Value	0.742	0.739	495.8	516.7	0.874	8877.743
	Δ in Follow-up	-0.002	0.007	11.7	11.0	0.012	369.3
	p	0.815	0.435	0.016*	0.070	0.106	0.061
Visit 4	Value	0.740	0.746	507.5	527.7	0.887	9247.1

		Legs Fat (%)	LS BMC (g)	LS BMD (g/cm^2)	LS BMD SDS	L14 BMC (g)	L14 BA (cm^2)	L14 vBMD (g/cm3)
Visit 1	Value	37.3	31.8	0.785	-1.1	39.2	44.8	0.256
	Δ in Observation	1.0	-0.3	0.010	0.0	1.0	0.3	0.004
	p	0.251	0.489	0.462	0.933	0.088	0.582	0.755
Visit 2	Value	38.2	32.0	0.795	-1.0	40.2	45.1	0.260
	Δ with WBVT	1.1	0.7	0.23	0.2	0.7	0.6	-0.008
	p	0.199	0.087	0.113	0.252	0.175	0.218	0.550
Visit 3	Value	39.3	32.7	0.818	-0.8	40.9	45.8	0.252
	Δ in Follow-up	0.4	0.0	0.005	-0.1	0.4	-0.0	0.007
	p	0.588	0.907	0.736	0.737	0.471	0.967	0.629
Visit 4	Value	39.8	32.8	0.823	-0.9	41.3	45.8	0.259

Note: Δ, change; TBMC, total bone mineral content; TBA, total bone area; TBMD, total bone mineral density; SDS, standard deviation score; TLTM, total body lean tissue mass; TFat, total body fat; BMD, bone mineral density; BMC, bone mineral content; BA, bone area; LTM, lean tissue mass; TF, tibia and fibula; LS, lumbar spine vertebrae 2-4; L14, lumbar spine vertebrae 1-4; vBMD, volumetric bone mineral density; R, region of distal femur scan.

Appendix B: pQCT Parameters

Table B.1. pQCT parameters for the CF cohort at each visit with changes between visits and associated p values from linear mixed models analysis. *p<0.05.

		4% Total BMC (mg/mm)	4% Total CSA (mm^2)	4% Total vBMD (mg/cm3)	4% Trabecular BMC (mg/mm)	4% Trabecular CSA (mm^2)	4% Trabecular vBMD (mg/cm3)
Visit 1	Value	226.3	765.4	300.200	71.9	344.3	210.406
	Δ in Observation	2.8	12.5	-2.013	-0.3	5.6	-3.244
	p	0.268	0.136	0.486	0.850	0.135	0.108
Visit 2	Value	229.1	777.9	298.187	71.7	350.0	207.162
	Δ with WBVT	7.5	6.6	6.056	1.2	2.9	0.456
	p	0.005*	0.430	0.040*	0.357	0.431	0.818
Visit 3	Value	236.6	784.4	304.244	72.9	352.9	207.619
	Δ in Follow-up	4.4	8.3	2.582	0.5	3.7	-0.654
	p	0.098	0.336	0.387	0.737	0.337	0.750
Visit 4	Value	241.0	792.689	306.826	73.4	356.6	206.965

		4% Trabecular vBMD SDS	4% Periosteal Circumference (mm)	20% Total BMC (mg/mm)	20% Total CSA (mm2)	20% Total vBMD (mg/cm3)	20% Cortical BMC (mg/mm)
Visit 1	Value	-1.0	97.2	192.1	318.4	615.081	162.7
	Δ in Observation	-0.1	1.1	7.3	7.5	7.213	7.6
	p	0.110	0.548	0.000*	0.000*	0.013*	0.000*
Visit 2	Value	-1.1	98.3	199.3	325.9	622.294	170.3
	Δ with WBVT	0.0	0.0	8.0	7.4	9.762	7.3
	p	0.744	0.996	0.000*	0.000*	0.001*	0.000*
Visit 3	Value	-1.1	98.3	207.3	333.3	632.056	177.6
	Δ in Follow-up	0.0	1.2	6.6	6.2	7.393	6.2
	p	0.858	0.533	0.000*	0.000*	0.014*	0.000*
Visit 4	Value	-1.1	99.4	213.9	339.5	639.450	183.8

		20% Cortical BMC SDS	20% Cortical CSA (mm^2)	20% Cortical CSA SDS	20% Cortical vBMD (mg/cm3)	20% Cortical vBMD SDS	20% Cortical Thickness (mm)
Visit 1	Value	0.2	147.3	-0.3	1099.694	1.3	2.7
	Δ in Observation	0.3	5.8	0.3	7.950	0.2	0.1
	p	0.000*	0.000*	0.000*	0.004*	0.007*	0.000*
Visit 2	Value	0.4	153.1	0.0	1107.644	1.5	2.8
	Δ with WBVT	0.3	5.6	0.3	7.069	0.2	0.1
	p	0.000*	0.000*	0.000*	0.011*	0.013*	0.000*
Visit 3	Value	0.7	158.7	0.3	1114.713	1.6	2.9
	Δ in Follow-up	0.3	4.2	0.2	9.332	0.2	0.1
	p	0.000*	0.000*	0.001*	0.001*	0.011*	0.009*
Visit 4	Value	1.0	162.9	0.6	1124.045	1.8	2.9

		20% Cortical Thickness SDS	20% Periosteal Circumference (mm)	20% Periosteal Circumference SDS	20% Cortical CSMI p (mm^4)	20% Cortical SS1p (mm^3)	20% Total CSMI p (mm^4)
Visit 1	Value	0.4	62.5	-0.9	11337.3	877.4	14490.0
	Δ in Observation	0.2	0.8	0.2	660.2	47.0	637.4
	p	0.003*	0.000*	0.000*	0.000*	0.000*	0.000*
Visit 2	Value	0.5	63.3	-0.8	11997.5	924.3	15127.4
	Δ with WBVT	0.2	0.8	0.2	649.7	43.6	807.9
	p	0.001*	0.000*	0.000*	0.000*	0.000*	0.000*
Visit 3	Value	0.7	64.1	-0.6	12647.2	967.9	15935.3
	Δ in Follow-up	0.1	0.6	0.2	457.2	44.5	519.4
	p	0.018*	0.000*	0.002*	0.001*	0.000*	0.002*
Visit 4	Value	0.9	64.7	-0.4	13104.4	1012.4	16454.6

		66% Cortical vBMD SDS	66% Cortical Thickness (mm)	66% Cortical Thickness SDS	66% Periosteal Circumference (mm)	66% Cortical CSMI p (mm^4)	66% Cortical CSMI p SDS
Visit 1	Value	0.8	3.2	-1.1	76.3	26296.4	-1.3
	Δ in Observation	0.2	0.0	0.1	1.6	1838.2	0.2
	p	0.135	0.208	0.205	0.000*	0.000*	0.000*
Visit 2	Value	1.0	3.2	-1.0	77.9	28134.5	-1.1
	Δ with WBVT	0.1	0.0	0.1	1.5	1967.0	0.2
	p	0.361	0.127	0.165	0.000*	0.000*	0.000*
Visit 3	Value	1.1	3.2	-0.9	79.4	30101.5	-0.9
	Δ in Follow-up	0.3	0.1	0.1	0.8	1201.6	0.2
	p	0.021*	0.090	0.095	0.043*	0.017*	0.004*
Visit 4	Value	1.3	3.3	-0.8	80.2	31303.2	-0.7

		66% Cortical SSI p (mm^3)	66% Cortical SSI p SDS	66% Soft Tissue Total CSA (mm^2)	66% Muscle CSA (mm^2)	66% Muscle CSA SDS	66% Fat CSA (mm^2)
Visit 1	Value	1417.3	-0.6	6538.1	4272.570	-0.8	1773.1
	Δ in Observation	88.3	0.3	183.8	82.530	0.1	81.4
	p	0.000*	0.000*	0.022*	0.204	0.125	0.042*
Visit 2	Value	1505.6	-0.3	6721.9	4355.100	-0.7	1854.5
	Δ with WBVT	77.7	0.3	64.6	57.010	0.1	-9.1
	p	0.000*	0.000*	0.407	0.378	0.166	0.815
Visit 3	Value	1583.3	0.0	6786.500	4412.1	-0.7	1845.4
	Δ in Follow-up	67.5	0.2	281.510	186.8	0.2	77.5
	p	0.001*	0.001*	0.001*	0.007*	0.004*	0.060
Visit 4	Value	1650.8	0.3	7068.010	4598.9	-0.5	1922.8

		66% Fat CSA:Muscle CSA (%)	66% Muscle CSA:Total CSA (%)	66% Cortical CSA:Muscle CSA (%)	66% Cortical BMC:Muscle CSA (mg/mm^2)	66% Cortical BMC:Muscle CSA SDS
Visit 1	Value	42.1	65.4	5.1	0.1	4.3
	Δ in Observation	0.8	-0.4	0.0	0.0	0.6
	p	0.384	0.415	0.652	0.382	0.358
Visit 2	Value	42.9	65.0	5.1	0.1	5.0
	Δ with WBVT	-0.5	0.1	0.1	0.0	0.7
	p	0.620	0.886	0.329	0.179	0.290
Visit 3	Value	42.4	65.1	5.2	0.1	5.7
	Δ in Follow-up	0.0	0.1	-0.1	0.0	-0.4
	p	0.984	0.798	0.488	0.801	0.587
Visit 4	Value	42.4	65.2	5.1	0.1	5.3

Note: Δ, change; 4%, referring to 4% tibial pQCT site; BMC, bone mineral content; CSA, cross-sectional area; vBMD, volumetric bone mineral density; SDS, standard deviation score; 20%, referring to the 20% tibial pQCT site; CSMI, cross-section moment of inertia; p, referring to the polar measurement; SSI, stress strain index; 66%, referring to the 66% tibial pQCT site.

Table B.2. pQCT parameters for the paediatric cohort MRCD cohort at each visit with changes between visits and associated p values from linear mixed models analysis. *p<0.05.

		4% Total BMC (mg/mm)	**4% Total CSA (mm^2)**	**4% Total vBMD (mg/cm3)**	**4% Trabecular BMC (mg/mm)**	**4% Trabecular CSA (mm^2)**	**4% Trabecular vBMD (mg/cm3)**
Visit 2	Value	125.989	549.749	241.935	33.721	247.274	142.427
	Δ with WBVT	9.238	20.011	6.181	1.324	9.046	1.023
	p	0.009*	0.089	0.277	0.406	0.088	0.801
Visit 3	Value	135.227	569.760	248.117	35.045	256.320	143.450
	Δ in Follow-up	-1.348	11.493	-7.517	-0.430	5.173	-3.550
	p	0.610	0.261	0.158	0.764	0.262	0.350
Visit 4	Value	133.878	581.253	240.600	34.615	261.493	139.900

		4% Trabecular vBMD SDS	4% Periosteal Circumference (mm)	20% Total BMC (mg/mm)	20% Total CSA (mm^2)	20% Total vBMD (mg/cm3)	20% Cortical BMC (mg/mm)
Visit 2	Value	-2.983	81.860	119.797	227.760	560.133	100.440
	Δ with WBVT	0.027	1.821	7.083	-1.867	26.883	8.718
	p	0.813	0.069	0.000*	0.719	0.005*	0.000*
Visit 3	Value	-2.956	83.781	126.880	225.893	587.017	109.158
	Δ in Follow-up	-0.097	0.955	2.388	3.013	0.967	1.993
	p	0.362	0.267	0.068	0.564	0.901	0.190
Visit 4	Value	-3.053	84.736	129.268	228.907	587.983	111.152

		20% Cortical BMC SDS	20% Cortical CSA (mm^2)	20% Cortical CSA SDS	20% Cortical vBMD (mg/cm3)	20% Cortical vBMD SDS	20% Cortical Thickness (mm)
Visit 2	Value	-0.321	91.707	-1.065	1092.717	3.396	2.039
	Δ with WBVT	0.692	6.827	0.562	13.433	0.377	0.155
	p	0.001*	0.000*	0.002*	0.049*	0.193	0.001*
Visit 3	Value	0.371	98.533	-0.504	1106.150	3.772	2.194
	Δ in Follow-up	0.119	1.840	0.123	1.000	0.045	0.020
	p	0.437	0.202	0.363	0.871	0.869	0.567
Visit 4	Value	0.490	100.373	-0.381	1107.150	3.818	2.214

		20% Cortical BMC SDS	20% Cortical CSA (mm^2)	20% Cortical CSA SDS	20% Cortical vBMD (mg/cm3)	20% Cortical vBMD SDS	20% Cortical Thickness (mm)
Visit 2	Value	-0.321	91.707	-1.065	1092.717	3.396	2.039
	Δ with WBVT	0.692	6.827	0.562	13.433	0.377	0.155
	p	0.001*	0.000*	0.002*	0.049*	0.193	0.001*
Visit 3	Value	0.371	98.533	-0.504	1106.150	3.772	2.194
	Δ in Follow-up	0.119	1.840	0.123	1.000	0.045	0.020
	p	0.437	0.202	0.363	0.871	0.869	0.567
Visit 4	Value	0.490	100.373	-0.381	1107.150	3.818	2.214

		20% Cortical Thickness SDS	20% Periosteal Circumference (mm)	20% Periosteal Circumference SDS	20% Cortical CSMI p (mm^4)	20% Cortical SS1p (mm^3)	20% Total CSMI p (mm^4)
Visit 2	Value	0.141	52.945	-1.499	4688.530	454.669	6277.490
	Δ with WBVT	0.561	-0.114	0.016	308.982	35.442	196.390
	p	0.008*	0.836	0.912	0.017*	0.002*	0.358
Visit 3	Value	0.702	52.831	-1.483	4997.512	490.111	6473.880
	Δ in Follow-up	0.057	0.382	0.058	183.673	16.132	167.378
	p	0.728	0.493	0.680	0.118	0.081	0.431
Visit 4	Value	0.759	53.213	-1.425	5181.185	506.243	6641.258

		20% Cortical Thickness SDS	20% Periosteal Circumference (mm)	20% Periosteal Circumference SDS	20% Cortical CSMI p (mm^4)	20% Cortical SS1p (mm^3)	20% Total CSMI p (mm^4)
Visit 2	Value	0.141	52.945	-1.499	4688.530	454.669	6277.490
	Δ with WBVT	0.561	-0.114	0.016	308.982	35.442	196.390
	p	0.008*	0.836	0.912	0.017*	0.002*	0.358
Visit 3	Value	0.702	52.831	-1.483	4997.512	490.111	6473.880
	Δ in Follow-up	0.057	0.382	0.058	183.673	16.132	167.378
	p	0.728	0.493	0.680	0.118	0.081	0.431
Visit 4	Value	0.759	53.213	-1.425	5181.185	506.243	6641.258

		66% Cortical BMC (mg/mm)	66% Cortical BMC SDS	66% Cortical CSA (mm^2)	66% Cortical CSA SDS	66% Cortical CSA:Total CSA (%)	66% Cortical vBMD (mg/cm^3)
Visit 2	Value	120.813	-2.023	113.600	-2.263	40.014	1063.083
	Δ with WBVT	5.528	0.298	3.680	0.192	0.157	17.067
	p	0.036*	0.029*	0.020*	0.021*	0.901	0.115
Visit 3	Value	126.342	-1.725	117.280	-2.072	39.857	1080.150
	Δ in Follow-up	0.443	0.053	1.600	0.099	-1.252	9.383
	p	0.850	0.662	0.256	0.190	0.332	0.365
Visit 4	Value	126.785	-1.672	118.880	-1.973	38.604	1070.767

		66% Cortical vBMD SDS	66% Cortical Thickness (mm)	66% Cortical Thickness SDS	66% Periosteal Circumference (mm)	66% Cortical CSMI p (mm^4)	66% Cortical CSMI p SDS
Visit 2	Value	2.045	2.275	-1.837	57.610	8084.868	-2.209
	Δ with WBVT	0.597	0.030	0.124	1.350	716.471	0.115
	p	0.118	0.611	0.455	0.133	0.021*	0.010*
Visit 3	Value	2.643	2.305	-1.713	58.960	8801.340	-2.094
	Δ in Follow-up	-0.339	-0.038	-0.080	1.906	778.948	0.145
	p	0.354	0.521	0.628	0.044*	0.014*	0.003*
Visit 4	Value	2.304	2.267	-1.793	60.866	9580.288	-2.209

		66% Cortical SSI p (mm^3)	66% Cortical SSI p SDS	66% Soft Tissue Total CSA (mm^2)	66% Muscle CSA (mm^2)	66% Muscle CSA SDS	66% Fat CSA (mm^2)
Visit 2	Value	617.598	-1.746	4801.227	2686.133	-1.029	1815.253
	Δ with WBVT	45.146	0.333	223.573	145.573	0.248	61.920
	p	0.000*	0.002*	0.017*	0.014*	0.015*	0.267
Visit 3	Value	662.744	-1.413	5024.800	2831.707	-0.781	1877.173
	Δ in Follow-up	25.420	0.168	149.760	2.187	0.041	129.120
	p	0.009*	0.060	0.084	0.965	0.636	0.034*
Visit 4	Value	688.164	-1.245	5174.560	2833.893	-0.739	2006.293

		66% Fat CSA:Muscle CSA (%)	66% Muscle CSA:Total CSA (%)	66% Cortical CSA:Muscle CSA (%)	66% Cortical BMC:Muscle CSA (mg/mm^2)	66% Cortical BMC:Muscle CSA SDS
Visit 2	Value	70.277	55.820	4.287	0.046	-3.140
	Δ with WBVT	-2.364	0.631	-0.120	-0.001	-0.623
	p	0.292	0.329	0.226	0.561	0.574
Visit 3	Value	67.913	56.451	4.167	0.045	-3.763
	Δ in Follow-up	4.939	-1.570	0.082	0.000	0.075
	p	0.043*	0.029*	0.402	0.743	0.946
Visit 4	Value	72.852	54.881	4.249	0.045	-3.688

Note: Δ, change; 4%, referring to 4% tibial pQCT site; BMC, bone mineral content; CSA, cross-sectional area; vBMD, volumetric bone mineral density; SDS, standard deviation score; 20%, referring to the 20% tibial pQCT site; CSMI, cross-section moment of inertia; x, referring to the measurement in the x plane y, referring to the measurement in the y plane; p, referring to the polar measurement; SSI, stress strain index; 66%, referring to the 66% tibial pQCT site.

Table B.3. pQCT parameters for the adult MRCD cohort at each visit with changes between visits and associated p values from linear mixed models analysis. *p<0.05.

		4% Total BMC (mg/mm)	**4% Total CSA (mm^2)**	**4% Total vBMD (mg/cm3)**	**4% Trabecular BMC (mg/mm)**	**4% Trabecular CSA (mm^2)**	**4% Trabecular vBMD (mg/cm3)**
Visit 2	Value	273.630	1076.840	257.550	94.427	484.493	197.250
	Δ with WBVT	-1.033	-0.760	-0.075	-0.431	-0.307	-0.125
	p	0.388	0.878	0.938	0.516	0.891	0.875
Visit 3	Value	272.597	1076.080	257.475	93.996	484.187	197.125
	Δ in Follow-up	0.843	0.227	0.708	0.170	0.067	0.242
	p	0.480	0.964	0.464	0.797	0.976	0.762
Visit 4	Value	273.441	1076.307	258.183	94.166	484.253	197.367

		4% Periosteal Circumference (mm)	4% Trabecular BMC (mg/mm)	20% Total BMC (mg/mm)	20% Total CSA (mm^2)	20% Total vBMD (mg/cm3)	20% Cortical BMC (mg/mm)
Visit 2	Value	116.066	94.427	225.665	341.720	663.750	206.971
	Δ with WBVT	-0.059	-0.431	-0.280	-0.693	1.092	0.076
	p	0.828	0.516	0.716	0.518	0.617	0.921
Visit 3	Value	116.007	93.996	225.385	341.027	660.842	207.047
	Δ in Follow-up	0.003	0.170	-0.430	0.213	-1.892	-0.803
	p	0.990	0.797	0.577	0.842	0.388	0.297
Visit 4	Value	116.010	94.166	224.955	341.240	662.950	206.243

		20% Cortical CSA (mm^2)	20% Cortical vBMD (mg/cm3)	20% Cortical Thickness (mm)	20% Periosteal Circumference (mm)	20% Cortical CSMI p (mm^4)	20% Cortical SS1p (mm^3)
Visit 2	Value	175.947	1176.358	3.178	65.390	15026.898	1182.136
	Δ with WBVT	-0.480	2.792	-0.001	-0.068	-109.071	-0.187
	p	0.521	0.226	0.951	0.508	0.189	0.984
Visit 3	Value	175.467	1179.150	3.177	65.322	14917.827	1181.949
	Δ in Follow-up	-0.400	-1.492	-0.011	0.021	-1.628	2.162
	p	0.593	0.512	0.532	0.840	0.984	0.811
Visit 4	Value	175.067	1177.658	3.166	65.343	14916.199	1184.111

		66% Total BMC (mg/mm)	66% Total CSA (mm^2)	66% Total vBMD (mg/cm3)	66% Cortical BMC (mg/mm)	66% Cortical CSA (mm^2)	66% Cortical CSA:Total CSA (%)
Visit 2	Value	341.704	582.293	594.992	289.793	251.773	43.808
	Δ with WBVT	-0.176	0.307	-0.867	0.237	0.947	0.105
	p	0.807	0.873	0.653	0.740	0.220	0.497
Visit 3	Value	341.528	582.600	594.125	290.029	252.720	43.913
	Δ in Follow-up	-0.347	1.627	-1.942	-1.013	-1.707	-0.387
	p	0.632	0.400	0.318	0.164	0.033*	0.019*
Visit 4	Value	341.182	584.227	592.183	289.016	251.013	43.526

		66% Cortical vBMD (mg/cm3)	66% Cortical Thickness (mm)	66% Periosteal Circumference (mm)	66% Cortical CSMI p (mm^4)	66% Cortical SSI p (mm^3)	66% Soft Tissue Total CSA (mm^2)
Visit 2	Value	1151.100	3.556	82.484	37210.932	2016.335	8726.307
	Δ with WBVT	-2.875	0.008	0.145	158.897	5.674	-32.413
	p	0.176	0.529	0.202	0.342	0.472	0.776
Visit 3	Value	1148.225	3.564	82.629	37369.82	2022.009	8693.893
	Δ in Follow-up	3.242	-0.031	0.073	-135.482	-0.514	-68.280
	p	0.129	0.015*	0.518	0.417	0.948	0.551
Visit 4	Value	1151.467	3.532	82.702	37234.346	2021.496	8625.613

		66% Muscle CSA (mm^2)	66% Fat CSA (mm^2)	66% Fat CSA:Muscle CSA (%)	66% Muscle CSA:Total CSA (%)	66% Cortical CSA:Muscle CSA (%)	66% Cortical BMC:Muscle CSA (mg/mm^2)
Visit 2	Value	5225.360	2874.013	56.701	60.252	4.885	0.056
	Δ with WBVT	-39.560	6.480	0.448	-0.171	0.016	0.000
	p	0.703	0.927	0.826	0.810	0.875	0.974
Visit 3	Value	5185.800	2880.493	57.149	60.081	4.901	0.056
	Δ in Follow-up	61.427	-131.307	-2.824	1.094	-0.053	0.000
	p	0.555	0.074	0.174	0.135	0.599	0.696
Visit 4	Value	5247.227	2749.187	54.325	61.174	4.848	0.056

Note: Δ, change; 4%, referring to 4% tibial pQCT site; BMC, bone mineral content; CSA, cross-sectional area; vBMD, volumetric bone mineral density; 20%, referring to the 20% tibial pQCT site; CSMI, cross-section moment of inertia; x, referring to the measurement in the x plane; y, referring to the measurement in the y plane; p, referring to the polar measurement; SSI, stress strain index; 66%, referring to the 66% tibial pQCT site.

Table B.4. pQCT parameters for the Leigh Syndrome cohort MRCD cohort at each visit with changes between visits and associated p values from linear mixed models analysis. *p<0.05.

		4% Total BMC (mg/mm)	**4% Total CSA (mm^2)**	**4% Total vBMD (mg/cm3)**	**4% Trabecular BMC (mg/mm)**	**4% Trabecular CSA (mm^2)**	**4% Trabecular vBMD (mg/cm3)**
Visit 2	Value	178.112	654.816	278.580	48.316	294.560	163.380
	Δ with WBVT	6.326	20.192	3.100	1.926	9.120	4.160
	p	0.071	0.106	0.608	0.145	0.105	0.164
Visit 3	Value	184.438	675.008	281.680	50.242	303.680	167.540
	Δ in Follow-up	1.826	4.320	-1.940	0.382	1.952	0.800
	p	0.565	0.707	0.747	0.757	0.706	0.776
Visit 4	Value	186.264	679.328	279.740	50.624	305.632	168.340

		4% Periosteal Circumference (mm)	20% Total BMC (mg/mm)	20% Total CSA (mm^2)	20% Total vBMD (mg/cm3)	20% Cortical BMC (mg/mm)	20% Cortical CSA (mm^2)
Visit 2	Value	87.760	160.536	248.416	654.920	144.767	126.944
	Δ with WBVT	1.844	3.620	1.728	10.820	4.894	3.712
	p	0.080	0.122	0.562	0.089	0.027*	0.070
Visit 3	Value	89.604	164.156	250.144	665.740	149.570	130.656
	Δ in Follow-up	0.571	0.368	2.528	-4.120	0.506	1.408
	p	0.551	0.865	0.402	0.482	0.786	0.450
Visit 4	Value	90.175	164.524	252.672	661.620	150.076	132.064

		4% Periosteal Circumference (mm)	20% Total BMC (mg/mm)	20% Total CSA (mm^2)	20% Total vBMD (mg/cm3)	20% Cortical BMC (mg/mm)	20% Cortical CSA (mm^2)
Visit 2	Value	87.760	160.536	248.416	654.920	144.767	126.944
	Δ with WBVT	1.844	3.620	1.728	10.820	4.894	3.712
	p	0.080	0.122	0.562	0.089	0.027*	0.070
Visit 3	Value	89.604	164.156	250.144	665.740	149.570	130.656
	Δ in Follow-up	0.571	0.368	2.528	-4.120	0.506	1.408
	p	0.551	0.865	0.402	0.482	0.786	0.450
Visit 4	Value	90.175	164.524	252.672	661.620	150.076	132.064

		20% Cortical vBMD (mg/cm3)	20% Cortical Thickness (mm)	20% Periosteal Circumference (mm)	20% Cortical CSMI p (mm^4)	20% Cortical SS1p (mm^3)	66% Total BMC (mg/mm)
Visit 2	Value	1122.740	2.657	54.842	8267.347	725.271	212.832
	Δ with WBVT	7.960	0.088	0.240	216.025	31.765	5.236
	p	0.282	0.040*	0.509	0.177	0.029*	0.035*
Visit 3	Value	1130.700	2.745	55.082	8483.372	757.036	218.068
	Δ in Follow-up	-5.600	0.017	0.348	143.372	6.004	2.688
	p	0.441	0.643	0.345	0.355	0.628	0.230
Visit 4	Value	132.064	1125.100	2.762	8626.743	763.040	220.756

		66% Total CSA (mm^2)	66% Total vBMD (mg/cm3)	66% Cortical BMC (mg/mm)	66% Cortical CSA (mm^2)	66% Cortical CSA:Total CSA (%)	66% Cortical vBMD (mg/cm3)
Visit 2	Value	389.184	565.240	173.260	158.400	42.978	1072.260
	Δ with WBVT	3.968	17.580	5.298	2.976	0.811	21.720
	p	0.452	0.144	0.033*	0.038*	0.402	0.070
Visit 3	Value	393.152	582.820	178.558	161.376	43.789	1093.980
	Δ in Follow-up	9.600	-8.060	2.054	2.240	-0.415	-2.540
	p	0.092	0.479	0.347	0.099	0.662	0.813
Visit 4	Value	402.752	574.760	180.612	163.616	43.374	1091.440

		66% Cortical Thickness (mm)	66% Periosteal Circumference (mm)	66% Cortical CSMI p (mm^4)	66% Cortical SSI p (mm^3)	66% Soft Tissue Total CSA (mm^2)	66% Muscle CSA (mm^2)
	Value	2.731	64.941	18037.238	1086.900	6364.096	3784.576
Visit 2	Δ with WBVT	0.058	0.413	323.269	29.749	205.408	28.000
	p	0.179	0.361	0.100	0.056	0.099	0.794
	Value	2.789	65.354	18360.507	1116.649	6569.504	3812.576
Visit 3	Δ in Follow-up	0.005	0.855	405.134	20.390	171.552	130.880
	p	0.910	0.080	0.048*	0.164	0.158	0.242
Visit 4	Value	2.794	66.209	18765.640	1137.039	6741.056	3943.456

		66% Fat CSA (mm^2)	66% Fat CSA:Muscle CSA (%)	66% Muscle CSA:Total CSA (%)	66% Cortical CSA:Muscle CSA (%)	66% Cortical BMC:Muscle CSA (mg/mm^2)
Visit 2	Value	2171.680	66.625	57.503	4.289	0.046
	Δ with WBVT	171.264	0.823	-0.422	-0.031	0.000
	p	0.198	0.832	0.747	0.779	0.556
Visit 3	Value	2342.944	67.448	57.081	4.259	0.046
	Δ in Follow-up	31.616	2.064	-0.255	0.009	0.000
	p	0.803	0.598	0.845	0.930	0.869
Visit 4	Value	2374.560	69.511	56.826	4.268	0.046

Note: Δ, change; 4%, referring to 4% tibial pQCT site; BMC, bone mineral content; CSA, cross-sectional area; vBMD, volumetric bone mineral density; 20%, referring to the 20% tibial pQCT site; CSMI, cross-section moment of inertia; x, referring to the measurement in the x plane y, referring to the measurement in the y plane; p, referring to the polar measurement; SSI, stress strain index; 66%, referring to the 66% tibial pQCT site.

Appendix C: Bone Turnover Markers

Table C.1 Serum biochemistry and bone turn-over marker parameters for the CF cohort at each visit with changes between visits and associated p values from linear mixed models analysis. *p<0.05.

		Alkaline Phosphatase (U/L)	**Calcium (mmol/L)**	**Phosphate (mmol/L)**	**Parathyroid Hormone**
	Value	213.2	2.3	1.5	4.6
Visit 1	Δ in Observation	-9.6	0.0	-0.1	-0.2
	p	0.356	0.714	0.140	0.781
	Value	203.6	2.3	1.4	4.4
Visit 2	Δ with WBVT	-19.7	-0.1	0.0	-0.4
	p	0.063	0.003*	0.740	0.556
	Value	183.9	2.2	1.4	4.0
Visit 3	Δ in Follow-up	8.0	0.1	0.0	-0.4
	p	0.458	0.000*	0.582	0.557
Visit 4	Value	191.8	2.4	1.5	3.6

		Osteocalcin (nmol/L)	Vitamin D (pmol/L)	Urinary Deoxypyridoline: Creatine Ratio (nmol/mmol creatinine)
Visit 1	Value	6.1	70.7	17.5
	Δ in Observation	-2.4	-8.5	-2.6
	p	0.013*	0.069	0.213
Visit 3	Value	3.8	62.2	14.9
	Δ with WBVT	0.8	7.1	-0.6
	p	0.383	0.133	0.774
Visit 5	Value	4.6	69.3	14.3
	Δ in Follow-up	-0.4	-7.5	1.5
	p	0.661	0.121	0.510
Visit 7	Value	4.2	61.8	15.8

Note: Δ, change; WBVT, whole body vibration training.

Table C.2. Serum biochemistry and bone turn-over marker parameters for the paediatric MRCD cohort at each visit with changes between visits and associated p values from linear mixed models analysis. *p<0.05.

		Alkaline Phosphatase (U/L)	**Calcium (mmol/L)**	**Phosphate (mmol/L)**	**Lactate (mmol/L)**
Visit 1	Value	155.286	2.416	1.453	3.357
	Δ in Observation	7.286	-0.011	0.087	-0.071
	p	0.556	0.753	0.061	0.866
Visit 2	Value	162.571	2.404	1.540	3.286
	Δ with WBVT	9.000	0.009	-0.017	0.243
	p	0.468	0.813	0.698	0.567
Visit 3	Value	171.571	2.413	1.523	3.529
	Δ in Follow-up	8.571	-0.016	-0.096	-0.257
	p	0.489	0.666	0.042*	0.544
Visit 4	Value	180.143	2.397	1.427	3.271

		Urinary Deoxypyridoline: Creatine Ratio (nmol/mmol creatinine)	Osteocalcin (nmol/L)	Vitamin D (pmol/L)
Visit 1	Value	17.229	6.386	106.000
	Δ in Observation	3.800	0.471	-22.571
	p	0.313	0.553	0.018*
Visit 2	Value	21.029	6.857	83.429
	Δ with WBVT	-4.829	-2.843	-3.714
	p	0.204	0.002*	0.670
Visit 4	Value	16.200	4.014	79.714
	Δ in Follow-up	3.014	-0.329	-8.000
	p	0.420	0.678	0.364
Visit 6	Value	19.214	3.686	71.714

Table C.3. Serum biochemistry and bone turn-over marker parameters for the adult MRCD cohort at each visit with changes between visits and associated p values from linear mixed models analysis. *p<0.05.

		Alkaline Phosphatase (U/L)	**Calcium (mmol/L)**	**Phosphate (mmol/L)**	**Lactate (mmol/L)**
Visit 1	Value	74.917	2.426	1.233	3.567
	Δ in Observation	-1.333	-0.058	0.028	-1.033
	p	0.669	0.063	0.567	0.153
Visit 2	Value	73.583	2.367	1.261	2.533
	Δ with WBVT	-3.500	0.002	-0.063	0.550
	p	0.266	0.935	0.206	0.442
Visit 3	Value	70.083	2.370	1.198	3.083
	Δ in Follow-up	5.834	0.008	0.047	0.908
	p	0.079	0.784	0.340	0.208
Visit 4	Value	75.917	2.378	1.245	3.992

		Urinary Deoxypyridoline: Creatine Ratio (nmol/mmol creatinine)	Osteocalcin (nmol/L)	Vitamin D (pmol/L)
Visit 1	Value	7.950	1.577	78.917
	Δ in Observation	-0.950	-0.495	-2.833
	p	0.450	0.070	0.706
Visit 2	Value	7.000	1.082	76.083
	Δ with WBVT	0.517	0.101	6.500
	p	0.680	0.691	0.389
Visit 3	Value	7.517	1.183	82.583
	Δ in Follow-up	-0.846	-0.229	7.000
	p	0.512	0.416	0.354
Visit 4	Value	6.671	0.954	89.583

Table C.4. Serum biochemistry and bone turn-over marker parameters for the Leigh Syndrome MRCD cohort at each visit with changes between visits and associated p values from linear mixed models analysis. *p<0.05.

		Alkaline Phosphatase (U/L)	**Calcium (mmol/L)**	**Phosphate (mmol/L)**	**Lactate (mmol/L)**
Visit 1	Value	155.800	2.432	1.302	1.540
	Δ in Observation	-2.200	-0.018	0.132	-0.180
	p	0.833	0.639	0.006*	0.627
Visit 2	Value	153.600	2.414	1.434	1.360
	Δ with WBVT	15.400	0.018	0.044	0.320
	p	0.157	0.639	0.291	0.393
Visit 3	Value	169.000	2.432	1.478	1.680
	Δ in Follow-up	2.800	-0.002	-0.214	0.120
	p	0.788	0.958	0.000*	0.745
Visit 4	Value	171.800	2.430	1.264	1.800

		Urinary Deoxypyridoline: Creatine Ratio (nmol/mmol creatinine)	Osteocalcin (nmol/L)	Vitamin D (pmol/L)
Visit 1	Value	13.400	4.560	93.000
	Δ in Observation	6.820	0.100	-5.200
	p	0.099	0.904	0.583
Visit 2	Value	20.220	4.660	87.800
	Δ with WBVT	-4.600	-1.160	10.400
	p	0.251	0.177	0.282
Visit 4	Value	15.620	3.500	98.200
	Δ in Follow-up	3.720	-1.500	-8.800
	p	0.349	0.088	0.359
Visit 6	Value	19.340	2.000	89.400

Note: Δ, change; WBVT, whole body vibration training.

Appendix D: Force plate Parameters

Table D.1 CF Cohort
Table D.2 Paediatric MRCD Cohort
Table D.3 Adult MRCD Cohort
Table D.4 Leigh Syndrome Cohort

Table D.1. Fore plate parameters for the CF cohort at each visit with changes between visits and associated p values from linear mixed models analysis. *p<0.05.

		CRT Time per test (s)	CRT Time per test SDS	CRT P max (W)	CRT Ave P max rel (W/kg)	CRT Ave P max rel SDS	CRT F max (kN)
Visit 1	Value	1.1	-0.3	558.1	12.6	-0.4	1.6
	Δ in Observation	-0.1	0.3	46.7	-0.1	-0.1	-0.1
	p	0.025*	0.025*	0.205	0.867	0.732	0.165
Visit 2	Value	1.1	0.0	604.8	12.5	-0.5	1.5
	Δ with WVBT	-0.1	0.5	52.0	0.4	0.1	0.1
	p	0.001*	0.001*	0.159	0.539	0.747	0.028*
Visit 3	Value	1.0	0.5	656.8	12.9	-0.4	1.6
	Δ in Follow-up	0.0	-0.2	27.5	0.3	0.1	0.0
	p	0.234	0.234	0.465	0.617	0.762	0.907
Visit 4	Value	1.0	0.3	684.4	13.2	-0.3	1.6

		CRT Ave F max/g (g)	CRT Ave F max/g SDS	S2LJ EFI (%)	S2LJ EFI SDS	S2LJ FE (%)	S2LJ FE SDS
Visit 1	Value	1.5	0.1	95.9	-0.3	103.6	0.3
	Δ in Observation	0.0	-0.1	-0.4	0.0	-1.9	-0.2
	p	0.707	0.707	0.832	0.805	0.378	0.404
Visit 2	Value	1.5	-0.1	95.5	-0.4	101.7	0.2
	Δ with WVBT	0.0	0.3	-0.4	0.0	-4.2	-0.4
	p	0.370	0.371	0.840	0.778	0.053	0.055
Visit 3	Value	1.5	0.2	95.2	-0.4	97.5	-0.2
	Δ in Follow-up	0.0	0.1	-1.2	-0.1	1.4	0.1
	p	0.748	0.775	0.532	0.485	0.533	0.543
Visit 4	Value	1.5	0.3	94.0	-0.5	98.9	-0.1

		S2LJ P max (kW)	S2LJ P max rel (W/kg)	S2LJ P max rel SDS	S2LJ F max (kN)	S2LJ F max/g (g)
Visit 1	Value	1.7	41.3	-0.4	0.9	2.3
	Δ in Observation	0.1	0.5	0.0	0.1	0.0
	p	0.010*	0.514	0.874	0.061	0.769
Visit 2	Value	1.9	41.8	-0.4	1.0	2.4
	Δ with WVBT	0.1	0.6	0.0	0.1	0.1
	p	0.061	0.495	0.943	0.027*	0.132
Visit 3	Value	1.9	42.4	-0.4	1.1	2.5
	Δ in Follow-up	0.1	0.2	-0.1	0.0	-0.1
	p	0.096	0.823	0.699	0.860	0.307
Visit 4	Value	2.0	42.6	-0.5	1.1	2.4

		S2LJ F max/g SDS	M1LJ F max (kN)	M1LJ F max/g (g)	M1LJ F max/g SDS
Visit 1	Value	-0.5	10.9	2.8	-1.8
	Δ in Observation	0.1	0.1	-0.1	-0.4
	p	0.769	0.827	0.004*	0.004*
Visit 2	Value	-0.5	10.9	2.6	-2.2
	Δ with WVBT	0.3	0.5	0.0	0.1
	p	0.132	0.050*	0.727	0.727
Visit 3	Value	-0.1	11.4	2.7	-2.2
	Δ in Follow-up	-0.2	-0.2	-0.2	-0.5
	p	0.307	0.302	0.002*	0.002*
Visit 4	Value	-0.3	11.2	2.5	-2.7

Note: Δ, change; WBVT, whole body vibration training; CRT, chair rise test; Ave V max, average maximum velocity; P max, maximum power; P max rel, maximum power in relation to body weight; Ave P max rel, average maximum power in relation to body weight; F max, maximum force; F max rel, maximum relative force in relation to body weight; F max/g, maximum relative force per force equivalent to body weight (Fg); Ave F max/g, average maximum relative force per force equivalent to body weight.

Table D.2. Fore plate parameters for the paediatric MRCD cohort at each visit with changes between visits and associated p values from linear mixed models analysis. *p<0.05.

		CRT Time per test (s)	CRT V max (m/s)	CRT Speed SDS	CRT P max (W)	CRT P max rel (W/kg)	CRT Ave P max rel (W/kg)	CRT Power SDS
Visit 1	Value	2.475	0.678	-6.410	234.059	8.221	7.134	-2.491
	Δ in Observation	0.055	0.045	0.250	42.079	1.290	0.483	0.127
	p	0.765	0.489	0.765	0.256	0.277	0.525	0.630
Visit 2	Value	2.530	0.722	-6.660	276.138	9.511	7.617	-2.363
	Δ with WBVT	-0.070	-0.036	-0.320	-27.246	-1.417	-0.696	-0.317
	p	0.703	0.578	0.703	0.457	0.234	0.363	0.238
Visit 3	Value	2.460	0.686	-6.341	248.892	8.094	6.921	-2.680
	Δ in Follow-up	-0.097	0.016	-0.440	3.069	-0.053	0.460	0.090
	p	0.601	0.809	0.601	0.933	0.964	0.545	0.733
Visit 4	Value	2.363	0.702	-5.901	251.961	8.041	7.381	-2.590

		CRT F max (kN)	CRT F max rel (N/kg)	CRT Ave F max rel (g)	CRT Force SDS	S2LJ EFI (%)	S2LJ EFI SDS	S2LJ FE (%)
Visit 1	Value	1.288	49.458	1.250	-2.201	38.215	-5.151	52.155
	Δ in Observation	0.013	-1.878	-0.021	-0.196	-1.652	-0.117	-1.813
	p	0.804	0.452	0.609	0.567	0.604	0.546	0.689
Visit 2	Value	1.302	47.580	1.230	-2.397	36.564	-5.269	50.342
	Δ with WBVT	-0.119	-6.278	-0.054	-0.492	-1.543	-0.070	-4.508
	p	0.037*	0.019*	0.196	0.160	0.591	0.689	0.284
Visit 3	Value	1.182	41.302	1.176	-2.889	35.020	-5.338	45.834
	Δ in Follow-up	0.079	1.749	0.081	0.632	2.349	0.108	-2.729
	p	0.153	0.482	0.058	0.076	0.418	0.538	0.510
Visit 4	Value	1.261	43.052	1.257	-2.257	37.370	-5.231	43.106

		S2LJ FE SDS	S2LJ P max (kW)	S2LJ P max rel (W/kg)	S2LJ Power SDS	S2LJ F max (kN)	S2LJ F max rel (N/kg)	S2LJ Force SDS
Visit 1	Value	-4.523	0.475	16.993	-4.798	0.497	18.520	-1.799
	Δ in Observation	-0.131	0.007	-0.493	-0.130	0.021	-0.624	-0.186
	p	0.745	0.812	0.672	0.570	0.662	0.787	0.788
Visit 2	Value	-4.653	0.482	16.500	-4.928	0.518	17.897	-1.985
	Δ with WBVT	-0.391	0.018	-0.059	-0.119	0.019	0.312	0.093
	p	0.293	0.494	0.954	0.568	0.650	0.883	0.910
Visit 3	Value	-5.045	0.500	16.441	-5.047	0.537	18.209	-1.892
	Δ in Follow-up	-0.311	0.023	1.043	0.172	0.100	4.242	1.272
	p	0.398	0.383	0.326	0.412	0.035*	0.067	0.067
Visit 4	Value	-5.356	0.523	17.485	-4.875	0.637	22.451	-0.620

Note: Δ, change; WBVT, whole body vibration training; CRT, chair rise test; SDS, standard deviation score; Ave V max, average maximum velocity; P max, maximum power; P max rel, maximum power in relation to body weight; Ave P max rel, average maximum power in relation to body weight; F max, maximum force; F max rel, maximum relative force in relation to body weight; F max/g, maximum relative force per force equivalent to body weight (Fg); Ave F max/g, average maximum relative force per force equivalent to body weight; S2LJ, single two-leg jump; EFI, Esslinger Fitness Index; FE, force efficiency; V max, maximum velocity.

Table D.3. Fore plate parameters for the adult MRCD cohort at each visit with changes between visits and associated p values from linear mixed models analysis. *p<0.05.

		CRT Time per test (s)	CRT V max (m/s)	CRT Speed SDS	CRT P max (W)	CRT P max rel (W/kg)	CRT Ave P max rel (W/kg)	CRT Power SDS
Visit 1	Value	2.806	0.650	-3.421	437.484	7.329	6.772	-2.684
	Δ in Observation	-0.162	0.039	0.244	24.854	0.411	0.391	0.226
	p	0.179	0.283	0.274	0.491	0.398	0.355	0.343
Visit 2	Value	2.644	0.688	-3.177	498.338	7.741	7.163	-2.458
	Δ with WBVT	-0.269	-0.012	-0.113	-29.499	-0.203	0.004	-0.057
	p	0.030*	0.732	0.610	0.414	0.675	0.992	0.811
Visit 3	Value	2.375	0.676	-3.290	468.839	7.537	7.167	-2.515
	Δ in Follow-up	-0.201	0.046	0.253	75.600	1.093	0.542	0.506
	p	0.099	0.205	0.257	0.042*	0.030*	0.202	0.038*
Visit 4	Value	2.174	0.722	-3.037	544.440	8.630	7.709	-2.009

		CRT F max (kN)	CRT F max rel (N/kg)	CRT Ave F max rel (g)	CRT Force SDS	S2LJ EFI (%)	S2LJ EFI SDS
Visit 1	Value	1.205	19.652	1.205	2.924	53.775	-2.776
	Δ in Observation	0.001	-0.103	-0.001	-0.025	-1.268	-0.132
	p	0.950	0.796	0.963	0.935	0.506	0.258
Visit 2	Value	1.206	19.549	1.204	2.898	52.507	-2.907
	Δ with WBVT	0.034	0.696	0.032	0.346	-0.357	-0.006
	p	0.142	0.088	0.110	0.266	0.854	0.959
Visit 3	Value	1.240	20.245	1.236	3.244	52.150	-2.914
	Δ in Follow-up	0.063	1.155	0.039	0.732	1.385	0.109
	p	0.009*	0.006*	0.052	0.022*	0.478	0.359
Visit 4	Value	1.303	21.400	1.275	3.976	53.535	-2.805

		S2LJ FE (%)	S2LJ FE SDS	S2LJ P max (kW)	S2LJ P max rel (W/kg)	S2LJ Power SDS
Visit 1	Value	65.805	-3.419	1.498	23.442	-2.957
	Δ in Observation	-0.896	-0.090	-0.082	-0.898	-0.118
	p	0.636	0.636	0.280	0.346	0.439
Visit 2	Value	64.909	-3.509	1.416	22.544	-3.075
	Δ with WBVT	-2.429	-0.243	-0.012	-0.083	-0.042
	p	0.214	0.214	0.881	0.932	0.789
Visit 3	Value	62.481	-3.752	1.404	22.461	-3.117
	Δ in Follow-up	3.125	0.312	0.42	0.612	0.097
	p	0.113	0.113	0.590	0.529	0.536
Visit 4	Value	65.606	-3.439	1.446	23.073	-3.020

		S2LJ F max (kN)	S2LJ F max rel (N/kg)	S2LJ Force SDS	M1LJ F max (kN)	M1LJ F max rel (g)	M1LJ Force SDS
Visit 1	Value	1.275	20.044	-1.230	11.895	1.937	-4.545
	Δ in Observation	-0.022	-0.244	-0.087	-0.208	-0.057	-0.191
	p	0.622	0.663	0.603	0.632	0.436	0.436
Visit 2	Value	1.253	19.800	-1.317	11.686	1.879	-4.736
	Δ with WBVT	0.038	0.689	0.208	-0.127	0.024	0.080
	p	0.416	0.233	0.232	0.761	0.734	0.734
Visit 3	Value	1.291	20.490	-1.110	11.560	1.903	-4.656
	Δ in Follow-up	-0.036	-0.630	-0.187	0.874	0.087	0.289
	p	0.438	0.275	0.282	0.051	0.241	0.241
Visit 4	Value	1.255	19.860	-1.296	12.433	1.990	-4.367

Note: Δ, change; WBVT, whole body vibration training; CRT, chair rise test; Ave V max, average maximum velocity; SDS, standard deviation score; P max, maximum power; P max rel, maximum power in relation to body weight; F max, maximum force; F max rel, maximum relative force in relation to body weight; F max/g, maximum relative force per force equivalent to body weight (Fg); S2LJ, single two-leg jump; EFI, Esslinger Fitness Index; FE, force efficiency; V max, maximum velocity; M1LH, multiple one-leg hop.

Table D.4. Fore plate parameters for the Leigh Syndrome MRCD cohort at each visit with changes between visits and associated p values from linear mixed models analysis. *p<0.05.

		CRT Time per test (s)	CRT V max (m/s)	CRT Speed SDS	CRT P max (W)	CRT P max rel (W/kg)	CRT Ave P max rel (W/kg)
Visit 1	Value	2.741	0.630	-4.081	326.370	7.190	6.395
	Δ in Observation	0.354	0.010	-0.702	31.759	1.056	0.094
	p	0.098	0.907	0.359	0.650	0.459	0.919
Visit 2	Value	3.095	0.640	-4.783	358.129	8.246	6.489
	Δ with WBVT	-0.173	-0.069	0.001	-85.541	-1.675	-0.960
	p	0.398	0.413	0.999	0.235	0.249	0.313
Visit 3	Value	2.922	0.570	-4.782	272.589	6.571	5.529
	Δ in Follow-up	-0.196	0.038	0.210	76.034	0.522	0.805
	p	0.341	0.652	0.779	0.288	0.712	0.395
Visit 4	Value	2.726	0.608	-4.572	348.623	7.093	6.334

		CRT Power SDS	CRT F max (kN)	CRT F max rel (N/kg)	CRT Ave F max rel (Fg)	CRT Force SDS
Visit 1	Value	-2.572	1.217	39.935	1.182	-1.776
	Δ in Observation	0.085	-0.029	-2.340	-0.036	-0.228
	p	0.804	0.617	0.2490	0.373	0.536
Visit 2	Value	-2.487	1.188	37.595	1.146	-2.004
	Δ with WBVT	-0.504	-0.064	-4.885	-0.047	-0.364
	p	0.160	0.275	0.163	0.245	0.329
Visit 3	Value	-2.992	1.124	32.710	1.098	-2.369
	Δ in Follow-up	0.295	0.136	1.941	0.151	1.106
	p	0.398	0.033*	0.566	0.002*	0.009*
Visit 4	Value	-2.697	1.260	34.651	1.249	-1.263

Note: Δ, change; WBVT, whole body vibration training; CRT, chair rise test; Ave V max, average maximum velocity; SDS, standard deviation score; P max, maximum power; P max rel, maximum power in relation to body weight; F max, maximum force; F max rel, maximum relative force in relation to body weight; F max/g, maximum relative force per force equivalent to body weight (Fg); S2LJ, single two-leg jump; EFI, Esslinger Fitness Index; FE, force efficiency; V max, maximum velocity; M1LH, multiple one-leg hop.

Appendix E: Exercise Parameters

Table E.1. Cardiopulmonary exercise test parameters for the CF cohort at each visit with changes between visits and associated p values from linear mixed models analysis. *p<0.05.

		Pre-exercise VO_2 (L/min)	**Pre-exercise VO_2/kg (ml/kg/min)**	**Pre-exercise HR (bpm)**	**Pre-exercise HR % Predicted (%)**	**Pre-exercise O_2 Pulse (ml/beat)**	**Pre-exercise VCO_2 (L/min)**
Visit 1	Value	0.3	7.1	96.8	46.7	3.0	0.3
	Δ in Observation	0.0	0.3	4.8	2.5	0.5	0.1
	p	0.203	0.629	0.253	0.226	0.116	0.061
Visit 2	Value	0.3	7.5	101.6	49.2	3.5	0.3
	Δ with WVBT	0.0	-0.6	-7.3	-3.4	-0.3	0.0
	p	0.567	0.375	0.086	0.095	0.400	0.279
Visit 3	Value	0.3	6.9	94.3	45.7	3.2	0.3
	Δ in Follow-up	0.0	-1.2	-9.0	-4.2	-0.2	-0.1
	p	0.123	0.079	0.039*	0.045*	0.522	0.086
Visit 4	Value	0.3	5.6	85.2	41.5	3.0	0.2

		Pre-exercise RQ	Pre-exercise VE (L/min)	Pre-exercise VE/VO_2	Pre-exercise VE/VCO_2	Pre-exercise Borg Score	AT VO_2 (L/min)	AT VO_2 (% Predicted)
Visit 1	Value	1.0	10.3	40.3	41.3	0.6	1.6	135.7
	Δ in Observation	0.0	1.0	-0.6	-1.6	0.1	-0.1	-7.4
	p	0.669	0.378	0.831	0.478	0.739	0.425	0.525
Visit 2	Value	1.0	11.3	39.6	39.7	0.7	1.5	128.2
	Δ with WVBT	0.0	-0.2	2.3	2.0	0.3	0.0	-11.5
	p	0.836	0.851	0.424	0.365	0.442	0.955	0.346
Visit 3	Value	1.0	11.1	41.9	41.8	0.9	1.5	116.7
	Δ in Follow-up	0.0	-1.4	-0.4	1.7	-0.2	0.0	3.4
	p	0.329	0.244	0.881	0.444	0.479	0.949	0.764
Visit 4	Value	1.0	9.7	41.5	43.5	0.7	1.5	120.2

		AT VO_2 (ml/kg/min)	AT VO_2 (ml/kg/min) % Predicted VO_2 (%)	AT VO_2 as a % of Peak VO_2 (%)	AT HR (bpm)	AT HR % Predicted (%)	AT O_2 Pulse (ml/beat)
Visit 1	Value	36.5	89.2	80.0	165.9	80.4	9.2
	Δ in Observation	-5.3	-8.8	-0.3	-4.5	-2.0	4.5
	p	0.075	0.223	0.953	0.301	0.350	0.154
Visit 2	Value	31.2	80.4	79.7	161.4	78.4	13.8
	Δ with WVBT	-0.4	-4.9	-4.3	-1.8	-0.6	-4.4
	p	0.900	0.509	0.385	0.679	0.777	0.188
Visit 3	Value	30.8	75.5	75.5	159.5	77.8	9.4
	Δ in Follow-up	-0.2	1.3	3.4	-0.5	-0.1	-0.2
	p	0.954	0.857	0.466	0.913	0.972	0.995
Visit 4	Value	30.7	76.8	78.8	159.0	77.7	9.3

		AT VCO_2 (L/min)	AT RQ	AT VE (L/min)	AT VE/VO_2	AT VE/VCO_2
Visit 1	Value	1.6	1.0	44.1	28.5	28.9
	Δ in Observation	-0.1	0.0	-1.4	1.5	0.7
	p	0.478	0.019*	0.750	0.067	0.286
Visit 2	Value	1.5	1.0	42.7	30.0	29.6
	Δ with WVBT	0.0	0.0	-0.8	0.1	0.9
	p	0.869	0.432	0.858	0.866	0.205
Visit 3	Value	1.4	1.0	41.9	30.2	30.5
	Δ in Follow-up	0.0	0.0	0.3	-0.5	-0.6
	p	0.989	0.395	0.947	0.542	0.411
Visit 4	Value	1.4	1.0	42.2	29.7	30.0

		Peak VO_2 (L/min)	Peak VO_2 (L/min) % Predicted (%)	Peak VO_2 (ml/kg/min)	Peak VO_2 (ml/kg/min) % Predicted (%)
Visit 1	Value	1.9	104.2	45.7	114.9
	Δ in Observation	-0.1	-8.7	-4.3	-9.8
	p	0.169	0.033*	0.030*	0.035*
Visit 2	Value	1.8	95.6	41.4	105.0
	Δ with WVBT	0.1	-0.7	0.2	0.3
	p	0.517	0.855	0.925	0.940
Visit 3	Value	1.8	94.8	41.6	105.4
	Δ in Follow-up	0.0	-3.2	-1.3	-3.8
	p	0.955	0.430	0.495	0.419
Visit 4	Value	1.8	91.6	40.3	101.6

		Peak HR (bpm)	Peak HR % Predicted (%)	Peak O_2 Pulse (ml/beat)	Peak O_2 Pulse % Predicted (%)	Peak VCO_2 (L/min)	Peak RQ
Visit 1	Value	181.6	87.7	10.4	111.9	1.9	1.0
	Δ in Observation	1.2	0.8	2.6	5.6	0.0	0.1
	p	0.728	0.662	0.315	0.716	0.631	0.020*
Visit 2	Value	182.8	88.5	13.0	117.5	1.9	1.1
	Δ with WVBT	-3.7	-1.6	-2.7	-16.5	0.0	-0.0
	p	0.301	0.361	0.305	0.294	0.982	0.093
Visit 3	Value	179.1	86.9	10.3	101.0	1.9	1.0
	Δ in Follow-up	1.9	1.2	-0.1	-6.2	0.0	0.0
	p	0.621	0.509	0.968	0.704	0.999	0.795
Visit 4	Value	181.0	88.1	10.2	94.8	1.9	1.0

		Peak VE (L/min)	Peak VE/VO_2	Peak VE/VCO_2	Exercise Time (mins)
Visit 1	Value	55.2	29.7	29.2	11.9
	Δ in Observation	0.9	2.7	1.4	-0.3
	p	0.787	0.001*	0.011*	0.312
Visit 2	Value	56.1	32.4	30.6	11.6
	Δ with WVBT	0.6	-0.6	0.6	0.2
	p	0.848	0.468	0.236	0.598
Visit 3	Value	56.7	31.8	31.2	11.8
	Δ in Follow-up	0.65	0.2	-0.2	0.1
	p	0.890	0.817	0.758	0.707
Visit 4	Value	57.1	32.0	31.0	11.9

		Peak Borg Score	Recovery VO_2 (L/min)	Recovery VO_2 (ml/kg/min)	Recovery HR (bpm)	Recovery HR % predicted (%)	Recovery O_2 Pulse (ml/beat)
Visit 1	Value	7.0	0.4	8.5	108.5	52.5	3.2
	Δ in Observation	0.0	0.0	-1.0	-1.4	-0.7	0.2
	p	0.956	0.252	0.075	0.662	0.671	0.511
Visit 2	Value	7.0	0.3	7.6	107.1	51.8	3.5
	Δ with WVBT	0.3	0.0	-0.3	-0.9	-0.3	-0.4
	p	0.658	0.971	0.589	0.778	0.864	0.221
Visit 3	Value	7.3	0.3	7.3	106.2	51.5	3.0
	Δ in Follow-up	0.2	0.0	-0.5	1.0	0.6	-0.2
	p	0.727	0.493	0.319	0.746	0.681	0.661
Visit 4	Value	7.5	0.3	6.8	107.2	52.2	2.9

		Recovery VCO_2 (L/min)	Recovery RQ	Recovery VE (L/min)	Recovery VE/VO_2	Recovery VE/VCO_2	Recovery Borg Score
Visit 1	Value	0.4	1.1	13.4	40.0	38.5	3.1
	Δ in Observation	0.0	0.0	-0.1	2.7	-0.1	-0.3
	p	0.783	0.052	0.964	0.164	0.916	0.621
Visit 2	Value	0.4	1.1	13.4	42.7	38.4	2.8
	Δ with WVBT	0.0	0.0	0.3	1.6	1.6	-0.1
	p	0.781	0.786	0.767	0.392	0.204	0.809
Visit 3	Value	0.4	1.1	13.7	44.3	40.0	2.7
	Δ in Follow-up	0.0	0.0	0.0	2.9	2.6	0.5
	p	0.546	0.682	0.978	0.122	0.047*	0.331
Visit 4	Value	0.3	1.1	13.7	47.2	42.6	3.2

		Pre-exercise SBP	Pre-exercise DBP	Pre-exercise SpO2	Peak SBP	Peak DBP	Peak SpO2
Visit 1	Value	107.1	66.9	97.5	124.5	68.1	95.9
	Δ in Observation	-0.5	-1.8	-0.1	6.2	3/7	0.2
	p	0.793	0.544	0.890	0.089	0.157	0.685
Visit 2	Value	106.6	65.1	97.4	130.7	71.8	96.1
	Δ with WVBT	2.2	1.1	-0.1	-2.9	0.5	0.6
	p	0.235	0.711	0.890	0.379	0.842	0.181
Visit 3	Value	108.8	66.1	97.4	127.9	72.2	96.7
	Δ in Follow-up	3.2	6.5	-0.1	5.0	2.5	-0.7
	p	0.091	0.031*	0.917	0.132	0.292	0.178
Visit 4	Value	112.0	72.7	97.3	132.9	74.8	96.0

		Recovery SBP	Recovery DBP	Recovery SpO2
Visit 1	Value	110.9	64.7	96.9
	Δ in Observation	2.3	3.4	0.1
	p	0.361	0.273	0.722
Visit 2	Value	113.1	68.1	97.1
	Δ with WVBT	2.0	0.2	0.1
	p	0.416	0.942	0.722
Visit 3	Value	111.2	68.3	97.2
	Δ in Follow-up	5.8	3.1	-0.1
	p	0.024*	0.297	0.725
Visit 4	Value	117.0	72.7	97.1

Note: Δ, change; WBVT, whole body vibration training; VO_2, oxygen uptake; HR, heart rate; bpm, beats per minute; O_2, oxygen; VCO_2, carbon dioxide production; RQ, respiratory quotient; VE, minute ventilation; VE/VO_2, ventilator equivalent of oxygen; VE/VCO_2, ventilator equivalent of carbon dioxide; AT, anaerobic threshold.

Table E.2. Six-minute walk test parameters for the paediatric MRCD cohort at each visit with changes between visits and associated p values from linear mixed models analysis. *p<0.05.

		6MWT Distance (m)	6MWT Percent Predicted (%)	6MWT Baseline HR (bpm)	6MWT Baseline Borg Score	6MWT Baseline SBP (mmHg)	6MWT Baseline DBP (mmHg)
Visit 1	Value	319.243	52.171	96.040	0.571	104.1	68.1
	Δ in Observation	-12.000	-2.685	7.817	0.143	5.0	-5.8
	p	0.380	0.214	0.149	0.700	0.130	0.279
Visit 2	Value	307.243	49.485	103.857	0.714	109.1	62.3
	Δ with WBVT	-8.643	-1.754	-10.429	-0.143	-4.6	6.3
	p	0.525	0.411	0.011*	0.700	0.113	0.189
Visit 3	Value	298.600	47.731	93.429	0.571	104.6	68.6
	Δ in Follow-	10.443	1.162	10.259	-0.143	2.4	-2.3
	p	0.443	0.584	0.017*	0.700	0.418	0.643
Visit 4	Value	309.043	48.893	103.668	0.429	107.0	66.3

		6MWT Baseline SpO2 (%)	6MWT Post HR (bpm)	6MWT Post Borg Score	6MWT Post SBP (mmHg)	6MWT Post DBP (mmHg)	6MWT Post SpO2 (%)
Visit 1	Value	99	106.7	3.0	116.6	71.3	99
	Δ in Observation	-1	26.8	-0.3	0.4	7.5	-2
	p	0.486	0.037*	0.437	0.938	0.187	0.276
Visit 2	Value	98	133.429	2.7	117.0	78.8	97
	Δ with WBVT	0	-27.9	-0.1	0.0	-5.2	1
	p	0.687	0.015*	0.696	0.998	0.259	0.534
Visit 3	Value	98	105.571	2.6	117.0	73.6	98
	Δ in Follow-up	0	23.0	-0.4	-1.8	-3.8	0
	p	0.759	0.038*	0.249	0.663	0.379	0.920
Visit 4	Value	98	129	2.1	115.2	69.8	98

		6MWT Recovery HR (bpm)	6MWT Recovery Borg Score	6MWT Recovery SBP (mmHg)	6MWT Recovery DBP (mmHg)	6MWT Recovery SpO2 (%)
Visit 1	Value	112.6	1.8	106.2	67.4	98
	Δ in Observation	-1.2	0.3	1.9	-2.0	1
	p	0.875	0.569	0.645	0.688	0.755
Visit 2	Value	111.4	2.1	108.1	65.4	99
	Δ with WBVT	-7.9	-1.3	0.8	-0.1	0
	p	0.148	0.018*	0.832	0.982	0.854
Visit 3	Value	103.6	0.9	108.9	65.3	99
	Δ in Follow-up	3.8	0.3	3.1	-0.8	-1
	p	0.499	0.569	0.385	0.856	0.497
Visit 4	Value	107.3	1.1	112.0	64.5	98

Table E.3. Six-minute walk test parameters for the adult MRCD cohort at each visit with changes between visits and associated p values from linear mixed models analysis. *p<0.05.

		6MWT Distance (m)	6MWT % Predicted (%)	6MWT Baseline HR (bpm)	6MWT Baseline Borg Score	6MWT Baseline SBP (mmHg)	6MWT Baseline DBP (mmHg)
Visit 1	Value	413.042	71.303	86.000	1.177	126.083	83.167
	Δ in Observation	-11.225	-1.307	-3.721	-0.093	-6.161	-8.195
	p	0.466	0.588	0.388	0.653	0.057	0.002
Visit 2	Value	401.817	69.996	82.279	1.083	119.922	74.972
	Δ with WBVT	19.175	3.258	-4.898	-0.417	5.328	0.028
	p	0.216	0.182	0.356	0.110	0.098	0.991
Visit 3	Value	420.992	73.254	77.382	0.667	125.250	75.000
	Δ in Follow-up	4.133	0.641	3.064	0.167	-2.096	1.710
	p	0.787	0.790	0.564	0.519	0.510	0.492
Visit 4	Value	425.125	73.895	80.446	0.833	123.154	76.710

		6MWT Baseline SpO2 (%)	6MWT Post HR (bpm)	6MWT Post Borg Score	6MWT Post SBP (mmHg)	6MWT Post DBP (mmHg)	6MWT Post SpO2 (%)
Visit 1	Value	98	106.8	2.7	138.8	84.4	98
	Δ in Observation	0	-1.4	-0.2	-0.4	-2.2	0
	p	0.514	0.797	0.610	0.287	0.193	0.673
Visit 2	Value	98	105.4	2.6	138.4	82.3	98
	Δ with WBVT	0	0.7	0.2	5.3	1.8	1
	p	0.259	0.894	0.582	0.134	0.243	0.201
Visit 3	Value	98	106.1	2.8	143.7	84.1	99
	Δ in Follow-up	0	-2.2	-0.2	-7.0	-1.6	0
	p	0.300	0.688	0.582	0.045*	0.295	0.653
Visit 4	Value	98	103.9	2.6	136.7	82.5	99

		6MWT Recovery HR (bpm)	6MWT Recovery Borg Score	6MWT Recovery SBP (mmHg)	6MWT Recovery DBP (mmHg)	6MWT Recovery SpO2 (%)
Visit 1	Value	86.9	1.4	127.7	78.9	97
	Δ in Observation	-2.7	0.0	-6.2	-2.8	1
	p	0.410	0.952	0.059	0.386	0.106
Visit 2	Value	84.2	1.4	121.5	76.2	98
	Δ with WBVT	0.7	-0.1	1.9	-1.5	0
	p	0.832	0.764	0.547	0.635	0.915
Visit 3	Value	84.9	1.3	123.4	74.7	98
	Δ in Follow-up	3.1	-0.3	-1.8	0.5	-1
	p	0.346	0.371	0.580	0.892	0.349
Visit 4	Value	88.0	1.1	121.7	75.1	97

Table E.4. Six-minute walk test parameters for the Leigh Syndrome MRCD cohort at each visit with changes between visits and associated p values from linear mixed models analysis. *p<0.05.

		6MWT Distance (m) Leigh	6MWT Percent Predicted	6MWT Baseline HR (bpm)	6MWT Baseline Borg Score	6MWT Baseline SBP (mmHg)	6MWT Baseline DBP (mmHg)
Visit 1	Value	341.580	56.765	94.5	0.4	113.1	78.7
	Δ in Observation	-66.700	-10.794	6.1	-0.2	-0.5	14.2
	p	0.035*	0.023*	0.282	0.387	0.906	0.014*
Visit 2	Value	274.880	45.971	100.6	0.2	112.6	64.4
	Δ with WBVT	24.020	3.306	-5.4	0.0	-1.8	7.4
	p	0.408	0.439	0.259	1.000	0.628	0.128
Visit 4	Value	298.900	49.278	95.2	0.2	110.8	71.8
	Δ in Follow-up	18.500	2.753	3.6	0.0	5.6	1.0
	p	0.521	0.518	0.443	1.000	0.150	0.829
Visit 6	Value	317.400	52.031	98.8	0.2	116.4	72.8

		6MWT Baseline SpO2 (%)	6MWT Post HR (bpm)	6MWT Post Borg Score	6MWT Post SBP (mmHg)	6MWT Post DBP (mmHg)	6MWT Post SpO2 (%)
Visit 1	Value	99	123.9	2.8	119.4	74.4	99
	Δ in Observation	0	-13.9	-0.6	-10.6	-3.8	-1
	p	0.781	0.360	0.068	0.342	0.613	0.611
Visit 2	Value	99	110.0	2.2	108.8	70.6	98
	Δ with WBVT	0	-10.4	0.8	17.8	1.8	0
	p	1.000	0.413	0.020*	0.071	0.762	0.936
Visit 3	Value	99	99.6	3.0	126.6	72.4	98
	Δ in Follow-up	0	20.4	-0.6	0.2	0.0	0
	p	0.781	0.128	0.068	0.974	1.000	0.680
Visit 4	Value	99	120.0	2.4	126.8	72.4	98

		6MWT Recovery HR (bpm)	6MWT Recovery Borg Score	6MWT Recovery SBP (mmHg)	6MWT Recovery DBP (mmHg)	6MWT Recovery SpO2 (%)
Visit 1	Value	112.6	1.2	122.1	75.6	99
	Δ in Observation	-8.0	0.2	-12.0	-7.7	0
	p	0.255	0.584	0.093	0.234	0.441
Visit 2	Value	104.6	1.4	110.1	67.9	99
	Δ with WBVT	0.8	-1.0	5.9	-3.1	0
	p	0.880	0.016*	0.373	0.609	0.794
Visit 3	Value	105.4	0.4	116.0	64.8	99
	Δ in Follow-up	-0.8	0.4	4.0	0.4	0
	p	0.880	0.283	0.467	0.937	0.794
Visit 4	Value	104.6	0.8	120.0	65.2	99

Table E.5. Cardiopulmonary exercise test parameters for the adult MRCD cohort at each visit with changes between visits and associated p values from linear mixed models analysis. *p<0.05.

		Pre-exercise VO_2 (L/min)	**Pre-exercise VO_2/kg (ml/kg/min)**	**Pre-exercise HR (bpm)**	**Pre-exercise HR % Predicted (%)**	**Pre-exercise O_2 Pulse (ml/beat)**	**Pre-exercise VCO_2 (L/min)**
Visit 1	Value	0.249	4.679	96	53.0	2.6	0.233
	Δ in Observation	-0.004	-0.388	-9	-3.8	0.2	-0.026
	p	0.889	0.465	0.141	0.273	0.585	0.266
Visit 2	Value	0.246	4.292	88	49.2	2.8	0.207
	Δ with WBVT	-0.008	-0.167	-2	-0.3	-0.1	-0.009
	p	0.738	0.714	0.773	0.928	0.723	0.660
Visit 3	Value	0.238	4.125	86	49.0	2.7	0.199
	Δ in Follow-up	-0.009	-0.052	1.000	0.9	-0.1	-0.003
	p	0.702	0.909	0.840	0.775	0.761	0.889
Visit 4	Value	0.229	4.073	87	49.8	2.6	0.196

		Pre-exercise RQ	Pre-exercise VE (L/min)	Pre-exercise VE/VO_2	Pre-exercise VE/VCO_2	Pre-exercise Borg Score	AT VO_2 (L/min)
Visit 1	Value	0.922	10.344	42.0	45.8	3.1	0.7
	Δ in Observation	-0.079	-1.402	-4.4	-1.3	-1.7	0.1
	p	0.053	0.286	0.158	0.684	0.005*	0.275
Visit 2	Value	0.843	8.943	37.6	44.5	1.4	0.8
	Δ with WBVT	-0.016	0.129	0.6	1.9	-0.3	0.0
	p	0.651	0.909	0.807	0.484	0.533	0.814
Visit 3	Value	0.827	9.071	38.3	46.4	1.1	0.8
	Δ in Follow-up	0.033	-0.071	1.6	0.5	-0.4	-0.2
	p	0.351	0.949	0.543	0.859	0.440	0.076
Visit 4	Value	0.860	9.000	39.9	46.8	0.8	0.6

		AT VO_2 % Predicted (%)	AT VO_2 (ml/kg/min)	AT VO_2 as a % of Peak VO_2 (%)	AT HR (bpm)	AT HR % Predicted (%)	AT O_2 Pulse (ml/beat)
Visit 1	Value	34.9	12.2	69.8	132.2	73.9	5.0
	Δ in Observation	5.7	1.7	5.4	-6.0	-2.9	0.9
	p	0.257	0.247	0.480	0.289	0.348	0.170
Visit 2	Value	40.5	13.8	75.2	126.1	71.0	5.9
	Δ with WBVT	2.0	-0.1	2.1	2.3	1.9	0.2
	p	0.630	0.929	0.760	0.632	0.466	0.755
Visit 4	Value	42.6	13.7	77.3	128.4	72.9	6.1
	Δ in Follow-up	-5.3	-2.5	-5.7	-4.2	-2.4	-1.1
	p	0.246	0.068	0.424	0.414	0.390	0.076
Visit 6	Value	37.2	11.2	71.5	124.2	70.5	5.0

		AT VCO_2 (L/min)	AT RQ	AT VE (L/min)	AT VE/VO_2	AT VE/VCO_2	AT Watts (W)
Visit 1	Value	0.7	1.0	22.8	36.2	37.0	34.7
	Δ in Observation	0.1	0.0	1.7	-2.6	-3.7	8.7
	p	0.246	0.260	0.623	0.310	0.143	0.144
Visit 2	Value	0.8	1.0	24.5	33.6	33.3	43.4
	Δ with WBVT	0.0	0.0	1.7	0.1	0.7	-1.0
	p	0.899	0.428	0.565	0.947	0.724	0.841
Visit 3	Value	0.8	1.0	26.2	33.8	34.1	42.4
	Δ in Follow-up	-0.1	0.0	-3.9	3.0	2.4	-6.0
	p	0.079	0.319	0.233	0.212	0.285	0.266
Visit 4	Value	0.6	1.0	22.3	36.8	36.5	36.4

		AT Watts (% Predicted)	AT Watts (W/kg)	AT Watts as a % of Peak Watts (%)	AT % Exercise Time (%)	Peak VO_2 (L/min)	Peak VO_2 % Predicted (%)
Visit 1	Value	24.9	0.7	55.0	56.0	1.0	54.2
	Δ in Observation	5.5	0.1	5.6	-2.5	0.0	1.1
	p	0.164	0.276	0.429	0.818	0.880	0.840
Visit 2	Value	30.4	0.8	60.6	53.5	1.0	55.3
	Δ with WBVT	0.2	0.0	2.7	-0.4	0.1	1.2
	p	0.944	0.945	0.650	0.964	0.651	0.788
Visit 3	Value	30.6	0.8	63.3	53.1	1.1	56.5
	Δ in Follow-up	-3.2	-0.1	-5.9	-8.4	-0.1	-2.4
	p	0.365	0.314	0.364	0.410	0.215	0.597
Visit 4	Value	27.4	0.7	57.4	44.7	1.0	54.1

		Peak VO_2 (ml/kg/min)	Peak HR (bpm)	Peak HR % Predicted (%)	Peak O_2 Pulse (ml/beat)	Peak O_2 Pulse % Predicted (%)	Peak VCO_2 (L/min)
Visit 1	Value	17.8	154.2	87.7	6.2	37.1	1.2
	Δ in Observation	-0.3	-1.8	-1.7	0.2	1.2	0.0
	p	0.842	0.799	0.636	0.721	0.765	0.977
Visit 2	Value	17.5	152.4	86.0	6.4	38.3	1.2
	Δ with WBVT	0.9	-3.0	-0.9	0.0	-0.2	0.0
	p	0.519	0.625	0.786	0.937	0.953	0.858
Visit 3	Value	18.4	149.4	85.1	6.4	38.1	1.2
	Δ in Follow-up	-1.5	2.1	1.0	-0.2	-1.2	-0.0
	p	0.278	0.726	0.748	0.695	0.727	0.945
Visit 4	Value	16.9	151.6	86.1	6.1	36.9	1.2

		Peak RQ	Peak VE (L/min)	Peak VE/VO_2	Peak VE/VCO_2	Peak Watts (W)	Peak Watts (% Predicted)
Visit 1	Value	1.3	340.6	42.9	36.7	69.3	50.3
	Δ in Observation	0.0	-1.4	2.8	-3.0	0.9	0.3
	p	0.559	0.791	0.555	0.162	0.744	0.922
Visit 2	Value	1.3	39.2	45.7	33.7	70.3	50.6
	Δ with WBVT	0.0	0.4	0.6	1.6	-1.3	-0.5
	p	0.979	0.930	0.887	0.383	0.600	0.839
Visit 3	Value	1.3	39.6	46.3	35.3	69.0	50.1
	Δ in Follow-up	0.1	0.3	5.1	1.0	-0.6	0.0
	p	0.261	0.953	0.213	0.576	0.815	0.994
Visit 4	Value	1.4	39.9	51.4	36.3	68.4	50.1

		Peak Watts (W/kg)	Exercise Time (mins)	Peak Borg Score	Recovery VO_2 (L/min)	Recovery VO_2 (ml/kg/min)	Recovery HR (bpm)
Visit 1	Value	1.2	5.7	7.4	0.4	6.4	111.3
	Δ in Observation	0.0	0.4	-0.1	-0.1	-0.7	-12.9
	p	0.684	0.241	0.941	0.263	0.334	0.013*
Visit 2	Value	1.2	6.1	7.3	0.3	5.7	98.4
	Δ with WBVT	0.0	0.0	-0.7	0.0	-0.2	0.4
	p	0.918	0.881	0.524	0.847	0.727	0.915
Visit 3	Value	1.2	6.1	6.6	0.3	5.5	98.9
	Δ in Follow-up	0.0	-0.2	-0.6	0.0	-0.6	1.0
	p	0.894	0.431	0.626	0.439	0.388	0.804
Visit 4	Value	1.2	5.9	6.0	0.3	4.9	99.9

		Recovery HR % predicted (%)	Recovery O_2 Pulse (ml/beat)	Recovery VCO_2 (L/min)	Recovery RQ	Recovery VE (L/min)	Recovery VE/VO_2	Recovery VE/VCO_2	Recovery Borg Score
Visit 1	Value	62.7	3.3	0.4	1.1	16.6	48.1	45.9	3.0
	Δ Observation	-7.3	0.1	-0.1	0.0	-2.8	-2.6	-2.4	-0.3
	p	0.009*	0.873	0.307	0.824	0.188	0.376	0.272	0.717
Visit 2	Value	55.4	3.2	0.3	1.1	13.8	45.6	43.6	2.7
	Δ with WBVT	0.7	-0.1	0.0	0.1	0.7	5.9	1.8	-0.4
	p	0.730	0.822	0.912	0.101	0.687	0.029*	0.321	0.574
Visit 3	Value	56.2	3.1	0.3	1.2	14.5	51.4	45.3	2.3
	Δ in Follow-up	0.7	-0.4	0.0	0.0	-0.3	-0.1	0.7	1.1
	p	0.738	0.355	0.531	0.486	0.856	0.954	0.707	0.227
Visit 4	Value	56.9	2.8	0.3	1.1	14.2	51.3	46.0	3.4

		Pre-exercise SBP (mmHg)	Pre-exercise DBP (mmHg)	Peak SBP (mmHg)	Peak DBP (mmHg)	Recovery SBP (mmHg)	Recovery DBP (mmHg)
Visit 1	Value	120.9	85.2	165.0	89.6	135.5	82.9
	Δ in Observation	0.8	-10.1	-13.4	-7.0	-6.1	-9.1
	p	0.861	0.033*	0.076	0.277	0.261	0.091
Visit 2	Value	121.7	75.1	151.6	82.6	129.4	73.9
	Δ with WBVT	-4.4	-1.0	1.1	6.3	-6.7	4.9
	p	0.261	0.741	0.855	0.271	0.164	0.288
Visit 4	Value	117.3	74.1	152.7	88.9	122.7	78.1
	Δ in Follow-up	4.8	0.8	-2.5	-1.7	9.2	2.3
	p	0.257	0.816	0.702	0.778	0.097	0.653
Visit 6	Value	122.0	74.9	150.2	87.2	131.9	81

Note: Δ, change; WBVT, whole body vibration training; VO_2, oxygen uptake; HR, heart rate; bpm, beats per minute; O_2, oxygen; VCO_2, carbon dioxide production; RQ, respiratory quotient; VE, minute ventilation; VE/VO_2, ventilator equivalent of oxygen; VE/VCO_2, ventilator equivalent of carbon dioxide; AT, anaerobic threshold.

Appendix F: Quality of Life Parameters

Table F.1 CF Cohort
Table F.2 Paediatric MRCD Cohort
Table F.3 Adult MRCD Cohort
Table F.4 Leigh Syndrome Cohort

Table F.1. Quality of Life parameters for the CF cohort at each visit with changes between visits and associated p values from linear mixed models analysis. *p<0.05.

		Physical Domain	Emotion Domain	Eating Domain	Body Domain	Treatment Burden Domain	Respiratory Domain
Visit 1	Value	81.9	80.5	81.3	82.0	61.1	73.4
	Δ in Observation	-1.1	1.9	5.6	2.1	3.5	2.6
	p	0.664	0.519	0.209	0.675	0.454	0.542
Visit 2	Value	80.8	82.4	86.8	84.0	64.6	76.0
	Δ with WVBT	3.0	2.2	0.0	4.9	-2.1	-2.3
	p	0.259	0.451	0.999	0.299	0.655	0.599
Visit 3	Value	83.8	84.6	86.8	88.9	62.5	73.8
	Δ in Follow-up	-3.3	0.5	4.5	-4.0	1.5	4.9
	p	0.218	0.857	0.320	0.405	0.757	0.262
Visit 4	Value	80.4	85.1	91.3	84.9	64.0	78.7

		Digestion Domain	Social Domain	Health Perceptions Domain (Adult Version)	Role Domain (Adult Version)	Weight Domain (Adult Version)	Body Image Domain (Parent Version)
Visit 1	Value	79.2	73.7	72.8	71.1	60.3	82.7
	Δ in Observation	10.4	2.4	0.6	6.3	10.2	-4.9
	p	0.017*	0.429	0.917	0.172	0.190	0.500
Visit 2	Value	89.6	76.1	73.3	77.5	70.5	77.8
	Δ with WVBT	-4.2	2.5	-4.1	-2.0	0.7	9.6
	p	0.327	0.408	0.453	0.665	0.922	0.215
Visit 3	Value	85.4	78.6	69.2	75.5	71.2	87.4
	Δ in Follow-up	3.3	-2.2	-2.6	3.2	-8.3	2.9
	p	0.443	0.490	0.617	0.454	0.252	0.758
Visit 4	Value	88.8	76.5	66.7	78.7	63.0	90.3

		Physical Domain (Parent Version)	Emotion Domain (Parent Version)	Vitality Domain (Parent Version)	School Domain (Parent Version)	Eating Domain (Parent Version)	Weight Domain (Parent Version)
	Value	92.6	91.1	74.1	80.3	70.4	51.9
Visit 1	Δ in Observation	-0.8	-4.5	-5.2	-8.7	-1.9	7.4
	p	0.813	0.067	0.346	0.323	0.834	0.518
	Value	91.8	86.7	68.9	71.6	68.5	59.3
Visit 2	Δ with WVBT	0.7	5.8	4.1	19.1	2.8	6.1
	p	0.843	0.026*	0.477	0.043*	0.762	0.611
	Value	92.5	92.5	72.9	90.7	71.4	65.3
Visit 3	Δ in Follow-up	-2.9	7.2	5.1	-12.6	19.5	21.4
	p	0.513	0.026*	0.452	0.247	0.099	0.153
Visit 4	Value	89.6	99.7	78.1	78.2	90.9	86.7

		Treatment Burden Domain (Parent Version)	Health Perceptions Domain (Parent Version)	Respiratory Domain (Parent Version)	Digestion Domain (Parent Version)
Visit 1	Value	72.8	86.4	87.0	74.1
	Δ in Observation	-9.3	-6.2	-10.5	3.7
	p	0.085	0.194	0.046*	0.570
Visit 2	Value	63.6	80.3	76.5	77.8
	Δ with WVBT	7.9	4.6	-2.2	-3.5
	p	0.159	0.350	0.668	0.612
Visit 3	Value	71.5	84.9	74.3	74.3
	Δ in Follow-up	2.2	-0.2	13.5	2.0
	p	0.749	0.979	0.037*	0.816
Visit 4	Value	73.6	84.7	87.9	76.3

Note: Δ, change; WBVT, whole body vibration training

Table F.2: Quality of life parameters for the paediatric MRCD cohort at each visit with changes between visits and associated p values from linear mixed models analysis. *p<0.05.

		NMPDS 1 Total	NMPDS 2 Total	NMPDS 3 Total	NMPDS 4 Total
Visit 1	Value	4.7	1.1	10.1	9.2
	Δ in Observation	1.9	0.1	1.3	1.1
	p	0.000*	0.696	0.077	0.115
Visit 2	Value	6.6	1.3	11.4	10.3
	Δ with WBVT	-0.3	0.4	-0.6	-1.2
	p	0.484	0.249	0.415	0.104
Visit 3	Value	6.3	1.7	10.9	9.2
	Δ in Follow-up	0.0	0.6	1.0	-1.2
	p	1.000	0.129	0.161	0.103
Visit 4	Value	6.3	2.3	11.9	8.0

Table F.3: Quality of life parameters for the adult MRCD cohort at each visit with changes between visits and associated p values from linear mixed models analysis. *p<0.05.

		NMDAS 1 Total	NMDAS 2 Total	NMDAS 3 Total	PF NBS SDS	RP NBS SDS	BP NBS SDS
Visit 1	Value	9.8	5.0	10.2	-1.5	-1.1	-1.0
	Δ in Observation	1.5	0.4	-0.5	-0.1	-0.3	0.5
	p	0.145	0.577	0.423	0.705	0.275	0.116
Visit 2	Value	11.3	5.4	9.7	-1.6	-1.4	-0.5
	Δ with WBVT	-1.8	-0.2	-1.7	-0.2	0.5	0.0
	p	0.083	0.780	0.010*	0.440	0.074	1.000
Visit 3	Value	9.5	5.2	8.0	-1.8	-0.9	-0.5
	Δ in Follow-up	0.7	0.8	0.9	0.0	-0.2	0.3
	p	0.490	0.269	0.155	1.000	0.361	0.339
Visit 4	Value	10.2	6.0	8.9	-1.8	-1.1	-0.2

		GH NBS SDS	VT NBS SDS	SF NBS SDS	RE NBS SDS	MH NBS SDS	PCS SDS	MCS SDS
Visit 1	Value	-1.3	-0.6	-0.6	-0.1	0.2	-1.7	0.3
	Δ in Observation	0.4	0.3	0.0	0.2	0.1	0.1	0.2
	p	0.175	0.311	1.000	0.460	0.801	0.681	0.497
Visit 2	Value	-0.9	-0.3	-0.6	0.1	0.2	-1.6	0.503
	Δ with WBVT	-0.2	-0.1	0.0	-0.2	-0.2	0.1	-0.2
	p	0.469	0.7933	1.000	0.327	0.453	0.612	0.376
Visit 3	Value	-1.1	-0.4	-0.6	-0.2	0.1	-1.5	0.3
	Δ in Follow-up	0.2	-0.3	-0.1	-0.1	-0.1	0.1	-0.2
	p	0.469	0.311	0.735	0.805	0.802	0.704	0.534
Visit 4	Value	-0.9	-0.7	-0.7	-0.2	0.0	-1.4	0.1

Note: Δ, change; WBVT, whole body vibration training; NMDAS, Newcastle Disease Adult Scale; PF, physical functioning domain; NBS, norm-based score; SDS, standard deviation score; RP, role limitation domain; BP, bodily pain domain; GH, general health.

Table F.4: Quality of life parameters for the Leigh Syndrome MRCD cohort at each visit with changes between visits and associated p values from linear mixed models analysis. *p<0.05.

		NMPDS 1 Total	NMPDS 2 Total	NMPDS 3 Total	NMPDS 4 Total
Visit 1	Value	4.0	0.6	9.6	8.0
	Δ in Observation	0.2	-0.2	0.4	0.3
	p	0.825	0.670	0.493	0.738
Visit 2	Value	4.2	0.4	10.0	8.3
	Δ with WBVT	0.4	-0.4	-0.6	-0.1
	p	0.659	0.401	0.310	0.953
Visit 3	Value	4.6	0.0	9.4	8.2
	Δ in Follow-up	0.2	0.4	1.8	0.4
	p	0.825	0.401	0.008*	0.679
Visit 4	Value	4.8	0.4	11.2	8.6

Note: Δ, change; WBVT, whole body vibration training; NMPDS, Newcastle Mitochondrial Paediatric Disease Scale.

Appendix G: Lung Function Parameters

Table G.1 CF Cohort

Table G.1. Lung function parameters for the CF cohort at each visit with changes between visits and associated p values from linear mixed models analysis. *p<0.05.

		FEV1 (L) (n=13)	**FEV1 (% predicted)**	**FVC (L)**	**FVC (% Predicted)**	**FEV1/FVC (%)**
Visit 1	Value	2.4	84.4	3.0	92.3	79.2
	Δ in Observation	0.3	-2.4	0.1	0.3	-2.6
	p	0.909	0.329	0.163	0.893	0.041*
Visit 2	Value	2.4	82.0	3.1	92.6	76.6
	Δ with WVBT	0.1	0.1	0.2	-0.5	0.2
	p	0.117	0.975	0.114	0.814	0.809
Visit 3	Value	2.5	82.1	3.3	92.1	76.8
	Δ in Follow-up	0.0	-1.9	0.0	-2.0	-1.0
	p	0.958	0.449	0.765	0.384	0.298
Visit 4	Value	2.5	80.2	3.3	90.1	75.8

		FEF25-75 (L)	FEF 25-75 (% predicted)	RV/TLC (%) (n=12)	LCI (n=16)
Visit 1	Value	2.3	73.5	27.5	7.8
	Δ in Observation	-0.2	-8.9	-0.8	0.1
	p	0.060	0.007*	0.669	0.632
Visit 2	Value	2.1	64.6	26.8	7.9
	Δ with WVBT	0.2	2.4	2.3	0.3
	p	0.121	0.443	0.188	0.151
Visit 3	Value	2.3	67.0	29.1	8.2
	Δ in Follow-up	0.0	-0.9	-0.6	0.2
	p	0.859	0.785	0.739	0.509
Visit 4	Value	2.3	66.2	28.5	8.4

Note: Δ, change; WBVT, whole-body vibration training; FEV1, forced expiratory volume in the first second; FVC, forced vital capacity; FEF, forced expiratory flow at 25-75% of lung volume; RV, residual volume; TLC, total lung capacity; LCI, lung clearance index.

Appendix H: Anthropometric Parameters

Table H.1 CF Cohort
Table H.2 Paediatric MRCD Cohort
Table H.3 Adult MRCD Cohort
Table H.4 Leigh Syndrome Cohort

Table H.1. Anthropometric parameters for the CF cohort at each visit with changes between visits and associated p values from linear mixed models analysis. *p<0.05.

		Height (cm)	Height SDS	Weight (kg)	Weight SDS	BMI (kg/m^2)	BMI SDS
Visit 1	Value	151.6	0.0	40.5	-0.6	17.1	-0.7
	Δ in Observation	2.6	0.0	2.7	0.1	0.5	0.1
	p	0.000*	0.509	0.000*	0.170	0.005*	0.135
Visit 2	Value	154.2	0.0	43.2	-0.5	17.6	-0.6
	Δ with WVBT	2.5	0.1	1.0	-0.1	0.0	-0.1
	p	0.000*	0.137	0.064	0.211	0.815	0.148
Visit 3	Value	156.7	0.0	44.1	-0.5	17.5	-0.7
	Δ in Follow-up	2.2	0.1	2.1	0.1	0.4	0.1
	p	0.000*	0.145	0.000*	0.369	0.041*	0.503
Visit 4	Value	158.9	0.1	46.2	-0.5	17.9	-0.7

Note: Δ, change; SDS, standard deviation score; BMI, body mass index.

Table H.2. Anthropometric parameters for the paediatric MRCD cohort at each visit with changes between visits and associated p values from linear mixed models analysis. *p<0.05.

		Height (cm)	Height SDS	Weight (kg)	Weight SDS	BMI (kg/m^2)	BMI SDS
Visit 1	Value	134.7	-2.1	29.4	-2.3	16.0	-1.4
	Δ in Observation	1.8	0.1	0.8	0.1	0.0	0.0
	p	0.017*	0.135	0.183	0.625	0.954	0.997
Visit 2	Value	136.4	-1.9	30.2	-2.2	16.0	-1.4
	Δ with WBVT	1.8	-0.1	1.3	-0.1	0.3	0.0
	p	0.017*	0.459	0.045*	0.701	0.366	0.907
Visit 3	Value	138.2	-2.0	31.5	-2.3	16.3	-1.4
	Δ in Follow-up	1.6	-0.1	0.8	-0.2	0.1	-0.2
	p	0.030*	0.283	0.190	0.160	0.730	0.421
Visit 4	Value	139.8	-2.1	32.3	-2.5	16.4	-1.56

Note: Δ, change; SDS, standard deviation score; BMI, body mass index.

Table H.3. Anthropometric parameters for the adult MRCD cohort at each visit with changes between visits and associated p values from linear mixed models analysis. *p<0.05.

		Height (cm)	Weight (kg)	BMI (kg/m^2)
Visit 1	Value	166.8	64.3	23.2
	Δ in Observation	0.2	0.6	0.2
	p	0.220	0.289	0.429
Visit 2	Value	166.9	64.9	23.4
	Δ with WBVT	-0.1	-0.2	-0.1
	p	0.300	0.684	0.651
Visit 3	Value	166.8	64.7	23.3
	Δ in Follow-up	0.1	-0.6	-0.3
	p	0.364	0.349	0.181
Visit 4	Value	166.9	64.1	23.0

Note: Δ, change; BMI, body mass index.

Table H.4. Anthropometric parameters for the Leigh Syndrome MRCD cohort at each visit with changes between visits and associated p values from linear mixed models analysis. *p<0.05.

		Height (cm)	Weight (kg)	BMI (kg/m^2)
Visit 1	Value	144.8	47.6	20.2
	Δ in Observation	1.6	0.6	-0.1
	p	0.094	0.340	0.680
Visit 2	Value	146.4	48.3	20.1
	Δ with WBVT	1.5	1.7	0.4
	p	0.117	0.019*	0.218
Visit 3	Value	148.0	50.0	20.5
	Δ in Follow-up	1.6	2.3	0.6
	p	0.101	0.004*	0.090
Visit 4	Value	149.6	52.3	21.1

Note: Δ, change; BMI, body mass index.

Appendix I: Participant Information Sheets for the CF Cohort

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CHILD INFORMATION SHEET (Outpatient Study)

Vibration Training in Children with Cystic Fibrosis: Function, Power, Bone

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We would like you to consider participating in a study that will be conducted in the Department of Endocrinology. This is an information sheet to help you decide whether you want to take part in a research study about vibration training in children with cystic fibrosis.

Who is doing the study?
This study is being done by doctors and physiotherapists from the Respiratory and Endocrine Departments at The Children's Hospital at Westmead.

What is the project about?
Children with cystic fibrosis often have muscles and bones that are not as strong as children without cystic fibrosis. It is important to keep muscles and bones strong so as to keep breathing as strong as possible and do every day activities. Some children with cystic fibrosis can also have high blood sugar levels which can lead to diabetes. In people without CF stronger muscles have been shown to improve the control of blood sugar levels. We are trying to find out if standing on a vibration platform can make muscles work better, help bones become stronger, improve breathing and help control blood sugar levels.

What will I have to do if I take part?

While in hospital for a 'tune up' or clinic appointment you will be asked if you want to be involved in this study. If you agree to be involved, you will have to stand on a vibration platform every day for 12 minutes for six months. One way to see if a new treatment like vibration training is useful is to look at what changes happen to your muscles, breathing and bones when you are not getting the vibration and compare them to what happens after you have had the vibration. To see how long any changes that occurred while on vibration last, we will also do some tests after you have been off vibration for 6 months. During the study you will continue to receive the normal treatment and have the normal tests that are done for CF patients.

To see if the vibration training is useful, you will be asked to do a number of simple tests. Most of these tests will be done at the start of the study and then you will need to return to the hospital every 3 months during the study. The blood tests and bone tests will be done every 6 months.

Muscle tests: You will jump, hop and get up from and sit on a chair while on a special machine called a force plate. This will look at how the muscles in you legs work. You will do these tests at each visit.

Breathing tests: As well as doing the normal breathing tests you are use to doing, you will also be asked to do other ones. These tests are not painful. You will do these tests at each visit.

Exercise Tests: You often do exercise tests as part of a tune up or clinic visit. You will do two formal exercise tests. The first one gets you to run on a treadmill which gradually increases in speed and slope every 3 minutes. The aim of the test is not to exhaust you but to make measurements when your heart beat has increased to a rate that indicates you are exercising well. The duration of the actual test is approximately 10 minutes, with setting up taking an additional 10 minutes. During the test we will measure how hard your heart is working using an ultrasound. This does not hurt. We will put some gel on your neck and use a small piece of equipment to look at the heart. The other exercise test will get you to cycle on a bike as hard as you can for 30s against a resistance. This test is hard work but doesn't last for long. You will do the tests with a mask on your face. You should wear sports shoes for these tests. You will do these tests at each visit.

Bone Tests: Some tests will be done to look at how strong your bones and muscles are. These tests are called Peripheral Quantitative Computer Tomography (pQCT) and duel energy x-ray absorptiometry (DXA). Both tests are painless and require you to remain still for short periods of time. You may have had these tests before. You will do these tests every 6 months, at the beginning of the study, before you start the vibration training, at the end of training and at the end of the study.

Blood Tests: You will have blood tests every 6 months - at the start of the study, before you start vibration, at the end of vibration and 6 months after the vibration is finished. One of the blood tests, called an oral glucose tolerance test will only be done twice (before you start vibration and at the end of vibration). This test looks at what happens to your blood sugar level when you have a very sweet drink called Lucozade™. The test will be done on Turner ward first thing in the morning before you have any breakfast. A special needle will be put in your arm so blood can be taken from you every 30 minutes for 2 hours. This test is done by nurses and doctors in the hospital who have lots of experience in doing them. At the same time, we will also take some blood to look at special markers that tell us if your bones are getting stronger. Approximately 15mls (3 teaspoons) of blood will be drawn at each visit.

Urine Tests: You will be asked to give us a urine sample so we can look at different markers that tell us if your bones are getting stronger. You will do these tests at the beginning of the study, before you start the vibration training, at the end of training and at the end of the study.

Questionnaires: At each visit you will be asked to complete three questionnaires. The first is the **Cystic Fibrosis Clinical Score** which assesses 5 symptoms (level of energy, production of sputum, cough, shortness of breath and appetite) as well as 5 physical findings (temperature, weight, degree of air entry, lung crackles and respiratory rate). This takes less than five minutes to complete. The second questionnaire is the **Cystic Fibrosis Questionnaire**. It is designed to measure quality of life, and has different versions, depending on how old you are. If you are over 13 years of age you can complete it yourself, but if you are younger, one of your family members will complete one too. This is a longer questionnaire, taking up to 20 minutes to complete. The third is a dietary questionnaire to look at your intake of some nutrients from food and supplements. This will be completed every 6 weeks, at each visit to the hospital and between visits over the phone. It will take about 10 minutes to complete. After you have finished the vibration training you will also be asked to fill in a short questionnaire on how you found the vibration training. This will take about 5 minutes.

Throughout the study period a physiotherapist will talk to you about the vibration training and you can ask any questions you need.

Do I have to take part in the research?
No, you don't. If you don't want to take part, that is okay. It's up to you. Even if you choose to take part at the beginning, you can change your mind and choose not to take part later on. All you need to do is tell the researcher that you don't want to take part in the research any more. If you choose not to be in the study you will get the normal treatment like all the other children.

Are there any benefits of participating in the study?
There are no benefits to participating in this study that we can be sure of, however we hope that the results of this will determine if vibration training improves the muscle and lung function of children with cystic fibrosis.

Are there any side-effects and risk associated with this study?
The risks of this study are minimal. During the study you will be exposed to a very small amount of radiation. As part of everyday living, everyone is exposed to naturally occurring background radiation. This is a dose of about **2 to 3 millsieverts (mSv)** each year. The effective dose from this study is about **0.012 mSv**. At this dose level, no harmful effects of radiation have been found so the risk to you is very small. Although the strength of the vibration will be gradually increased, you may find the feeling uncomfortable. At all times while on the vibration platform you will be supervised by a physiotherapist or parent and the vibration will be stopped if you ask. When the vibration finishes, some people have a tingling feeling in their legs and feet. While not painful, you may not like this feeling. The blood tests may cause some local pain and bruising, but we will apply some numbing cream to the area. Very occasionally the Lucozade™ drink can make you feel a little unwell in the stomach. This only lasts a short time and settles by itself.

Will I be given something for taking part?
At the end of the study you will be given a certificate of participation to show our appreciation.

If you have any questions about the study or you want to talk about it, please contact us.

This project has been approved by The Children's Hospital at Westmead Ethics Committee. If you have any concerns about the conduct of this study, please do not hesitate to contact the Research Ethics Manager, Secretary of the Human Research Ethics Committee (02 9845 3017).

This Information Sheet is for you to keep.

the children's hospital at Westmead

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PARENT INFORMATION SHEET (Outpatient Study)

Vibration Training in Children with Cystic Fibrosis: Function, Power, Bone

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We would like you to consider participating in a study that will be conducted in the Department of Endocrinology. This is an information sheet to help you decide whether you want your child to take part in a research study about vibration training in children with cystic fibrosis.

Who is doing the study?
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Children with cystic fibrosis often have muscles and bones that are not as strong as children without cystic fibrosis. It is important to keep muscles and bones strong so as to keep breathing as strong as possible and do every day activities. Some children with cystic fibrosis can also have high blood sugar levels which can lead to diabetes. In people without CF stronger muscles have been shown to improve the control of blood sugar levels. We are trying to find out if standing on a vibration platform can make muscles work better, help bones become stronger, improve breathing and help control blood sugar levels.

Who can participate in the study?
Children with cystic fibrosis who are admitted to The Children's Hospital at Westmead for a chest 'tune up' or visiting for a clinic appointment will be asked if they want to be involved in the study.

What will the study involve?
While in hospital for a 'tune up' or clinic appointment you will be asked if you want your child to be involved in this study. If you agree for your child to be involved, your child will be asked to stand on a vibration platform daily for 12 minutes for a period of six months. The speed of the vibration will be gradually increased over the first few days. One way to see if a new treatment like vibration training is useful is to look at what changes happen to your child's the muscles, breathing and bones when not getting the vibration and compare them to what happens after having had the vibration. To see how long any changes that occurred while on vibration last, we will also do some tests after they have been off vibration for 6 months. In this study, your child will be observed for the first six months of the study, will receive vibration training for the second six months and be observed again for the final six months of the study. Your child will continue to receive the normal treatment and have the normal tests that are done for the management of CF as an inpatient or outpatient.

To see if the vibration training is useful, your child will be asked to do a number of simple tests. Most of these tests will be done before the start of the study and you will need to return to the hospital every 3 months for the duration of the study period (18 months) for testing. The blood tests and bone tests will be done every 6 months.

Muscle tests: Your child will jump, hop and get up from and sit on a chair while on a special machine called a force plate. This will look at the function or the leg muscles. This test will be done on all visits.

Breathing tests: As well as doing the normal breathing tests your child is use to doing such as spirometry and lung volumes, more detailed tests will be done. They involve normal breathing through a mouthpiece and are easy to perform in children of all ages. Multiple breath washout involves breathing different mixtures of air to see how well the body can take up the air breathed in. This test is not painful and should cause no discomfort. These tests will be done at all visits.

Exercise tests: Exercise testing is one of the normal assessments done as an inpatient and outpatient. Your child will do two types of formal exercise testing. The first test involves your child running on a treadmill which gradually increases in speed and slope every 3 minutes. The aim of the test is not to exhaust your child but to make measurements when your child's heart beat has increased to a rate that indicates they are exercising adequately. The duration of the actual test is approximately 10 minutes, with setting up taking an additional 10 minutes. During the test we will measure the amount of blood your child's heart is pumping using ultrasound. This requires a small piece of equipment and some gel to be placed at the top of the sternum (breastbone). The second test involves your child cycling on a bike as fast as they can for 30 seconds against a resistance. This test is hard work but does not last long. During the tests your child will wear a mask to analyse the amount of oxygen your child uses and the concentration of gases in the air they breathe out. Your child should wear sports shoes for this test. Exercise testing will be performed on all visits.

Bone Tests: Two tests will be done to look at the strength of your child's bones and the amount and quality of your child's muscle. These teats are called Peripheral Quantitative Computer Tomography (pQCT) and duel energy x-ray absorptiometry (DXA). Both tests are painless and require your child to remain still for short periods of time. Your child may have had these tests as part of usual clinical care. These tests will be done every 6 months, at the start of the study, before the start of vibration training, after 6 months of training and at the end of the study.

Blood Tests: Your child will have blood tests every 6 months - at the start of the study, before you start vibration, at the end of vibration and 6 months after the vibration is finished. **Insulin sensitivity** will be measured by an Oral Glucose Tolerance Test (OGTT) and test will only be done twice (before the start and at the end of vibration). This will involve bringing your child to Turner Day Stay ward at The Children's Hospital at Westmead at 8am after an overnight fast. After height and weight an IV cannula will be placed in one arm. Local anaesthetic cream will be used so that discomfort will be minimised. Your child will be asked to drink a glass Lucozade™ (a sugary drink). The cannula will be used to take blood at the beginning of the study and every 30 minutes for 2 hours. During the test your child can watch a video, listen to music, read a book etc. At the end of the test your child will be given lunch on the ward. This test will conducted by a doctor and an experienced nurse. At the same time blood is taken for the glucose test some blood will be taken to look at bone markers that show if your child's bones are getting stronger. Approximately 15mls (3 teaspoons) of blood will be drawn at each visit.

Urine Tests: A urine test will be collected from your child at the start of the study, before the start of vibration training, after 6 months of training and at the end of the study. The urine sample will give more information about whether your child's bones are getting stronger.

Questionnaires: At each visit your child will be asked to complete three questionnaires. The first is the **Cystic Fibrosis Clinical Score** which assesses 5 symptoms (level of energy, production of sputum, cough, shortness of breath and appetite) as well as 5 physical findings (temperature, weight, degree of air entry, lung crackles and respiratory rate). This takes less than five minutes to complete. The second questionnaire is the **Cystic Fibrosis Questionnaire**. This questionnaire is designed to measure quality of life, and has different versions, depending on the age of your child. If your child is over 13 years of age they can complete it themselves, but if they are younger, then you will be needed to complete a version of it in addition to us completing a child specific one with your child. This is a longer questionnaire, taking up to 20 minutes to complete. The third is a dietary questionnaire to look at your child's intake of some nutrients from food and supplements. This will be completed every 6 weeks, at each visit and between visits over the phone. It will take about 10 minutes to complete. After completing the vibration training your child will also be asked to fill in a short questionnaire on how he/she found the vibration training. This will take about 5 minutes.

Throughout the study period a physiotherapist will be in regular phone contact with you and your child.

Does my child have to take part in the research?
No, your child does not. If you don't want your child to take part, that is okay. It's up to you. Even if you choose for your child to take part at the beginning, you can change your mind and choose withdraw your child later on. All you need to do is tell the researcher that you don't want to take part in the research any more. If you choose not to be in the study, your child will get normal treatment like all the other children.

Are there any benefits for my child participating in the study?
There are no benefits to participating in this study that we can be sure of, however we hope that the results of this will determine if vibration training improves the muscle, bone and respiratory function of children with cystic fibrosis and helps prevent the development of diabetes in CF.

Are there any side-effects and risk associated with this study?
The risks of this study are minimal. This research study involves exposure to a very small amount of radiation. As part of everyday living, everyone is exposed to naturally occurring background radiation and receives a dose of about **2 to 3 millsieverts (mSv)** each year. The effective dose from this study is about **0.012 mSv**. At this dose level, no harmful effects of radiation have been demonstrated the risk is negligible. Although the strength of the vibration will be gradually increased, some children may find the feeling uncomfortable. Your child's first sessions on the vibration platform will be supervised by a physiotherapist and the vibration will be stopped if they find it uncomfortable. When the vibration finishes, some people have

a tingling feeling in their legs and feet. While not painful, some children may not like this feeling. The blood tests may cause some local pain and bruising, but we will apply some numbing cream to the area. Very occasionally the Lucozade™ drink can make the child feel a little unwell in the stomach. This only lasts a short time and settles by itself.

Other Information

The privacy of your child is of utmost importance to us. When enrolled in the study, your child will receive a study number. This is the way they will be identified through out the study. At no time will information that would identify your child be made available to people outside they study team or be written any paper that might result from this study. All data collected as part of the study will be kept in a secure data base.

If you have any questions about the study or you want to talk about it, please contact us. You can reach us on the phone numbers at the top of this information sheet.

This project has been approved by The Children's Hospital at Westmead Ethics Committee. If you have any concerns about the conduct of this study, please do not hesitate to contact the Research Ethics Manager, Secretary of the Human Research Ethics Committee (02 9845 3017).

This Information Sheet is for you to keep.

Appendix J:

Participant Information Sheets for the MRCD Cohort

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PARENT INFORMATION SHEET

Whole Body Vibration Training for Mitochondrial Respiratory Chain Disorders

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We would like you to consider participating in a study that will be conducted by the Genetic Metabolic Disorders Service, Department of Endocrinology, the Department of Surgery, the Department of Nuclear Medicine and Neuromuscular Research Institute. This is an information sheet to help you decide whether you want your child to take part in a research study about vibration training in children with mitochondrial respiratory chain disorders.

Who is doing the study?

This study is being done by staff from the Genetic Metabolic Disorders Service, Department of Endocrinology, the Department of Surgery, the Department of Nuclear Medicine and Neuromuscular Research Institute at The Children's Hospital at Westmead.

What is the project about?

Children with mitochondrial respiratory chain disorders often have muscles that are not as strong as children without mitochondrial respiratory chain disorders. It is important to keep muscles as strong as possible to permit every day activities. In addition, because of reduced muscle activity they are at risk of reduced bone density and strength. We are trying to find out if standing on a vibration platform can make muscles work better and strengthen bones. The best way to see if a new treatment like vibration training is useful, is to look at what happens to children who receive the treatment for six months (treatment period) and compare

our findings with the same tests performed on the same child over a three month period before the vibration therapy was started (baseline period). It is also important to see how long any changes that occurred during the treatment period last. To do this we will also compare our findings from the treatment period with the same tests performed on the same child over a six month period after the vibration training has finished (follow-up period).

Who can participate in the study?
All children with mitochondrial respiratory chain disorders in whom reduced muscle strength is a major component, and who are patients of The Children's Hospital at Westmead, will be asked if they want to be involved in the study.

What will the study involve?
You will be asked if you want your child to be involved in this study. If you agree for your child to be involved, your child will receive their usual treatment and will be monitored for a period of three months. They will then participate in the vibration training, which consists of standing on the platform for 9 minutes per day. The speed of the vibration will be gradually increased each day.

To see if the vibration training is useful all children will be asked to do a number of simple tests during the baseline period (at the beginning of the monitoring period and after 3 months) and after three and six months of the vibration therapy.

Blood and urine tests: Blood and urine samples will be collected at baseline, before the start of vibration, at the end of vibration and 6 months after the vibration is finished. Approximately 10-15mls (2-3 teaspoons) of blood will be drawn at each visit. These samples will be used to measure the effectiveness of the treatment on measures of bone turnover.

Muscle tests: Your child will jump and get up from and sit on a chair while on a special machine called a force plate. This will look at the function of the leg muscles. We will also get your child to squeeze a handheld gauge as hard as they can. This will look at the strength of the forearm muscles. We will also take accurate measures of height, weight, arm circumference and skin fold thickness. The muscle tests will be performed at 3 monthly intervals for the duration of the study.

Exercise tests: your child will be asked to do two different exercise tests. The first is the 6-minute walk test. In this test your child will be asked to walk as far as they can on a walking circuit for a period of 6 minutes. This test will give us an idea of how well your child participates in their daily activities. The second test is a formal exercise test. It involves walking on a treadmill which gradually increases in speed and slope every 3 minutes or cycling on a bike that gets harder every minute. The test is performed using a mask to analyse the amount of oxygen your child uses and the concentration of gases in the air they breathe out. The aim of the test is not to exhaust your child but to make measurements when your child's heart beat has increased to a rate that indicates they are exercising adequately. During the test we will measure the amount of blood your child's heart is pumping using ultrasound. This requires a small piece of equipment and some gel to be placed at the top of the sternum (breastbone). The duration of the actual test is approximately 10 minutes, with setting up taking an additional 10 minutes. Your child should wear sports shoes for this test. This test will tell us how well your child's muscles use energy during exercise. Exercise testing will be performed at 3 monthly intervals for the duration of the study.

Bone Tests: Two tests will be done to look at the strength of your child's bones and the amount and quality of your child's muscle. These teats are called Peripheral Quantitative Computer Tomography (pQCT) and duel energy x-ray absorptiometry (DXA). Both tests are painless and require your child to remain still for short periods of time. Your child may have had these tests as part of usual clinical care. These tests will be at the start of the study, before the start of vibration training, after 6 months of training and at the end of the six month follow up.

Questionnaires: We will ask you to complete some questionnaires when the DXA measurements are taken to look at your child's intake from food and supplements of some nutrients. We do not want you to change the diet during the study period. At the end of the 6 months you or your child will also be asked to fill in a short questionnaire on how he/she found the vibration training.

We will give you and your child a book to note when the vibration training is done and any problems that occur.

Does my child have to take part in the research?
No, your child does not. If you don't want your child to take part, that is okay. It's up to you.
Even if you choose for your child to take part at the beginning, you can change your mind and choose to withdraw your child later on. All you need to do is tell the researcher that you don't want to take part in the research any more. If you choose not to be in the study, your child will continue to receive the usual treatment as all the other children.

Are there any benefits for my child participating in the study?
There are no benefits to participating in this study that we can be sure of, however we hope that the results will determine if vibration training improves the muscle function of children with mitochondrial respiratory chain disorders.

Are there any side-effects and risk associated with this study?
The risks of this study are minimal. Although the strength of the vibration will be gradually increased, some children may find the feeling uncomfortable. We will ask that while your child is on the vibration platform you will supervise the vibration therapy after you have been instructed by a physiotherapist, and will be able to stop the vibration if they find it uncomfortable. When the vibration finishes, some people have a tingling feeling in their legs and feet. While not painful, some children may not like this feeling.

This research study involves exposure to a very small amount of radiation due to having scans for bone strength. As part of everyday living, everyone is exposed to naturally occurring background radiation and receives a dose of about **2 to 3 millisieverts** each year (**0.008 millisieverts** per day). Four DXA and three pQCT scans will be done over the course of this study, and the effective dose from this study is **0.011 millisieverts**.

Apart from the pain at the time that the blood is taken, a small bruise may develop at the site of blood collection.

Measuring skin fold thickness involves pinching up the skin at a few different sites of the body. It can be a little uncomfortable but is for a very short time.

Other Information
The privacy of your child is of utmost importance to us. When enrolled in the study, your child will receive a study number. This is the way they will be identified throughout the study. At no time will information that would identify your child be made available to people outside the study team or be written in any paper that might result from this study. All data collected as part of the study will be kept in a secure database.

If you have any questions about the study or you want to talk about it, please contact us. You can reach us on the phone numbers at the top of this information sheet.

This project has been approved by The Children's Hospital at Westmead Ethics Committee. If you have any concerns about the conduct of this study, please do not hesitate to contact the Research Ethics Manager, Secretary of the Human Research Ethics Committee (02 9845 3017).

This Information Sheet is for you to keep.

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PATIENT INFORMATION SHEET

Whole Body Vibration Training for Mitochondrial Respiratory Chain Disorders

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We would like you to consider participating in a study that will be conducted by the Genetic Metabolic Disorders Service, Department of Endocrinology, the Department of Surgery, the Department of Nuclear Medicine and Neuromuscular Research Institute. This is an information sheet to help you decide whether you want to take part in a research study about vibration training in children with mitochondrial respiratory chain disorders.

Who is doing the study?
This study is being done by staff from the Genetic Metabolic Disorders Service, Department of Endocrinology, the Department of Surgery, the Department of Nuclear Medicine and Neuromuscular Research Institute at The Children's Hospital at Westmead.

What is the project about?
Children with mitochondrial respiratory chain disorders often have muscles that are not as strong as children without mitochondrial respiratory chain disorders. It is important to keep muscles as strong as possible to permit every day activities. In addition, because of reduced muscle activity they are at risk of reduced bone density and strength. We are trying to find out if standing on a vibration platform can make muscles work better and strengthen bones. The best way to see if a new treatment like vibration training is useful, is to look at what happens to children who receive the

treatment for six months (treatment period) and compare our findings with the same tests performed on the same child over a three month period before the vibration therapy was started (baseline period). To see how long any changes that occurred while on vibration last, we will also do some tests after you have been off vibration for 6 months (follow-up period).

What will I have to do if I take part?
You will be asked if you want to be involved in this study. If you agree to be involved, you will receive your usual treatment and will be monitored for a period of three months. You will then participate in the vibration training, which consists of standing on the platform for 9 minutes per day. The speed of the vibration will be gradually increased each day.

To see if the vibration training is useful you will be asked to do a number of simple tests during the baseline period (at the beginning of the monitoring period and after 3 months) and after three and six months of the vibration therapy.

Blood and urine tests: Blood and urine samples will be collected at baseline, before the start of vibration, at the end of vibration and 6 months after the vibration is finished. Approximately 10-15mls (2-3 teaspoons) of blood will be drawn at each visit. These samples will be used to measure the effectiveness of the treatment on measures of bone turnover.

Muscle tests: You will jump and get up from and sit on a chair while on a special machine called a force plate. This will look at the function of the leg muscles. We will also get you to squeeze a handheld gauge as hard as you can. This will look at the strength of the muscles in your arm. We will also take accurate measures of height, weight, arm circumference and skin fold thickness. The muscle tests will be done every 3 months during the study.

Exercise tests: You will be asked to do two different exercise tests. The first is the 6-minute walk test. In this test you have to walk as far as you can in 6 minutes. This test will give us an idea of how easy it is for you to do your daily activities. The second test is a formal exercise test. It gets you to walk on a treadmill which gradually increases in speed and slope every 3 minutes or to cycle on a bike that gets harder every minute. You will do the test with a mask on your face. The aim of the test is not to exhaust you but to make measurements when your heart beat has increased to a rate that indicates you are exercising well. During the test we will measure how hard your heart is working using an ultrasound. This does not hurt. We will put some gel on your neck and use a small piece of equipment to look at the heart. The duration of the actual test is approximately 10 minutes, with setting up taking an additional 10 minutes. You should wear sports shoes for this test. This test will tell us how your muscles use energy during exercise. We will do these tests every 3 months during the study

Bone Tests: Some tests will be done to look at how strong your bones and muscles are. These tests are called Peripheral Quantitative Computer Tomography (pQCT) and dual energy x-ray absorptiometry (DXA). Both tests are painless and require you to remain still for short periods of time. You will do these tests at the beginning of the study, before you start the vibration training, after 6 months of training and at the end of the six month follow up.

Questionnaires: We will ask you to complete some questionnaires when the DXA measurements are taken to look at your intake from food and supplements of some nutrients. We do not want you to change the diet during the study period. At the end of the 6 months you will also be asked to fill in a short questionnaire on how you found the vibration training.

We will give you a book to note when the vibration training is done and any problems that occur.

Do I have to take part in the research?
No, you don't. If you don't want to take part, that is okay. It's up to you.
Even if you choose to take part at the beginning, you can change your mind and choose not to take part later on. All you need to do is tell the researcher that you don't want to take part in the research any more. If you choose not to be in the study you will get the normal 'tune up' treatment as all the other children.

Are there any benefits of participating in the study?
There are no benefits to participating in this study that we can be sure of, however we hope that the results of this will determine if vibration training improves the muscle function of children with mitochondrial respiratory chain disorders.

Are there any side-effects and risk associated with this study?
The risks of this study are minimal. Although the strength of the vibration will be gradually increased, you may find the feeling uncomfortable. At all times while on the vibration platform you will be supervised by a physiotherapist or parent and the vibration will be stopped if you ask. When the vibration finishes, some people have a tingling feeling in their legs and feet. While not painful, you may not like this feeling.

This research study involves exposure to a very small amount of radiation due to having scans for bone strength. As part of everyday living, everyone is exposed to naturally occurring background radiation and receives a dose of about **2 to 3 millisieverts** each year (**0.008 millisieverts** per day). Four DXA and three pQCT scans will be done over the course of this study, and the effective dose from this study is **0.011 millisieverts**.

Apart from the pain at the time that the blood is taken, a small bruise may develop at the site of blood collection.

Measuring skin fold thickness involves pinching up the skin at a few different sites of the body. It can be a little uncomfortable but is for a very short time.

Will I be given something for taking part?
At the end of the study you will be given a certificate of participation to show our appreciation.

If you have any questions about the study or you want to talk about it, please contact us.

This project has been approved by The Children's Hospital at Westmead Ethics Committee. If you have any concerns about the conduct of this study, please do not hesitate to contact the Research Ethics Manager, Secretary of the Human Research Ethics Committee (02 9845 3017).

This Information Sheet is for you to keep.

the children's hospital at Westmead

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ADULT INFORMATION SHEET

Whole Body Vibration Training for Mitochondrial Respiratory Chain Disorders

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We would like you to consider participating in a study that will be conducted by the Genetic Metabolic Disorders Service, Department of Endocrinology, the Department of Surgery, the Department of Nuclear Medicine and Neuromuscular Research Institute. This is an information sheet to help you decide whether you want to take part in a research study about vibration training in individuals with mitochondrial respiratory chain disorders.

Who is doing the study?
This study is being done by staff from the Genetic Metabolic Disorders Service, Department of Endocrinology, the Department of Surgery, the Department of Nuclear Medicine and Neuromuscular Research Institute at The Children's Hospital at Westmead, and the Department of Neurology and the Department of Respiratory Medicine at Royal North Shore Hospital.

What is the project about?
Individuals with mitochondrial respiratory chain disorders often have muscles that are not as strong as individuals without mitochondrial respiratory chain disorders. It is important to keep muscles as strong as possible to permit every day activities. In addition, because of reduced muscle activity you are at risk of reduced bone density and strength. We are trying to find out if standing on a vibration platform can make muscles work better and strengthen bones. The best way to see if a new treatment like vibration training is useful, is to look at what happens to those who receive the treatment for six months (treatment period) and compare our findings with the same tests performed on the same person over a three month period before the vibration therapy was started (baseline period). It is also important to see how long any changes that occurred during the treatment period last. To do this we will also compare our findings from the treatment period with the same tests performed on the same person over a six month period after the vibration training has finished (follow-up period).

Who can participate in the study?
All individuals with mitochondrial respiratory chain disorders in whom reduced muscle strength is a major component, and who are seen by the Western Sydney Genetics Program, will be asked if they want to be involved in the study.

What will the study involve?
You will be asked if you want to be involved in this study. If you agree to be involved, you will receive your usual treatment and will be monitored for a period of three months. You will then participate in the vibration training, which consists of standing on the platform for 9 minutes per day. The speed of the vibration will be gradually increased each day.

To see if the vibration training is useful you will be asked to do a number of simple tests during the baseline period (at the beginning of the monitoring period and after 3 months), after three and six months of the vibration therapy and three and six months after the vibration therapy has finished. All of these tests will occur at the Children's Hospital Westmead under the Genetic Metabolic Disorders Service except the formal exercise test which will be conducted at the exercise laboratory at Royal North Shore Hospital.

Blood and urine tests: Blood and urine samples will be collected at baseline, before the start of vibration, at the end of vibration and 6 months after the vibration is finished. Approximately 10-15mls (2-3 teaspoons) of blood will be drawn at each visit. These samples will be used to measure the effectiveness of the treatment on measures of bone turnover.

Muscle tests: You will jump and get up from and sit on a chair while on a special machine called a force plate. This will look at the function of the leg muscles. We will also get you to squeeze a handheld gauge as hard as you can. This will look at the strength of the muscles in your forearm.

We will also take accurate measures of height, weight, arm circumference and skin fold thickness. The muscle tests will be performed at 3 monthly intervals for the duration of the study.

Exercise tests: you will be asked to do two different exercise tests. The first is the 6-minute walk test. In this test you will be asked to walk as far as they can on a walking circuit for a period of 6 minutes. This test will give us an idea of how well you participate in your daily activities. The second test is a formal exercise test. It involves walking on a treadmill which gradually increases in speed and slope every 3 minutes or cycling on a bike that gets harder every minute. The test is performed using a mask to analyse the amount of oxygen you uses and the concentration of gases in the air you breathe out. The aim of the test is not to exhaust you but to make measurements when your heart beat has increased to a rate that indicates you are exercising adequately. During the test we will measure the amount of blood your heart is pumping using ultrasound. This requires a small piece of equipment and some gel to be placed at the top of the sternum (breastbone). The duration of the actual test is approximately 10 minutes, with setting up taking an additional 10 minutes. You should wear sports shoes for this test. This test will tell us how well your muscles use energy during exercise.These exercise tests will be conducted at the exercise laboratory at Royal North Shore Hospital. Exercise testing will be performed at 3 monthly intervals for the duration of the study.

Bone Tests: Two tests will be done to look at the strength of your bones and the amount and quality of your muscle. These teats are called Peripheral Quantitative Computer Tomography (pQCT) and duel energy x-ray absorptiometry (DXA). Both tests are painless and require you to remain still for short periods of time. You may have had these tests as part of usual clinical care. These tests will be at the start of the study, before the start of vibration training, after 6 months of training and at the end of the six month follow up.

Questionnaires: We will ask you to complete some questionnaires when the bone measurements are taken to look at you intake from food and supplements of some nutrients. We do not want you to change the diet during the study period. At the end of the 6 months you will also be asked to fill in a short questionnaire on how you found the vibration training.
We will give you a book to note when the vibration training is done and any problems that occur.

Do I have to take part in the research?
No, you do not. If you don't want to take part, that is okay. It's up to you.
Even if you choose to take part at the beginning, you can change your mind and choose to withdraw later on. All you need to do is tell the researcher that you don't want to take part in the research any more. If you choose not to be in the study, you will continue to receive the usual treatment.

Are there any benefits for me participating in the study?
There are no benefits to participating in this study that we can be sure of, however we hope that the results of this will determine if vibration training improves the muscle function of individuals with mitochondrial respiratory chain disorders.

Are there any side-effects and risk associated with this study?
The risks of this study are minimal. Although the strength of the vibration will be gradually increased, some people may find the feeling uncomfortable. You will be able to stop at any time if you feel it is too uncomfortable. We will ask that while on the vibration platform you are supervised by a friend or carer who has been instructed by a physiotherapist in the use of the vibration platform. When the vibration finishes, some people have a tingling feeling in their legs and feet. While not painful, some people may not like this feeling.

This research study involves exposure to a very small amount of radiation due to having scans for bone strength. As part of everyday living, everyone is exposed to naturally occurring background radiation and receives a dose of about **2 to 3 millisieverts** each year (**0.008 millisieverts** per day). Four DXA

and three pQCT scans will be done over the course of this study, and the effective dose from this study is **0.011 millisieverts**.

Apart from the pain at the time that the blood is taken, a small bruise may develop at the site of blood collection.

Measuring skin fold thickness involves pinching up the skin at a few different sites of the body. It can be a little uncomfortable but is for a very short time.

Other Information
Your privacy is of utmost importance to us. When enrolled in the study, you will receive a study number. This is the way you will be identified through out the study. At no time will information that would identify you be made available to people outside they study team or be written any paper that might result from this study. All data collected as part of the study will be kept in a secure data base.

If you have any questions about the study or you want to talk about it, please contact us. You can reach us on the phone numbers at the top of this information sheet.

This project has been approved by The Children's Hospital at Westmead Ethics Committee. If you have any concerns about the conduct of this study, please do not hesitate to contact the Research Ethics Manager, Secretary of the Human Research Ethics Committee (02 9845 3017).

This Information Sheet is for you to keep.

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