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The Muscle-Bone Unit in Children and Adolescents With and Without Cystic Fibrosis

Amy Riddell

MRC Human Nutrition Research

&

St Catharine’s College

University Of Cambridge

2016

Dissertation submitted for the degree of Doctor of Philosophy
Abstract

University of Cambridge

School of the Biological Sciences

The Muscle-Bone Unit in Children and Adolescents with Cystic Fibrosis and Healthy Controls

By Amy Riddell

Introduction - Puberty is a crucial period for rapid changes in bone mineral, size, geometry, and microarchitecture. The mechanostat theory postulates that increased mechanical loading will affect bone phenotype and strength during development and in later life (Frost, 1996, Frost, 1987). Individuals with cystic fibrosis (CF) have an increased risk of developing osteoporosis and fragility fractures in young adulthood, which may be caused by poor growth. The aim was to investigate whether sex and disease status modified the relationship between: 1) puberty and bone, and 2) muscle and bone. This would contribute to the understanding of how sex (males vs. females) and disease group (CF vs. controls) alters the relationship between bone and muscle in children and adolescents as they transition through puberty and who, on a population level, differ in the prevalence of osteoporosis and risk of fracture in later life.

Methods and Analyses - This observational study used novel imaging and muscle assessment techniques to measure bone and muscle parameters in White Caucasian children and adolescents, aged 8 to 16 years, living in the UK, with children with CF (n=65) and controls (n=151). Anthropometry and pubertal status were assessed. Dual energy x-ray absorptiometry, peripheral quantitative computed tomography (pQCT), high-resolution pQCT, and jumping mechanography were used to measure bone and muscle outcomes. ANCOVA with Scheffé post hoc and multiple linear regression tests were performed. Data were adjusted according to the research aims and included covariates; sex, disease group, pubertal stage, age, quadratic age, height, weight, maximum force (Fmax), and maximum power (Pmax). Data are presented as beta-coefficient (%) and p-value, with the significance level set to p<0.05.

Results – In height adjusted analyses, among healthy participants, females had smaller bones and lower bone density compared to males. With pubertal maturation, females had lower apparent gains in the distal and proximal total area (Tt.Ar and CSA), distal cortical porosity (Ct.Po) and proximal bone strength (SSI) but higher apparent gains in distal and proximal cortical bone density (Ct.BMD, Ct.TMD, vBMD). Females had consistently lower distal total area (total CSA) and density (total vBMD), distal trabecular density (BV/TV) and number (Tb.N), and proximal cortical area (CSA) compared to males, across all stages of puberty. With increasing muscle force (Fmax), females had
higher apparent gains in total body less head bone mineral (TBLH BMC) and bone area (BA), distal total and trabecular density (total and trab vBMD) compared to males. In contrast, with increasing muscle power (Pmax), females had higher apparent gains in distal total and cortical densities (D100, Ct.BMD and Ct.TMD), and distal trabecular thickness (Tb.Th), and proximal cortical density (cortical vBMD) but lower apparent gains in distal cortical porosity (Ct.Po) and trabecular number (Tb.N) compared to males.

In height adjusted analyses, participants with CF had smaller bones and lower bone density compared to controls. With increasing pubertal maturation, participants with CF had lower apparent gains in total body less head bone mineral (TBLH BMC) and bone area (TBLH BA), and in distal trabecular density (BV/TV), cortical porosity (Ct.Po), and trabecular thickness (Tb.Th) compared to controls. Participants with CF had consistently lower distal total and cortical area (total CSA, Tt.Ar, and Ct.Ar), distal total and trabecular densities (total and trab vBMD and D100) and proximal bone strength (SSI) compared to controls, across all stages of puberty. With increasing muscle force (Fmax), participants with CF had lower apparent gains in total body less head bone mineral (TBLH BMC) and bone area (BA), distal total density (D100), trabecular density (BV/TV), and trabecular number (Tb.N). In contrast, with increasing muscle power (Pmax), participants with CF had higher apparent gains in distal trabecular density (BV/TV) and trabecular number (Tb.N) compared to controls.

**Conclusion** – These findings suggest that sex and disease status do modify the relationships between puberty and bone, and between muscle function and bone. Skeletal adaptation to muscle differs between sexes and in populations with chronic disease, which may explain sex and disease group differences in risks of osteoporosis and fracture. Bone adaptation to muscle in children with CF is altered, which may lead to narrow, under-mineralised bones, with lower bone strength in later life. Understanding better impairments in muscle functions may provide targets for intervention to improve skeletal health in later life.
Preface

This dissertation is the result of my own work and includes nothing which is the outcome of work done in collaboration except as declared in the Preface and specified in the text. It is not substantially the same as any that I have submitted, or, is being concurrently submitted for a degree or diploma or other qualification at the University of Cambridge or any other University or similar institution except as declared in the Preface and specified in the text. I further state that no substantial part of my dissertation has already been submitted, or, is being concurrently submitted for any such degree, diploma or other qualification at the University of Cambridge or any other University of similar institution except as declared in the Preface and specified in the text.

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**Date of submission:** June 2016

Amy Riddell

St Catharine’s College, University of Cambridge
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PhD study outputs


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### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviations</th>
<th>Meaning</th>
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<tbody>
<tr>
<td>Ach</td>
<td>Acetylcholine</td>
</tr>
<tr>
<td>AP</td>
<td>Action potential</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
</tr>
<tr>
<td>ADP</td>
<td>Adenosine diphosphate</td>
</tr>
<tr>
<td>aBMD</td>
<td>Areal bone mineral density</td>
</tr>
<tr>
<td>BA</td>
<td>Bone area</td>
</tr>
<tr>
<td>BMAD</td>
<td>Bone mineral apparent density</td>
</tr>
<tr>
<td>BMC</td>
<td>Bone mineral content</td>
</tr>
<tr>
<td>BMD</td>
<td>Bone mineral density</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>BMU(s)</td>
<td>Basic multicellular unit(s)</td>
</tr>
<tr>
<td>$\text{Ca}_{10}\left(\text{PO}_4\right)_6\left(\text{OH}\right)_2$</td>
<td>Hydroxyapatite</td>
</tr>
<tr>
<td>CF</td>
<td>Cystic fibrosis</td>
</tr>
<tr>
<td>CNS</td>
<td>Central nervous system</td>
</tr>
<tr>
<td>CSMA</td>
<td>Cross-sectional muscle area</td>
</tr>
<tr>
<td>CSMI</td>
<td>Cross-sectional moment of inertia</td>
</tr>
<tr>
<td>CT</td>
<td>Computed tomography</td>
</tr>
<tr>
<td>CTh</td>
<td>Cortical thickness</td>
</tr>
<tr>
<td>CV</td>
<td>Coefficient of variation</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>DPA</td>
<td>Dual photon absorptiometry</td>
</tr>
<tr>
<td>DXA</td>
<td>Dual energy X-ray absorptiometry</td>
</tr>
<tr>
<td>E</td>
<td>Young’s (or elastic) modulus</td>
</tr>
<tr>
<td>e-</td>
<td>Electron</td>
</tr>
<tr>
<td>FN</td>
<td>Femoral Neck</td>
</tr>
<tr>
<td>Fx</td>
<td>Fracture</td>
</tr>
<tr>
<td>GH</td>
<td>Growth hormone</td>
</tr>
<tr>
<td>GP</td>
<td>General practitioner</td>
</tr>
<tr>
<td>HR-pQCT</td>
<td>High resolution Peripheral quantitative computed tomography</td>
</tr>
<tr>
<td>HU</td>
<td>Hounsfield units</td>
</tr>
<tr>
<td>IGF</td>
<td>Insulin-like growth factor</td>
</tr>
<tr>
<td>ISCD</td>
<td>The International Society for Clinical Densitometry</td>
</tr>
<tr>
<td>LM</td>
<td>Lean mass</td>
</tr>
<tr>
<td>LMS</td>
<td>Lambda, mu, sigma</td>
</tr>
<tr>
<td>LS</td>
<td>Lumbar spine</td>
</tr>
<tr>
<td>MMPs</td>
<td>Matrix metalloproteinases</td>
</tr>
<tr>
<td>MES</td>
<td>Minimum effective strain</td>
</tr>
<tr>
<td>MESm</td>
<td>Minimum effective strain threshold which activates modelling drifts</td>
</tr>
<tr>
<td>MESp</td>
<td>Minimum effective strain threshold which activates microdamage repair</td>
</tr>
<tr>
<td>MESr</td>
<td>Minimum effective strain threshold which activates remodelling</td>
</tr>
<tr>
<td>mSv</td>
<td>MilliSieverts</td>
</tr>
<tr>
<td>PBM</td>
<td>Peak bone mass</td>
</tr>
<tr>
<td>PHV</td>
<td>Peak height velocity</td>
</tr>
<tr>
<td>pQCT</td>
<td>Peripheral quantitative computed tomography</td>
</tr>
<tr>
<td>PS(s)</td>
<td>Pubertal stage(s)</td>
</tr>
<tr>
<td>QCT</td>
<td>Quantitative computed tomography</td>
</tr>
<tr>
<td>ROI(s)</td>
<td>Region(s) of interest</td>
</tr>
<tr>
<td>SR</td>
<td>Sarcoplasmic reticulum</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
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</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SDS</td>
<td>Standard deviation score</td>
</tr>
<tr>
<td>SPA</td>
<td>Single photon absorptiometry</td>
</tr>
<tr>
<td>SE</td>
<td>Standard error</td>
</tr>
<tr>
<td>SSI</td>
<td>Strength strain index</td>
</tr>
<tr>
<td>sV</td>
<td>Sieverts</td>
</tr>
<tr>
<td>T-tubules</td>
<td>Transverse tubule system</td>
</tr>
<tr>
<td>Tb.N</td>
<td>Trabecular number</td>
</tr>
<tr>
<td>Tb.Sp</td>
<td>Trabecular separation</td>
</tr>
<tr>
<td>Tb.Th</td>
<td>Trabecular thickness</td>
</tr>
<tr>
<td>UK</td>
<td>United Kingdom</td>
</tr>
<tr>
<td>vBMD</td>
<td>Volumetric bone mineral density</td>
</tr>
<tr>
<td>WB</td>
<td>Whole body</td>
</tr>
<tr>
<td>Z</td>
<td>Atomic number</td>
</tr>
<tr>
<td>Z_{eff}</td>
<td>Effective atomic number</td>
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1 Introduction

Childhood and adolescence are pivotal stages of life for bone and muscle development. The mechanostat theory postulates that increasing muscle force during growth or in response to increased mechanical loading will affect bone mineral, geometry, microarchitecture, and consequently strength in later life (1, 2). Individuals with chronic illness such as cystic fibrosis (CF) are known to have low bone mineral content (BMC) compared to healthy peers as a result of slow growth and/or delayed puberty. If BMC are lower after catch-up growth, it is likely that there would be an increased risk of developing osteoporosis and fragility fracture in adolescence and early adulthood.

Dual energy X-ray absorptiometry (DXA) is the most common technique to assess bone and body composition. DXA measurements (i.e. areal bone mineral density (aBMD) and bone mineral content (BMC) are both size dependent and may lead to under- or over-estimation of bone mineral when body size (i.e. height) is not accounted for. This can be problematic when comparing individuals with CF, who tend to have a shorter stature compared to their healthy peers. Failure to account adequately for delayed growth leads to inaccurate conclusions as short, thin individuals will have a lower aBMD compared to tall, wider individuals. New and advanced technologies such as peripheral quantitative computed tomography (pQCT), high resolution pQCT (HR-pQCT), and jumping mechanography are available and can assess many more aspects of bone strength and muscle function than DXA alone.

The aim of this thesis was to investigate whether sex and disease status modified the relationship between: 1) puberty and bone, and 2) muscle and bone. The primary objective was to characterise the muscle-bone unit by measuring volumetric BMD, bone geometry, cortical and trabecular microarchitecture, and muscle function in children aged 8-16 years, using novel bone densitometry and muscle assessment techniques.

This would ultimately contribute to the understanding of how sex alters the relationship between bone and muscle in healthy males and females as they transition through puberty and who, on a population level, differ in the prevalence of osteoporosis and risk of fracture in later life.

Secondly, fractures in young adults with CF are common, however the aetiology and timing of CF-related bone disease remain unclear. This study aimed to contribute to the understanding of the aetiology of CF-related bone disease and whether CF status alters the relationship between puberty and bone and body composition outcomes and the role that muscle plays in bone development.

The hypotheses are that sex (males vs. females) and disease group (CF vs. controls) do: 1) modify the relationships between puberty and bone outcomes. Healthy females have lower apparent gains in
bone area but have greater apparent gains in cortical thickness and bone density (as a consequence of more bone within their periosteal envelope), as puberty proceeds. Participants with CF have smaller difference in bone area but have lower difference in bone density (as a consequence of less bone within their periosteal envelope), as puberty proceeds, and 2) modify the relationship between muscle function (i.e. Pmax and Fmax) and bone outcomes as the mechanostat theory states that bone adapts to changes in loading (i.e. widening the bone). Healthy females and participants with CF would have lower apparent gains in bone area, as muscle force and power increase.

This thesis begins by describing musculoskeletal health through the life course, highlighting the importance of genetics, hormones, nutrition, general health, and physical exercise in the developing skeleton and for reducing the risk of developing osteoporosis in later life. An overview of the relevant research areas including bone anatomy, growth and development, the muscle-bone unit, critically evaluating bone imaging and muscle assessment techniques, and aetiology of CF-related bone disease are described to set the background for the main research aims of thesis. To set the study scene, the primary and secondary aims, objectives, hypotheses of this thesis are presented before describing the study set-up, methods, and recruitment. The results and discussion for sex and disease group differences have been described in two parts (i.e. Chapter 5.0 and Chapter 6.0). The final part of the thesis concludes with the study evaluations and final research conclusions.

Understanding how muscle relates to bone strength may explain why healthy females and individuals with CF tend to have lower BMC and a higher risk of low trauma fracture compared to healthy males/non-CF individuals in later life.
2 Literature review

2.1 Musculoskeletal health through the life course
Childhood and adolescence are critical periods of longitudinal and appositional bone growth. Bone mass, a composite measure including contributions from bone volume and volumetric bone mineral density (vBMD), increases during growth, from 70-95 g at birth to 2400-3300 g in adulthood (Cooper et al., 2006, Trotter and Hixon, 1974). There are two distinct phases of bone development which result in a net increase in bone tissue. The first is skeletal patterning which occurs during the embryonic period, when the position and shape of the various skeletal elements is determined by the expression of numerous regulatory genes and by local factors. The second phase (post-embryonic) begins with mineralisation, the location of which is influenced by mechanical strain and determines bone mineral content, geometry, and microarchitecture (Davies et al., 2005).

Throughout life, bone is modelled and remodelled for growth, maintenance, and repair. Bone modelling is the process whereby bone is formed without necessarily being preceded by resorption, whereas remodelling requires bone osteoclastic resorption prior to osteoblastic bone formation. Bone accrual during the life course is illustrated in Figure 2.1. In early bone development, bone formation is accelerated causing bone size and mass to increase. At the end of puberty, when peak adult height has been reached, the growth plates close and growth ceases. Mineralisation of the bone continues until peak bone mass (PBM) has been achieved. Equal rates of formation and resorption (remodelling) maintain PBM during adulthood. The exact chemical and/or mechanical signals that coordinate local rates of formation and resorption are not fully understood. In later stages of life (>50 years of age), resorption begins to exceed formation, bone cross-sectional area (CSA) increases but the bone tissue mass slowly decreases. Genetic factors account for 60-80% of the population variance in PBM with the remainder due to diet, exercise, disease and other environmental factors (Rizzoli R, 2001, Gueguen et al., 1995). Those who achieve a lower PBM will be predisposed to osteoporosis and greater risk of fragility fractures in later life (Matkovic et al., 1998).

2.1.1 Osteoporosis and fracture
Osteoporosis is a skeletal disorder characterised by low bone density and deterioration of bony microarchitecture, leading to enhanced bone fragility and increased risk of fragility (low trauma) fracture (Eastell, 2004, Christiansen, 1991). The most common form of osteoporosis is known as either “primary osteoporosis” or “juvenile / idiopathic / age-related osteoporosis” as it is not caused by a specific disorder. Bone loss caused by specific chronic diseases such as cystic fibrosis (CF) and/or use of certain medications like glucocorticoids is referred to as “secondary osteoporosis” and can occur at any stage during the life course.
Fragility fractures are common in later life and are associated with considerable morbidity, mortality and economic cost. In the UK, approximately 3 million people have been diagnosed with osteoporosis and this is expected to increase dramatically with an aging population. Osteoporosis leads to nearly 9 million fractures annually worldwide (Johnell and Kanis, 2006), with over 300,000 patients presenting with fragility fractures to hospitals in the UK each year (Darowski, 2007). One in two women and one in five men will suffer a fragility fracture after the age of 50 in the UK (van Staa et al., 2001). The most common fracture sites are the hip, wrist, and vertebrae. Current projections suggest that, in the UK, hip fracture incidence will rise from 91,500 in 2015 to 101,000 in 2020 (Darowski, 2007). Hip fractures are the cause of greatest morbidity and mortality. Individuals with osteoporosis are at high risk of suffering one or more fractures, injuries that can often have devastating consequences which can be physically debilitating and potentially lead to a downward spiral in physical and mental health. There are many other risk factors for developing osteoporosis such as age, genetics, weight, growth disturbances, low PBM, hormonal imbalance, menopause, nutrition, physical activity, smoking and alcohol, use of certain medication (e.g. glucocorticoids, co-morbidities, and neuromuscular dysfunction).

Figure 2.1 - Bone mass during the life course.

The blue line denotes male, pink line female and dotted the consequence of not achieving peak bone mass and increased fracture risk in later life. Source: Diagram adapted from (Ward, 2012).

Osteoporosis has been clinically defined on the basis of areal bone mineral density (aBMD) assessment. According to the World Health Organisation diagnostic classification, adult osteoporosis and osteopenia should be based on a comparison of an individual’s aBMD to the mean aBMD of
healthy pre-menopausal young adult (25-30-years of age). The diagnosis of osteoporosis is classed as having a T-score (defined as [aBMD – mean reference aBMD]/SD\(^1\) and used in adults) of less than or equal to 2.5 SD and osteopenia a T-score between -1.0 and -2.5 SD (WHO, 1994, Kanis et al., 1994). The most common sites for assessing the risk of osteoporosis are the hip and spine using dual-energy X-ray absorptiometry (DXA), which is a two dimensional imaging technique used to measure aBMD. An odds ratio for lifetime risk of hip fracture is 2.6 for every SD decrease in aBMD of postmenopausal women (Marshall et al., 1996). Therefore, it is essential that people who are of high risk of developing or already diagnosed with osteoporosis are regularly monitored for bone health status and provided advice and medical care if necessary.

Among healthy children, as many as half of all boys and a third of girls will fracture by age 18, and one-fifth will have 2 or more fractures (Bishop et al., 2014). Most fractures are of the upper limb (i.e. radius) and the timing of fracture usually coincides with pubertal growth spurt. Healthy children and adolescents who have fractured have been reported to have a lower aBMD compared to those without fracture (Goulding et al., 1998, Goulding et al., 2000, Goulding et al., 2001). However, The International Society for Clinical Densitometry (ISCD) guidelines state that the diagnosis of primary or juvenile osteoporosis in children and adolescents should not be made on the basis of densitometric criteria alone. Assessment should include significant clinical fracture history of two or more fractures of the upper extremities and low aBMD (i.e. a SD less or equal to -2.0) adjusted for age, gender, and body size, as appropriate (Bishop et al., 2014). Evidence has shown that in addition to low aBMD, aspects of bone geometry or microarchitecture together with physical activity contribute to increased fracture incidence during normal pubertal growth (Cooper et al., 2004, Baxter-Jones et al., 2008b, Clark et al., 2008, Nishiyama et al., 2012a). Factors which compromise bone strength and increase fracture risk include: 1) low bone mineral content (BMC) or aBMD, 2) small bone CSA, 3) disturbed microarchitecture, and 4) increased bone turnover.

Over the last decade, the focus of research in bone health has shifted from treatment to prevention of bone loss and strength by ensuring adequate bone accrual during growth. It has become increasingly important to understand bone development in early life as disruptions during the transition from childhood to adulthood can result in lower PBM and increased risk of fracture in later life. The underlying bone biology and aetiology of osteoporosis is described in more detail in Chapter 2.6.2.

2.2 Bone

The principal role of the skeleton is to provide structural support for the body. The human adult skeleton is comprised of 213 bones of various shapes and sizes, for example long, short, flat,
irregular, and sesamoid bones. Depending on its location, each bone supports one or more specific functions, including structural support and movement, protection of vital organs, and maintenance of mineral homeostasis (Dempster D.W, 2006). A healthy bone needs to be structurally stable to withstand forces generated through daily loading (without fracturing). Accommodation of changes in metabolic and mechanical demands is achieved through alterations in turnover, mineral content and morphology (Ward, 2012).

2.2.1 Bone composition
Bone is a heterogeneous and anisotropic material, composed of two phases, an organic phase and an inorganic phase. The organic phase (osteoid) accounts for 20% to 40% (dry weight) of bone tissue and is mainly composed of collagen type I (85-90%) (Clarke, 2008), which is the most abundant fibrous collagen in the body. The basic unit of collagen type I is the tropocollagen molecule. Each molecule is composed of two identical \( \alpha_1 \) and one \( \alpha_2 \) polypeptide chains. The three chains are bound together in a tight right-handed triple helix and cross-linked in the extracellular space to form collagen fibrils (Figure 2.2 A). The inorganic phase accounts for 50-70% of the bone’s dry weight and is composed of small crystals (<0.1 nm in dimension) of calcium and phosphate, known as hydroxyapatite \( \text{Ca}_10[\text{PO}_4]_6[\text{OH}]_2 \), with small amounts of phosphorus, magnesium, manganese, and zinc (Clarke, 2008). Water accounts for up to 25% of the bone’s total weight. In the extracellular matrix, collagen molecules are staggered in an axial direction with a gap zone of 35 nm (Fonseca, 2012), where hydroxyapatite crystals are deposited (Figure 2.2 B).

![Figure 2.2](image)

**Figure 2.2 - The structure of a typical collagen molecule.**
(A) Represents a single collagen molecule, which consists of three subunits of collagen, known as tropocollagen, wound into a triple helix; (B) Collagen molecules are assembled in a head-to-tail alignment with a small gap that separates the 'head' of one molecule with the 'tail' of the next molecule, and in a staggered side-by-side arrangement. Adjacent collagen molecules are displaced about 67 nm, the side-by-side interactions are stabilised by covalent bonds (red bars). Source: (Campbell and Reece, 2001).
The size and orientation of these crystals are influenced by collagen organisation, which gives rise to the anisotropic properties of bone (Nair et al., 2013). Bone strength is influenced by crystal size, shape, arrangement, and volume, and by collagen spacing, orientation, length, and the strength of intermolecular interactions. The combination of collagen and hydroxyapatite crystals allows the bone to be stiff and be also to bend when loaded. The biomechanics of bone are described in detail in Chapter 2.2.4.

2.2.2 Anatomy of bone

Whole bone level - Long bones are highly responsible for the structural support and movement of the skeleton as they serve as levers for muscles, supporting locomotion. The shaft of a long bone is known as the diaphysis, which expands at the ends to form the cone-shaped metaphyses and the rounded edge of the bone, known as the epiphyses (Figure 2.3). The ends of epiphyses are covered with articular cartilage, which allows movement at joints to occur by acting as a smooth surface for articulation and a shock absorber (Cohen et al., 1998). The growth plate is located between the metaphysis and epiphysis and is described in greater detail in Chapter 0. The skeleton is composed of two different types of bone; cortical (compact) and trabecular (spongy) bone.

Cortical and trabecular bone - Cortical bone represents approximately 80% of skeletal mass and forms a protective, dense outer shell (cortex) around every bone. Cortical bone surrounds the bone marrow at the diaphysis of long bones and the trabecular bone at the epiphysis of long bones, and in the femoral neck and vertebrae (Genuth, 1998). Trabecular bone consists of a honeycomb-like network of interconnected trabecular plates and rods, with a thickness between 50-150 µm (Boutroy et al., 2005). Trabecular bone has five times as much total surface area as cortical bone and has a high turnover rate suggesting that turnover is driven more by its role in mineral metabolism than structural adaptations due to strains (Genuth, 1998). Cortical bone and trabecular bone have the ability to withstand and adapt to the forces placed upon them, with cortical bone experiencing the most strain at the diaphysis of long bones and trabecular bone experiencing strain at the epiphyses of long bone, in the femoral neck and vertebrae. Cortical bone is largely responsible for withstanding the force placed on the bone, however, the orientation of the rods and plates of trabecular bone do adapt to the strains placed on the bone and contribute to the bone’s structural integrity and bone strength (Bell et al., 1967, Silva and Gibson, 1997). Trabecular microarchitecture is generally characterised by the number of trabeculae (Tb.N) in a given volume, the mean trabecular thickness (Tb.Th) and mean trabecular separation (Tb.Sp), and the degree of connectivity between trabeculae (Bouxsein, 2005). Studies have shown that the loss of whole trabecular elements had a 2 to 5 times more deleterious effect on bone strength compared to the same amount of bone loss through trabecular thinning. Thus, the connectivity of the trabecular network is critical to bone strength (Silva and Gibson, 1997). Bell et al. proposed that trabecular structures failed when the trabecular network
connectivity declined (Bell et al., 1967). The emphasis was placed upon the loss of horizontal trabecular elements, as these elements provide support for vertical trabecular elements. Therefore, when horizontal trabeculae fail, the vertical trabeculae start to buckle, and bone strength is reduced.

Different bones and skeletal sites within bones have different ratios of cortical to trabecular bone (e.g. cortical to trabecular bone ratio of 25:75 in the vertebrae, 50:50 in the femoral head, and 95:5 in the radial diaphysis) (Clarke, 2008). Cortical and trabecular bone can be considered as one material with porosity varying over a wide range; in cortical bone 5-30% and in trabecular bone from 30-90% (Samuel et al., 2009). Porosity is considered a normal part of bone architecture which enables the bone to be structurally strong but light in weight. However, abnormal porosity can significant affect the biomechanics of bone; a high degree of porosity as seen in osteoporosis can lead to bone fragility and higher fracture risk, whereas a low degree of porosity as seen in osteopetrosis can lead to very dense, brittle bone, and again higher fracture risk. Factors affecting porosity include age, gender, genetics, hormones, nutrition, adequate mineralisation and bone turnover.

**Periosteum and endostem** - Long bones are considered to have two membrane surfaces; the periosteum and the endostem. The periosteum is a thin, fibrous membrane which surrounds the whole bone, except for the joint space. This membrane plays an important role in growth, development, modelling and remodelling, and fracture repair of bone as it contains an array of skeletal cell types, including cells of the entire osteoblastic lineage (bone forming cells). This accounts for its broad potential to create and shape the bone throughout growth. The periosteum in adults has two layers; an outer fibrous sheath containing fibroblasts and mesenchymal cells, elastin, and collagen (types I, III, and VI), and an inner cambium layer which is highly osteogenic and contributes to the appositional growth of bone throughout life. The periosteum is 70 µm to 150 µm thick (Squier et al., 1990) but thins with age in adulthood (Moore et al., 2014). With age, the membrane thins and becomes less cellular but the ability to respond to mechanical stimulus through apposition of new bone throughout life is not affected. Periosteal apposition occurs in adult males and females as they age, although the amount of apposition that occurs in females is insufficient to offset the large losses of bone during the menopause (Riggs et al., 2004).

The endostem lines the surfaces of trabeculae and the central medullary cavity, which is filled with two types of bone marrow; 1) red marrow, which is haematopoietic (blood cells) and 2) yellow marrow, which consists of fat cells (Britannica, 2014). At birth and until about the age of seven years, all bone marrow is red due to high blood cell formation. With increasing age, bone marrow gradually becomes fattier as blood formation decreases (Britannica, 2014). The endostem is composed mainly of osteoprogenitor cells. During growth, the periosteum forms bone on the outer cortex while the endostem resorbs bone, resulting in widening the bone.
2.2.3 Bone growth and development

Skeletal development occurs in two phases; embryonically and post-embryonically. During embryonic development, mesenchymal cells, which originate from the mesoderm layer (middle germ layer), differentiate into three distinct cell lineages; 1) the somites generate the axial skeleton along with skeletal muscle and connective tissues, 2) the lateral plate mesoderm generates the appendicular skeleton (limbs), and 3) the cranial neural crest gives rise to the branchial arch and craniofacial bones and cartilage. Mesenchymal cells also give rise to smooth tissue, dermis, and blood.

Osteogenesis is the process of forming new bone and occurs through two distinct pathways: intramembranous ossification and endochondral ossification. Intramembranous ossification occurs in the flat bones of the skull and involves embryonic mesenchymal cells differentiating into the osteoblasts and directly forming bone. In contrast, endochondral ossification, which occurs in the long bones, involves the replacement of a hyaline cartilage model with bone tissue.

Figure 2.3 - Midsection of the upper femur.
Bone is heterogeneous. Cortical bone is significantly thicker around the shaft (diaphysis) and trabecular bone is located in the proximal and distal (metaphysis) regions of the bone. Source: (Gray, 1918).
2.2.3.1 Intramembranous ossification

Intramembranous ossification is first seen around the eighth week of gestation and is illustrated in Figure 2.4.

![Figure 2.4 - Intramembranous ossification.](image)

Source: (Campbell and Reece, 2001).

The process starts when mesenchymal stem cells cluster together to form a nodule in the center of the fibrous connective tissue membrane, forming an ossification center (Figure 2.4 A). The mesenchymal stem cells differentiate into osteoblasts and start to secrete osteoid, which is then mineralised. Osteoblasts become entombed in the mineralised matrix and transform into osteocytes (Figure 2.4 B). Osteoid is continuously laid down randomly around the embryonic blood vessels, forming the trabecular compartment (Figure 2.4 C). The vascularised mesenchyme tissue on the outside of the trabecular bone condenses and forms the periosteum. As the trabeculae thicken, the osteoblasts on the periphery will continue to lay down osteoid. This osteoid will condense to form lamellar bone (cortical/compact bone) around the trabecular bone. Finally, the vascular tissue is replaced with red blood marrow, which is contained in the medullary cavity and between trabeculae (Figure 2.4 D).

2.2.3.2 Endochondral ossification

Endochondral ossification involves replacing hyaline cartilage with bone tissue, forming all bones in the skeleton except for the skull and clavicles. Mesenchymal stem cells proliferate and differentiate into chondroblasts (cartilage forming cells), which secrete a cartilaginous matrix, resulting in a hyaline cartilage model (Figure 2.5 A-B). The cartilage model acts as a template for bone formation and consists of a diaphysis, newly-forming epiphyses, and a primary ossification centre (located in middle of diaphysis). The dense membrane surrounding the model is called the perichondrium and
contains fibroblasts and chondroblasts. As the cartilage template grows in size, chondroblasts become entombed in the matrix and transform into chondrocytes (Figure 2.5 C). At the diaphysis, cartilage is resorbed and lacunae (cavities) are formed. The cartilage model is invaded by blood vessels, occupying the spaces left by resorbed cartilage. The perichondrium develops into the periosteum, which gives rise to osteogenic cells. Osteoblasts generate a layer of bone on the calcified cartilage model, giving rise to woven bone (Figure 2.5 D-E). During this process, secondary centres of ossification rise between the metaphysis and epiphysis at either end of the long bone, producing the growth plates (epiphyseal cartilage). These ossification centres allow the epiphyses to expand in bone length and diameter, and are responsible for the post-embryonic longitudinal growth (Figure 2.5 F-H).

**Figure 2.5 - Endochondral ossification in long bone.**
Source: (Gilbert, 2006).

### 2.2.3.3 Post-embryonic bone growth

Skeletal growth during childhood and adolescence involves interstitial and appositional growth. Interstitial growth occurs at the epiphyseal growth plate and is responsible for bone lengthening, while appositional growth occurs at diaphysis of long bones and results in bone widening. The two different types of growth will be discussed in this section.

**1) Interstitial growth at the growth plate**

The growth plate, also known as epiphyseal cartilage, is composed of gelatinous hyaline cartilage tissue and is located between the metaphysis and epiphysis of long bones. The function of a growth plate is longitudinal growth through endochondral ossification. The growth plate exhibits five distinct zones as illustrated in Figure 2.6.
Nearest to the epiphysis is the zone of reserve (resting) cartilage. This zone contains small, randomly oriented chondrocytes which exhibit no cellular proliferation or active matrix production and do not contribute to growth. In the zone of proliferation, chondrocytes undergo division and are organised to form stacked columns. These cells interact with each other and with the extracellular matrix to actively produce collagen (mainly types II and XI) and other cartilage matrix proteins. In the zone of hypertrophy, chondrocytes are continually kept in stacked columns and start to enlarge. The chondrocytes remain metabolically active, secreting type I collagen, while increasing secretion of type X collagen. Hypertrophic chondrocytes initiate vascular invasion by secreting vascular endothelial growth factor. In the zone of calcified cartilage, the hypertrophied cells begin to degenerate and the cartilage matrix becomes calcified. The calcified cartilage then serves as an initial scaffold for deposition of new bone. Chondrocytes positioned in the most proximal part of this zone (nearest to metaphysis) undergo apoptosis. The last zone, the zone of resorption, small blood vessels
invade the region previously occupied by the dying chondrocytes. Osteoclasts reabsorb the most mature ends of the bone spicules creating a honey-comb structure as calcified cartilage and the dead chondrocytes are removed. Osteoblasts then replace the calcified cartilage with osteoid, which will later become trabecular bone.

2) Appositional bone growth

During growth, bone needs to grow in length as well as in width for bone strength to be maintained. Bone at the diaphysis grows in width through appositional growth, which occurs at a slower pace compared to interstitial growth (Rauch, 2012). This process involves cellular activity on both the periosteum and endosteum. Periosteal apposition involves osteoblasts forming layers of new bone on the periosteal surface, resulting in an increase in bone CSA (Rauch, 2007). At the same time, endosteal resorption occurs, which involves osteoclasts resorbing bone on the endosteal surface, resulting in the medullary cavity and trabecular compartment expanding/contracting in diameter (Rauch, 2007). Periosteal apposition involves bone modelling, whereas endosteal resorption involves bone remodelling. These two processes serves to increase the whole-bone diameter and to expand the marrow cavity, necessary for the formation of blood, and to increase mechanical strength of the bone, while at the same time not increasing the bone’s weight (Hochberg et al., 2014). The rate of periosteal apposition and endosteal resorption differ in males and females during puberty, resulting in males having a wider bone compared to females in adulthood. Unlike interstitial growth, appositional growth has not been well characterised. However, studies have shown that the rates of apposition change throughout the life course can alter bone strength and can be affected by mechanical and non-mechanical factors (Rauch, 2005, Rauch, 2007, Rauch, 2012). This will be described in Chapter 2.6.

2.2.3.4 Bone modelling and remodelling

Bone modelling and remodelling are dynamic processes which require coordinated cellular activities, involving osteoclasts, osteoblasts, and osteocytes, to form new bone during growth and to continually renew bone throughout life.

Bone modelling and resorption drifts - Bone modelling, the process whereby bone is formed without necessarily being preceded by resorption, occurs during growth to alter the bone’s size, geometry, and structure. This process occurs during longitudinal growth of long bones, modelling of flat bones (e.g. the skull), and correction of bone deformities.
Figure 2.7 - Metaphyseal in-waisting during longitudinal bone growth.
The younger bone profile is on the left and the older is on the right. The outline of the earlier bone shape is shown as a superimposed dotted line on the right side of the figure. The bone has grown in length by endochondral ossification at the growth region beneath articular cartilage and at the diaphysis. The general shape of the bone is maintained by bone resorption and formation drifts; bone resorption occurs on some surfaces while bone deposition occurs on different surfaces. Source: (Campbell and Reece, 2001).

During growth, the geometry and structure of new bone is continuously being modelled by two modelling drifts; formation and resorption drifts. The bone modelling drifts act on the periosteum and endosteum of cortical bone, with formation drifts occurring on the periosteum to form new bone and resorption drifts occurring on the endosteum to resorb bone. This allows bone to grow in size and length, whilst maintaining the structural integrity of bone and bone strength. The modelling process during longitudinal growth of long bones allows the bone to change shape by modelling the wider metaphysis into a narrower shape, to form part of the diaphysis, therefore making the bone a more efficient load-bearing structure. This process is known as metaphyseal in-waisting and is illustrated in Figure 2.7.

Bone remodelling and mineralisation - Bone remodelling, the process of repairing bone micro-damage, maintaining mineral homeostasis, and maintaining the strength and integrity of the skeleton, occurs throughout life. Bone remodelling takes place in the basic multicellular unit (BMU) and occurs in four distinct phases, 1) activation, 2) resorption, 3) reversal, and 4) formation (Figure 2.8 A).
Figure 2.8 - Bone remodeling.
(A) The four phases of bone remodelling. (B) In the trabecular bone, the osteoclasts create Howship’s lacunae, and (C) in the cortical bone, the osteoclasts erode bone tissue forming cutting cones. Osteoblasts will form new bone in these resorption pits. Sources: (A) (Idris, 2010) and (B & C) (Parra-Torres et al., 2013).

The **activation phase** involves the recruitment of mononucleated osteoclast precursors, infiltration of the bone lining cell layer, and fusion of the mononuclear cells to form multinucleated osteoclasts (bone degrading cells). The osteoclasts affix themselves to the bone matrix and create a ring-shaped sealed zone, creating a unique microenvironment for resorbing bone. During the **resorption phase**, osteoclasts pump protons into the sealed compartment, resulting in a significant drop in the pH level (pH 4.0). The acidic environment allows digestive enzymes such as cathepsin K and matrix metalloproteinases (MMPs), which are most active at a low pH, to effectively dissolve and digest the collagen and mineral components of bone. Saucer-shaped resorption cavities, known as Howship’s lacunae are created on the surface of trabecular bone while cylindrical tunnels forming “cutting cones” are formed within the cortex (Figure 2.8 B). The depth of the resorption sites can vary between 40 - 60 μm (Tang et al., 2009). The resorption phase ends with osteoclast apoptosis and is followed by the reversal phase. During the **reversal phase**, macrophage, monocytes, and pre-osteoblasts are recruited to clean the resorption pit, which is covered with undigested demineralised collagen matrix, in preparation for the final phase. The **formation phase** is carried out in two parts,
first the osteoblast synthesis the new collagen matrix, which is then followed by mineralisation of the collagen matrix. As bone formation continues, osteoblasts become entombed in the matrix as osteocytes. Osteocytes sense mechanical changes in bone and communicate with one another using their cytoplasmic processes that extend through the small tubes in the matrix, known as canaliculae. Therefore, these cells are thought to be responsible for orchestrating osteoclast and osteoblast activity (Martin and Seeman, 2008). The remodelling sequence is tightly regulated by both local (cytokines and growth factors) and systemic (hormones) factors to ensure that resorption and formation are at equilibrium. Any significant deviations from the neutral balance between resorption and formation would result in accelerated bone loss or bone gain. In normal bone, the resorption phase takes 2-3 weeks but it takes at least 3 months to rebuild it.

2.2.4 Bone biomechanics

2.2.4.1 Bone mechanical properties

Functionally, the most important biomechanical properties of bone are its strength and stiffness which are based on the bone’s material and structural properties. Bone health is usually assessed by measuring aBMD and it is widely accepted that low aBMD is a powerful predictor of osteoporosis and fracture in children, adolescents, and adults. However, aBMD over-simplifies the assessment of bone strength and does not encapsulate other contributing components of strength such as bone size, geometry, microarchitecture, and the loading conditions placed on the bone (Ward, 2012). A bone must be strong to withstand the variety of loads placed upon it but also to be light in weight to allow skeletal movement with little energy expenditure (Figure 2.9).

**Figure 2.9 - Types of loading and stresses on bone.**

**Tension:** when forces pull the bone in two opposite directions, increasing bone length; **Compression:** when forces load the bone in the same direction causing bone to decrease in length; **Torsion:** when forces twist the bone around its axis in opposite directions; **Shear:** when forces are applied parallel to each other in opposite direction, causing the bone to move in two different directions; **Bending:** when forces are applied in a manner that causes the bone to bend about an axis, and **Combined:** when bone experiences a combination of different stresses in different directions. Source: (Nordin and Frankel, 1989).
The mechanical properties of a bone can be assessed by measuring its behaviour when stress is imposed upon it. Stress is defined as force (load) per unit area that develops on a plane surface within a structure in response to externally applied loads (Nordin and Frankel, 1989). Strain is the deformation (change in dimension) that develops within bone in response to externally applied forces (loads) (Nordin and Frankel, 1989). Stress and strain values of bone can be measured by using a testing vice and loading the bone specimen until it deforms and fractures Figure 2.10 A). These values can plotted on a stress-strain curve which can be used to calculate bone strength, stiffness, and other biomechanical behaviour during loading Figure 2.10 B).

![Figure 2.10 - Assessing stress-strain in bone.](image)

A) A bone specimen is placed in a testing vice and different loads can be placed upon the specimen until it breaks/fracture. B) Stress-strain deformation curve. Sources: (Skurla, 2008) and (Huei-Ming, 2005).

The stress-strain curve can be divided into two regions; elastic and plastic. During loading, strain increases linearly with increasing stress and deforms slightly. When the load is removed before the yield point (i.e. where stress and strain exceed the elastic threshold and bone begins to behave plastically), the bone is able to return to its original shape (elastic deformation). If the load continues to increase past the yield point, the bone becomes deformed, micro-damage begins to accumulate, and the bone will not return to its original shape (plastic deformation). If the load continues to increase, eventually the bone will fracture (failure point) (i.e. when ultimate stress and strain have been reached). From the curve, biomechanical properties can be measured such as:

**Strength** – Bone strength can be described and measured by assessing the yield point, ultimate stress, and ultimate strain. The higher the failure point (i.e. where fracture occurs) the stronger the bone is to withstand the force placed upon it.

**Stiffness (Young’s modulus)** – is the gradient of the slope in the elastic region. Stiffness is calculated by dividing stress by strain at the same point on the gradient. This value is known as Young’s modulus. Stiffer materials have higher moduli.
**Toughness** – is the amount of energy absorbed by the bone before a fracture occurs. Toughness can be measured by assessing the area under the curve. A larger area under the curve means the bone is tougher and is able to withstand fracture at higher loads.

Bone strength, stiffness, and toughness can vary and depend on the material and structural characteristics of the bone.

### 2.2.4.2 Bone material properties

Material properties of bone are determined by the biological composition (refer to Chapter Bone composition). As stated earlier, the bone matrix is composed of organic (collagen type I) and inorganic phases (bone mineral hydroxyapatite, \( \text{Ca}_5(\text{PO}_4)_3(\text{OH}) \)), which together allow bone to be strong and stiff. Under loading conditions, the collagen is able to absorb and disperse the energy (elasticity) induced through loading. Therefore, the bone is able to deform and then return to its original shape and length (**Figure 2.11 A**). The stiffness of bone is determined by the quantity of hydroxyapatite crystals embedded in the collagen matrix and is important for skeletal support. However, when the amount of mineralisation exceeds the required amount needed for strength, the bone becomes too stiff. This results in the bone becoming increasingly brittle and consequently starts to behave plastically at lower loads than usual, by reducing its ability to deform and absorb the energy (low toughness). Therefore, bone accumulates micro-damage and will fracture easily (e.g. osteopetrosis). When stiffness is reduced due to low mineralisation, toughness increases as more strain is placed upon the collagen fibrils. Evidently, there is a trade-off between stiffness and toughness as abnormally high stiffness (and low toughness) can increase fracture risk, and abnormally toughness (and low stiffness) can result in softening of bone tissue. Children with vitamin D/calcium deficiency rickets have bowed legs due to decreased stiffness (as a consequence of low mineralisation) of their lower limbs (Pettifor and Prentice, 2011b) (**Figure 2.11B**). This leads to “softening” of the skeleton and poor skeletal support but not increased fragility fracture risk. However, these children may develop pseudo-fractures or Loosers zones, a thickening of the periosteum and formation of new bone over what looks like an incomplete fracture and unmineralised matrix.
**Figure 2.11 - Stress-strain curve.**

(A) Stress-strain curve to demonstrate how bone phenotype may respond to changes in stiffness as a result of BMD. The black line denotes a healthy bone. ‘1’ represents the elastic portion of the curve where the strain does not create micro-damage, and when the load is removed, the bone can return to its original form. ‘2’ represents the plastic region where damage starts to accumulate and the bone can no longer return to its original form. ‘FX’ is when the bone fractures. The red line is a bone with high stiffness (and low toughness) due to increased BMD and the blue line is a bone with low stiffness (and high toughness) due to decreased BMD. The gradient of the elastic region indicates the stiffness, whereas the length indicates the flexibility of the bone. The toughness can be calculated from the total area under the curve. (B) Consequences of reduced bone stiffness can be seen in the radiograph of a child with untreated vitamin D deficiency rickets. The growth plates in the lower limbs become undermineralised, resulting in bone softening (i.e. reduced bone stiffness). Under normal levels of stress and strain, the growth plates widen and severely bowing of the legs occurs. Source: (A) Adapted from (Ward, 2012) and (B) (Pettifor and Prentice, 2011b).

2.2.4.3 Bone geometry

The geometry of bone is an essential component of bone strength and may change in response to growth, ageing, changes in mechanical stimulus, and general health. **Figure 2.12** illustrates three tubular structures of bone composed of the same bone material. The resistance of each bone to compressive and tensile loading is proportional to the CSA of bone. However, the resistance to bending and torsional loading is proportional to the cross-sectional moment of inertia (CSMI = \( \pi r^4/4 \))\(^2\), which is profoundly influenced by the distribution of bone mineral placed away from the centre. Although the bones contain identical amounts of bone mineral, the bone on the right would have an eight-fold higher bending strength due to the more favourable distribution of bone mass (Bouxsein, 2005). In instances where stiffness is decreased due to low mineralisation (e.g. vitamin D deficiency), strain may be over-estimated leading to altered bone geometry. Bone mineral is distributed further away from the bone centre, therefore widening the bone CSA (i.e. periosteal

\(^2\) CSMI formula symbols: \( \pi \) denotes Pi (3.14) and \( r \) denotes radius.
apposition). This increases CSMI as the resistance to bending and torsion forces increases, therefore preventing the bone from fracturing.

<table>
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<td>1.7</td>
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<tr>
<td>Bending Strength</td>
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**Figure 2.12** - The effects of bone geometry on compressive and bending strength.
Source: (Bouxsein, 2005).

### 2.3 Skeletal muscle

Skeletal muscle is the most abundant tissue in the human body, accounting for 40 to 45% of the total body weight (Nordin and Frankel, 1989). Muscle consists primarily of water (~75%), but protein and collagen, which constitutes most of the non-aqueous portion of muscle accounts for 20% of total tissue weight. Beyond the basic action of muscle contraction and movement, skeletal muscle is a tissue involved in many other functions including: maintaining posture and body position, support of soft tissues and internal organs, maintenance of body temperature, acting as a nutrient reservoir for protein, and is the site of metabolic activities such as glucose, glycogen and lipid metabolism, as well as endocrine and immunogenic activities (Nielsen and Pedersen, 2008, Hargreaves, 2004, Jensen and Richter, 2012, Pedersen and Febbraio, 2008). Skeletal movement is the result of complex interactions between the nervous, muscular, and skeletal systems. To understand the biomechanics of muscle function and the relationship muscle with bone, the gross anatomy of muscle will first be described in the following sections.

#### 2.3.1 Muscle biology and anatomy

Skeletal muscle is a very organised and structured unit (**Figure 2.13 A and B**). The structural unit of muscle is the fibre, a long cylindrical cell with many hundreds of nuclei. Muscle fibres range in thickness from 10 to 100 µm and in length from about 1 to 30 cm (Pitman and Peterson, 1989). Each fibre is encompassed by a loose connective tissue, known as the endomysium, and the fibres are organised into bundles, or fascicles, which are in turn encased in a dense connective tissue sheath.
known as the perimysium. The epimysium surrounds the entire muscle. Each muscle fibre is composed of a large number of muscle strands, known as myofibrils. Myofibrils lie parallel to each other within the sarcoplasm of the muscle fibre and are composed of a subunit known as a sarcomere (Figure 2.15 A).

![Figure 2.15 - Skeletal muscle anatomy.](image)

Figure 2.15 - Skeletal muscle anatomy.
(A) Cross-section of the whole muscle, and (B) The gross anatomy of a muscle fascicle. Source: (Campbell and Reece, 2001).

The sarcomere is subunit of skeletal muscle and is the functional unit of the contractile system. Each myofibril is composed of fibrous filaments of two types; thin filament (5 nm in diameter) composed of protein actin and thick filaments (15 nm in diameter) composed of the protein myosin (Figure 2.14) (Pitman and Peterson, 1989). The thick filaments are located in the central region of the sarcomere, where their orderly, parallel arrangement gives rise to dark bands, known as A-band. The thin filaments are attached at either end of the sarcomere to the Z line, which consists of short elements that link the thin filaments of adjacent sarcomeres. The thin filaments extend from the Z line toward the centre of the sarcomere, where they overlap with the thick filaments. Nebulin is an actin-binding protein which regulates thin filament length during sarcomere assembly but also functions in the regulation of muscle contraction, and has a role in calcium homeostasis. These novel functions indicate that nebulin might have evolved in vertebrate skeletal muscles to develop high levels of muscle force efficiently (Labeit et al., 2011). Titan is a large protein that functions as a molecular spring and plays an important role in stabilising the sarcomere and the myosin filament. Titin, also known as connectin, is able to regulate muscle force by changing the muscle’s stiffness in an activation/force dependent manner and by binding to actin, thereby adjusting its free spring length and allowing for the passive elasticity of muscle (Herzog et al., 2012). Actin and myosin filaments give rise to the striated appearance (light and dark banding pattern) of skeletal muscle when viewed under an electron microscope (Figure 2.15A).
Figure 2.14 - Myosin and actin.
(A) Myosin, and (B) Actin. Source: (Campbell and Reece, 2001).

2.3.2 Muscle contraction
The mechanism of muscle contraction is known as the excitation-contraction coupling or the sliding filament hypothesis and requires the central nervous system (CNS) and neurotransmitters to produce electrical signals which result in the cyclic sliding of myosin and actin filaments. This process is very complex but takes milliseconds from the CNS to the movement of limbs. The process of excitation-contraction coupling is illustrated in Figure 2.16.

A muscle contraction is initiated by an action potential (AP), which originates in the CNS. The AP causes an electrical event to occur by activating voltage-gated sodium channels downstream along the axon. When the AP reaches the neuromuscular junction, the plasma membrane of the nerve depolarises, opening voltage-gated calcium channels and resulting in an influx of calcium ions into the terminal axon, where vesicles of neurotransmitters (containing acetylcholine [Ach]) are stored.

Figure 2.15 - Image of skeletal muscle.
(A) A schematic diagram and (B) electron microscope. The images represent multiple myofibrils aligned in parallel. The A-band (dark band) consists mainly of thick myosin filaments (joined at the M-line), whereas the I-band (light band) are composed of thin actin filaments. Each sarcomere is flanked by a Z-disc region. Source: (Luther, 2014).
The calcium influx triggers fusion of these vesicles with the membrane of the terminal axon, where Ach is released into the synaptic cleft. Ach diffuses across the synapse and binds to Ach receptors on the motor end plate causing depolarisation and initiates an end plate potential which is then propagated along the sarcolemma (muscle cell membrane) of the muscle fibre. The AP spreads through the sarcolemma, down the extensive network of interconnecting tubular channels (i.e. the sarcoplastic reticulum [SR] and the transverse tubule system [T tubules]), depolarising the inner portion of the muscle fibre. Depolarisation of the T tubule membrane triggers the release of calcium ions from terminal cisternae, which are located in the SR, into the sarcoplasm.

Under resting conditions, the sarcoplasm is free from calcium ions and the tropomyosin molecule on the actin thin-filament obstructs the myosin receptor sites on the actin. However, when the calcium ions are present in the sarcomere they bind to tropinin with high affinity, modifying the actin molecule by allowing tropomyosin to move, unblocking the binding site. Once the site is free, myosin heads are able to form cross-bridges to actin molecules within reach. The energy for cross-bridge movement is provided by the hydrolysis of adenosine triphosphate (ATP) to diphosphate (ADP + Pᵢ) by ATPase on the myosin head. The attachment of the myosin head to the actin binding site pulls the Z-bands together and shortens the sarcomere unit initiating contraction.

To release the myosin head (relaxation), energy from ATP is required. The muscle relaxes when ATP binds to myosin, which promptly dissociates actin from myosin, breaking the cross-bridge bond between them. The tropomyosin returns to its original state and once more inhibits the actin from interacting with myosin. During muscle contractions, the cyclic forming and breaking of cross-bridges repeat as long as ATP is available and calcium is freely bound within the actin filament.
2.4 The muscle-bone unit

2.4.1 The mechanostat theory

A healthy bone is one that is considered to be ‘fit for purpose’ and does not fail during physiological loading (Ward, 2012). Evolutionary mechanisms have given bone the ability to adapt to changes in the environment by responding to strains placed upon it. The relationship between muscle and bone is described by Frost’s mechanostat theory, which postulates that increasing maximal muscle force during growth or in response to increased mechanical loading will affect bone mass, size, and length (Frost, 1996, Frost, 1987) (Figure 2.17). When a muscle contracts, the force generated creates a strain on the bone, changing the shape and length of the bone (Ward, 2012). Osteocytes, the most abundant cells found in bone, can sense strain and orchestrate the spatial and temporal recruitment of bone cells to adapt to changes in strain (Frost, 1996).
The modulators are non-mechanical factors which can affect the mechanostat set points through alterations in the ability for muscle to generate forces (load) and the ability of bone to detect or respond to changes in load (Refer to section 2.4.2). The red arrows represent a ‘healthy’ developing mechanostat where bone formation is greater than bone resorption so increasing bone mass and strength. The blue arrows represent a ‘disturbed’ developing mechanostat. This situation may occur when one or more of the modulators are deficient or defected. For example, cystic fibrosis causes chronic lung infections and malabsorption of vital nutrients, which disrupts growth and may cause muscle weakness, which would reduce strain sensed by the osteocytes and reduce osteoblast bone formation. For example, Vitamin D deficiency also causes under-mineralisation, which would in turn affect the bone architecture, stiffness and mass. Consequent failure to change bone phenotype in response to this would result in reduced bone strength compared to a healthy child with an adequate vitamin D status.

The fine-tuning of the mechanostat is achieved by physiological set points that act as thresholds for initiation or inhibition of bone modelling and remodelling (Figure 2.18). The changes in bone strength via mechanostat strain set points, rely on both cortical and trabecular structures adapting to the strains placed upon them. When strains increase (e.g. during exercise), the mechanostat may alter bone strength in several ways, first by increasing BMC, secondly by widening the bone through periosteal apposition (Ward, 2012), and thirdly, by increasing trabecular thickness, creating a dense interconnecting network. This allows strain to be distributed over a larger area throughout the bone. However, decreased strains (i.e. due to ageing or physical inactivity) result in loss of mineral, increasing cortical porosity, continuing periosteal apposition, trabecular thinning, and a deterioration of the trabecular connectivity. When osteocytes detect strains above the ‘steady state’, osteoblasts are recruited to form bone. However, if strains fall below the ‘steady state’, osteoclasts are recruited to resorb bone through hydrolytic enzymes (e.g. cathepsin K) (Ross, 2006). When bone is maintained in a steady state, bone formation and resorption are in equilibrium, therefore no bone is lost or
gained. The mechanostat set points are genetically determined (Schoenau and Fricke, 2008) and are regulated by non-mechanical modulators, which can alter the sensitivity of the mechanostat (refer to section 2.4.2).

Figure 2.18 - Schematic diagram of the mechanostat thresholds for bone modelling and remodelling.
(A) Osteocytes are responsible for detecting changes in mechanical strain. When strain exceeds the formation threshold, the osteocytes stimulate bone formation (dotted line), and when strain falls below the resorption threshold, resorption occurs (dashed line). BMC, geometry, and microarchitecture of the whole bone are altered therefore influencing bone strength. (B) Overloading and disuse (under-loading) both stimulate an increase in remodelling rates. When strain exceeds the threshold, the remodelling rate favours bone formation, resulting in an increase in bone mass. Bone microdamage from repetitive loading begins to accumulate and stimulates remodelling. When strain falls below the threshold, the remodelling rate favours bone resorption, resulting in a decrease in bone mass and an increase in bone porosity. Source: (Hughes and Petit, 2010).

2.4.2 The modulators which influence the mechanostat thresholds
The mechanostat continually adapts to optimise resistance to changes in muscle mass and function by altering BMC, bone geometry and microarchitecture. When growth ceases and the epiphyseal plates close, bone loses its ability to change significantly. Therefore, the structure and integrity of bone is set for life. Modulators such as nutrition, physical activity/loading, genetics, hormones, medications, and environmental factors can alter the mechanostat’s threshold set points during childhood and puberty, which can either be beneficial or have detrimental consequences for bone and muscle health in later life.

In this thesis, the muscle-bone unit in participants with and without CF will be investigated to understand whether sex (females vs. males) and disease group (CF vs. controls) modify the relationships between puberty and bone outcomes, as well as the relationship between muscle function and bone outcomes. In healthy individuals, the mechanostat theory postulates that increases in loads on bone increase strain within the bone and when they are above the threshold set points will trigger a cellular bone response by recruiting osteoblasts to increase BMC, bone size and bone geometry. Therefore, the bone will widen and will have adequate bone mineral with an
aim to resist bending and fracture. Individuals with complex chronic illnesses such as cystic fibrosis (CF) may have a disrupted mechanostat as the threshold set points are altered either directly by the disease or indirectly via disease complication. CF can affect many aspects of health including; nutritional deficiencies due to pancreatic insufficiency and malabsorption of fats and fat-soluble vitamins, delayed puberty, reduced physical activity due to chronic lung infections, and use of glucocorticoids (Sermet-Gaudelus et al., 2011). The mechanostat thresholds in patients with CF may be altered, resulting in reduced muscle strength/contractions, reduced bone mineral, and altered bone microarchitecture (Figure 2.17). This will be investigated in this thesis.

Modulators which effect bone

- **Nutrition** - An appropriate balanced diet is essential for developing and maintaining bone integrity and bone strength. In children with CF, deficiencies in bone minerals (calcium, phosphorus, magnesium, and zinc), and vitamins involved in bone mineralisation (Vitamins D, K, and C) are known to disrupt bone metabolism. This may alter the biomechanics of the bone as the bone becomes less mineralised, less stiff, and easier to fracture (Ward, 2012, Winzenberg and Jones, 2013).

- **Physical activity / loading** – Physical exercise/loading of bone creates strain which is sensed by the osteocytes and stimulates bone adaptation by increasing BMC, bone widening, and increasing bone strength. During periods of immobilisation, BMC and bone strength can be significantly reduced due to reduced skeletal loading (Buckingham and Jeffcott, 1991, Ceroni et al., 2013). This has been shown in long-term exposure to microgravity (Orwoll et al., 2013), in individuals who are non-ambulatory/severe physical disability (e.g. cerebral palsy) (Mergler et al., 2009), and long-term bed rest (Armbrecht et al., 2011, Miokovic et al., 2012). Individuals with CF have reduced physical activity due to reduced lung capacity and can be exposed to long-term bed rest due to chest infections, therefore have reduced physical loading on bone compared to their healthy peers. As a consequence of reduced strains on bone, there may be less bone formation compared to healthy children, as the recruitment of osteoclasts increases, which would increase bone resorption and bone may become less resistant to fracture.

- **Genetics** – Genetic are an important determinant of bone health and determine bone mineral, shape, and size. For example, differences have been found between Black, Asian, and Caucasian populations, which may explain differences in bone strength and fracture risk in these populations (Laskey et al., 2010, Bhudhikanok et al., 1996, Zengin et al., 2015, Barrett-Connor et al., 2005). In CF, studies in humans and mice bone cells have shown that
cystic fibrosis transmembrane chloride channels are present (refer to Chapter 2.7.6). This may directly alter bone turnover by increasing osteoclast activity, which reduces bone mineralisation, bone stiffness, and resistances to fracture.

- **Hormones** – Sex hormones released during puberty influence the geometry of bone (e.g. testosterone in males increases periosteal apposition (increasing bone width) and oestrogen in females increases endosteal apposition (increasing cortical thickness and smaller bone width compared to males) (Seeman, 2001). During ageing, bone fragility becomes more common in females due to the menopause. The lack of oestrogen increases bone turnover and bone loss, leading to osteoporosis (Clarke and Khosla, 2010). Deficiencies in thyroid hormone, growth hormone (GH), and insulin-like growth factor 1 (IGF-1) can cause growth disturbances and alter bone structure and integrity (Saggese et al., 2002). In growing individuals with CF, slow growth and delayed puberty may occur, which would disrupt bone mineralisation and bone widening during growth and potentially result in small, narrower, less mineralised bones in adulthood.

- **Medication** – Glucocorticoids (used in CF), anti-seizure medicines, and some contraceptive pills can affect bone strength by disrupting bone turnover and reducing BMD (NOF, 2014). Therefore, it is essential that these drugs are monitored and not used in the long-term. Osteoporotic medication such as bisphosphonates, an antiresorptive drug, can be used to increase bone mass by reducing osteoclast activity. However, long-term use can cause an accumulation of micro-damage and increase the stiffness (i.e. more brittle), which can increase the risk of fracture (Haworth and Webb, 2012).

- **Environmental factors** – Lifestyle choices such as smoking have long been acknowledged to be risk factors for poor bone health and strength during all stages of life. Smoking can effect bone health by: a) altering the production of hormones (i.e. reduces parathyroid hormone and oestrogen concentrations, and increasing cortisol concentration) which can alter bone turnover and reduce bone strength, b) reducing vitamin D levels and calcium absorption leading to reduced mineralisation, c) increasing oxidative stress, and D) reducing blood circulation (Kapoor and Jones, 2005, Brot et al., 1999). As a consequence, smokers are at high risk of developing osteoporosis and have a 25% increase in fracture risk compared to non-smokers (Kanis et al., 2005). Smoking can also reduce the rate of healing, especially for operations to repair fractures, which can increase the risk of post-operative infections and result in long-term mobility issues (Haverstock and Mandracchia, 1998).
Modulators which effect muscle

- **Nutrition** – Protein, fat, and minerals (calcium and phosphate) are essential for muscle development and function. Deficiencies in protein and fat can result in reduced muscle mass and strength, whereas deficiencies in calcium and phosphorus can disrupt muscle contractions and reduce muscle strength. Nutrient deficiencies are common in CF due to malabsorption of fats, essential fatty acids, and fat soluble vitamins and due to marked increases in energy expenditure during periods of infection. Therefore, individuals with CF tend to have lower muscle mass and muscle strength, which could lead to reduced strains on bone and disruption of muscle and bone development.

- **Physical activity / loading** – Individuals who take part in moderate to high intensity sport/exercise regimes have been reported to have increased muscle mass/area in gymnastics compared to controls (Ward et al., 2007, Ward et al., 2005). Individuals who live an active lifestyle or assigned an exercise regime have higher muscle mass, higher BMD and wider bones compared to controls as muscle contractions transmit greater strains on bone (Khan et al., 2000, McKay et al., 2011, Rideout et al., 2006, Duncan et al., 2002, Johannsen et al., 2003, Specker et al., 2004). Like bone, muscle strength can be significantly reduced during periods of immobilisation, exposure to microgravity, and severe physical disability. Individuals with CF tend to have reduced activity due to poor lung functions, poor health, and lower muscle strength. Therefore, muscle contractions are likely to be weaker in individuals with CF compared to healthy peers, which reduces strain on bone.

- **Genetics** – Genetically mediated ethnic differences in muscle/lean mass and muscle strength do exist. Studies have shown black populations to have a higher muscle mass and muscle strength compared to non-black populations. Black women have higher musculoskeletal mass and the loss of muscle mass with age may be lower in black women than in white women, which may explain why black populations have lower risks of sarcopenia and fracture rates compared to non-black populations (Aloia et al., 2000). However, variations in muscle strength between people from different countries may be attributed in part to differences in socio-economic status. In CF, studies in mice have shown that the CFTR mutation may be directly involved in the CF-related muscle disease. Dif et al. found that mice with the CFTR mutation had severe osteopenia and muscle mass was significantly reduced (Figure 2.49). Decreased BMD may be a result of decrease muscle mass and physical loading on the bone, which causes alterations in bone turnover. These mice were well nourished and free from therapeutic interventions (Dif et al., 2004).
• **Hormones** – During childhood and puberty, hormones such as GH, IGF-1, and testosterone have a significant impact on muscle development, especially in males. Deficiencies in these hormones will result in reduced muscle mass and strength. In CF, disruption in GH may contribute to the muscle impairment (Ciro et al., 2013).

• **Environmental factors** – Like bone, smoking has been associated with poor muscle health and strength (Rom et al., 2012). Smoking has been shown to disrupt protein synthesis, increase oxidative stress, reduced blood circulation to muscle, and reduced skeletal muscle contractile endurance due to impaired oxygen delivery (Rom et al., 2012, Degens et al., 2015). As a consequence, smokers develop sarcopenia and are at higher risk of falls and fractures (Degens et al., 2015).
2.5 Bone densitometry and muscle assessment techniques
Since bone strength can only be measured by material testing (breaking) of the bone in-vitro, surrogate measures of bone strength must be used for in-vivo assessment. Bone densitometry is a non-invasive, quantitative measurement technique used to quantify bone mineral, size, geometry, and microarchitecture to help understand bone strength. In this chapter radiation physics in imaging and the advantages and limitations of imaging techniques used in this study will be described.

2.5.1 Physics of absorptiometry
Radiation is a form of energy transmitted through space (vacuum) as an electromagnetic wave. There are many forms of radiation, which can be categorised into non-ionising and ionising radiation depending on the wavelength and energy produced. Non-ionising radiation (e.g. sound waves, visible light, and microwaves) does not carry enough energy per quantum to ionise (i.e. remove an electron) from atoms. Ionising radiation (e.g. x-rays and gamma rays) is highly energetic and has the ability to remove tightly bound electrons from atoms, thus creating ions. Ionising radiation has many practical applications in medical imaging such as x-rays and computed tomography (CT) scans to assess bone, muscle, fat, and other soft-tissues.

2.5.1.1 X-ray
X-rays are generated inside a vacuum tube called a Coolidge tube (Figure 2.19), which contains a tungsten target and two electrodes: a cathode (negatively charged) and an anode (positively charged).

![Figure 2.19 - X-ray glass tube.](image)
Source: (Gautam, 2014).

A high voltage is applied across the cathode, causing the filament to heat. Electrons are repelled and accelerated towards the tungsten target, which is located in front of the anode. The anode has a slightly higher voltage compared to the cathode, resulting in an electrical potential difference. The
electrons strike the tungsten target at high speed and undergo sudden deflection producing X-rays. The X-rays pass through the beryllium window, a thin metal which has little effect on the photon beam and can effectively maintain the vacuum, and through the collimator.

2.5.1.2 X-rays interaction with tissues and attenuation
The intensity of an X-ray beam is reduced by interaction with the tissue it encounters, resulting in photoelectric absorption and scattering (Figure 2.20 A). Photoelectric absorption (or effect) involves the interactions of an X-ray photon with an inner shell electron (usually the K shell) in the absorbing tissue atom that has a binding energy similar to but less than the energy of the X-ray photon (Seibert and Boone, 2005, Perkins, 1995). The X-ray photon transfers its energy to the electron, resulting in the ejection of the electron from its shell. This process makes the tissue atom become electrically unstable and the vacated electron shell is subsequently filled by an electron from an outer shell with less binding energy (from the L or M shell), releasing energy in the form of light and heat (Seibert and Boone, 2005, Perkins, 1995).

![Figure 2.20 - X-ray interaction.](image)

(A) Illustrates the interaction of X-ray with tissue atoms: a) X-ray photon does not interact with atom, b) Photoelectric absorption is when an X-ray photon removes an electron from inner-shell, which is then replaced with an electron from an outer-shell, c) Rayleigh scattering occurs when an X-ray photon interacts with an electron in which no energy is exchanged. The X-ray photon energy equals the scattered X-ray energy with small angular change in direction and no electron is removed, and d) Compton scattering interactions occur with unbound electrons, with transfer of energy shared between recoil electron and scattered photon. (B) Linear attenuation coefficients for various human tissue types. Source: (Seibert and Boone, 2005) and (Viana et al., 2011).

Photoelectric absorption is greater in materials with a high atomic number (Z) (i.e. a greater number of protons found in the nucleus). Tissues in the human body contain mostly low atomic number elements (e.g. hydrogen, Z = 1; carbon, Z = 6; nitrogen, Z = 7; and oxygen, Z = 8) (Seibert and Boone, 2005). The average Z for compound materials is known as the effective atomic number (Z_eff) (i.e. 52
bone, $Z_{eff} = 13$; muscle, $Z_{eff} = 7.4$; and fat, $Z_{eff} = 6$). Bone has a higher photoelectric effect compared to muscle, and muscle has a higher effect compared to fat. Bone attenuates the most X-rays compared to muscle and fat and therefore will appear brighter (i.e. less dark) on the X-ray image.

Attenuation is the removal of photons from an X-ray beam as it passes through matter. Attenuation is caused by both absorption and scattering of the X-ray photons (Bushberg et al., 2011). The fraction of photons removed from a monoenergetic beam of X-rays per unit thickness of material is called the linear attenuation coefficient ($\mu$), typically expressed in units of inverse centimetres (cm$^{-1}$) (Bushberg et al., 2011). The $\mu$ values are converted to create the CT images. The value of $\mu$ is affected by the material’s $Z_{eff}$ value, the energy of X-ray photon beam, and the thickness of the material. The effect of increasing X-ray beam photon energy on linear attenuation coefficients for cortical bone, air, adipose tissue, muscle, and soft tissue are illustrated in Figure 2.20 B (Viana et al., 2011).

2.5.1.3 Ionising radiation exposure
The International System (SI) of units for radioactivity throughout Europe is the Becquerel (Bq). Bq is a measure of the amount of ionising radiation emitted by a substance and can be defined as one Bq unit is equivalent to the decay of one atom of a radioisotope per second (Perkins, 1995). However, to understand the effect of the radiation dose on living tissue, the type of radiation (i.e. alpha, beta or gamma/X-ray) and the amount of energy absorbed (also known as Gray units [Gy]) need to be considered. The combination of radiation type, dose, and absorption is known as the equivalent dose and it is expressed in Sieverts (Sv). This is a measure of the amount of potential damage to the body from a given amount of radiation. Since one sievert is a very large value, radiation is usually expressed in smaller values (i.e. millisieverts, mSv (1/1000 sievert) or microsieverts, $\mu$Sv (1/100000 sievert))(Little, 2003).

The biological effects of ionising radiation depend upon the type, amount and rate of exposure as well as the tissue through which the radiation passes. High doses (greater than 50,000 - 100,000 $\mu$Sv) (HealthPhysicsSociety, 2001) of ionising radiation can damage living cells and DNA by splitting water molecules, producing free radicals which are then able to react with other cells. The biological damage may take many years to take effect, resulting in the development of cancer and production of permanent inheritable changes which may be passed onto future generations (Perkins, 1995, Little, 2003). The typical range of doses from bone densitometry techniques used in this thesis are between 0.2 and 3.0 $\mu$Sv.
2.5.2 Bone densitometry

2.5.2.1 A brief history of bone densitometry

- Early methods involved measurement of radiographic cortical morphometry, usually of the second metacarpal (metacarpal index) and single and dual energy photon absorptiometry (SPA and DPA) using radionuclide sources. These techniques had a long scanning time and poor precision. The limitations of these techniques are that the radiation source would often decay and require regular replacement (Bonnick and Lewis, 2013).

- In the 1980s, DXA was introduced to assess aBMD, with a shorter scanning time, lower doses of radiation, more stable due to use of X-rays (rather than radionuclide sources), and increased spatial resolution compared to SPA and DPA. This technique is widely available in both clinical and research institutes and large reference datasets for bone in children and adults are now available. However, there are some limitations with measuring aBMD and vertebral fracture assessment which are discussed later in this chapter.

- Axial quantitative CT (QCT) was first developed in the 1970s to assess vBMD at the lumbar spine. However, with the introduction of DXA in 1988, the use of QCT declined. In the 1990s, peripheral QCT (pQCT) become widely available and has advantages over DXA, which include: measurement of vBMD, cortical and trabecular bone can be assessed separately, measurements of biomechanical parameters, and relatively short scanning time.

- Most recently, advances in QCT have led to the development of high resolution pQCT (HR-pQCT) which in addition to the advantages of single-slice pQCT is able to quantify the trabecular microarchitecture and cortical porosity with good precision, and with the addition of finite element modelling it can also measure the predicted stress and strain of a bone. This technique has a relatively short scanning time but is prone to movement artefacts.

2.5.2.2 Definitions of bone measurements

During growth, the skeleton increases in size, shape, and mass, which alters bone strength. However, when bone strength is assessed, it is often these parameters which are neglected, especially in growing children. Rauch and Schoenau (Rauch and Schoenau, 2001) describe the biological organisation of bone in terms of three levels;

1) Material bone mineral density (BMD\textsubscript{material}) - The degree of mineralisation of the bone matrix, excluding the marrow spaces, osteonal canals, lacunae, and canaliculi. This biological level can only
be measured via histomorphometry using biopsies. HR-pQCT has the resolution to measure in-vivo cortical tissue mineral density at this micro-level in-vivo (Figure 2.21 A and C).

2) **Compartment bone mineral density (BMD<sub>compartment</sub>)** - The amount of mineral contained within the trabecular or cortical compartments. Densitometry techniques such as pQCT and HR-pQCT, which measure vBMD, have the ability to quantify the two bone compartments separately (Figure 2.21 B and D).

3) **Total bone mineral density (BMD<sub>total</sub>)** – The mineral density of all the tissue contained within the periosteal envelope. DXA and QCT measurements have the ability quantify bone at the macro-level (Figure 2.21 E).

![Figure 2.21 - The three different biological levels for assessing bone mineral.](image)

Source: (Rauch and Schoenau, 2001).

Advances in bone densitometry techniques have meant that different levels of bone organisation can now be measured. The current techniques available and the level of which bone can be assessed in-vivo are summarised in Table 2.1. Imaging techniques such as magnetic resonance imaging (MRI) and quantitative ultrasound can measure parameters related to bone strength but cannot measure BMD.
Table 2.1 - A summary of bone densitometry techniques and the bone organisational level measured.

Source: Adapted from (Ward et al., 2007b)

<table>
<thead>
<tr>
<th>Bone densitometry technique*</th>
<th>Biological organisational level of bone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BMD&lt;sub&gt;material&lt;/sub&gt;</td>
</tr>
<tr>
<td>Radiogrammetry</td>
<td>No</td>
</tr>
<tr>
<td>DXA</td>
<td>No</td>
</tr>
<tr>
<td>pQCT</td>
<td>No</td>
</tr>
<tr>
<td>HR-pQCT</td>
<td>Yes&lt;sup&gt;5&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Key: DXA – dual-energy x-ray absorptiometry, pQCT – peripheral QCT, HR-pQCT – high resolution-pQCT. ¹ Cortical microstructure including cortical porosity. ² Trabecular microstructure including trabecular number, thickness, and separation and the marrow spaces. ³ Only femoral cortical total BMD. ⁴ Trabecular density including bone marrow. ⁵ Tissue mineral density.

2.5.2.3 Precision of densitometry techniques

Normal changes of BMC proceed at a relatively slow pace ranging from 0.5% to 2% per annum for most healthy adults and from 2% to 5% in early postmenopausal women (Harris and Dawson-Hughes, 1992, Reeve et al., 1999). To detect these small changes, bone densitometry techniques need to have good precision. In-vivo precision of a technique is the degree to which the device gives the same bone parameter value when repeated at the same site on the same participant. Precision is expressed as a standard deviation (SD) or as coefficient of variance (CV), which is the SD expressed as a percent of measured quantity or its average value. To calculate precision of a bone imaging technique, a participant group of at least 14 individuals should be assessed and the same parameter measured at the same site should be repeated three or four times, and then the values compared to calculate the difference between measurements (Gluer et al., 1995).

The precision of a technique can be affected by:

1. **Device issues** – a) Image resolution, b) change in room temperature, c) vibration of the scanner.
2. **Operator issues** – a) operator experience with technique, b) operator attention to precision, and operator bias during the analysis of scans.
3. **Operating device issues** – a) Inadequate quality assurance (QA) and quality control (QC) monitoring with the manufacture’s phantom, b) incorrect positioning of the reference line prior to scanning, and c) inappropriate threshold applied.
4. **Biological issues** – a) Participants with small, under-mineralised bones, b) participants with large body mass which can affect the attenuation of X-ray beam, and c) participant movement.
2.5.3 Dual energy X-ray absorptiometry (DXA)

DXA was made commercially available in the late 1980s. In 2010, there were estimated to be over 50,000 whole body DXA systems in use worldwide (IAEA, 2010), which has led to a significant increased interest in bone research (Crabtree et al., 2007). DXA is an X-ray imaging technique which uses photons of two different energies to allow discrimination between fat, muscle and bone; high-energy photons are attenuated by bone and low-energy photons by soft tissue. During scanning, the X-ray beam is passed through the participant in a posterior-anterior direction and projected upwards to the scintillation detector. The scanning arm sweeps over the region of interest until scanning has completed (Figure 2.22).

![Figure 2.22 - Lunar Prodigy whole-body DXA scanner (GE Medical Systems, Madison, WI).](image)

The function of the collimator is to direct the X-ray beams in the direction of the participant being scanned, to reduce scattered radiation, and to improve image resolution. Originally, imaging devices used a highly collimated beam of X-rays, known as pencil beams, in conjunction with a single detector that moved in a raster pattern (i.e. in a series of thin parallel lines across the participant) (Figure 2.23). The pencil beam systems produced an image of high quality with minimal magnification but the scan takes a long time (Crabtree et al., 2007). Advances in imaging techniques have led to the developments of fan and cone beams. The fan beam system uses a slit collimator to generate a beam that diverges in two directions in conjunction with a linear array of detectors whereas the cone beam system does not use a collimator and has planar detectors (Crabtree et al., 2007). These systems can produce images with a higher resolution as a higher energy photon intensity and greater photon flux is used compared to the pencil beam system. This reduces the scanning time but a relatively higher radiation dose is a result and the images can be affected by magnification. The degree of magnification is lower in participants who are thin as the body is closer to the X-ray source compared to participants who are larger (Crabtree et al., 2007). A schematic
diagram of a DXA scanner and the differences between pencil, fan, and cone beams are illustrated in Figure 2.23.

Figure 2.23 - Illustrations of a DXA scanner and types of collimators used in imaging. Left, image shows the components of a DXA scanner with the radiation source located below the table and patient. The x-ray beam passes through the patient and is received by the detector above the patient. Right, (A) Pencil beam, (B) Fan beam, and (C) Cone beam. Source: Left diagram (IAEA, 2010) and right diagram (Ketcham, 2012).

2.5.3.1 DXA image analysis
The pixel-by-pixel attenuation data are converted into electronic signals to produce a two-dimensional (2D) image of the projected area. After acquisition, an edge-detection algorithm is used to calculate the bone area (BA) by locating the bone edge and summing the pixels within the bone perimeter. This is converted into areal BMD (aBMD, g/cm²) when values are compared with a bone mineral phantom (Ward et al., 2007b). BMC is calculated by multiplying mean aBMD by BA (Ward et al., 2007b).

2.5.3.2 DXA measurement sites and parameters
Clinically, the most common DXA measurement sites are the lumbar spine and hip in adults. Whereas in children, the lumbar spine and the total body (less head) are the most common (Figure 2.24). The choice of measurement site depends on the purpose of the scan. To assess BMD and fracture risk, measurements are taken at sites which are more prone to osteoporotic fractures (i.e. the lumbar spine and femoral neck, which are rich in trabecular bone). In osteoporosis, bone turnover increases,
especially in the trabecular compartment, therefore increasing bone fragility and increased fracture risk (IAEA, 2010). To assess body composition (bone, lean mass, and fat mass), a total body scan is required. The parameters and scan sites are summarised in Table 2.2.

![Figure 2.24 - The most common DXA sites.](image)

(A) Lumbar spine L1 to L4, (B) Femoral neck, (c) Forearm, and (D) Total body. Source: (IAEA, 2010).

### 2.5.3.3 Strengths and Limitations of DXA

The strengths and limitations of DXA are summarised in Table 2.7. A brief description of the main points are described below:

The main strengths of DXA:

1) **Radiation dose** – Participants are subjected to a low radiation dose in comparison to other imaging techniques and in comparison with daily background radiation.

2) **Scanning time** – The quality of the image is highly dependent on the participant remaining still throughout the scanning procedure. DXA techniques have improved the scanning time from 15 minutes to 2-3 minutes as modern DXA operates using a fan beam instead of pencil beam X-rays. A short scanning time has improved the quality of the images for participants who are very young, old, and those with medical conditions or learning difficulties as these groups may find it difficult to remain still for long periods of time.

3) **Measurements of whole body composition** – DXA is able to measure lean soft tissue mass (LSTM) (i.e. a surrogate for muscle) and FM. This technique allows the relationship between body composition and whole skeleton to be explored.
4) **Precision** – The precision of aBMD varies with a coefficient of variance (CV) of 1-3%. This allows relatively small changes in aBMD within individuals to be detected with confidence. Precision is machine, operator and site specific. During longitudinal studies, precision needs to be considered when assessing the changes in bone measurements.

5) **Available reference data for adults and children** – Bone mineral, size, and shape are known to be different between sexes, ethnic groups, and different stages of life. Therefore, it is essential that participants are compared to a suitable reference dataset to draw correct conclusions.

### Table 2.2 - Parameters measured by DXA.

<table>
<thead>
<tr>
<th>DXA parameter</th>
<th>Description of parameter</th>
<th>Measurement site</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone mineral content (BMC)</td>
<td>The mineral mass component of bone in the form of hydroxyapatite, and does not include the mass of any of the organic components of bone (marrow, collagen, etc.) (IAEA, 2010).</td>
<td>Lumbar spine, hip, forearm, and total body.</td>
</tr>
<tr>
<td>Bone area (BA)</td>
<td>The projected area of the bone onto the image plane, in cm² (IAEA, 2010).</td>
<td>Lumbar spine, hip, forearm, and total body.</td>
</tr>
<tr>
<td>Areal bone mineral density (aBMD)</td>
<td>The mineral mass of bone per unit image area in g/cm². aBMD = BMC/BA (g/cm²).</td>
<td>Lumbar spine, hip, forearm, and total body.</td>
</tr>
<tr>
<td>Bone mineral apparent density (BMAD)</td>
<td>A size adjusted measurement of aBMD to obtain a measure of vBMD. This measurement is important when assessing bone density in children or in patients with short stature (Carter et al., 1992)</td>
<td>Lumbar spine</td>
</tr>
<tr>
<td>Fat mass (FM)</td>
<td>The sum of all lipid mass including phospholipids, marrow, and subcutaneous adipose tissue. FM is measured in unit of g or kg (IAEA, 2010).</td>
<td>Total body</td>
</tr>
<tr>
<td>Lean mass (LM)</td>
<td>The sum of all soft lean tissue and excluding bone and fat. LM is measured in units of g or kg (IAEA, 2010).</td>
<td>Total body</td>
</tr>
<tr>
<td>Fat free mass (FFM)</td>
<td>The sum of all the non-body lipid (IAEA, 2010). FFM = LSTM +BMC (g)</td>
<td>Total body</td>
</tr>
<tr>
<td>Soft tissue mass (STM)</td>
<td>The sum of lean soft tissue and FMs (IAEA, 2010). STM = LSTM (g) + FM</td>
<td>Total body</td>
</tr>
<tr>
<td>Total body mass (TBM)</td>
<td>The equivalent measure to scale weight in g or kg. TBM = FM + FFM = FM + BMC + LSTM</td>
<td>Total body</td>
</tr>
<tr>
<td>Percent fat mass (PCTFM)</td>
<td>A regions FM divided by its total mass, multiplied by 100. PCTFM = FM / TBM X 100</td>
<td>Total body</td>
</tr>
</tbody>
</table>
The main limitations of DXA are:

1) **DXA measurements are size-dependent** – Measurement of BMC and aBMD are size dependent as these parameters are based on a 2D projection of a 3D structure. These measurements rely on the projected area of the bone and do not account for the depth (thickness) of the bone (Ward et al., 2007b). This can be detrimental when assessing children or patients with small statures (Warner et al., 1998, van Rijn and Van Kuijk, 2009). The rate and timing of growth in children and adolescents varies, therefore children of the same age may have different bone sizes, making comparisons difficult. Children with small bones will be reported to have a low aBMD (underestimated) and those with large bones to have a high aBMD (over-estimated) (Figure 2.25). With increasing awareness of these problems with BMC and aBMD, methods to determine vBMD (e.g. pQCT and HR-pQCT) have been used to overcome issues with size (Burrows et al., 2010a). vBMD is defined as the mass of mineral divided by the volume enclosed by the periosteal envelope (g/cm3), and therefore is not size-dependent. There are several methods to correct DXA data for size dependence (Carter et al., 1992)

2) **Bone geometry or microarchitecture cannot be assessed** – DXA can only measure bone mineral, aBMD, and projected bone area, which accounts for 70% of bone strength. DXA is unable to discriminate between cortical and trabecular bone. Therefore, it is not possible to determine whether a treatment or disease affects one bone compartment more than the other, which may be helpful in understanding disease aetiology.

3) **Changes in bone mineral and body composition on bone detection algorithms** – The attenuation of the X-ray beams and detection of the bone edge depends on the mineralisation of the bone and body thickness of the participant, which may cause some inaccuracies in the measurement (Crabtree et al., 2007). Thin individuals with small or under mineralised bones will not attenuate the X-ray beam (i.e. low X-ray attenuation), therefore BMD will be underestimated. In large or obese participants, the lean and fat tissues absorb more X-rays (i.e. high X-ray attenuation compared to thin participants), resulting in an over estimation of BMD and bone size due to the large projected area, and poor image quality with blurred outer bone edges (Figure 2.25).
2.5.4 Peripheral quantitative computed tomography (pQCT)

pQCT scanners first became commercially available in the early 1990s and measures vBMD at the peripheral skeletal sites. The most prominent part of the scanner is the gantry; a circular, rotating frame with an X-ray tube mounted on one side and a detector on the opposite side (Figure 2.26). During the scanning procedure, the participant’s limb is placed within the gantry, the rotating frame spins the X-ray tube and detector around the participant’s limb. A fan beam is used to generate a single 2D cross-sectional CT slice (1-2 mm thick) of the limb with a resolution of 0.2 – 0.8 mm (Burrows et al., 2010c). The CT scanner utilises the X-ray beam and produces an image based on the linear X-ray absorption coefficients of the tissues through which it passes. X-ray attenuation values are converted into Hounsfield units (HU) (i.e. water is 0 HU, air is -1000 HU, fat is -60 to -120 HU, and cortical bone is +1000 HU). Algorithms are used to reconstruct the attenuation data into 3D images. A hydroxyapatite phantom is used to calibrate the data, providing a measurement of vBMD that is independent of bone size. The image produced is a physiological cross-section of the bone, which allows the capacity for cortical and trabecular bone compartments to be assessed separately.
Figure 2.26 - pQCT scanner (Stratec XCT2000L®, Pforzheim, Germany).
The x-ray tube and the detector rotate around the gantry where the participant’s limb or calibration phantom is positioned. Source: (Writers, 2007).

2.5.4.1 pQCT image analysis
The manufacturer’s software is used to measure trabecular and cortical bone, muscle, and fat of each CT slice. The software has the ability to segregate cortical and trabecular bone by using varies modes, which are described below (Ferretti, 1999):

a) **ContMode** – separates the soft tissue from the outer edge of the bone. A pre-selected threshold is applied, which eliminates any voxels with an attenuation value below this threshold (i.e. the surrounding soft tissue is stripped away from bone).

b) **PeelMode** – separates the trabecular region from the cortical shell. This mode strips a selected proportion of the bone from the outside bone edge inwards and assigns this as cortical-subcortical. The remaining bone is classed as trabecular bone.

c) **CortMode** – separates the cortical bone from the subcortical bone to obtain a cortical bone value. The cortical shell is separated by applying a threshold, which peels away every voxel showing an attenuation value below the set threshold.

These modes define the trabecular and cortical regions of interest, which are then quantified by the software functions known as CalcBD and CortBD to measure total, trabecular, cortical-subcortical, cortical vBMD, and the CSA of the bone (Ferretti, 1999). Cortical thickness, periosteal and endosteal circumferences can be assessed using two different modes, non-circular mode and circular ring mode. The former assumes that the bone area is not circular and the latter assumes that bone is circular-shaped. The manufacturer recommends using data extracted from the circular ring mode to improve precision.
2.5.4.2 pQCT measurement sites and parameters

Unlike DXA, pQCT is able to measure bone at the metaphysis and the diaphysis regions of the radius and tibia. Therefore, site-specific measurements of trabecular and cortical bone can be assessed, which is important as the two bone compartments react differently during growth, physical exercise, disease state, medication, and ageing (Zemel et al., 2008, Ward et al., 2007b). The most common sites include a combination of 4%, 14%, 38%, and 66% of the tibia length and the 4%, 50%, and 65% of the radius length (Ward et al., 2007b). Trabecular bone is measured at the 4% site, cortical bone at 14%, 20%, 38%, 50% and 66% sites, and muscle and fat at the 66% and 65% sites.

The parameters and scan sites are summarised in Table 2.3. Parameters related to bending and torsional bone strength are derived at the mid-diaphyseal site and include stress-stain index (SSI), polar moment of inertia, and section modulus. These parameters are closely related and are defined in Figure 2.27. The polar moment of inertia is the distribution of bone mineral around the centre of the bone (i.e. the sum of the bone-filled voxel areas multiplied by the square of the distance between voxel and centre of bone) (21, 27). The section modulus is the ratio between the polar moment of inertia and the maximal distance of a bone-filled voxel from the centre. SSI is a combination of polar moment of inertia and vBMD of the cortex (21, 27).

![Figure 2.27 - Definition of bone strength parameters measured by pQCT.](image)

In the formulas: $A = \text{CSA of the voxel}$, $d = \text{distance of the voxel from the centre of gravity}$, $vBMD_{\text{vox}} = \text{vBMD in the voxel (mg/cm}^2\text{)}$, $d_{\text{max}} = \text{maximum distance of any voxels of the cortical cross-section from the centre of gravity}$, $vBMD_{\text{max}} = \text{maximum vBMD under physiological conditions (1200 mg/cm}^3\text{)}$. Source: (Rauch and Schoenau, 2008).
### Table 2.3 - Parameters measured by pQCT.

<table>
<thead>
<tr>
<th>pQCT parameter</th>
<th>Description of parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bone mineral parameters</strong></td>
<td></td>
</tr>
<tr>
<td>Total BMC (mg/mm)</td>
<td>Mass of mineral per unit of axial bone length (i.e. a BMC value of 80 mg/mm represents a bone CT slice of 1 mm thickness contains 80 mg of mineral).</td>
</tr>
<tr>
<td>Total vBMD (mg/cm³)</td>
<td>Total BMC divided by total CSA.</td>
</tr>
<tr>
<td>Trabecular BMC (mg)</td>
<td>The mean trabecular BMC of the 45% central area of the total CSA.</td>
</tr>
<tr>
<td>Trabecular vBMD (mg/cm³)</td>
<td>Usually measured as the mean BMD of the 45% central area of the total CSA. This includes some margin of safety to exclude admixture of cortical bone to the trabecular region of interest. The actual relative CSA of the trabecular compartment is larger than 45%, but the resolution of the pQCT system is not able to identify the endosteal border (Rauch and Schoenau, 2008). Depends on the peel mode settings.</td>
</tr>
<tr>
<td>Cortical BMC (mg/mm)</td>
<td>Total BMC minus Trabecular BMC.</td>
</tr>
<tr>
<td>Cortical vBMD (mg/cm³)</td>
<td>Total vBMD minus Trabecular vBMD.</td>
</tr>
<tr>
<td><strong>Geometric parameters</strong></td>
<td></td>
</tr>
<tr>
<td>Total CSA (mm²)</td>
<td>The surface area of the whole bone cross-section, including cortical and trabecular bone. This is directly measured.</td>
</tr>
<tr>
<td>Trabecular CSA (mm²)</td>
<td>Usually measured as 45% central area of the total CSA. Depends on the peel mode settings.</td>
</tr>
<tr>
<td>Cortical CSA (mm²)</td>
<td>The surface area of the cortical bone cross-section. This is equivalent to total CSA minus the cross-sectional size of the marrow cavity.</td>
</tr>
<tr>
<td>Cortical thickness (mm)</td>
<td>The average thickness of the cortical shell.</td>
</tr>
<tr>
<td>Marrow CSA (mm²)</td>
<td>Total CSA minus cortical shell (i.e. trabecular and bone marrow).</td>
</tr>
<tr>
<td>Periosteal circumference (mm)</td>
<td>The outer diameter of bone which is mathematically derived from total CSA by assuming total CSA is circular.</td>
</tr>
<tr>
<td>Endosteal circumference (mm)</td>
<td>The inner diameter of bone which is mathematically derived from trabecular CSA.</td>
</tr>
<tr>
<td>Cross-sectional moment of inertia [CSMI] (mm⁴)</td>
<td>A measure of bending strength (Figure 2.27).</td>
</tr>
<tr>
<td>Polar moment of inertia (mm⁴)</td>
<td>A measure of strength in torsion (Figure 2.27).</td>
</tr>
</tbody>
</table>
### pQCT parameter Description of parameter

<table>
<thead>
<tr>
<th>pQCT parameter</th>
<th>Description of parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Section modulus (mm$^3$)</td>
<td>Polar moment of inertia / max distance to the centroid. A measure of shearing strength (Figure 2.27).</td>
</tr>
<tr>
<td>Strain strength index [SSI] (mm$^3$)</td>
<td>The density-weighed polar section modulus of a cross-section and reflects the strength of the long bone with respect to torsion (Figure 2.27).</td>
</tr>
</tbody>
</table>

#### Muscle and fat parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle CSA (mm$^2$)</td>
<td>Measurement taken at the widest CSA of the limb and is a surrogate marker of muscle force. Bone area was subtracted from whole image.</td>
</tr>
<tr>
<td>Muscle mass</td>
<td>Bone mass and fat mass subtracted from whole image to derive muscle mass.</td>
</tr>
<tr>
<td>Muscle density (g)</td>
<td>Muscle density derived by dividing total muscle mass by MCSA.</td>
</tr>
<tr>
<td>Subcutaneous Fat (g)</td>
<td>Bone mass and muscle mass subtracted from whole image to derive subcutaneous fat mass.</td>
</tr>
</tbody>
</table>

### 2.5.4.3 Strengths and limitations of pQCT

The strengths and limitations of pQCT are summarised in Table 2.7. Brief descriptions of the main points are described below:

The main strength of pQCT are:

1) **Measurements are size-independent** – Unlike DXA, pQCT is able to measure vBMD. This measurement is a 3D physiologic cross-section of the bone, which overcomes difficulties when assessing children and patients with short stature.

2) **Measurements of bone size, geometry, and bone compartments can be measured** – Bone size and geometry have been found to differ between different populations and patient groups, which may help explain differences in bone strength and fracture risk (ISCD, 2013, Zemel et al., 2008). Trabecular and cortical bone play important roles in the prediction of bone strength and are affected by age, sex, and metabolic conditions and have a varying response to therapy (Burghardt et al., 2011).

3) **Measurements of muscle and fat** – pQCT is able to assess muscle and fat. Muscle CSA is used as a surrogate measurement for muscle strength and this has been used to assess the relationship between bone and muscle. Subcutaneous fat and fat within muscle (i.e. muscle density can also be assessed to understand limb composition).

4) **Measurements of bone strength** – Measurements of SSI and section modulus are highly associated with bone strength measurements carried out by three-point bending tests. Therefore, SSI and section modulus are good surrogates for bone strength in in vivo studies.
The limitations of pQCT are:

1) **Difficulty in positioning for longitudinal measurements** - pQCT can be challenging in children and adolescents as they are continuously growing. The bone presents a “moving target” as long bone length increases differentially at proximal and distal ends at different time points. Positioning of the reference line is extremely important as bone is heterogeneous. Small errors in positioning will mean that the site of interest is incorrect and will result in large errors, especially when measuring trabecular bone (Marjanovic et al., 2009).

2) **Only peripheral regions of the skeleton can be assessed** – pQCT is limited to the peripheral skeletal sites and provides no direct measure of bone strength at other skeletal sites (i.e. the lumbar spine or femoral neck, which are common sites for osteoporotic fractures in older people). However, there are some advantages of assessing the peripheral sites; 1) the most common site of fracture in children is at the distal radius which pQCT can measure vBMD and bone geometry and 2) cortical bone is assessed at the mid-diaphyseal site as this region is exposed to the greatest stress and strains, resulting in a thicker cortex.

3) **Long scanning time** – The total scanning time depends on the number of sites being measured. A single CT slice takes approximately 1 minute to be obtained. Usually, three or four sites are measured along the tibia length (total scan time = 3-5 minutes) and two sites along the radius (total scan time = 2-3 minutes). However, the time taken to correctly position the participant within the scanner, perform the scanogram (i.e. the initial short scan to locate the end plate and place the reference line), and then the scan, in total the procedure can take up to 10-15 minutes.

4) **Sensitive to participant movement** - Participant movement during scanning results in movement artefacts (refer to Chapter 4.3.2.2). The quality of the image is highly dependent on the participant’s age and maturity. The participant is required to remain still throughout the scanning procedure which can be difficult for young children (<8 years), the elderly (>80 years), and for patient groups with learning difficulties or involuntary body movements which can make it hard to remain still. Distraction techniques such as playing relaxing music, dimming the lights, or giving the participant something to do in silence (e.g. read a book) can help to reduce movement.

5) **Inconsistencies in pQCT methods** – With the introduction of DXA in 1988, the use of QCT had declined until recently (Ward et al., 2007b). pQCT has been widely used in research centres (e.g. Cologne, Manchester, Birmingham, Cambridge) and has not yet been accepted as a clinical technique for assessing bone strength (Adams et al., 2014). There are inconsistencies in pQCT methods therefore reference datasets differ in device settings and scanning sites of interest (Adams et al., 2014).
6) Partial volume effect (PVEs) - PVEs can be defined as an imaging artefact due to incomplete filling of voxels with bone tissue, resulting in an underestimation of vBMD (Figure 2.28) (Zemel et al., 2008). The attenuation values of a voxel depend on the content of bone and soft tissue within the voxel. Voxels that are close to the edge of the bone are likely to be comprised of both bone and soft tissue, resulting in a lower attenuation value compared to voxels that are located within the bone envelope. Participants with thin cortices (<2.5 mm) will have a higher proportion of voxels close to the edge of bone, which will be detected as non-bone (i.e. lower vBMD) and therefore are more likely to be affected by PVEs (Prevral et al., 1999). Using appropriate and consistent thresholds values with a CT voxel size of 0.4 mm can minimise error due to PVEs but it is important to consider PVE’s when interpreting cortical vBMD measurements (Ward et al., 2005a). Despite the limitations, vBMD measured by pQCT has been shown to closely correlate with ashed samples, area to whole samples, and the estimated biomechanical values are very similar to those reported in material testing (Ebbesen et al., 1999). Therefore, this technique provides good measures of parameters related to bone strength in-vivo.

![Figure 2.28 - A schematic diagram demonstrating the effect of PVEs.](image)

The voxels on the bone edge will contain bone and soft tissue, resulting in a lower attenuation and underestimated of vBMD. Source: (Zemel et al., 2008).
2.5.5 High resolution peripheral quantitative tomography (HR-pQCT)

HR-pQCT became commercially available in the mid-2000s as a powerful imaging technique, which allows direct and indirect evaluation of cortical and trabecular bone architecture. HR-pQCT acquires images based on the same principles as pQCT but can achieve a much higher resolution of 82 μm, which enables the assessment of parameters such as cortical porosity, trabecular number (Tb.N), trabecular thickness (Tb.Th) and trabecular separation (Tb.Sp) are possible with this technique.

Figure 2.29 - HR-pQCT scanner (Xtreme CT, SCANCO Medical AG, Basserdorf, Switzerland).

The HR-pQCT scanner consists of a 0.08 mm micro-focus X-ray source and high-resolution charge-coupled device detector (Figure 2.29), which rotate around the 15 cm length gantry. The participant’s limb is immobilised in a carbon fibre cast to correctly position the limb within the gantry and to reduce participant movement. The X-ray cone beam is able to rotate and move along the length of the immobilised limb producing a helical scanning pathway. The total scanning time is 3 minutes. Compared to HR-pQCT, pQCT has a lower resolution of 0.2 to 0.5 mm, only acquires one CT slice at each site of interest, and takes 1-2 minutes to obtain a CT slice (Lala et al., 2014). The standard HR-pQCT protocol utilises the following settings: an X-ray tube potential of 60 kVp, X-ray tube current of 95 mA, matrix size of 1536×1536 and slice thickness and in-plane voxel size of 82 μm (Cheung et al., 2013).
Figure 2.30 - Scanogram and 3D image of the distal tibia and distal radius of an adult.

(A) and (B) at distal tibia and (C) and (D) distal radius. The solid green line denotes the reference line and the area between the dotted lines is the scanning ROI. The landmark used for the tibia is the distal plafond and for the radius is the bisection of the end plate. (B and D) the 3D output image which clearly show the cortical and trabecular compartments. Sources: Scanograms from (Boyd, 2008) and 3D images from (Cheung et al., 2013).

2.5.5.1 HR-pQCT measurement sites - The standard acquisition protocol in adults

The HR-pQCT standard protocol was designed to measure bone microarchitecture at the distal tibia and radius in adults. A single scout projection image of the distal tibia or radius is acquired to define the tomographic scan region. This scout image is acquired at an oblique (45°) anterolateral-posteromedial orientation at the ankle and at an anterior posterior orientation at the wrist (Burghardt et al., 2011). For the tibia, the reference line is placed on the tibial plafond and the ROI is located 22.5 mm proximal from the reference line and for the radius, the reference line is placed on the through the end plate of the radius and the ROI is located 9.5 mm from the reference line, regardless of limb length (Figure 2.30 A and C) (Boyd, 2008, Cheung et al., 2013, Sekhon et al., 2009). The tomographic region spans 9.02 mm in length with 110 CT slices, each 82 µm thick. Using the manufacturer’s analysis software, the CT slices are collated to produce a 3D image of the bone (Figure 2.30 B and D) (Cheung et al., 2013).
2.5.5.2 HR-pQCT measurement sites - Adapted protocol for children and adolescents

Several studies have adapted the standard protocol to assess bone in children and adolescents and are summarised in Table 2.4. During growth, the bone presents a “moving target” as long bone length increases differentially at proximal and distal ends at different time points. Positioning of the reference line is extremely important as bone is heterogeneous. Small errors in positioning will mean that the site of interest is incorrect and will result in large errors, especially when measuring trabecular bone (Marjanovic et al., 2009). Therefore, the standard protocol has been adapted to account for bone size and length, and to avoid radiation exposure to the active growth plate.

Kirmani et al. and Chevalley et al. adapted the protocol to identify and place the reference line on the most proximal epiphysis, positioning the ROI 1 mm proximal to this reference line (Kirmani et al., 2009, Chevalley et al., 2011). This approach eliminates the need to measure bone length but introduces more difficulty associated with identifying the most proximal epiphysis and whether a distance of 1 mm from the epiphysis is sufficient to avoid radiation exposure to this area.

Burrows and McKay have reported that a 5% ROI site of the tibial length would miss the epiphyseal plate in approximately 94% of participants (aged 15 to 20 years), while an 8% ROI would miss the growth plate in 100% of participants (Burrows et al., 2010a). Therefore, Burrows and McKay developed a protocol to scan the 8% site of the tibia from the tibial plafond and the 7% site of the radius from the distal medial edge. The location of the ROI differs from the manufacturer’s standard protocol, with the tibia ROI on average 7 mm proximal from the standard protocol site and for the radius, on average 1.8 mm proximal from the standard protocol site (Liu et al., 2010). Other research groups have adapted the protocol to use a “relative” (i.e. percentage length from a fixed anatomical landmark) rather than a fixed distance (Burrows et al., 2010a, Burrows et al., 2010c, Nishiyama et al., 2012a). These protocols require the researcher to measure the participant’s limb length and adjust the scanning control files prior to scanning, setting the scanning ROI at the percentage distance.

Wang et al. placed the reference line on the proximal to the centre of the tibial joint surface of the ankle and the tibial ROI at the 7% of the lower leg length and for the radius, the reference line was placed on the proximal to the lateral edge of the radial joint surface of the wrist and the radius ROI at the 4% of the forearm length (Wang et al., 2011, Wang et al., 2010).
Table 2.4 - Summary of HR-pQCT protocols for assessing bone in children and adolescents.
Source: Modified from (Adams et al., 2014).

<table>
<thead>
<tr>
<th>Authors (Year)</th>
<th>Study population</th>
<th>Age (Years)</th>
<th>Position of reference line</th>
<th>Scanning ROI</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Kirmani et al., 2009)</td>
<td>USA M &amp; F (n = 127)</td>
<td>6 - 21</td>
<td>Unfused: Identify the most proximal epiphyseal plate; Fused: use epiphyseal line</td>
<td>Place the ROI 1 mm proximal to the reference line.</td>
</tr>
<tr>
<td>(Chevalley et al., 2011)</td>
<td>Switzerland M (n = 176)</td>
<td>7 - 15</td>
<td>Unfused: Identify the most proximal epiphyseal; If the radial epiphyseal plates have fused but the remnant of the plate is still visible, the reference line should be placed from the proximal epiphyseal.</td>
<td>Place the ROI 1 mm proximal to the reference line.</td>
</tr>
<tr>
<td>(Wang et al., 2010)</td>
<td>Australia M &amp; F (n = 130)</td>
<td>5 - 18</td>
<td>Proximal to the lateral edge of the radial joint surface of the wrist.</td>
<td>Proximal to the centre of the tibial joint surface of the ankle. 4% of the forearm length.</td>
</tr>
<tr>
<td>(Wang et al., 2011)</td>
<td>Australia F (n = 133)</td>
<td>7 - 20</td>
<td>Proximal to the lateral edge of the radial joint surface of the wrist.</td>
<td>Proximal to the centre of the tibial joint surface of the ankle. 7% of the tibial length.</td>
</tr>
<tr>
<td>(Burrows et al., 2010a)</td>
<td>Canada M &amp; F (n = 278)</td>
<td>15 - 20</td>
<td>Tibial plafond.</td>
<td>8% of the tibial length.</td>
</tr>
<tr>
<td>(Burrows et al., 2010c)</td>
<td>Canada M &amp; F (n = 328)</td>
<td>9 - 21</td>
<td>Distal medial edge of radius.</td>
<td>7% of the radius length.</td>
</tr>
<tr>
<td>(Liu et al., 2010)</td>
<td>Canada M &amp; F (n = 323)</td>
<td>9 - 21</td>
<td>Tibial plafond.</td>
<td>8% of the tibial length and 7% of the radius length.</td>
</tr>
<tr>
<td>(Nishiyama et al., 2012a)</td>
<td>Canada M &amp; F (n = 398)</td>
<td>9 - 22</td>
<td>Tibial plafond.</td>
<td>8% of the tibial length and 7% of the radius length.</td>
</tr>
</tbody>
</table>
2.5.5.3 HR-pQCT image analysis

Image analysis occurs in two stages; 1) Standard imaging analysis for assessing bone density and trabecular microarchitecture, and 2) An additional cortical imaging analysis for assessing cortical density and microarchitecture.

1) Standard imaging analysis

The manufacturer’s standard software is used to analysis each CT slice. First, a contour line is applied around the periosteal boundary of the cortex in the first CT slice (most distal slice) to separate the bone from the surrounding soft tissue. The software runs a semi-automatic contour script which applies a contour line to all CT slices within the stack. Each CT slice is manually checked and amended if required. To segment the cortical and trabecular regions, an automatic segmentation algorithm is implemented. The threshold used to discriminate cortical from trabecular bone is based on the assumption that trabecular bone is one-third of the apparent cortical vBMD (Burrows et al., 2010b, Burrows et al., 2010c). The CT slices are converted into grey scale images (binarised) and the cortex is segmented from the trabecular bone by using a Gaussian filter and threshold. The Gaussian filter is used to remove thin trabeculae and noise from the images. The cortical and trabecular compartments are then analysed separately.

The linear attenuation values of the CT slices are converted into hydroxyapatite mineral densities using a calibration phantom and a beam-hardening correction (Chapter 2.5.5.5). The phantom consists of five cylindrical rods containing a mixture of hydroxyapatite (densities of 0, 100, 200, 400, and 800 mg HA/cm$^3$) and resin (a soft-tissue equivalent) (Boutroy et al., 2005). This allows whole, cortical, and trabecular vBMD to be measured directly. Total bone area (Tt.Ar) is calculated by averaging the total area of each CT slice. Cortical thickness (Ct.Th) is calculated as mean cortical volume divided by the periosteal surface.

The assessment of trabecular microarchitecture can be challenging as the average Tb.Th is between 100 to 150 µm (i.e. approximately 1-2 voxels). PVEs can occur if the bone is under mineralised, inducing inaccuracies. It is important to note that in the first generation scanners, all trabecular parameters with the exception of Tb.N are derived. Tb.N is calculated by using a 3D ridge extraction algorithm which measures the inter-trabecular distances between the “centre points of the trabeculae” (i.e. ridges), by placing a sphere between the ridges (Figure 2.31) (Laib et al., 1997, Laib and Rüegsegger, 1999). Tb.N is defined as the inverse of the mean spacing of the mid-axes and does not depend on the assumptions of the plate-and-rod model (Boutroy et al., 2005). The HR-pQCT measurements of cortical and trabecular bone are summarised in Table 2.5.
Figure 2.31 - Schematic diagram of HR-pQCT trabecular measurements.
Tb.N is calculated by placing a sphere between two trabecular ridges and measures the inter-trabecular distance. The black arrow denotes Tb.N, blue is Tb.Sp, and red is Tb.Th. Source: Modified from (Laib and Rüegsegger, 1999).

2) An additional cortical imaging analysis – To assess cortical microarchitecture, Burghardt et al. developed an additional cortical imaging script with the aim to measure cortical density, thickness, and porosity (Burghardt et al., 2010). Like the standard protocol, a semi-automatic contour algorithm is performed to locate the periosteal surface but then a contour is also placed within the bone to locate the endosteal surface. The image analysis consists of three stages, which are briefly described below;

a) Cortical compartment segmentation – A Laplace-Hamming filter is applied to enhance the image. An automated contour algorithm is used to segregate the cortex from the surrounding soft-tissue, trabecular bone, and marrow cavity. To identify the surface of the periosteum, a 3D morphological dilation (15 voxels, 2.46 mm) is performed to close all cortical pores and smooth the periosteum surface (Burghardt et al., 2010). To identify the surface of the endosteum, the binarised image is inverted and a 2D connectivity algorithm is used to locate the connecting trabecular-network. Thin trabeculae with a thickness of less than 2 voxels thick are removed and then a dilation step occurs to smooth the endosteal surface (Burghardt et al., 2010).

b) Cortical porosity segmentation – A mask is applied to the binary image to identify the background voxels (i.e. voxels which contain no bone), which may represent Haversian canals and other voids, are identified within the cortical shell. A 2D connectivity algorithm is used to identify the connectivity between the background voxels. Voids with a volume smaller than 5 mutually connected voxels are discarded and filled in as these voids are too small to be Haversian canals and may represent noise (Burghardt et al., 2010).
c) **Cortical mask refinement** – The intracortical porosity segmentation (generated in stage 2) is subtracted from the cortical shell (generated in stage 1) to produce a refined image of the cortical compartment. The periosteum and endosteum surfaces can be identified and measured in finer detail compared to the standard imaging analysis (Burghardt et al., 2010).

2.5.5.4 **HR-pQCT measurement parameters**

HR-pQCT is a powerful imaging tool used to measure bone density and bone microarchitecture at the distal tibia and radius. Multiple bone parameters can be measured by HR-pQCT and are summarised in Table 2.5 and Table 2.6.

**Table 2.5 - Summary of HR-pQCT parameters from standard analysis.**

Sources: (Boutroy et al., 2005), (Burghardt et al., 2010), and (Liu et al., 2010).

<table>
<thead>
<tr>
<th>Bone parameters</th>
<th>Description of parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Area</strong></td>
<td></td>
</tr>
<tr>
<td>Total bone area (Tt.Ar, mm$^2$)</td>
<td>Calculated as an average of total area defined by the imaging contours of the middle 104 slices analysed (the first three and last three slices are excluded from the analysis as per the manufacturer’s default). Tt.Ar includes all bone tissue and marrow contained within the periosteal envelope.</td>
</tr>
<tr>
<td>Cortical area (CtAr, mm$^2$)</td>
<td>Tt.Ar minus trabecular area.</td>
</tr>
<tr>
<td>Trabecular bone area (Tb.Ar, mm$^2$)</td>
<td>Tt.Ar minus cortical area.</td>
</tr>
<tr>
<td><strong>Densities</strong></td>
<td></td>
</tr>
<tr>
<td>Total vBMD (D100, mg HA/cm$^3$)</td>
<td>Representing both trabecular and cortical compartments and quantified based on the periosteal segmentation. The mean value of all voxels within the whole bone.</td>
</tr>
<tr>
<td>Cortical vBMD (DComp1, mg HA/cm$^3$)</td>
<td>Whole bone minus trabecular compartment and is a direct measure</td>
</tr>
<tr>
<td>Trabecular bone volume to tissue volume (BV/TV)</td>
<td>Derived from trabecular density assuming fully mineralised bone to have a mineral density of 1.2 g HA/cm$^3$ (i.e. BV/TV (%) = 100 x (Dtrab (mg HA/cm$^3$)/1200 mg HA/cm$^3$).</td>
</tr>
<tr>
<td><strong>Structure</strong></td>
<td></td>
</tr>
<tr>
<td>Trabecular number (Tb.N, mm$^{-1}$)</td>
<td>The inverse of the mean spacing of the mid-axes. Does not depend on a priori assumptions regarding the plate- or rod-like nature of the underlying structure.</td>
</tr>
<tr>
<td>Trabecular thickness (Tb.Th, mm)</td>
<td>Derived from BV/TV and TbN$^<em>$ using standard methods from histomorphometry (i.e. TbTh = (BV/TV) /TbN$^</em>$).</td>
</tr>
<tr>
<td>Trabecular separation (Tb.Sp, mm)</td>
<td>Derived from BV/TV and TbN$^<em>$ using standard methods from histomorphometry (i.e. TbSp = (1 - BV/TV)/TbN$^</em>$).</td>
</tr>
<tr>
<td>Cortical thickness (Ct.Th, mm)</td>
<td>Defined as the mean cortical volume divided by the outer bone surface.</td>
</tr>
</tbody>
</table>
Table 2.6 - Summary of HR-pQCT parameters from segmentation algorithm analysis.
Sources: (Boutroy et al., 2005), (Burghardt et al., 2010), and (Liu et al., 2010).

<table>
<thead>
<tr>
<th>Bone parameters</th>
<th>Description of parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Volume</strong></td>
<td></td>
</tr>
<tr>
<td>Cortical total volume (Ct.TV, mm$^3$)</td>
<td>The volume of all voxels (i.e. bone and pore) contained within the cortical volume of interest.</td>
</tr>
<tr>
<td>Cortical bone volume (Ct.BV, mm$^3$)</td>
<td>The volume of all bone voxels within the cortical volume of interest.</td>
</tr>
<tr>
<td><strong>Area</strong></td>
<td></td>
</tr>
<tr>
<td>Total bone area (Tt.Ar, mm$^2$)</td>
<td>The average cross-sectional area of the whole bone circumscribed by the periosteal contour.</td>
</tr>
<tr>
<td>Cortical bone area (Ct.Ar, mm$^2$)</td>
<td>The average cross-sectional area of the cortical compartment between the periosteal and endosteal contours.</td>
</tr>
<tr>
<td>Trabecular bone area (Tb.Ar, mm$^2$)</td>
<td>The average cross-sectional area of the trabecular compartment circumscribed by the endosteal contour.</td>
</tr>
<tr>
<td><strong>Density</strong></td>
<td></td>
</tr>
<tr>
<td>Cortical bone mineral density (Ct.BMD, mg HA/cm$^3$)</td>
<td>Mean mineralisation of the cortical volume of interest.</td>
</tr>
<tr>
<td>Cortical tissue mineral density (Ct.TMD, mg HA/cm$^3$)</td>
<td>Mean mineralization of the segmented cortical bone voxels after exclusion of pores.</td>
</tr>
<tr>
<td><strong>Structure</strong></td>
<td></td>
</tr>
<tr>
<td>Cortical thickness (Ct.Th, mm)</td>
<td>Mean cortical thickness.</td>
</tr>
<tr>
<td>Cortical pore volume (Ct.Po.V, mm$^3$)</td>
<td>Direct voxel-based measure of the volume of the intracortical pore space.</td>
</tr>
<tr>
<td>Cortical porosity (Ct.Po, %)</td>
<td>Relative voxel-based measure of the volume of the intracortical pore space normalised by the sum of the pore and cortical bone volume.</td>
</tr>
</tbody>
</table>

2.5.5.5  The strengths and limitations of HR-pQCT
The strengths and limitations of HR-pQCT are summarised in Table 2.7. Brief descriptions of the main points are described below. The main strengths of HR-pQCT are:

1) **Measurements are size-independent** – Like pQCT, vBMD is measured (Refer to Section Strengths and limitations of pQCT).

2) **Measurements of bone microarchitecture** – The assessment and quantification of trabecular bone by pQCT is limited due to the resolution. HR-pQCT has an extremely high resolution of 82 µm which is able to measure trabecular structures and cortical porosity, therefore, providing a greater insight into bone microarchitecture and bone strength.

3) **Precision** – Boutroy et al. reported the precision of HR-pQCT measurements to be between 0.7-1.5% for total, trabecular, and cortical densities and 2.5-4.4% for trabecular microarchitecture.
(Boutroy et al., 2005). Burghardt et al. reported cortical porosity to be between 6.0-13.5% (Burghardt et al., 2010).

The main limitations of HR-pQCT are:

1) Sensitive to participant movement – HR-pQCT is extremely sensitive to participant movement. Movement artefacts can result in inaccuracies in bone measurements. It is essential that the participant is made as comfortable as possible and instructed to remain still throughout the scanning procedure. Images of movement artefacts are shown in Figure 4.15.

2) The scanner can only scan the distal regions of the peripheral skeleton – The scanning region is limited to the distal regions of the peripheral skeleton (i.e. ankle and wrist) as the gantry is only 15 cm in length. Although conventional pQCT is also limited to the peripheral skeleton, sites at the diaphysis and metaphysis can be measured by HR-pQCT.

3) Difficulty in positioning for longitudinal measurements – Assessing growing bone longitudinally is difficult and error is exacerbated by using a fixed distance from the reference line. For example, consider an average 10-year-old boy whose tibial length is 312 mm. The fixed 22.5 cm distance from the distal tibial represents a site 7% of the total tibial length. Compare this to an average 17-year-old boy whose tibial length is 410 mm. The fixed distance represents a measurement site 5% of the total tibial length (Burrows et al., 2010a). Therefore, the same region of bone is not measured and cannot be compared.

4) Bone parameters are measured indirectly - With the current version of HR-pQCT, it is not possible to directly measure all microarchitecture parameters. While the reconstructed voxel size is 82 μm, the actual spatial resolution of the image is approximately 130 μm near the centre of the field of view, and less off-centre (140–160 μm) (Cheung et al., 2013). Consequently, structures less than 100 μm (i.e. trabecular thickness), are not typically resolved from in vivo images (Cheung et al., 2013). Therefore, these parameters need to be derived from other measurements.

5) PVEs – Measuring trabecular microarchitecture can be challenging due to PVEs, therefore, resulting in an underestimation of trabecular bone parameters. Refer to Chapter 2.5.4.3 and Figure 2.28.

6) Ring artefacts – Ring artefacts are prominent in third generation CT scanners which use multiple detectors. If one of the detectors is out of calibration, the detector will give a consistently inaccurate reading at each angular position, creating a ring artefact (Barrett and Keat, 2004).
7) **Beam-hardening effect** – Beam-hardening results from preferential attenuation of low-energy radiation, which alters (hardens) the energy spectrum of the beam as it passes through the limb, shifting the energy spectrum towards a higher energy photons (Sekhon et al., 2009). The image becomes distorted as it causes the edges of the bone to appear brighter than the centre, even if the material is the same throughout (Ketcham and Carlson, 2001). vBMD varies between the cortex and trabecular compartments therefore it is difficult to differentiate between beam hardening artefacts and actual material variations. This can have a significant impact on geometric and densitometric measurements (Sekhon et al., 2009, Sidky et al., 2004, Barrett and Keat, 2004).

8) **Limb composition cannot be assessed** – The scanner was designed to only measure bone. The high resolution is used to assess the cortical and trabecular compartments. Currently, there are no imaging scripts to assess soft-tissue using this scanner.

![Image analysis](image.png)

**Figure 2.32 - HR-pQCT image analysis.**
The software applies a semi-automatic contour line around the perimeter of the endosteal border. However, the contours may deviate from the apparent endosteal margin, minor semi-manual adjustments are required to correct. Source: (Burghardt et al., 2010).

9) **Incorrect contouring** – During the analysis, the software applies a semi-automated contour algorithm to select the outer cortical edge of the whole bone. Contour algorithms are designed to reduce the contouring time and are based on the assumption that the bone is oval shaped and has a complete outer cortex. However, the contouring algorithm does not always perform as desired and may attach contours to unwanted regions (**Figure 2.32**) (Buie et al., 2007). This is a common problem in participants with a thin (<2 mm), porous cortex as the automated contour will pass through the cortical pore and select trabecular bone. To overcome this issue, the researcher must verify each slice to ensure that the correct contour has been selected and if not, the researcher must amend.
This amendment can introduce more errors, affecting precision and bias. Therefore, all scans should ideally be analysed by one researcher (Buie et al., 2007).

2.5.6 Summary of strengths and limitations of imaging techniques
A summary of the main advantages and limitations of bone densitometry technique used in this study are described in Table 2.7.

Table 2.7 - A summary of the main advantages and limitations of bone densitometry technique used in this study.
Source: Adapted from (Ward et al., 2007b).

<table>
<thead>
<tr>
<th>Densitometry Technique</th>
<th>Advantages</th>
<th>Limitations</th>
<th>Radiation*</th>
</tr>
</thead>
</table>
| DXA                    | 1. Rapid scan time.  
2. Low cost.  
3. High precision.  
4. Low ionising radiation.  
5. Clinical ‘gold standard’.  
6. Availability of paediatric reference data.  
2. Sensitivity to body composition changes.  
4. Unable to quantify trabecular and cortical bone characteristics. | <1µSv per site. |
| pQCT                   | 1. Size independent.  
2. Low radiation.  
3. Separate measurements of cortical and trabecular bone.  
4. Measures geometry.  
5. Measures of bone strength.  
7. Low radiation dose.  
8. Low cost. | 1. Long scanning time.  
2. Only applicable to peripheral sites.  
3. Sensitive to movement.  
4. More commonly used as a research tool.  
5. Limited paediatric reference data.  
6. Imaging artefacts.  
7. PVEs.  
8. Reproducibility of scan location. | <0.2 µSv per site (including scout view). |
| HR-pQCT                | 1. Same advantages as 1-5 for pQCT.  
2. Very detailed images due to high resolution (82µm and in plane spatial is 130µm).  
3. Imaging of trabecular bone structure is feasible. | 1. Same limitations as 1-6 for pQCT.  
2. Imaging artefacts.  
3. PVEs.  
4. High cost and maintenance.  
5. No paediatric reference data. | <3 µSv per site. |

Key: *The radiation exposure from these scanning techniques are very low. These figures should be compared to natural background radiation in Cambridge, UK, of 7 µSv per day or a return transatlantic flight, of 80 µSv (England, 2011).
2.5.7 Muscle assessment techniques

Until recently, the interaction between bone and muscle had received little attention due to methodological difficulties in quantifying muscle phenotype and strength. Muscle strength cannot be directly measured in-vivo without using invasive methods such as force transducer implants. Instead, two proxy markers of intrinsic muscle strength can be used: a) muscle size/mass (volume, cross-sectional area or lean tissue mass) and/or b) torque or ground reaction force.

2.5.7.1 Muscle strength assessed by muscle size

The use of muscle size as a surrogate of maximum intrinsic muscle force and power is limited. Techniques such as DXA and pQCT imaging, which are able to measure lean mass/muscle area as a proxy for muscle strength, are easy to use, non-invasive, good precision and have a large reference datasets.

DXA - DXA can measure total body composition by measuring LSTM, FM, and bone from a two dimensional image. LSTM measurements are based on the assumption that the hydration of fat-free mass remains constant at 73%. However, hydration can vary from 67%–85%, and can be variable in certain disease states and therefore not a reliable measurement when comparing different groups.

pQCT - pQCT can be used to assess muscle CSA. The CSA of muscle is measured by subtracting the bone CSA from the combined muscle and bone CSA. Mean muscle density can then be calculated from the muscle CSA. Although, pQCT provides skeletal measures along with muscle measures, the sensitivity of measuring muscle CSA has been questioned. In adults, CSA has not been consistently been shown to differ with age, whereas functional tests (e.g. chair raising and jumping mechanography) have shown a decline in muscle force (Ward, 2012). In research, it is essential that new technologies are critically assessed in order to interpret the data correctly. However, neither DXA nor pQCT provide information about the composition or structure of the muscle (i.e. muscle fibre length and diameter), intramuscular fat, organs, or neurological function making these new techniques of relevance to study.

2.5.7.2 Muscle strength assessed by muscle contraction

Several techniques using dynamometers have been developed. Skeletal muscle strength can be assessed from isometric (no movement), dynamic (movement against fixed resistance) or isokinetic (movement against fixed angle speed) muscle contractions (Saey and Troosters, 2008).

Muscle mass and structure are key determinants in how much force and power a muscle can generate. Muscle force is the measure of how much load is applied to bone, whereas power is a measure of function (Ward, 2012). Muscle function tests are very specific to the muscle group tested, the type of contraction, the velocity of the muscle motion, the type of equipment and the joint range.
of motion (Saey and Troosters, 2008). Therefore, results of any test are specific to the procedure being used.

2.5.7.3 Jumping mechanography
Dynamic muscle function of the lower limbs can be assessed using a Leonardo Mechanography® Ground Reaction Force Plate (Novotec Medical, Pforzheim, Germany). This is a force platform with a length of 66 cm, a width of 66 cm and a height of 7 cm (Figure 2.33). The platform is composed of two symmetrical force plates that separate the platform into a left and a right half. The force plate has eight sensors (four on each side), with each sensor recording force at a sampling frequency of 800 Hz. Force is detected by a deformation of the detector that is proportional to the applied force. The detector changes its electrical resistance proportionally to the deformation, the change of force is measured over time is recorded by the computer.

Figure 2.33 - Leonardo Mechanography® Ground Reaction Force Plate.

2.5.7.4 Jumping mechanography tests and measurements
The two most common tests carried out in paediatric studies are the single two legged jump (S2LJ) and the multiple one legged hop (M1LH) (Fricke et al., 2008, Rittweger et al., 2004). Briefly, the S2LJ involves the participant to perform a counter-movement jump to achieve maximum jump height (Figure 2.34) and the M1LH involves the participant hopping on the forefoot as hard and as fast as possible (Figure 2.35). These tests are able to assess a number of important parameters, which are described in Table 2.8.
Table 2.8 - Summary of jumping mechanography parameters for the single two legged jump (S2LJ) and the multiple one legged hop (M1LH) tests.
Source: (Veilleux and Rauch, 2010)

<table>
<thead>
<tr>
<th>Muscle parameters</th>
<th>Description of parameter</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak power (P.max, watts/kg)</td>
<td>The amount of energy stored and released during a muscle contraction to propel the body vertically in a jump.</td>
<td>S2LJ</td>
</tr>
<tr>
<td>Esslinger fitness index (EFI %)</td>
<td>This value compares the participant’s measurement to a healthy German reference dataset. A value of 100% is equal to the statistical mean value of the same age and sex group.</td>
<td>S2LJ</td>
</tr>
<tr>
<td>Jump height (max E pot, m)</td>
<td>The jump height is proportional to the potential energy of the lifted body. Kinetic energy is transferred into potential energy when the participant leaves the plate, which is then used to calculate jump height.</td>
<td></td>
</tr>
<tr>
<td>Jump velocity (V.max)</td>
<td>V.max = acceleration multiplied by time.</td>
<td>S2LJ</td>
</tr>
<tr>
<td>Peak force (F.max, Newtons/g)</td>
<td>Typically approx 3 times the body weight and is limited by ligaments. Fmax = mass multiplied by acceleration</td>
<td>M1LH</td>
</tr>
</tbody>
</table>

The strengths and limitations of jumping mechanography are summarised in Table 2.9. Brief descriptions of the main points are described below. The main strengths of jumping mechanography are:

1) **High reproducibility** - Low variability in inter-day test-retest assessment of the main outcome parameters (varying 3.4-7.5% in healthy children (Veilleux and Rauch, 2010)). Therefore could be a potential method for screening musculoskeletal impairment (Sumnik et al., 2013).

2) **User friendly** – The plate and software are relatively cheap, easy to use, and relatively portable in comparison to dyna-metric techniques.

3) **Natural body measurements** – Jumping and hopping are normal body movements in most populations and therefore the measurements reflect everyday forces experienced on the body.

The main limitations of jumping mechanography are:

1) **Participant’s motivation** - Validity and reliability is highly dependent upon the motivation of the participant performing the maximum voluntary contraction.
2) Lack of paediatric reference data - The use and correct interpretation of any method in children and adolescents relies on the availability of appropriate reference data matched for sex, age, and weight. The original reference dataset was derived from 312 German Caucasian children and adolescents (F = 177 and M = 135) (Fricke et al., 2006), which only published data on single two legged jump (S2LJ) and subjects were not equally distributed by age, and some age groups were under represented. Sumnik et al. created a large paediatric reference dataset for S2LJ and one legged hopping, subjects were Caucasian from Czech Republic, 796 (F = 432 and M = 364), aged 6 – 19 yrs (Sumnik et al., 2013).

2.5.7.5 Summary of advantages and limitations of muscle assessment techniques
A summary of the main advantages and limitations of bone densitometry technique used in this study are described in Table 2.9.

Table 2.9 - A summary of the main advantages and limitations of each muscle assessment technique.

<table>
<thead>
<tr>
<th>Muscle Assessment Technique</th>
<th>Advantages</th>
<th>Limitations</th>
</tr>
</thead>
</table>
| Jumping mechanography       | 1. Measures lower body muscle function in the legs.  
                              2. Good precision.  
                              3. Reference data are available. | 1. Trained operator.  
                              2.Protocols can differ between studies.  
                              3. Can only be used for volunteers who are able to jump. |
| DXA                         | 1. Measures LBM which can be crude measure for muscle mass.  
                              2. Reference data are available.  
                              3. Good precision. | 1. Does not give an accurate measure of muscle and includes organs and all soft-tissue in the body. |
| pQCT                        | 1. Measure CSA of muscle.  
                              2. Measures muscle density.  
                              3. Reference data are available.  
                              2. Peripheral scanning only. |
Figure 2.34 - Single two-legged jump.
The jumping mechanography automatically calculates jump force-time, speed-time, and power-time. (A) At rest, (B) Lowest point of the counter-movement, (C) Take-off, (D) Highest point of jump, (E-F) landing, (G) At rest. The graph lines represent; blue = Force, green =Velocity, and red = Power. Source: (Veilleux and Rauch, 2010).

Figure 2.35 - Multiple one-legged hopping.
Ten hops on one forefoot and with stiff knees are recorded. A graph showing the process of one hop (A) Take-off, (B) Highest point of the hop, (C) Landing, (D) Lowest point after landing. The heel should not touch the platform (as highlighted by the red wedge between the heel and the plate), (E) Take-off, (F) Highest point of the hop, (G) Landing. The asterisk on the Force-Time curve indicates max force for this test. Source: (Veilleux and Rauch, 2010).
2.6 Growth and bone development

2.6.1 Stages of growth

There are three stages of postnatal growth; 1) infancy, 2) pre-puberty, and 3) puberty. Each stage is determined by different factors which affect growth rate and bone development.

2.6.1.1 Growth during infancy and childhood (Pre-pubertal growth)

At birth, an infant’s size is more dependent on maternal nutrition and intrauterine and placental factors than genetic makeup (Rogol et al., 2000). At this early stage of life, growth velocity is slightly slower in females compared to males, therefore males have a longer total body length and are heavier compared to females (Ireland et al., 2014a, Tanner, 1989). During pre-puberty, growth velocity and weight gain of both sexes increases at similar rates, with an average rate of 5-6 cm/year for height and 2.5 kg/year for weight (Tanner, 1989). Appendicular growth velocity is twice that of axial growth velocity from 1 year of age and continues until puberty. Therefore, the majority of growth in height before puberty is driven proportionally more by the rapid growth of the legs than trunk (Seeman, 2002). At puberty, axial skeletal growth predominates.

Rogol et al. suggested that pre-pubertal growth in both sexes, based on an American Caucasian population, follows a general growth pattern (i.e. a child usually grows on average 25 cm in the first year of life, half that in the second year (12-13 cm), and then 5-6 cm each year until puberty) (Rogol et al., 2000). However, many studies have reported growth as non-linear and that some children experience a mid-growth spurt between the ages of 6 to 7 years (Tillmann et al., 1998, Wales and Gibson, 1994, Hermanussen et al., 1988). Thalange et al. reported growth in children between the ages of 5 to 8 years to be a biphasic process comprising intense growth spurts, which last an average 8 weeks, separated by periods of slow growth or stasis over 2 to 3 weeks. Over a one year period, some children had experienced 3 to 6 unpredictable growth spurts (Thalange et al., 1996).

During pre-puberty, growth is controlled by thyroid hormone (TH), growth hormone (GH), and insulin-like growth factor 1 (IGF-1). These hormones play a vital role in muscle tissue growth via protein synthesis and glycosylation. IGF-1 promotes the growth of connective tissue, cartilage, and bone through the stimulation of cartilage growth and the formation of collagen (Wang and Seeman, 2013).

2.6.1.2 Growth during puberty

Puberty, the transition between childhood and adulthood, is a dynamic period of development marked by rapid changes in body size, shape, and composition, all of which are sexually dimorphic (Rogol et al., 2002). Puberty takes place in several sequential stages within each sex. These steps are known as Tanner stages (TS), which help to define an adolescent’s pubertal status and are associated with the release of sex hormones; oestrogen in females and testosterone in males (Marshall and
Tanner staging is the most common method to identify the pubertal status of a child or adolescent and ranges from stages one to five (i.e. TS1 = pre-pubertal, TS5 = end stage of puberty). The physical assessment is different for males and females; for males, pubic hair, testicular volume, and age of voice break, and for females; pubic hair, breast development, and age of menarche (refer to Appendices 8.17 and 8.18). At TS1 (known as adrenarche), the body starts to produce adrenal androgens in males and females, which are responsible for body odour and pubic hair development. This usually occurs two years before end of puberty or before TS2.

As children progress into puberty, sexual maturation begins to occur. In females, breast development (thelarche) occurs between 8-12 years (TS2), which is then followed by menarche two years later (11-13 years, TS3-TS4) (Rogol et al., 2002). In males, pubertal maturation is more difficult to distinguish as changes in penis and testicular size/ volume are much less overt. Testicular volume increases from 3-4 ml in pre-pubertal males (11-12 years, TS1-TS2) to 20-25 ml by the end of puberty (TS4-TS5) (Marshall, 1975). Sperm production and ejaculatory capability are present early in puberty but do not correlate well with testicular size (Rogol et al., 2002).

2.6.2 Bone growth and development in children and adolescents

2.6.2.1 Peak bone mass

Peak bone mass (PBM) can be defined as the volume of bone mineral at the end of skeletal maturation and is a key determinant in skeletal health throughout the life course (Bonjour et al., 1994). Childhood and adolescence is a crucial time for maximising bone mass as the skeleton continuously grows through modelling and remodelling processes, resulting in increased bone size and mineral content, as described in Chapter 2.6.

Osteoporotic fractures are associated with reduced aBMD, which can result from failure to achieve PBM and/or from age-related changes (Javaid and Cooper*, 2002). Achieving PBM is essential for bone strength in later life as bone mass has been reported to track throughout life (Kalkwarf et al., 2010, Cooper et al., 1997, Wren et al., 2014). Ferrari et al. reported familial resemblance for BMC, aBMD, and bone size (measured by DXA) between pre-pubertal daughters and their pre-menopausal mothers (Ferrari et al., 1998). The authors concluded that an individual with a high BMC and aBMD during early life (i.e. high end of normal population distribution) is likely to have a high BMC and aBMD during the later stages of life and that there is a genetic component (Cooper et al., 2006). In the British Birth Cohort study followed to age 64 years, positive associations between birth weight and height growth with bone size, amount of cortical bone, and strength were reported, which suggests that intrauterine conditions and early growth have an effect on bone health in later life (Kuh et al., 2014). Pre-set factors such as genetics, sex, and modifiable environmental factors such as
intrauterine conditions, nutrition, hormones, and general health play a role in an individual’s ability to achieve their PBM (Cooper et al., 1997). The timing of PBM is still debated as most studies have reported the timing to be in the late teens to early twenties (Sabatier et al., 1996, Matkovic et al., 1994, Henry et al., 2004, Cvijetic et al., 2008). However, other studies have reported bone accrual to continue into the fourth decade of life (Rodin et al., 1990, Recker et al., 1992, Baxter-Jones et al., 2011). The rate of bone accrual has been shown to vary at different skeletal sites, occurring earliest at the hip, followed by the spine and whole body (Theintz et al., 1992, Javaid and Cooper*, 2002).

2.6.2.2 Bone mineral and bone accrual
During infancy (<12 months), males tend to be longer in length and have a higher total BMC, and total vBMD compared to females (Ireland et al., 2014a, WHO, 2006). The average total body BMC (TB BMC) and total body aBMD (TBa BMD), measured by DXA, increased by 389% and 157% respectively, with weight and height being the dominant predictors of aBMC and aBMD between birth and age of 1 years. (Koo et al., 1998). Currently, there are few studies on vBMD in infants as pQCT and HR-pQCT are not suitable due to movement and constraints of scanner design. Ireland et al. reported longitudinal measurements, using pQCT, at the tibia total vBMD (at the 20% site) in new-born babies (mean age at baseline = 0.3 months, follow-up =14.8 months) and showed vBMD to increase significantly with age, especially in infants with early onset of walking. The authors concluded that mechanical stimuli may increase vBMD during early stages of life and therefore increase bone strength (Ireland et al., 2014b).

The majority of cross-sectional and longitudinal studies using DXA have reported there to be no sex differences in bone accrual during pre-puberty (Martin et al., 1997, Ferretti et al., 1998, Theintz et al., 1992, Maynard et al., 1998). However, some studies have reported pre-pubertal males to have a higher bone accrual rate compared to females. Specker et al. reported the relationship between sex, body size, total body bone area (TB BA) and TB BMC in males and females aged 3-5 years (Specker et al., 2001). Males were significantly taller and heavier compared to females. TB BMC and TB BMC adjusted for TB BA were significantly higher in males compared to females but TB BA was similar in both sexes. TB BA positively correlated with height, weight, percent body fat, and calcium intake. TB BMC positively correlated with TB BA, age, and weight and inversely correlated with height and percent body fat (Specker et al., 2001). Whiting et al. reported females to gain less bone mineral at every age compared to males during pre-puberty as females grow at a slower rate compared to males (Whiting et al., 2004). Other studies have also reported similar findings (Horlick et al., 2000, Whiting et al., 2004, Mølgaard et al., 1999).

During puberty, rapid gains in height, BMC, bone CSA and lean mass occur in both sexes. Longitudinal studies using DXA, have reported 26-30% of final adult BMC to be gained within the two years surrounding PHV (Bailey et al., 1999, Baxter-Jones et al., 2003). Timing of PHV and TBBMC velocity
have been reported to be ~2 years earlier in females compared to males (Figure 2.36). In the Saskatchewan Paediatric Bone Mineral Accrual Study (PBMAS) in Canada, PHV in females was achieved by mean age of 11.77 years and peak TBBMC velocity by mean age 12.54 years and in males, PHV was achieved by mean age of 13.44 years and peak TBBMC velocity by mean age of 14.05 years (Bailey et al., 1999). During this time, BMC and aBMD at the lumber spine and proximal femur increased by four- to six-fold over the pubertal growth spurt (Bailey et al., 1999). The timing of peak BMC velocity in females coincided with the onset of menarche (average age 12 years). After menarche, the rate of bone accrual decreased significantly and ceased by mean age of 16 years (Theintz et al., 1992). In males, bone accrual continues up to 18 years of age, which resulted in males gaining 22% more BMC than did females (Figure 2.36) (Theintz et al., 1992). Longitudinal follow-up of PBMAS reported similar findings as the average age of PHV was 11.8 (±1.0) years in females and 13.5 (±1.0) years in males (Baxter-Jones et al., 2011).

Differences in periosteal apposition and endosteal contraction have been attributed to the differential effects of testosterone and oestrogen. Periosteal apposition places bone on the outer cortex, which is further from the neutral axis, increasing total CSA and resistance to bending forces. This process is thought to be stimulated by testosterone as males have significantly higher periosteal apposition and larger total CSA compared to females. Oestrogen is thought to reduce or inhibit periosteal apposition in pubertal females and stimulate endosteal contraction. As a result, bone is placed within the medullary cavity (i.e. closer to the neutral axis), producing a smaller, narrower bone compared to males. Endosteal contraction does not have any significant beneficial effects on bone strength and may be an evolutionary mechanism for women to store calcium as a reserve for pregnancy and lactation (Kontulainen et al., 2006).
Figure 2.36 - Bone accrual during childhood and adolescence in males and females.
Left, TBBMC (g) increases steadily during childhood with males gaining more bone compared to females. Sex differences in bone accrual become more apparent when puberty starts. Right, offset between TBBMC and PHV Sources: (Whiting et al., 2004) and (Javaid and Cooper*, 2002).

A summary of previous studies assessing total body and other body regions using DXA have been summarised in Table 2.10. Although attaining a high PBM at the end of growth may reduce the risk of osteoporosis and fracture in later life, other components of bone strength including bone size, geometry, and microarchitecture need to be considered as part of PBM as these components also determine the mechanical competence of bone.
Table 2.10: A summary of previous studies using DXA to assess total body and other body regions from developed countries.

<table>
<thead>
<tr>
<th>Authors (Year)</th>
<th>Study population &amp; sample size</th>
<th>Sex &amp; Age (Years)</th>
<th>Imaging technique</th>
<th>Scan site</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Rauch et al., 2004)</td>
<td>Canada (same cohort for all studies) (n = 175)</td>
<td>M &amp; F 8 - 14</td>
<td>DXA</td>
<td>Total body, femoral neck and lumbar spine</td>
</tr>
<tr>
<td>(Bailey et al., 1999)*</td>
<td>(n = 113)</td>
<td>M &amp; F 8 - 20</td>
<td>DXA</td>
<td>Total body, lumbar spine, and proximal femur</td>
</tr>
<tr>
<td>(Baxter-Jones et al., 2003)*</td>
<td>(n = 152)</td>
<td>M &amp; F 8 - 19</td>
<td>DXA</td>
<td>Total body, femoral neck and lumbar spine</td>
</tr>
<tr>
<td>(Baxter-Jones et al., 2011)*</td>
<td>(n = 375)</td>
<td>M &amp; F 8 - 30</td>
<td>DXA</td>
<td>Total body, lumbar spine, total hip, and femoral neck</td>
</tr>
<tr>
<td>(Horlick et al., 2000)</td>
<td>USA (n = 336)</td>
<td>M &amp; F 6 - 11</td>
<td>DXA</td>
<td>Total body</td>
</tr>
<tr>
<td>(Specker et al., 2001)</td>
<td>USA (n = 239)</td>
<td>M &amp; F 3 - 5</td>
<td>DXA</td>
<td>Total body</td>
</tr>
<tr>
<td>(Ferretti et al., 1998)</td>
<td>Argentina (n = 778)</td>
<td>M &amp; F 2 - 20</td>
<td>DXA</td>
<td>Total body</td>
</tr>
<tr>
<td>(Martin et al., 1997)</td>
<td>Canada (n = 228)</td>
<td>M &amp; F 9 - 20</td>
<td>DXA</td>
<td>Total body</td>
</tr>
<tr>
<td>(Maynard et al., 1998)</td>
<td>USA (n = 148)</td>
<td>M &amp; F 8 - 18</td>
<td>DXA</td>
<td>Total body, spine, head, hip, arms and legs</td>
</tr>
<tr>
<td>(Zanchetta et al., 1995)</td>
<td>Argentina (n = 778)</td>
<td>M &amp; F 2 - 20</td>
<td>DXA</td>
<td>Total body, anterior-posterior and lateral lumbar spine, radius, femoral neck, trochanter, and Ward's triangle</td>
</tr>
<tr>
<td>(Kalkwarf et al., 2007)</td>
<td>USA (n = 1554)</td>
<td>M &amp; F 6 - 16</td>
<td>DXA</td>
<td>Total body, lumbar spine, hip, and forearm</td>
</tr>
<tr>
<td>(Theintz et al., 1992)</td>
<td>Switzerland (n = 198)</td>
<td>M &amp; F 9 - 19</td>
<td>DXA</td>
<td>Lumbar spine, femoral neck, and mid-femoral shaft</td>
</tr>
<tr>
<td>(Ward et al., 2007a)</td>
<td>UK (n = 442)</td>
<td>M &amp; F 6 - 19</td>
<td>DXA</td>
<td>Total body, lumbar spine and hip</td>
</tr>
</tbody>
</table>

**Key:** Longitudinal studies are denoted with an asterisk*. M – Males, F – Females, DXA – dual-energy x-ray absorptiometry, and pQCT – peripheral quantitative computed tomography.
2.6.3 Peripheral bone development during puberty

When making comparisons between different studies of bone during growth, the imaging techniques and the regions of interest used must be considered because the metaphysis and diaphysis of long bones may adapt differently during growth and to mechanical and environmental stimulus. Research has mainly focused on the radius as it is the most common site of fracture during growth compared to the tibia. The similarities and differences in pQCT and HR-pQCT have been described in Chapter 2.5.2. A summary of previous studies assessing growth and development at the radius and tibia are summarised in Table 2.11. In this section, bone growth and development of the radius and tibia at the metaphyseal and diaphyseal sites are described in detail.

2.6.3.1 Radius – Metaphyseal sites

**Total CSA** – Neu et al. reported total CSA at the 4% site using pQCT to be significantly higher in pre-pubertal males compared to pre-pubertal females (p<0.01). During TS3 and TS4, sex differences did not exist but then re-emerged at TS5, with males having a significantly larger CSA compared to females (p<0.001) (Neu et al., 2001). Other pQCT studies have reported similar findings (Rauch and Schoenau, 2005). Studies using HR-pQCT have reported total CSA to be significantly greater in males at pre-, late, and post puberty compared to females (Kirmani et al., 2009). However, females experienced a period of ‘catchup’ during early- to mid- puberty. After menarche, bone accrual and bone expansion started to plateau in females but total CSA continued to increase in males until the age of 18-20 years. Wang et al. reported similar results with males having a significantly greater total CSA during pre-puberty (+26%, p<0.05) and post puberty (+36%, p<0.05) (Figure 2.38) (Wang et al., 2010). However, Nishiyama et al. reported total CSA to be similar between males and females during pre-puberty and early puberty. At TS4, total CSA increased significantly in males and remained significantly higher into adulthood compared to females (Nishiyama et al., 2012a). Differences in methodology and scanning site may be responsible for these conflicting findings.

**Total bone vBMD** – Neu at al. reported males to have a significantly higher total vBMD at the 4% site, using pQCT, compared to females at TS1 (p<0.01). Total vBMD remained stable during puberty in both sexes until 15 years of age, then increases of 30% and 46% in females and males were observed (Neu et al., 2001). Wang et al. reported no sex differences in total vBMD using HR-pQCT, at pre- and post-puberty. During puberty, total vBMD diminished by 20% and 15% at TS3 compared to TS1 in males and females, respectively (Figure 2.38) (Wang et al., 2010). This coincides with achieving PHV and significant increases in bone length and diameter, resulting in a time lag between increase in bone size and mineralisation. However, Nishiyama et al. reported similar significant increases in total vBMD during peri and post puberty in males and females (Nishiyama et al., 2012a).
**Cortical vBMD** - Neu at al. reported cortical vBMD at the 4% site using pQCT to be significantly higher in males at TS1 (p<0.01) compared to females. However, there were no differences at TS2, TS3, to TS5 (Neu et al., 2001). Wang et al. reported no sex difference in cortical vBMD during puberty except at TS3, where males had significantly lower vBMD compared to females (Wang et al., 2010). Other studies have also reported similar findings (Nishiyama et al., 2012a).

**Cortical thickness** – Sex differences in Ct.Th did not appear until puberty, when females showed a significant decrease in Ct.Th in mid-puberty before increasing sharply at the end of puberty (Kirmani et al., 2009). Males maintained Ct.Th from pre- to mid-puberty before showing marked increases during late puberty. At TS5, there were no sex differences for Ct.Th (Kirmani et al., 2009). Wang et al. reported cortical thickness to be significantly lower in males at TS3 and TS4 compared in females, and no sex difference at TS1, TS2, and TS5 (Figure 2.38) (Wang et al., 2010). Nishiyama et al. reported similar findings to Wang et al. despite differences in scanning site (Nishiyama et al., 2012a).

**Cortical porosity** – HR-pQCT has a resolution of 82 µm (in plane spatial resolution of 130 µm) and is the only technique that can measure cortical porosity in-vivo. Nishiyama et al. reported there to be no sex difference in Ct.Po at the distal radius before puberty (Nishiyama et al., 2012a). Peri- and post pubertal females were shown to have a significantly higher Ct.vBMD (+9.4% and +7.4% respectively) and a significantly lower Ct.Po (-118% and -56% respectively) compared to peri- and post pubertal males (Figure 2.37) (Nishiyama et al., 2012a). Kirmani et al. reported no sex difference in Ct.Po during pre-puberty at the distal radius but there was a significant increase in males at mid- to late puberty, whereas Ct.Po increased slightly in females in early puberty and decreased with a subsequent increase in Ct.vBMD in late puberty (Kirmani et al., 2009).

![Figure 2.37 - Cortical porosity in males and females at the distal radius and tibia.](image)

Error bars represent standard error. A significant difference between females and males within the same puberty group; a = p<0.001, b = p<0.01 and c = p<0.05. A significant difference between puberty group and the pre-puberty group within sex; d = p<0.001 and e = p<0.01. Source: (Nishiyama et al., 2012a).
**Trabecular compartment** – Trabecular bone is known to contribute to bone strength in the vertebrae, hip, and the metaphysis of long bones, which are all common sites of fracture in later life. Therefore, it is essential to understand how trabecular bone changes during childhood and adolescence. Measurements of the trabecular compartment in-vivo are very challenging due to the average trabecular thickness of 50µm and 150µm. Techniques used to measure the trabecular compartment are 1) pQCT, which can measure trabecular BMC and vBMD, and 2) HR-pQCT, which can measure trabecular bone to tissue volume (BV/TV), number (Tb.N), thickness (Tb.Th), and separation (Tb.Sp). It is important to note that vBMD measured by pQCT also includes the marrow compartment due to the limited resolution.

*Figure 2.38 - Total and medullary/the endocortical area CSA, total vBMD, cortical vBMD, and cortical thickness at the distal radius and distal tibia.*
Full line denote males and dotted line for females (mean ±SE), † refers to a sex difference and Δ refers to a difference relative to Tanner stage I within a sex (p<0.05). Source: (Wang et al., 2010).
Studies using pQCT have reported trabecular vBMD to have similar increases with age in males and females during growth at the 4% site (Fujita et al., 1999). However, Neu et al. reported trabecular vBMD to be significantly higher in females at TS1 and TS5 compared to males but no sex differences were observed between TS2 and TS4 (Neu et al., 2001). The measurement of trabecular vBMD using pQCT is very variable to the site of measurement and has been shown to be highly variable at the distal radius and distal tibia in children (Lee et al., 2007, Rauch et al., 2001b). Therefore, it is essential that a strict protocol is adhered to (Zemel et al., 2008).

Studies using HR-pQCT have reported there to be no sex differences in trabecular parameters during pre-puberty (Figure 2.39) (Wang et al., 2010). There were no significant differences in BV/TV, Tb.N, Tb.Th, or Tb.Sp in females throughout puberty. However, BV/TV and Tb.Th increased significantly in males in the early- to- later stages of puberty. The authors concluded that trabecular parameters in females may be programmed in early life and may play a role in calcium storage for pregnancy and lactation in later life, whereas in males, trabecular changes are driven by testosterone and IGF-1 (Kirmani et al., 2009). Other studies have reported similar findings (Wang et al., 2010, Nishiyama et al., 2012a).

Figure 2.39 - Trabecular BV/TV was higher in boys than girls throughout pubertal at both sites. Trabecular number did not differ by sex or puberty. Full line denotes males and dotted line for females, † = p<0.05 for sex difference (mean ±SE). Source: (Wang et al., 2010).
2.6.3.2 Radius – Diaphyseal sites

Total CSA - Kontulainen et al. reported males to have higher rates of periosteal apposition and a wider total CSA at the 20% site, using pQCT, from pre-, peri- and post- puberty compared to females. No sex differences in endosteal contraction were observed (Kontulainen et al., 2005). Neu et al. reported total CSA at the 65% site using pQCT to increase significantly between the ages of 6 and 11 years and ceased at age 15 years in females, whereas males had a linear increase in total CSA between the ages of 6 and 20 years. During this time, total CSA increased by 50% and 116% in females and males, respectively (Rauch et al., 2001b).

Total bone vBMD – De Schepper et al. reported vBMD at the 33% site using pQCT to increase steadily by 9.8% with age in males and females during puberty. No sex differences in total vBMD were observed during pre-puberty and puberty (De Schepper et al., 1996). Neu et al. reported no sex differences between vBMD between TS1 and TS2. However, females had a significantly higher vBMD compared to males (p=<0.05) at late puberty (Rauch et al., 2001b).

Cortical vBMD - Schoenau et al. reported no sex differences in vBMD at the 65% site using pQCT during pre-puberty but was significantly greater (+3-4%) in females from mid- to post-puberty compared to maturity- and age-matched males (Schoenau et al., 2002b). This suggests that females may have a lower intracortical remodelling rate resulting in higher cortical vBMD and lower cortical porosity compared to males (Schoenau et al., 2002b). Ashby et al. reported vBMD at the 50% site to increase in males and females during puberty. At TS4, vBMD was greater in males compared to females but was similar in both sexes at TS5 (Ashby et al., 2011).

Cortical thickness - De Schepper et al. reported cortical thickness at the 33% site to increase linearly with age in males and females. However, males had a significantly higher cortical thickness compared to females during pre-puberty and puberty (De Schepper et al., 1996). Neu et al. reported the cortex to thicken by 1.33 mm in females and by 1.39 mm in males (an increase of 116% and 96%, respectively) (Rauch et al., 2001b). In females, cortical areas and thickness appeared to increase until the age of 14–15 years whereas in males, the increase in cortical area and thickness was relatively slow until age of 12 years and more rapid between 13-18 years, with a maximum at ~16 years. Marrow area did not change in females, but increased significantly in males. This suggests a sex difference in periosteal expansion males, which resulted in males having a wider bone with slightly thicker cortex compared to females in adulthood (Rauch et al., 2001b).

SSI – Schoenau et al. reported cortical vBMD and SSI at the 65% site to be significantly higher in males during pre- and after puberty compared to females (Schoenau et al., 2001). During puberty, no sex differences in cortical vBMD were observed but polar moment of inertia, section modulus, and SSI were significantly higher in males compared to females, as males had higher rates of periosteal
apposition and increases in total CSA gains. SSI increased with age by 106% and 130% in females and males, respectively (Schoenau et al., 2001).

2.6.3.3 Tibia – Metaphyseal site

**Total CSA** – Macdonald et al. reported total CSA at the 8% site using pQCT to be significantly larger (4-6%, p=<0.001) in males compared to females at pre- and early puberty (Macdonald et al., 2006). Nishiyama et al. reported total CSA at the 4% site using HR-pQCT to be similar during pre-puberty but total CSA increased significantly in males during peri- and post puberty (p<0.001) (Nishiyama et al., 2012a). However, Wang et al. reported total CSA at the 4% using HR-pQCT to be significantly larger in males at pre-, peri-, and post puberty compared to females (Figure 2.38) (Wang et al., 2010). Total CSA was significantly higher in males before puberty (+11%) and post-puberty (+31%) compared to females (Wang et al., 2005).

**Total bone vBMD** – Macdonald et al. reported total vBMD at the 8% site using pQCT to be significantly higher (6%, p=<0.001) in males compared to females at pre-puberty (Macdonald et al., 2006). Wang et al. reported sex differences at TS4 only, where males has significantly lower total vBMD compared to females but no sex differences were reported at the end of puberty (Figure 2.38) (Wang et al., 2010). However, Nishiyama et al. reported no sex difference in total vBMD at any stage of puberty (Nishiyama et al., 2012a).

**Cortical vBMD** – Wang et al. reported no sex difference in Ct.vBMD at the 7% site using HR-pQCT at TS1 and TS2 but at TS3 males were significantly lower, which remained at post puberty (Figure 2.38) (Wang et al., 2010).

**Cortical thickness** – Kirmani et al. reported Ct.Th (1mm proximal from growth plate) to significantly decrease during mid-puberty before increasing at the end of puberty. In males, cortical thickness was maintained from pre- to mid-puberty, before increasing sharply at the end of puberty, resulting in no sex differences by end of puberty (Kirmani et al., 2009). Wang et al. reported no sex differences at the 7% site at TS1 to TS3. At TS4, males had significantly lower Ct.Th which then become significantly higher at TS5 compared to females (Figure 2.38) (Wang et al., 2010). However, Nishiyama et al. reported pre-pubertal males to have significantly higher Ct.Th at the 4% site compared to females but no sex differences were found during mid-puberty. Differences emerged at the end of puberty with males having a significantly thicker cortex (Nishiyama et al., 2012a).

**Cortical porosity** – There is only one study which has reported Ct.Po at the distal tibia in children and adolescents (Nishiyama et al., 2012a). No sex differences in Ct.Po at the 7% site were found in pre-puberty. During puberty, early, peri- and post-pubertal females were shown to have a significantly lower Ct.Po compared to early, peri- and post-pubertal males (Figure 2.37) (Nishiyama et al., 2012a).
**Trabecular compartment** – Wang et al. reported BV/TV to significantly increase in males at peri- and post-puberty due to increases in Tb.Th and not Tb.N (Figure 2.37). Tb.N is thought to be genetically programmed and therefore should not increase during the growth (Boutroy et al., 2005, Kirmani et al., 2009). However, Nishiyama et al. reported Tb.N to increase significantly (p<0.005) in males throughout puberty compared to females. This may be the result of increased intracortical remodelling in males as bone is placed on the outer cortex to expand the bone CSA rather than in the medullary cavity therefore increasing Tb.N in males as the bone becomes wider.

### 2.6.3.4  Tibia – Diaphyseal sites

**Total CSA** – Binkley et al. reported total CSA at the 20% site using pQCT and found no sex differences in pre-pubertal children (aged 3-4 years) (Binkley and Specker, 2000). Macdonald et al. reported total CSA at the 50% site to be significantly higher in males at each stage of puberty (8.5 – 11.1%) compared to females and sex difference were associated with greater increases in height and lean mass in males (Macdonald et al., 2005, Macdonald et al., 2006). Other studies have reported similar findings (Leonard et al., 2004, Macdonald et al., 2005)

**Cortical vBMD** – Kontulainen et al. reported Ct.vBMD at the 50% site to increase on average by 1.9%, 2.8%, and 1.5% in early, peri, and post pubertal females compared with males, respectively (Kontulainen et al., 2006).

**Cortical thickness** – Binkley et al. reported Ct.Th at the 20% site and found no sex differences in pre-puberty (aged 3 to 4 years) and height was the only significant predictor (Binkley and Specker, 2000). These findings must be viewed with caution as the mean Ct.Th was 1.2 mm, which is too thin to get an accurate measurement (Prevral et al., 1999).

**SSI** – Macdonald et al. reported the greatest increase in SSI at the 50% site in females to occur at early and peri-puberty (+19% and +16.5%, respectively) compared to post pubertal females (+11.7%). These increases were associated with increases in total CSA (Macdonald et al., 2005). Males had a significantly higher SSI in early puberty compared to females and this remained throughout puberty. The authors concluded that males had a higher total CSA compared to females at early puberty, which increased by 31.5% across 20 months, resulting in an average 32% increase in SSI (Macdonald et al., 2005).

### 2.6.3.5  Relationship between bone mineral, geometry and microarchitecture with fracture risk

Epidemiological studies in the UK have reported differences in fracture rates between adolescent females (102.9/10,000 persons/year) and males (161.6/10,000 persons/year) (Cooper et al., 2004). The peak incidence of fracture occurs approximately between ages 10-12 years in females and 12-14
years in males, which coincides with age of PHV (Cooper et al., 2004). The most common site of fracture in children and adolescents is at the distal radius. By contrast, the incidence of tibia fractures is low and does not differ by sex (Landin, 1983). Males are more likely to participate in high injury risk activities and therefore are more likely to sustain a radius fracture due to trauma compared to females (Landin, 1983). Several studies have strongly suggested that the higher incidence of radial fracture around the age of PHV can result from a dissociation between growth in size and mineral accrual, inducing a transient phase where bone is under mineralised (Kirmani et al., 2009, Nishiyama et al., 2012a, Wang et al., 2010, Chevalley et al., 2011, Rauch et al., 2001a). Pritchett et al. reported changes in bone length and bone accrual during puberty at the radius and tibia and concluded that 90% of the increase in radial length and 30% of the increase in tibial length occur at the distal growth plate compared to the proximal growth plate (Pritchett, 1991, Pritchett, 1992). Therefore, bone modelling and remodelling are significantly higher at the distal sites making them vulnerable to fracture. Studies using DXA have demonstrated that children with fracture(s) tend to have significantly lower CSA and aBMD at multiple sites compared to non-fracture children (Manias et al., 2006, Dimitri et al., 2010, Goulding, 2007). Other studies using pQCT and HR-pQCT have also reported lower vBMD in fracture groups but not all fracture cases present with low vBMD and therefore other components of bone strength including sex differences in rate of bone accrual, bone size, geometry, and microarchitecture must be considered (Skaggs et al., 2001).

Studies have combined HR-pQCT with finite element analysis to understand how changes in bone microarchitecture during growth may alter bone strength (Kirmani et al., 2009). During puberty, males have a higher total fracture load compared to females so changes in fracture load, or in the factor of risk, fail to provide an explanation as to why males have a higher incidence of fracture. Bone growth in length and width is higher in males compared to females, resulting in higher cortical porosity and a longer transient phase between the growth of the bone and mineralisation in males (Nishiyama et al., 2012b, Schoenau et al., 2002b). A long time lag between increases in bone size and mineralisation can compromise bone strength and increase risk of fracture.
Table 2.11 - Summary of previous studies using pQCT and HR-pQCT to assess vBMD, geometry, and microarchitecture in healthy children and adolescents.

<table>
<thead>
<tr>
<th>Authors (Year)</th>
<th>Study population &amp; sample size</th>
<th>Sex &amp; Age (Years)</th>
<th>Imaging technique</th>
<th>Scan site</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Ashby et al., 2009a)</td>
<td>UK (n = 629)</td>
<td>M &amp; F 6 - 19</td>
<td>pQCT</td>
<td>Radius (4% and 50% sites)</td>
</tr>
<tr>
<td>(Schoenau et al., 2000)¹</td>
<td>Germany (n = 318)</td>
<td>M &amp; F 6 - 22</td>
<td>pQCT</td>
<td>Radius (65% site)</td>
</tr>
<tr>
<td>(Neu et al., 2001)</td>
<td>Germany (n = 473)</td>
<td>M &amp; F 6 - 40</td>
<td>pQCT</td>
<td>Radius (65% site)</td>
</tr>
<tr>
<td>(Schoenau et al., 2002b)¹</td>
<td>Germany (n = 469)</td>
<td>M &amp; F 6 - 40</td>
<td>pQCT</td>
<td>Radius (65% site)</td>
</tr>
<tr>
<td>(Rauch et al., 2001a)¹</td>
<td>Germany (n = 278)</td>
<td>M &amp; F 6 - 40</td>
<td>pQCT</td>
<td>Radius (4% site)</td>
</tr>
<tr>
<td>(Kalkwarf et al., 2011)</td>
<td>USA (n = 424)</td>
<td>M &amp; F 5 - 16</td>
<td>pQCT</td>
<td>Radius (4% and 20% sites)</td>
</tr>
<tr>
<td>(Leonard et al., 2004)</td>
<td>USA (n = 150)</td>
<td>M &amp; F 6 - 21</td>
<td>pQCT, DXA</td>
<td>Tibia (20% site), Total body</td>
</tr>
<tr>
<td>(Binkley and Specker, 2000)</td>
<td>USA (n = 101)</td>
<td>M &amp; F 3 - 4</td>
<td>pQCT</td>
<td>Tibia (20% site)</td>
</tr>
<tr>
<td>(Moyer-Mileur et al., 2008)</td>
<td>USA (n = 416)</td>
<td>M &amp; F 5 - 16</td>
<td>pQCT</td>
<td>Tibia (4% and 66% sites)</td>
</tr>
<tr>
<td>(Macdonald et al., 2005)¹</td>
<td>Canada (n = 128)</td>
<td>M &amp; F 11 - 13</td>
<td>pQCT</td>
<td>Tibia (50% site)</td>
</tr>
<tr>
<td>(Macdonald et al., 2006)¹</td>
<td>Canada (n = 424)</td>
<td>M &amp; F 9 - 11</td>
<td>pQCT</td>
<td>Tibia (8% and 50% sites)</td>
</tr>
<tr>
<td>(Chevalley et al., 2011)</td>
<td>Switzerland (n = 176)</td>
<td>M 7 - 15</td>
<td>HR-pQCT</td>
<td>Radius and tibia (1mm proximal to growth plate)</td>
</tr>
<tr>
<td>(Burrows et al., 2010b)¹</td>
<td>Canada (n = 279)</td>
<td>M &amp; F 15 - 20</td>
<td>HR-pQCT</td>
<td>Tibia (8% site)</td>
</tr>
<tr>
<td>(Nishiyama et al., 2012a)¹</td>
<td>Canada (n = 398)</td>
<td>M &amp; F 9 - 22</td>
<td>HR-pQCT</td>
<td>Radius (7% of ulnar site) and tibia (8% site)</td>
</tr>
<tr>
<td>(Wang et al., 2010)</td>
<td>Australia (n = 129)</td>
<td>M &amp; F 5 - 18</td>
<td>HR-pQCT</td>
<td>Radius (4% site) and tibia (7% site)</td>
</tr>
<tr>
<td>(Kirmani et al., 2009)</td>
<td>USA (n = 121)</td>
<td>M &amp; F 6 - 21</td>
<td>HR-pQCT</td>
<td>Radius (1mm proximal to growth plate)</td>
</tr>
</tbody>
</table>

Key: Longitudinal studies are denoted with an asterisk* and studies with the same population are denoted with ‘1’. M – Males, F – Females, DXA – dual-energy x-ray absorptiometry, and pQCT – peripheral quantitative computed tomography.

Kirmani et al. reported the percent load borne by cortical bone, which reflects the relative strength of cortical compared to trabecular bone, and the cortical to trabecular bone volume ratio, was at its lowest at mid-puberty in females and late puberty in males, before returning to the original value measured at the start of puberty (Kirmani et al., 2009). During puberty, total CSA increases
significantly, resulting in cortical expansion and reduced Ct.vBMD, therefore placing more strain on the trabecular compartment in both male and female adolescents (Figure 2.40) (Kirmani et al., 2009). Studies have shown trabecular vBMD and thickness to be significantly lower in fracture groups compared to non-fracture groups during growth (Chevalley et al., 2011). These changes in cortical and trabecular bone correspond with peak fracture incidence. Although adult males have a thinner cortex and similar vBMD compared to adult females, the longer duration of periosteal growth results in expansion of the CSA, making the bone more resistant to bending forces and less likely to fracture compared to adult females (Kirmani et al., 2009).

Figure 2.40 - Three dimensional reconstructions of trabecular and cortical bone of the distal radius in girls and boys during puberty using Finite Element Analysis.
Low strain energy density (SED) values indicating low strains (relatively strong areas) and high SED values indicating high strains (relatively weak areas). The arrows are pointing at each age-group and indicate thinning of the cortex relative to the amount of trabecular bone present in the adolescents during mid-puberty (group III) compared to pre- and post-puberty (groups I and II). Source: (Kirmani et al., 2009).
2.6.3.6 Body composition in children and adolescents during puberty

During growth, sexual dimorphism occurs in lean and fat mass, especially at puberty due to the release of sex hormones. DXA is the most common technique for assessing lean mass and fat mass in children and adolescents. Sex differences have been reported in pre-puberty with males having a higher total body lean mass (approx. 3 kg) and a lower total body fat mass compared to females (Rogol et al., 2002, Boot et al., 1997). Both sexes show relative decreases in body fat between the ages of 1 to 6 years (Figure 2.41). After the age of 6 years, females accrue approx. 6% more fat compared to males whereas males continue to increase lean mass (Rogol et al., 2002).

During puberty in males there is a decrease in the percentage of body fat by 1.15 kg/year with fat mass remaining relatively constant, whereas in females there is an increase in fat mass at a rate of 1.14 kg/year (Rogol et al., 2002). Lean mass accrual in males continues to significantly increase into the third decade of life, whereas lean mass begins to plateau at age 15 years in females (Rogol et al., 2002).

Figure 2.41 - The relationship between age and body composition.
(A) Age relative to fat-free mass and (B) Age relative to fatness during growth. Lean and fat mass were adjusted for height (i.e. data divided by height squared). The kink in the profile of fat mass index at ages 10-12 years may be a result of merging two datasets together. Sources: (Wells, 2007).

2.6.4 The muscle-bone unit

The concept of the muscle-bone unit is based on Frost’s mechanostat theory, which proposed a negative feedback system to explain how mechanical stimuli drive adaptation in BMC and bone geometry to maintain strength. Schiessl et al. were one of the first groups to publish data on the muscle-bone relationship in the first two decades of life, using DXA data taken from Zanchetta et al.’s study measuring TB aBMC and total body lean body mass (LBM) (Zanchetta et al., 1995). During pre-puberty, bone and lean mass increased similarly in both males and females. Between the ages of 12 to 15 years, BMC increased earlier in relation to lean mass in females compared to males and then
both BMC and lean mass plateau (due to growth rate slowing down). Similar but smaller increases in BMC and TB LBM are seen in males, resulting in adult males having more lean mass and bone compared to adult females but BMC increases disproportionately to LBM in females (Schiessl et al., 1998, Zanchetta et al., 1995). Schiessl et al. suggested that sex difference in BMC:LBM ratio in females is affected by oestrogen, placing more bone within the medullary cavity for the purpose of pregnancy and lactation (Schiessl et al., 1998).

A longitudinal study (using DXA) assessing BMC and LBM in males and females, aged 8 to 14 years, showed that the peak in LBM accrual preceded the peak in BMC accretion by an average of 0.51 years in females and by 0.36 years in males (Figure 2.42). The authors concluded that bone development is driven by muscle development but could not rule out genetic influences on growth (Rauch et al., 2004). Ashby et al. assessed total body BMC and LBM in children aged 5 to 18 years using DXA and reported females to accrual more BMC in relation to LBM compared to males, confirming Schiessl et al.’s previous findings. These sex differences were independent of body size and differential fat mass accrual in females (Ashby et al., 2011). Other studies have reported similar findings (Crabtree et al., 2004, Pludowski et al., 2005, Schiessl et al., 1998).

Using pQCT, Schoenau et al. reported a linear relationship between muscle CSA and BMC at the radial diaphysis in males and females ($R^2=0.91$ and $R^2=0.88$, respectively). BMC:Muscle CSA ratio did not vary significantly between the ages of 6 to 19 years in males but increased significantly in females at age 13 years and older. Sex differences in BMC:Muscle CSA did not appear until TS3, when BMC increased in females and became significantly higher in late puberty compared to males (Schoenau et al., 2002a). Further studies using the same population have shown that a linear relationship between muscle CSA and cortical area at the radial diaphysis in pre-pubertal males and females ($R^2=0.92$ and $R^2=0.89$ respectively) but at late puberty, females had a greater cortical area:Muscle CSA ratio compared to males (Figure 2.43). There were no sex differences between muscle CSA and periosteal circumference but sex differences did exist for muscle CSA and endosteal circumference (Figure 2.43) (Schoenau et al., 2000). These findings support the theory that puberty and the associated rise in oestrogen alters the muscle-bone unit in females.
Figure 2.42 - Velocities of total body lean body mass (TB LBM) and bone mineral content (BMC) accretion during pubertal growth spurt. The age of peak height velocity (PHV) is shown as a reference point of pubertal development. Source: (Rauch et al., 2004).

Macdonald et al. assessed with pQCT, longitudinally, cortical area: muscle CSA ratio at the tibial diaphysis (50% site). In early puberty, there were no sex differences in total CSA, cortical area, or cortical area: muscle CSA ratio (Macdonald et al., 2005). During puberty, total CSA and cortical area increased significantly (8.5-11.1%) in males at every stage of puberty compared to females, resulting in a significant increase in bone strength in males (13.8-15.6%). Cortical area:Muscle CSA ratio decreased in females during early to peri-puberty, whereas the ratio remained stable in males as a result of greater gains in cortical area in males. At post puberty, cortical area: muscle CSA ratio remained stable in females but decreased in males as a result of increased muscle CSA in males compared to females (Macdonald et al., 2005). Ashby et al. reported males and females to accrue more BMC in relation to LM at multiple skeletal sites, with females having a higher BMC:LM ratio compared to males, which supports Macdonald et al.’s findings. However, BMC:LM ratio at the 50% site of radius was higher in males compared females at late puberty (Ashby et al., 2011). These studies support the mechanostat hypothesis and suggest that there is a sex-specific pattern of development in the muscle-bone unit. Differences in methodology (i.e. cross-sectional vs. longitudinal), classification of puberty, and regions of interest (i.e. radius vs. tibia) may result in discrepancies and conflicting conclusions.
2.6.5 Muscle force, power, and bone

There are a number of devices which can assess muscle strength including hand and leg dynamometers and jumping mechanography. These devices are able to assess isometric and isokinetic muscle strength and may provide more accurate measurements of muscle force and power compared to muscle CSA, as previously described in Chapter 2.5.7. Recent studies have used these muscle assessment techniques in combination with DXA and pQCT to provide a new perspective on the muscle-bone unit. Schoenau et al. assessed grip force and radial bone strength index (BSI), measured by pQCT, in participants aged 3 to 62 years and reported a significant correlation between BSI and grip strength ($R^2=0.87$). Males had a greater grip force compared to females, which peaked after puberty at ages 25 to 30 years. Grip force increased moderately in females after puberty (Schonau et al., 1996). Chan et al. reported grip force in children aged 10 to 12 years to have a strong positive correlation with BMC and aBMD at the hip, spine, and total body (data for the radius was absent). Grip force of the non-dominant hand was an independent predictor of BMC and aBMD at all

Figure 2.43 - Muscle-bone unit at the radius in male and females during growth.

(A) Relationship between muscle CSA and cortical area and (B) Relationship between muscle CSA and periosteal and endosteal circumferences. Source: (Schoenau et al., 2000).
sites, therefore supporting the mechanostat theory in children. Grip strength is related to physical activity and normal activity produces strains throughout the skeleton, therefore a relationship between muscle force and BMD of nearby skeletal structures is not surprising (Chan et al., 2008).

Wang et al. assessed the relationship between TB BMC and maximal isometric voluntary contraction (MVC) of the left elbow flexor and knee extensors using a dynamometer in females aged 10 to 13 years and reported MVC was highly associated with BMC in the arm and leg ($R^2=0.54$ and $R^2=0.50$, respectively), indicating muscle force is associated with BMC (Wang et al., 2007). However, BMC:MVC ratio was significantly higher (30%) in the leg compared to the arm. The legs will generate more force compared to the arms as the legs are weight-bearing, however other factors in addition to muscle force might be responsible for the differences in BMC:MVC between the limbs. Fat mass constitutes an additional load on the skeleton, which may drive the mechanostat to increase BMC in the legs compared to the arms (Wang et al., 2007). These findings have also been reported in obese children who have a greater leg and arm BMC compared to controls (46% and 21%, respectively). The authors concluded that the skeleton in obese children had adapted to extra mechanical loading but the additional fat mass did not provide additional protection and may increase risk of fracture due to the additional load on the bone during a fall (Manzoni et al., 1996). However, other studies have shown obesity to have a detrimental effect on the skeleton as a high fat-mass has been associated with a smaller total CSA (Skaggs et al., 2001), lower BMAD and BMC (Goulding et al., 2001), and increased risk of falls due to abnormalities in gait compared to non-overweight children (Goulding et al., 2003, McGraw et al., 2000, Dimitri et al., 2010).

Jumping mechanography provides an alternative method for assessing muscle force and power by measuring ground reaction forces using a jumping plate. Binkley et al. assessed the muscle-bone unit in children and adolescents, aged 7 to 16 years old, using pQCT at the 66% site of the tibia and jumping mechanography (Binkley and Specker, 2008). Muscle power and force were positively correlated with polar strength strain index ($R^2=0.85$ and $R^2=0.66$, respectively). Muscle power and force were predictors of total and cortical areas but not for cortical vBMD (Binkley and Specker, 2008). Anliker et al. assessed the relationship between tibial vBMC and muscle force, using pQCT (14% site) and jumping mechanography (one legged hopping), and reported muscle force predicted 84% of the BMC. During growth, muscle force and vBMC both increased but plateaued after puberty, remained stable in young adulthood, and then decreased with age. Muscle force never increased above the values reached at the end of puberty, suggesting muscle force is set for life during growth (Anliker et al., 2011). It can be concluded that many studies have described the muscle-bone unit in children and adolescents but there is still an incomplete understanding of how muscle force and power change through growth, and subsequently how this related to changes in bone, particularly in
bone microarchitecture. A summary of studies assessing muscle-bone unit during puberty are summarised in **Table 2.12**.

### 2.6.6 Factors that influence bone and muscle development

There are many mechanical and non-mechanical factors which can influence bone and muscle development. The most relevant factors related to this study are described.

**Nutrition**

Adequate nutrition is essential for growth and development. Evidence has suggested that PBM and later fracture risk are influenced by the pattern of growth in childhood and by nutritional influences during the prenatal, infant, child, and adolescent periods. Many nutrients have been associated with bone strength including: bone-forming minerals (e.g. calcium, magnesium, phosphorus, and zinc), vitamins (e.g. vitamins D, E, and K), amino acids, and ions (e.g. copper, manganese, carbonate, and citrate) (Prentice A et al., 2011, Ward, 2012). Deficiencies in these nutrients alter bone metabolism, growth and development, and maintenance (Ward, 2012). Research into nutrition and bone health has mainly focused on vitamin D and calcium as deficiencies of these nutrients have the most detrimental effect on the skeleton. The effects of vitamin D, vitamin K, and calcium deficiencies have been described in greater detail below:

**Vitamin D** – is a fat-soluble hormone that promotes skeletal mineralisation by optimising calcium absorption from the gut and ensuring normal calcification of the growth plates and mineralisation of osteoid on trabecular and cortical bone surfaces. The Global Consensus panel on management of nutritional rickets and osteomalacia have recommended vitamin D status, based on serum 25-hydroxyvitamin D (25(OH)D) levels, to be classified as; sufficiency (>50 nmol/L), insufficiency (30–50 nmol/L), and deficiency (<30 nmol/L) (Munns et al., 2016). A consequence of vitamin D deficiency is the development of rickets and/or osteomalacia in children and osteomalacia in adults (Pettifor and Prentice, 2011a). Ward et al. reported in healthy post-menarchal females (aged 12-14 years) that improving vitamin D status did not directly affect BMD, bone geometry or strength (Ward et al., 2009, Ward et al., 2010). The effect of vitamin D2 supplementation on muscle function in adolescent girls (aged 12-14 years) significantly increased mean (±SD) baseline serum 25(OH)D concentration in the intervention group (18.1±8.0 to 56±8.9 nmol/L) compared to the control group (17.9±7.4 to 15.7±6.6 nmol/L).
Table 2.12 - A summary of the previous studies assessing the muscle-bone unit in healthy children and adolescents.

<table>
<thead>
<tr>
<th>Authors (Year)</th>
<th>Study population</th>
<th>Age (Years)</th>
<th>Imaging technique</th>
<th>Scan site/ muscle technique</th>
<th>Muscle-bone assessment</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Zanchetta et al., 1995)</td>
<td>Argentina (n = 778)</td>
<td>M &amp; F</td>
<td>DXA</td>
<td>Total body, anterior-posterior and lateral lumbar spine, radius, hip, trochanter, and Ward's triangle LBM</td>
<td>aBMD : LBM</td>
</tr>
<tr>
<td>(Schissel et al., 1998)</td>
<td>Argentina (n = 778)</td>
<td>M &amp; F</td>
<td>DXA</td>
<td>Total body, anterior-posterior and lateral lumbar spine, radius, hip, trochanter, and Ward's triangle LBM</td>
<td>aBMD : LBM</td>
</tr>
<tr>
<td>(Hogler et al., 2003)</td>
<td>Australia (n = 459)</td>
<td>M &amp; F</td>
<td>DXA</td>
<td>Total body, anterior-posterior and lateral lumbar spine, radius, hip, trochanter, and Ward's triangle LBM</td>
<td>aBMD : LBM</td>
</tr>
<tr>
<td>(Crabtree et al., 2004)</td>
<td>UK (n = 646)</td>
<td>M &amp; F</td>
<td>DXA</td>
<td>Total body, anterior-posterior and lateral lumbar spine, radius, hip, trochanter, and Ward's triangle LBM</td>
<td>aBMD : LBM</td>
</tr>
<tr>
<td>(Pludowski et al., 2005)</td>
<td>Poland (n = 562)</td>
<td>M &amp; F</td>
<td>DXA</td>
<td>Total body, anterior-posterior and lateral lumbar spine, radius, hip, trochanter, and Ward's triangle LBM</td>
<td>aBMD : LBM</td>
</tr>
<tr>
<td>(Rauch et al., 2004)</td>
<td>Canada (n = 175)</td>
<td>M &amp; F</td>
<td>DXA</td>
<td>Total body, anterior-posterior and lateral lumbar spine, radius, hip, trochanter, and Ward's triangle LBM</td>
<td>aBMD : LBM</td>
</tr>
<tr>
<td>(Ashby et al., 2011)</td>
<td>UK (n = 442)</td>
<td>M &amp; F</td>
<td>DXA</td>
<td>Total body, anterior-posterior and lateral lumbar spine, radius, hip, trochanter, and Ward's triangle LBM</td>
<td>aBMD : LBM</td>
</tr>
<tr>
<td>(Schoenau et al., 2000)</td>
<td>Germany (n = 318)</td>
<td>M &amp; F</td>
<td>pQCT</td>
<td>Radius (65% site)</td>
<td>CtA: MCSA</td>
</tr>
<tr>
<td>(Schoenau et al., 2002a)</td>
<td>Germany (n = 349)</td>
<td>M &amp; F</td>
<td>pQCT</td>
<td>Radius (65% site)</td>
<td>BMC: MCSA</td>
</tr>
<tr>
<td>(Schoenau et al., 1996)</td>
<td>Germany (n = 168)</td>
<td>M &amp; F</td>
<td>pQCT</td>
<td>Radius (20%)</td>
<td>GS: BSI</td>
</tr>
<tr>
<td>(Chan et al., 2008)</td>
<td>Hong Kong (n = 169)</td>
<td>M &amp; F</td>
<td>DXA</td>
<td>Total body, anterior-posterior and lateral lumbar spine, radius, hip, trochanter, and Ward's triangle LBM</td>
<td>aBMD : LBM</td>
</tr>
<tr>
<td>(Wang et al., 2007)*</td>
<td>Finland (n = 258)</td>
<td>F</td>
<td>DXA</td>
<td>Total body, anterior-posterior and lateral lumbar spine, radius, hip, trochanter, and Ward's triangle LBM</td>
<td>aBMD : LBM</td>
</tr>
<tr>
<td>(Macdonald et al., 2005)*</td>
<td>Canada (n = 128)</td>
<td>M &amp; F</td>
<td>pQCT</td>
<td>Tibia (50% site)</td>
<td>CtA: MCSA</td>
</tr>
<tr>
<td>(Macdonald et al., 2006)</td>
<td>Canada (n = 424)</td>
<td>M &amp; F</td>
<td>pQCT</td>
<td>Tibia (8% site and 50% site)</td>
<td>All total and cortical bone parameters: MCSA</td>
</tr>
<tr>
<td>(Binkley and Specker, 2008)</td>
<td>USA (n = 105)</td>
<td>M &amp; F</td>
<td>pQCT</td>
<td>Tibia (66% site)</td>
<td>Total and cortical bone areas and vBMD : muscle force and power</td>
</tr>
<tr>
<td>(Anliker et al., 2011)</td>
<td>Switzerland (n = 223)</td>
<td>M &amp; F</td>
<td>pQCT</td>
<td>Tibia (4%, 14%, 38%, and 66% sites)</td>
<td>vBMC : Force (1LH)</td>
</tr>
</tbody>
</table>

Key: Longitudinal studies are denoted with an asterisk*, Dual-energy X-ray absorptiometry (DXA), peripheral quantitative computed tomography (pQCT), jumping mechanography (JM), aBMD: areal bone mineral density, LBM: Lean body mass, MCSA: Muscle cross-sectional area, CtA: Cortical area, GS: Grip strength, BSI: Bone strength index, MVC: Maximal isometric voluntary contraction.
Vitamin D status was significantly associated with change in muscle power and force, and therefore could increase bone strength indirectly through improvements in muscle. The authors did conclude that early interventions of increasing vitamin D status in pre-puberty may have a greater effect on bone development. Efficiency of movement increased significantly (by 5%; p=0.02) in the intervention group. An interaction was also found between baseline serum 25(OH)D concentration and jump velocity in the intervention group (p=0.02) with greater change in those with lower concentrations. There were no improvements in muscle force or power (Ward et al., 2010). Patients who present with myopathy (muscle weakness and waddling gait) as a result of vitamin D deficiency have been shown to respond to vitamin D supplementation as muscle function increased (Pettifor and Prentice, 2011a). Supplementation trials in children have produced inconclusive results for bone (Winzenberg and Jones, 2013b).

**Calcium** - is essential for mineralisation of the skeleton, muscle contractions and signal transmission in the nervous system. About 99% of total body calcium is present in the form of hydroxyapatite within bone and teeth (Vokes, 2006). Calcium requirements vary during growth, with the greatest calcium need for bone during puberty (Winzenberg and Jones, 2013a). In early development, bone modelling is the predominant skeletal process promoting rapid longitudinal growth and periosteal expansion. Modelling requires mineralisation, hence calcium requirements are increased. Approximately 20-30% of total body calcium is accrued during puberty (Vokes, 2006). In the UK, the reference nutrient intake for children adolescents is between 500 to 1000 mg of calcium per day during rapid bone development (SACN, 2014). Calcium intake is a weak but positive predictor of bone mineral status in children and adolescents in the Caucasian UK population but this might be due to differences in size (Prentice, 2004). Low calcium intakes are associated with low PBM and increased fracture risk in later life (Vatanparast et al., 2010, Vatanparast et al., 2005, Whiting et al., 2004). However, the effects of calcium supplementation to increase calcium intake and bone accrual have shown either no sustained effect or has resulted in unintended consequences on size, BMC and bone strength (Prentice et al., 2012, Ward et al., 2014, Winzenberg et al., 2006a, Winzenberg et al., 2006b).

**Vitamin K** - Vitamin K functions as a cofactor for the vitamin K-dependent carboxylase, a microsomal enzyme that facilitates the post-translational conversion of glutamyl residues to γ-carboxyglutamyl residues in vitamin K-dependent proteins such as prothrombin and osteocalcin (OC). OC is produced by osteoblasts and is the main non-collagenous protein in bone. In its carboxylated form, OC binds to hydroxyapatite in bone and is believed to play a regulatory role in bone formation, mineralisation and resorption, positively affecting bone strength (Fewtrell et al., 2008). Dietary sources of vitamin K include green leafy vegetables, margarine, and plant oils. The FAO recommendation for vitamin K
intake for children and adolescents is 20-55 µg/day and for adults is 55-65 µg/day (FAO, 2001). The concentration of uncarboxylated OC (ucOC), a sensitive marker of vitamin K nutritional status, has been associated with low BMD and hip fracture risk in the elderly (Sokoll and Sadowski, 1996, Booth, 2007, Booth et al., 2003, Booth et al., 2000). Kalkwarf et al. investigated the effect of vitamin K intake on bone turnover and bone mass in healthy females aged 3–16 years in the USA. Females with a higher vitamin K status (measured by phylloquinone and unOC [%]) were associated with decreased bone turnover. ucOC was not associated with 4-year changes in BMC of the hip or TBLH but was positively associated with 4-year changes in lumbar spine BMC. Further research is needed in vitamin K and bone health in children and adolescents.

**Physical activity**

Mechanical strain associated with physical activity plays a key role in the regulation of the mechanostat. Increasing muscle contractions will drive the mechanostat to remodel the bone by altering BMC, bone geometry, and bone microarchitecture to increase the resistance to forces placed upon it. Many studies have reported physical activity to be beneficial to bone strength at all stages of life (Ward et al., 2005b, Wang et al., 2007, Bell et al., 2014, Kelley et al., 2012, Marques et al., 2012, McKay et al., 2005, Morris et al., 1997). Strenuous repetitive exercise has been shown in a cross-sectional study to have site specific effects on cortical and trabecular bone in pre-pubertal gymnasts compared to controls (Ward et al., 2005b). At the 50% radius diaphysis gymnasts had larger bones with greater cortical area (8.2%) and SSI than controls (13.6%). At the 65% tibia diaphysis, gymnasts had greater cortical area (5.3%) and thickness (6.2%) than controls. There were no significant differences in cortical vBMD at the radius or tibia diaphysis between the groups. However, at the distal radius and distal tibia, total bone and trabecular vBMD were significantly greater in gymnasts compared to controls despite similar skeletal size. The authors concluded that gymnasts had a significantly higher bone strength due to increased bone area at the diaphysis as a result of bone adaptation to exercise (Ward et al., 2005b).

Longitudinal studies have reported a positive relationship between physical activity and bone health in children and adolescents. The influence of physical activity on bone and LM accrual during adolescence was assessed in the PBMAS (Baxter-Jones et al., 2008a, Bailey et al., 1999).

Adolescents who had been physically active throughout the two years surrounding PHV had a 9% to 17% greater BMC at the total body, lumbar spine and femoral neck compared to less active peers (Bailey et al., 1999). The skeletal benefits were shown to continue into adulthood, with active adolescent males and females having 8% to 10% more BMC at the hip and femoral neck (Baxter-Jones et al., 2008b). The authors continued to follow these subjects, using pQCT at the tibia (4% and
66% sites) reported male subjects who had been physical active during adolescence had a significantly greater bone strength index and total bone area (13% and 10%, respectively) at the tibia diaphysis compared to less active peers. Females who were more active in adolescence had a significantly larger cortical area and cortical mineral content at the tibia diaphysis (10% and 12%, respectively), and a significantly greater (3%) trabecular mineral content at the distal tibia compared to less active peers (Duckham et al., 2014). However, only vigorous day-to-day physical activity has been positively associated with BMC and geometry in adolescents, whereas light or moderate physical activity has no detectable association. However, physical activity increased LM in males and females but males for the same level of activity accrued 21-120% more absolute LM compared to females. The authors concluded that physical activity had a significant independent influence on the growth of LM, once maturity and body size were controlled (Baxter-Jones et al., 2008a). Greater muscle mass and strength will increase strain on the bone, which will increase bone strength as described by the muscle-bone unit. Therefore, promoting high-impact physical activities performed in childhood and adolescence is likely to benefit skeletal development and improve bone strength in later life (Sayers et al., 2011).

Nogueira et al. conducted a systematic review and meta-analysis on the effects of exercise interventions in school aged children and BMC. Jumping-focused interventions were associated with small increases in TB BMC, femoral neck BMC, and lumbar spine BMC (Nogueira et al., 2014). The authors concluded that physical activity during growth is beneficial for bone health in later life and may help reduce the risk of fracture (Sayers et al., 2011, Nogueira et al., 2014).
2.7 Cystic fibrosis

Cystic fibrosis (CF) is the most common lethal autosomal recessive genetic disease in the White Caucasian population. CF is caused by a mutation in a gene that encodes for CF transmembrane conductance regulator (CFTR) protein, which is expressed in the cells of the lung epithelium, liver, pancreas, reproductive tract and sweat glands. Although CFTR functions mainly as a chloride channel, it has many other regulatory roles including the modulation of trans-epithelial ion transport, hydration of epithelial lining fluids, pH regulation, and inflammation. The mutation of CFTR results in thick sticky dehydrated secretions that cause chronic obstructive and infected airways, pancreatic insufficiencies, biliary disease, malnutrition, elevated sweat chloride concentration and fibrosis of many organs. The applications of new anti-microbial therapies and nutritional knowledge have seen the outlook for CF patients’ survival improve substantially in the past 50 years. In this section, the aetiology of CF and CF-bone disease is discussed in detail.

2.7.1 A brief of history of CF: Witchcraft to science

Although the entire clinical spectrum of CF was not fully recognised until the 1930s, historical searches, mainly by Busch R. whose research involved medieval manuscripts, have suggested that the characteristic symptoms of the disease may have been recognised and associated with morbidity in eastern European before the Middle Ages (Quinton, 1999, Busch, 1990). The earliest accurate medical description of the pancreatic lesion was given in an autopsy report on a supposedly "bewitched" 11-year-old girl in 1595 by Pieter Pauw, a Professor of Botany and Anatomy at Leiden, The Netherlands. He described “the child as having strange symptoms for eight years and during the autopsy the heart was floating in a poisonous liquid, sea green in colour, and the pancreas was swollen, white, and appeared to look cancerous. The little girl was very thin, worn out by a fluctuating fever which persisted” (Figure 2.44) (Busch, 1990, Quinton, 1999).

In 1606, Juan Alonso de los Ruices y de Fontecha, a professor of Medicine at Henares in Spain, first described the salty taste of infants with CF. He wrote that it was known that the fingers tasted salty after rubbing the forehead of the bewitched child. Other references recognising the association between the salt loss in CF and illness can been found in 18th century Germen and Swiss literature warning “Wehe dem Kind, das beim Kuß auf die Stirn salzig schmeckt, er ist verhext und muss bald sterbe” or "Woe to the child who tastes salty from a kiss on the brow, for he is cursed and soon must die," (Busch, 1990, Quinton, 1999). In 1953, Paul di Sant’Agnese, a physician and founder of the CF Foundation in New York, was the first to discover that sweat was abnormal in CF (Di Sant’Agnese et al., 1953) and was responsible for the development of the sweat test, which is now used to confirm the diagnosis of CF (Coury et al., 1983, Gibson and Cooke, 1959, Kessler and Andersen, 1951).

It was not until the 19th and early 20th century that symptoms such as steatorrhoea (presence of excess fat in faeces, caused by abnormal liver and pancreatic function), meconium ileus (bowel
blockage), pancreatic lesions and lung disease were reported together. In 1928, Guido Fanconi in Zurich, was one of the first to report on the sporadic cases of pancreatic infantilism which he called the ‘coeliac syndrome’, describing a connection between coeliac disease (disease of the digestive system and intolerance to gluten), fibrosis of the pancreas, and bronchiectasis (lung disease). Fanconi’s findings would later be confirmed two years later by Dorothy Andersen in New York, who described the autopsy findings of 20 infants with widespread lung infection, and destructive and cystic changes in the lungs with fibrosis in the pancreas itself (Andersen, 1938). Andersen used the term ‘cystic fibrosis of the pancreas’ to describe the disease and was the first to use pancreatic enzyme replacement therapy in patients. This term was later altered by Dr Sydney Farber, Chief of Pathology at the Children’s Hospital, Boston in 1945, who realised that CF affected various organs (not just the pancreas) and was linked to a build-up of sticky mucus secretions due to dehydration of the organ surface. Farber introduced the term “mucoviscidosis” meaning thick, sticky mucus condition.

Figure 2.44 - History of cystic fibrosis.
Left, Pieter Pauw performing an autopsy in the Anatomical Theatre in Leiden (From The Paradox of the Pancreas. Modlin IM, Kidd M (Eds). 2003:280.), and Right, A photo taken in 1989 at the presentation of the Paul di Sant’Agnese award to the leaders of the teams who identified the CF gene – from left to right are Lap-Chi Tsui, Paul di Sant’Agnese, Evelyn Graub, Milton Graub (President of the CF Foundation), Francis Collins and Jack Riordan. Source: (Littlewood, 2009).

One of the greatest achievements in CF history was the discovery of the genetic cause of CF. In 1946, Andersen and her colleague Hodges, were the first to report that CF was caused by an autosomal recessive genetic mutation but the location of the gene and the gene product were unknown. It was not until the 1980s when genetic breakthroughs started to emerge that Eiberg and colleagues (1985) discovered that a polymorphic serum paroxonase marker (an enzyme that catalyses the hydrolysis of organophosphates) was inherited with high frequency with the CF gene, indicating that the CF gene may be located on chromosome 7. This was later confirmed in 1989 by Professor Lap-Chi Tsui, Dr Francis Collins, and Professor Jack Riordan, who successfully cloned the CF gene and reported that
the gene was predominantly expressed in the affected organs (Figure 2.44) (Riordan et al., 1989). They were able to identify the first CF genotype, ΔF508, which is now known to be the most common CF genotype. Subsequent research has found over 1,900 different mutations of CF gene (Bobadilla et al., 2002, Lap-Chee, 2011) and shown that the gene is responsible for producing CFTR protein which regulates chloride and water transport across epithelial cells.

Despite the advances in medical research and management of CF there is still no cure. In the UK, the mean survival age in CF patients has increased from a median age of 6 months in 1959 (Barr et al., 2011) to possible survival into the third and fourth decade of life in 2009 (Dodge et al., 2007). Medical improvements have led to early diagnosis, genetic carrier testing, better nutritional knowledge, dangers of cross-infection of microorganisms, discovery of antibiotics and vaccinations, advances in medication (bronchodilators, corticosteroids and pancreatic enzyme replacement), advances in physiotherapy and airway clearance, and organ transplant, which have dramatically increased patient survival rates and quality of life. Consequently, co-morbidities such as CF-related diabetes and osteoporosis have started to emerge as the CF population grows older. These co-morbidities add to the complexity of disease and have now become the new challenge in CF research.

2.7.1.1 What do we know today
In the UK, the estimated prevalence of CF is 1 in 2500 live births and a carrier frequency of 1 in 25 people. Currently, over 9,000 people have CF in the UK (CF_Trust, 2008). CF is predominately found in the White Caucasian population but there are reports of CF in other ethnic groups (e.g. Asians from the Indian subcontinent constitute 1 in 60 of the UK CF population and 1 in 15000 in African Americans) but the aetiology and prevalence are not well described (McCormick et al., 2002a, McCormick et al., 2005a). The Republic of Ireland has the world’s highest incidence of CF with 1 in 1353 (Farrell et al., 2007, Farrell, 2008).

CF is a very complex disease and affects many organs in the body. According to the CF Trust, 10-15% of infants with CF present symptoms within 24 hours of birth (CF_Trust, 2014). The most common and strong indicators of CF include salty tasting skin, poor weight gain (despite adequate food intakes), accumulation of sticky mucus in the lungs, and difficulties in breathing. One in ten babies born with CF develops meconium ileus, a bowel obstruction, either at birth or within the first few days of life. The meconium is usually very thick and sticky causing an obstruction of the bowel and often requires an urgent bowel operation to remove the blockage. The different pathology and severities of symptoms are not fully understood and can be expressed in milder/severer forms and/or at different stages of life. The main signs and symptoms of CF are described in Table 2.13.
In the last two decades, advances in early CF diagnosis have helped to identify patients during infancy, which has improved survival rates. Methods for early diagnosis include newborn screening (NBS), pilocarpine sweat test, and genetic testing. In the UK, NBS for diagnosing CF was first introduced in 2002 and it has since been reported that patients who were screened and diagnosed at birth had a better weight gain, an older age of acquiring *P. aeruginosa* in sputum, and superior lung function in comparison with non-screened patients (Dijk et al., 2011, Farrell et al., 2001, Dankert-Roelse and Merelle, 2005, Grosse et al., 2006). However, having an abnormal NBS test needs to be
investigated further before CF is diagnosed. A sweat test is conducted at 2-4wks of age to assess the amount of chloride in the sweat. A sweat chloride level of >60 mmol/L is considered abnormal and an indicator of CF (Farrell et al., 2008). Babies with an abnormal NBS and sweat test will then undergo a genetic testing to confirm CF and the genotype. Adults with a family history of CF will be offered genetic testing to confirm whether they are a carrier of CF. NBS and sweat tests can help those who are CF carriers to prepare for the future in terms of family planning. A delay in recognising CF symptoms can lead to long-term problems, decreased quality of life and reduced life expectancy.

2.7.2 Genetics and pathophysiology of cystic fibrosis

The CFTR protein is a member of the ATP-binding cassette (ABC) transporter family of membrane proteins and is located in the apical membranes of epithelial cells. Most ABC proteins use ATP hydrolysis to actively pump substrates across cellular membranes, usually against a concentration gradient, to transport amino acids, sugars, drugs and proteins, and are required for metabolite transport, signal transduction, protein secretion, and antigen presentation (Higgins, 2001). However, CFTR is the only known member of the ABC family that acts as an ion channel (Cant et al., 2014).

CF results from a point mutation in the CFTR gene which is found on the long arm of chromosome 7. The homozygous deletion of a phenylalanine at amino acid position 508 (ΔF508) is the most frequent mutation. Approximately 70% of individuals with CF are homozygous for ΔF508 mutation, and almost 90% of all CF patients have at least one ΔF508 allele (Ameen et al., 2007). The ΔF508 mutation causes the CFTR protein to fold incorrectly and therefore is recognised by the endoplasmic reticulum as abnormal and becomes targeted for proteasomal degradation. The ΔF508-CFTR protein fails to be trafficked to the Golgi and cell surface to achieve chloride ion transport. CFTR mutation causes negatively charged chloride ions to build up with the cell, creating a difference in electrical potential inside and outside of the cell. This leads to positively charged sodium ions being drawn into the cell via the epithelial Na⁺ channel (ENaC). A combination of intracellular sodium and chloride ions creates salt crystals which are later lost in sweat and this forms the basis for the CF sweat test. The electrical ion imbalance results in a reduction in airway surface fluid (dehydrated) and submucosal glands are stimulated to secrete viscous mucus to prevent further dehydration. The differences between non-CF and CF airway function is illustrated in Figure 2.45 (Zeitlin, 2008).
Figure 2.45 - Airway surface in non-CF and CF individuals.
(A) In non-CF airways, CFTR, outwardly rectifying Cl⁻ channel (OOCC), Ca²⁺-activated Cl⁻ channel (CaCC), and ENaC function normally, transporting chloride and sodium ions across the membrane, to maintain a normal electrical balance, depth of airway surface liquid, enabling effective ciliary beat-driven mucociliary clearance. Mucus is secreted and is propelled towards the throat where it is swallowed or coughed-up. (B) In CF airways, CFTR is absent or dysfunctional and ENaC is no longer regulated, leading to hyperabsorption of Na⁺, causing dehydration. The airway surface liquid depth is reduced, the submucosal glands are hypertrophied, excessive mucus is secreted, and mucociliary clearance becomes impaired. This produces an environment which favours bacterial growth and causes acute and chronic lung infections. The P2Y₂ receptor actively pumps Ca²⁺ into the cell, which stimulates Cl⁻ secretions through the CaCC pathway. Source: (Zeitlin, 2008).

Since the discovery of the CFTR mutation in 1989, over 1900 different genotypes have been discovered. These genotypes have been classified into five groups (Class I to V) with regards to disease aetiology and severity (Table 2.14) (Cant et al., 2014, Prickett and Jain, 2013). Class I to III cause serious disease phenotypes and increased morbidity, whereas class IV and V are generally mild-disease causing mutations. These classifications are used as a guidance to predict a patient’s CF phenotype and the level of care a patient will need. However, uncontrollable environmental factors such as the presence of pathogens and frequency of infection, and patient compliance with treatment and physiotherapy will also play a role in determining the severity of the disease.
Table 2.14 - Classification of the CFTR gene mutations.
Source: (Rowntree and Harris, 2003).

<table>
<thead>
<tr>
<th>Class</th>
<th>Consequences</th>
<th>World frequency</th>
<th>Severity</th>
<th>Example of genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Mutations cause a defect in CFTR protein synthesis producing a short, non-functional polypeptide chain (i.e. premature termination of protein synthesis).</td>
<td>10%</td>
<td>Severe</td>
<td>W1282X</td>
</tr>
<tr>
<td>II</td>
<td>Mutations are translated into full-length nascent polypeptide chains but are defective in folding and therefore are targeted for degradation rather than trafficked to the plasma membrane.</td>
<td>70%</td>
<td>Severe</td>
<td>ΔF508 (Most common genotype)</td>
</tr>
<tr>
<td>III</td>
<td>Mutations produce polypeptides chains which are trafficked to the plasma membrane but have channel gating defects that decrease channel opening time and decrease chloride flux.</td>
<td>3-4%</td>
<td>Severe</td>
<td>G551D (second most common mutation)</td>
</tr>
<tr>
<td>IV</td>
<td>Mutations reach the plasma membrane but have decreased channel conductance even when the gate is open.</td>
<td>&lt;2%</td>
<td>Mild</td>
<td>R117H</td>
</tr>
<tr>
<td>V</td>
<td>Mutations represent a fully functional CFTR protein at the plasma membrane but with reduced abundance due to defective mRNA splicing.</td>
<td>&lt;1%</td>
<td>Variable</td>
<td>A455E</td>
</tr>
</tbody>
</table>

The evolutionary origins of CF have been studied to understand why and how this lethal gene mutation has been able to exist in the population with a high level of frequency. The origins of ΔF508 have been studied extensively and it has been reported that this gene mutation occurred more than 52,000 years ago, in a population genetically distinct from any present European group, and spread throughout Europe in chronologically distinct expansions, which are responsible for the different frequencies of ΔF508 in Europe (Bertranpetit and Calafell, 2007, Morral et al., 1994). Like other genetic mutations such as sickle cell anaemia, CF may have a heterozygous advantage to those who are carriers of the disease. It has been suggested that carriers of CF may have resistance to cholera and to other dehydrating intestinal diseases, as a normal host CFTR channel is required for these infections to affect the host. However, this has not been proven as CF has not been linked with populations who have high incidences of cholera. Another theory proposed by Poolman and Galvani is that CF carriers may have a degree of protection against tuberculosis (TB) (Poolman and Galvani, 2007). TB is extremely contagious and requires the host to have functional arylsulphatase enzyme activity to survive. Sulphate is produced by the host, which is then utilised by the TB bacteria to build cell walls and for cell replication. Patients with CF have diminished arylsulphatase activity, therefore
carriers may have some protection against one of the world’s greatest pandemics and the gene has been selectively conserved.

2.7.3 Cystic fibrosis – related bone disease
Low aBMD in CF has become a topic of widespread interest and impact in the CF population and for clinicians treating those with CF. As life expectancy in the CF population has increased, the prevalence of osteopenia and osteoporosis in adolescents and adults has increased significantly. During childhood, fracture risk in CF children is similar to children without CF. However, multiple fractures of the arms, legs, ribs, and vertebrae are common in young CF adults (18 years old) (Paccou et al., 2010). Fractures of the spine and rib cage can be debilitating and can interfere with lung function and mucus clearance, as well as daily physiotherapy, significantly reducing quality of life. A meta-analysis by Paccou et al. reported that the prevalence of osteoporosis and osteopenia (diagnosed using age-matched T-scores from DXA) in young CF adults (mean age 28 years) was 23.5% and 38%, respectively (Paccou et al., 2010). Fracture prevalence in this study was 14% vertebral and 20% non-vertebral fracture. For comparison, lifetime risk of fracture of a 60 year old woman is 3% for vertebral and 16% for forearm fractures (van Staa et al., 2001). Multiple cross-sectional studies have demonstrated an increased incidence of fractures in individuals with CF, with vertebral fractures being the most common followed by rib fractures (Aris et al., 1998, Conway et al., 2008, Elkin et al., 2001, Rossini et al., 2004, Stephenson et al., 2006). Other skeletal problems can be reported as early as the third decade of life such as kyphosis due to vertebral fractures (Aris et al., 1998, Denton et al., 1981, Henderson and Specter, 1994, Tattersall and Walshaw, 2003) and scoliosis (Paling and Spasovsky-Chernick, 1982).

Advances in medication and interventions to improve pulmonary function and nutrition status have increased the age of survival in CF patients but aBMD remains significantly lower compared to a healthy population. Putman et al. compared DXA-derived aBMD of two CF cohorts (i.e. a historic cohort from 1995-1999 and a present-day cohort from 2011-2013) and reported aBMD of the spine, lateral spine, and distal radius to be similar between the two cohorts despite significant improvements in pulmonary function and vitamin D status (Putman et al., 2015). The causes of low aBMD in CF are likely to be multifactorial due to poor nutritional intake, malabsorption, chronic inflammation and infection, reduced levels of physical activity, glucocorticoid use, poor growth and delayed puberty all contributing to varying degrees. Further research is needed to help understand CF-related bone disease.
2.7.3.1 Growth and development in cystic fibrosis

Growth disturbances are common in children and adolescents with CF (Johannesson et al., 1997, Landon and Rosenfeld, 1987, Reiter et al., 1981). Delayed puberty is said to occur when no signs of puberty have appeared at a chronological age 2 SD above the mean for the onset of puberty (13.4 years in girls and 13.8 in boys) (Albanese and Stanhope, 1995). Slow growth and delayed puberty are associated with delayed skeletal maturation and reduced accrual of bone mineral, likely to result in low PBM and increased risk of developing osteoporosis and fracture in later life (Albanese and Stanhope, 1995, Bonjour et al., 1994). Morison et al. reported height, weight and body mass index (BMI) of 3056 (1604 males) UK patients with CF in the 1990s (Morison et al., 1997). Children and adolescents with CF had age-and- gender matched standard deviation (SD) scores of -0.75 to -1.25 for all anthropometric measures. Improvements occurred between the ages of 5 and 10 years of age indicating a period of ‘catch-up’ in growth but were still below normal. However, after 10 years of age, height, weight, and BMI were at lower percentiles of reference population. A decrease from population norms occurred earlier in girls than boys, but was less steep and more protracted. The authors suggested that males may have delayed puberty due to slow increases in BMI after 10 years of age but pubertal timing in females was not mentioned. Other studies have shown CF females to have delayed menarche (i.e. 13.9 years in CF females compared to 12.8 years in healthy females). During the pubertal spurt, CF children do not have the ability to grow as fast as their peers and therefore are shorter and lighter at the end of maturity.

Despite the advances in treatments and management of CF, growth disturbances are still commonplace in 2012. The UK CF Trust’s annual data report for 2012 present the most recent dataset for median height and weight of 4256 children and adolescents with CF relative to percentiles of the healthy population (Figure 2.46) (CF_Trust, 2013). The growth trend is similar to that reported by Morison et al. in the 1990s. Children aged 2-10 years tracked between the 30-40th percentile for height and 30-50th percentile for weight. As puberty begins, there is a period of ‘catch-up’ in height and weight for females aged 10-12 years and for males aged 11-13 years. However, this is followed by rapid decline and the phase of rapid pubertal growth is inadequate and cannot compensate for the slow growth during pre-puberty.

Reduced aBMD was first described in patients with CF in 1979 but the full extent of the problem was not realised until the 1990s, when several cross-sectional BMD studies were performed using Dxa in children and adults with CF (Mischler et al., 1979). Low aBMD is highly prevalent in adults with CF and is often correlated with disease severity, chronic lung infections, weight loss, malnutrition (fats, calcium, vitamins D and K) and use of corticosteroid. Haworth et al. prospectively measured the

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3 SD scores = standard deviation score from the mean height and weight of an age-and-gender matched control population.
aBMD of 114 adults with CF (Haworth et al., 2002). In 55 patients with a mean age of 19 years, in whom aBMD would normally be expected to increase annually before reaching PBM, aBMD was stable in the lumbar spine but decreased by more than 2% per year in the proximal femur. In 59 patients with a mean age of 30 years, in whom aBMD would normally be expected to remain stable having reached PBM, there were no significant changes in the lumbar spine but there were significant annual reductions in aBMD in the proximal femur (-1.5%) and distal forearm (-0.8%). Haworth et al. concluded that reduced rates of bone accretion and early rates of bone loss explained the high prevalence of low BMD in adults with CF. Data were not adjusted for body size or compared to a control group.

Figure 2.46 - Median height and weight percentiles for children and adolescents aged 2 to 19 years. Puberty in healthy females occurs between the ages of 10 to 12 years and in healthy males at the ages of 12 to 16 years. Delayed puberty is common in children with CF and tend to grow at a slower rate, achieving lower height and weight compared to their healthy peers. Source: (CF_Trust, 2013).

Research into the causes of bone loss in CF has shifted from observing older patients to children and adolescents to pin-point when bone disease begins to occur and whether the cause is due to poor growth during early life or progression of lung disease in early adulthood. Currently, the timing and aetiology of the onset of bone disease in patients is unknown and studies with children are inconclusive as reports have provided conflicting results. This is mainly due to small sample sizes and the limitations of DXA with regards to adjusting for body size (as described in Chapter Strengths and
Limitations of DXA. Therefore, more research is needed using more robust imaging techniques (i.e. pQCT and HR-pQCT), which can measure vBMD, bone geometry, and bone microarchitecture, independently of bone size.

In healthy children, total body aBMD increases with age on a trajectory, which parallels linear growth. However, in CF, it appears that aBMD begins to deviate from normal development around the time of puberty. Some studies have reported normal SD scores for height, weight, and total body aBMD in pre-puberty, with a decline from normality occurring during adolescence, which without recovery is likely to progress to a significant bone deficit in adulthood (52-57). This suggests that the disease severity, malnutrition and hypogonadism (delayed puberty) are responsible for lower bone accrual in CF. Hardin et al. reported that there was no difference in aBMD between children with CF and controls, and suggested that well-nourished children with CF had smaller but normally mineralised bones (Hardin et al., 2001). The authors concluded that low aBMD in CF may be caused more by malnutrition and chronic use of corticosteroids. Buntain et al. performed a case/control cross-sectional study involving 152 CF patients and 149 controls (Buntain et al., 2004). Compared to controls, mean aBMD was not significantly different in children aged 5-10 years with CF. An aBMD deficit appeared to evolve during adolescence when a reduced (-33%; but non-significant) total body and radius aBMD was observed after adjusting for age, sex, and height. The reduction in aBMD became more marked in adults at the lumbar spine, total body, and femoral neck (Buntain et al., 2004).

Other studies have reported differences in height, weight, and aBMD in pre-pubertal stages (<8 years old). Differences during the prepubertal stages of life would indicate that CF mutation may have a direct influence on bone, affecting bone formation and resorption rates, and the later bone deficit, as the disease severity is very mild during childhood and the effects of hypogonadism and delayed puberty would not have occurred at this early time (Lucidi et al., 2009, Henderson and Madsen, 1996, Humphries et al., 1998, Douros et al., 2008, Bhudhikanok et al., 1998, Morison et al., 1997).

DXA has been the most common technique used to assess CF-related bone disease. There are only two studies that have used pQCT and one study with HR-pQCT to measure bone parameters in CF patients (Louis et al., 2009, Putman et al., 2014, De Schepper et al., 2012). Sood et al. reported normal BMC, decreased bone mineral apparent density (measured by DXA), and higher vertebral (T12-L3) trabecular vBMD (measured by QCT) together these data suggest that the vertebral cortical thickness or cortical density may be reduced in CF children (Sood et al., 2001). Louis et al. used pQCT to assess the radius of 48 young adults with CF (Louis et al., 2009). The authors reported vBMD, total CSA, of the radius and body composition of the forearm to be normal. However, the cortical thickness was significantly reduced in males (SD score; -1.22, P<0.05), and females (SD score; 1.61,
P<0.05). The conclusions drawn from these studies should be viewed with caution as the sample size was small but these studies provide an insight into potential compartmental differences in bone in individuals with CF. De Schepper et al. reported adolescents with CF to have bones with a small CSA but with normal vBMD at the 4% and 66% sites of the radius. The authors concluded that older females were at greater risk of fracture due to having thin forearm bones (De Schepper et al., 2012).

To date, only one study has used HR-pQCT to investigate bone health in young adults with CF (aged 18-40 years) (Putman et al., 2014). The authors concluded that CF patients had smaller bone CSA, lower total vBMD, and lower trabecular vBMD in the tibia and radius (Putman et al., 2014). These findings should be viewed with caution as the standard protocol, which uses a fixed scanning ROI was used to assess bone despite significant height differences between CF and controls (Ward et al., 2015). Therefore, the ROI in CF patients may be positioned more proximal (i.e. in the diaphysis region where the bone is more narrow and there is less trabecular bone and thicker cortices) whereas the ROI in the controls is more distal (i.e. in the metaphysis where the bone is wider and more trabecular bone). Future studies must account for differences in bone length when assessing patients with poor growth or stunted height by using a relative (i.e. percentage length from fixed anatomic landmark) rather than a fixed distance. This will ensure that the ROI is positioned relatively in the same place in both the patient and control group (Nishiyama et al., 2012a, Burrows et al., 2010a, Burrows et al., 2010b, Burrows et al., 2010c). All the evidence from pQCT and HR-pQCT has demonstrated children and adults with CF to have a smaller CSA with lower bone mineral. Further investigations into the timing and nature of the onset of bone disease and the characterisation of the bone phenotype (i.e. the amount of bone mineral, bone geometry, and bone microarchitecture) needs to be carried out, especially in children and adolescents to understand why CF patients develop osteoporosis and the aetiology of increased fracture risk in early adulthood.

2.7.3.2 Abnormal bone turnover in cystic fibrosis

Most studies measuring serum and urinary bone turnover markers indicate that CF patients have a combination of accelerated bone resorption and inadequate bone formation. Cobanoglu et al. studied 16 CF children, aged 4-8 years old, with an age and sex matched control group and found that serum osteocalcin (OC), a marker of bone formation, was lower in CF but total alkaline phosphatase (ALP), also a marker of bone formation, was higher in CF compared to controls (Cobanoglu et al., 2009). Urinary hydroxyproline levels (a marker of bone resorption) were similar in both groups. However, ALP activity may be a less specific formation marker in CF because it measures total alkaline phosphatase, which is liver and bone derived, because biliary obstruction may raise liver alkaline phosphate levels. It is important to consider liver function in CF patients when using ALP as a marker as it is difficult to specify the exact origin of ALP (Aris et al., 2002). In this study,
CF children did not have liver disease but the small sample size may have limited power to detect differences. Grey et al. reported increased bone resorption in 16 CF patients aged 17-42 years old, as they had high urinary hydroxyproline levels but normal 24 hour urinary calcium excretion compared to the controls (Grey et al., 1993).

Baroncelli et al. investigated 59 young adults with CF and 72 age-and-gender matched controls and found increased values in serum cross-linked carboxy-terminal telopeptide of type I collagen (CTx) and urinary values of cross-linked N-telopeptides of type I collagen (NTx) in prepubertal, pubertal, and young adults with CF, suggesting that CF have increased levels of bone resorption (Baroncelli et al., 1997). The reduced levels of OC and carboxy-terminal propeptide of type I procollagen (PICP, formation marker) in pubertal patients and the reduced values of OC in young adults suggests that reduced bone formation also contributes to disordered bone metabolism in CF. Aris et al. reported bone turnover markers in 50 clinically stable adults with CF and 53 matched controls. Patients with CF had higher urinary NTx and free deoxypyridinoline levels than controls (Aris et al., 2002). Serum OC levels were similar in the two groups. These data suggest that adults with CF have increased bone resorption with relatively normal levels of bone formation.
### 2.7.3.3 Summary of cystic fibrosis-related bone disease

Studies in CF-related bone disease are summarised in **Table 2.15**.

**Table 2.15 - A summary of previous studies assessing CF-related bone disease in children, adolescents and young adults.**

<table>
<thead>
<tr>
<th>Authors (Year)</th>
<th>Study population &amp; CF sample size</th>
<th>Sex &amp; Age (Years)</th>
<th>Imaging technique</th>
<th>Use of controls</th>
<th>Scan site</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Bhudhikanok et al., 1998)*</td>
<td>USA (n = 41)</td>
<td>M &amp; F 9 - 50</td>
<td>DXA</td>
<td>No</td>
<td>Total body, lumbar spine &amp; femoral neck</td>
</tr>
<tr>
<td>(Buntain et al., 2004)</td>
<td>Australia (n = 153)</td>
<td>M &amp; F 5 - 56</td>
<td>DXA</td>
<td>Yes (149 age &amp; sex matched)</td>
<td>Total body, lumbar spine, femoral neck, cortical wrist (R33%) &amp; distal wrist</td>
</tr>
<tr>
<td>(Buntain et al., 2006)*</td>
<td>(n = 85)</td>
<td>M &amp; F 5 - 18</td>
<td>DXA</td>
<td>Yes (100 age &amp; sex matched)</td>
<td>Total body, lumbar spine &amp; femoral neck</td>
</tr>
<tr>
<td>(Bianchi et al., 2006)*</td>
<td>Italy (n = 136)</td>
<td>M &amp; F 3 - 24</td>
<td>DXA</td>
<td>No</td>
<td>Total body &amp; lumbar spine</td>
</tr>
<tr>
<td>(Conway et al., 2000)</td>
<td>UK (n = 114)</td>
<td>M &amp; F 16 - 30</td>
<td>DXA</td>
<td>No</td>
<td>Total body, lumbar spine &amp; femoral neck</td>
</tr>
<tr>
<td>(Cobanoglu et al., 2009)</td>
<td>Turkey (n = 16)</td>
<td>M &amp; F 4 - 8</td>
<td>DXA</td>
<td>Yes (16 controls)</td>
<td>Lumbar spine, femoral neck and total hip</td>
</tr>
<tr>
<td>(De Schepper et al., 2012)</td>
<td>Belgium (n = 64)</td>
<td>M &amp; F 12 - 26</td>
<td>DXA</td>
<td>No</td>
<td>Total body</td>
</tr>
<tr>
<td>(Donovan et al., 1998)</td>
<td>USA (n = 30)</td>
<td>M &amp; F 17 - 52</td>
<td>DXA</td>
<td>No</td>
<td>Lumbar spine, femoral neck, total hip &amp; radius</td>
</tr>
<tr>
<td>(Elkin et al., 2001)</td>
<td>UK (n = 107)</td>
<td>M &amp; F 18 - 60</td>
<td>DXA</td>
<td>No</td>
<td>Lumbar spine &amp; femoral neck</td>
</tr>
<tr>
<td>(Fewtrell et al., 2008)</td>
<td>UK (n = 32)</td>
<td>M &amp; F 8 - 12</td>
<td>DXA</td>
<td>No</td>
<td>Total body &amp; lumbar spine</td>
</tr>
<tr>
<td>(Greer et al., 2003)</td>
<td>Australia (n = 149)</td>
<td>M &amp; F 5 - 56</td>
<td>DXA</td>
<td>Yes (141 controls)</td>
<td>Lumbar spine</td>
</tr>
<tr>
<td>(Grey et al., 2008)</td>
<td>Canada (n = 81)</td>
<td>M &amp; F 9 - 17</td>
<td>DXA</td>
<td>No</td>
<td>Total body &amp; lumbar spine</td>
</tr>
<tr>
<td>(Gronowitz et al., 2003)</td>
<td>Sweden (n = 70)</td>
<td>M &amp; F 6 - 49</td>
<td>DXA</td>
<td>No</td>
<td>Lumbar spine &amp; femoral neck</td>
</tr>
<tr>
<td>(Grey et al., 1993)</td>
<td>Australia (n = 22)</td>
<td>M &amp; F 17 - 42</td>
<td>DXA</td>
<td>No</td>
<td>Total body, lumbar spine &amp; femoral neck</td>
</tr>
<tr>
<td>(Hardin et al., 2001)</td>
<td>USA (n = 41)</td>
<td>M &amp; F 17 - 42</td>
<td>DXA</td>
<td>Yes (17 controls)</td>
<td>Total body, lumbar spine, femoral neck &amp; trochanter</td>
</tr>
<tr>
<td>(Henderson and Madsen, 1996)</td>
<td>USA (n = 62)</td>
<td>M &amp; F 4 - 18</td>
<td>DXA</td>
<td>No</td>
<td>Lumbar spine &amp; proximal femur</td>
</tr>
<tr>
<td>(Haworth et al., 1999)</td>
<td>UK (n = 151)</td>
<td>M &amp; F 15 - 52</td>
<td>SXA</td>
<td>Distal and ultra-distal forearm</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>QCT</td>
<td>Lumbar spine</td>
<td></td>
</tr>
</tbody>
</table>

124
Table 2.15 – Continued - A summary of previous studies assessing CF-related bone disease in children, adolescents and young adults.

<table>
<thead>
<tr>
<th>Authors (Year)</th>
<th>Study population &amp; CF sample size</th>
<th>Sex &amp; Age (Years)</th>
<th>Imaging technique</th>
<th>Use of controls</th>
<th>Scan site</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Haworth et al., 2002)*</td>
<td>UK (n = 114)</td>
<td>M &amp; F 16 - 35</td>
<td>DXA</td>
<td>No</td>
<td>Lumbar spine &amp; proximal femur</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>SXA</td>
<td></td>
<td>Distal and ultra-distal forearm</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>QCT</td>
<td></td>
<td>Lumbar spine</td>
</tr>
<tr>
<td>(Haslam et al., 2001)</td>
<td>Australia (n = 22)</td>
<td>M &amp; F 7 - 13</td>
<td>DXA</td>
<td>No</td>
<td>Total body</td>
</tr>
<tr>
<td>(Mortensen et al., 2000)</td>
<td>USA (n = 11)</td>
<td>M &amp; F 8 - 12</td>
<td>DXA</td>
<td>Yes</td>
<td>Radius and ulna</td>
</tr>
<tr>
<td>(Ujhelyi et al., 2004)</td>
<td>Hungary (n = 38)</td>
<td>M &amp; F 4 - 30</td>
<td>DXA</td>
<td>No</td>
<td>Lumbar spine &amp; femoral neck</td>
</tr>
<tr>
<td>(Sood et al., 2001)</td>
<td>UK (n = 29)</td>
<td>M &amp; F 5 - 16</td>
<td>DXA</td>
<td>Yes</td>
<td>Total body, lumbar spine &amp; total hip</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>QCT</td>
<td></td>
<td>T12-L3 vertebral bodies</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>BUA</td>
<td></td>
<td>Calcaneal</td>
</tr>
<tr>
<td>(Louis et al., 2009)</td>
<td>Brussel (n = 48)</td>
<td>M &amp; F 13 - 30</td>
<td>DXA</td>
<td>No</td>
<td>Total body</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>pQCT</td>
<td></td>
<td>Radius (4% &amp; 66% sites)</td>
</tr>
<tr>
<td>(Putman et al., 2014)</td>
<td>USA (n = 30)</td>
<td>M &amp; F 18 - 40</td>
<td>HR-pQCT</td>
<td>Yes</td>
<td>Distal radius and distal tibia</td>
</tr>
</tbody>
</table>

Key: Longitudinal studies are denoted with an asterisk*, male (M), female (F), Dual-energy X-ray absorptiometry (DXA), peripheral quantitative computed tomography (pQCT), High resolution pQCT (HR-pQCT), Single x-ray absorptiometry (SXA), and Broadband ultrasound attenuation (BUA).

2.7.4 Cystic fibrosis-related muscle disease

Sarcopenia, the loss of skeletal muscle mass and strength, and exercise intolerance has long been recognised as a feature in CF disease. Reasons for limited exercise capacity are complex and multifactorial; including poor pulmonary function, malnutrition, gonadal dysfunction, inflammation, glucocorticoid therapy, and the cardio-respiratory system’s ability to meet the metabolic demands associated with exercise, all of which can also be detrimental to bone strength. To date, characterisation of muscle in children and adults with CF has not been well described as research into muscle health has been scarce. This is mainly due to a lack of interest in muscle research in CF but also muscle assessment techniques vary and this has led to inconsistencies in measuring muscle strength, thus making it difficult to compare conclusions. Bone adapts to the stress and strains of muscle contractions, therefore it is essential to measure muscle to understand how muscle affects bone strength. There are limited reference datasets and muscle measurements are often not used in clinical practice despite the knowledge that muscle strength is tightly coupled with bone strength.
2.7.4.1 Muscle mass in patients with cystic fibrosis

Studies have reported LBM and FFM measured by DXA to be significantly lower in children (Sheikh et al., 2014, Stallings et al., 2005, Reix et al., 2010, Mughal et al., 2006, Bianchi et al., 2006, Sood et al., 2003) and adults (Ionescu et al., 2003, Ionescu et al., 2002, Ionescu et al., 2000b, King et al., 2014) with CF compared to their peers, with increasing muscle loss with growth (Figure 2.47) (Elkin et al., 2000). The loss of LBM or FFM has been shown to be a significant negative predictor of survival, independently of pulmonary function and gas exchange (Sharma et al., 2001).

![Figure 2.47 - Changes in percentage of lean body mass (LBM) with age in children with CF and size-matched controls.](image)

The regression slope was 70.8 (95% CI: 71.6 to 70.1) and for controls 0.16 (95% CI: 70.4 to 0.72) Source: (Mughal et al., 2006). Graph based on data from (Elkin et al., 2000).

2.7.4.2 Peripheral muscle strength in patients with cystic fibrosis

Low peripheral muscle strength is common in patients with CF (Troosters et al., 2009, Elkin et al., 2000, Pinet et al., 2003, Lands et al., 1993, De Meer et al., 1999, Jong et al., 2001). Troosters et al. reported isometric quadriceps force (QF, %) to be significantly reduced in 56% of stable CF adults (Troosters et al., 2009). Studies have reported QF, peak torque and muscle CSA to be reduced by 25-35% in CF patients compared to age-matched controls (Elkin et al., 2000, Pinet et al., 2003, Troosters et al., 2009). However, three studies have reported no differences in peripheral muscle strength between patients with CF and controls (Alison et al., 1997, Hanning et al., 1993, Sahlberg et al., 2005). This may be due to the small sample size and mild severity of CF. Elkin et al. reported CF patients to have significant lower QF, LBM, and leg aBMD compared to controls (Elkin et al., 2000). The authors concluded that CF patients were significantly weaker due to lower muscle mass rather than reduced force-generating capacity of the muscle. De Jong et al. reported lower muscle force as a result of airflow obstruction and the relationship was significant when FEV1 was correlated with
predicted knee extensor strength and percent predicted elbow flexor strength (Jong et al., 2001). Moser et al. reported mid-thigh muscle CSA, measured by MRI, did not differ between CF and non-CF children (Moser et al., 2000). However, CF children had a lower peak oxygen consumption which suggests that there is a muscle-related abnormality in oxygen metabolism in CF. It remains unclear whether patients with CF have muscle weakness and what factors are associated with poor muscle growth and low muscle strength.

2.7.4.3 Respiratory muscles in patients with cystic fibrosis
Respiratory muscle weakness is often reported in CF and is associated with hyperinflation and poor nutrition (Lands et al., 1990, Szeinberg et al., 1985). However, some studies have shown that respiratory and abdominal muscles are preserved despite peripheral muscle wasting and hyperinflation (Mier et al., 1990, Pinet et al., 2003). Pinet et al. reported twitch transdiaphragmatic pressure to be 23% lower and twitch gastric pressure to be 22% greater in 18 stable CF adults compared to controls (Pinet et al., 2003). Diaphragm mass and abdominal muscle thickness were similar in both groups. The authors concluded that the diaphragm and abdominal muscles may be conserved as a result of a training effect (i.e. physiotherapy and lung clearance).

2.7.5 Factors affecting bone and muscle in patients with cystic fibrosis
CF is a complicated disease and there are many factors which are likely to affect the muscle-bone unit. These factors are briefly described below:

2.7.5.1 Endocrine complications and malabsorption
In CF, intestinal malabsorption occurs in approximately 95% of patients due to exocrine pancreatic insufficiency and altered bile salt metabolism, which leads to malabsorption of fat and fat-soluble vitamin deficiency (vitamins A, D, E, and K) (CF_Trust, 2002). Poor nutritional status is associated with lower pulmonary function and a deterioration of overall health in CF patients. The CF Foundation clinical guidelines recommend BMI to be maintained above the 50th percentile of growth reference in children and above a BMI score of 22 in adults to prevent worsening of the disease (Stallings et al., 2008). Nutritional advice and supplements have been widely used to improve the nutritional status of CF patients. It is now recommended that patients with CF are encouraged to eat a high-fat, high-protein diet with appropriate pancreatic enzyme supplements and additional vitamin supplements to help prevent malnutrition, poor growth and stunting. In the following section, vitamin D, calcium, and vitamin K will be considered as these nutrients are essential for bone and muscle strength.

A) Vitamin D - CF represents the “perfect storm” for vitamin D deficiency as depicted in Table 2.16 (Hall et al., 2010). The Global Consensus panel on management of nutritional rickets and osteomalacia have recommended vitamin D status, based on serum 25-hydroxyvitamin D (25[OH]D)
levels, to be classified as; sufficiency (>50 nmol/L), insufficiency (30–50 nmol/L), and deficiency (<30 nmol/L) (Munns et al., 2016). Vitamin D deficiency in CF has been well documented. Boyle et al. studied 111 patients with CF and reported that 66% had a 25(OH)D concentration below 75nmol/L (30ng/ml) (Boyle et al., 2005). These individuals had a significantly higher serum PTH concentration (>50pg/ml) than those with adequate vitamin D, suggesting chronic deficiency and possibly increased bone turnover in CF. There were no control participants in this study.

Solomons et al. in 1981 were among the first to document low 25(OH)D in young CF patients, despite good therapeutic management (Solomons et al., 1981). Most supplementation studies with high doses of vitamin D have failed to improve 25(OH)D status. Lark et al. reported that adults with CF absorbed less than one-half of a single dose of vitamin D\textsubscript{2} (whole dose was 100,000IU) compared to control subjects (Lark et al., 2001). Whereas, the control subjects showed a significant increase in serum 25(OH)D. When analysed individually, large variations in vitamin D absorption were recorded, with some CF subjects absorbing virtually none of the administered dose. Other large UK based studies have also supported the findings of Lark et al. Haworth et al. reported 38% (53/139) of adults with CF had 25(OH)D concentration below <37.6 nmol/L (15ng/ml) despite supplementation of 900IU vitamin D/day (Haworth et al., 1999), and Conway et al. reported that 39.8% of adolescents and adults had 25(OH)D concentration below <37.6 nmol/L (15ng/ml) despite 800IU of vitamin D/day (Conway et al., 2000). Some studies have reported low 1,25(OH\textsubscript{2})D despite normal 25(OH)D and PTH concentrations in CF patients, which would suggest that conversion of 25(OH)D to its active form 1,25(OH\textsubscript{2})D is disrupted. Reported values for PTH concentrations in CF adults are inconsistent between studies, with reports of PTH being normal (Donovan et al., 1998, Papaioannou et al., 2008, Haworth et al., 2002, Haworth et al., 1999), high (Aris et al., 2002, Mortensen et al., 2000) and subnormal (Greer et al., 2003, Grey et al., 2008).

Chronic vitamin D deficiency, especially during winter, may contribute to increased bone turnover and bone deficits seen in CF patients. To circumvent the problems with intestinal malabsorption in CF patients, studies have used alternative methods of administrating vitamin D which bypass the gastrointestinal system. Only one trial has been conducted in CF patients involving an administration of 500,000 IU vitamin D\textsubscript{2} via intramuscular injection (Ontjes, 2000). This failed to increase calcium absorption and 25(OH)D concentration two weeks post-injection. This suggests that deficiency cannot be solely blamed on malabsorption of fat, and that vitamin D metabolism may be impaired (Ontjes, 2000). A number of possible defects in vitamin D metabolism have been suggested, which include; genes that encode for enzymes and proteins involved in the different stages of vitamin D metabolism and transport (i.e. altered membrane phospholipid composition in the skin which may affect the transformation of pre-vitamin D\textsubscript{3} into vitamin D\textsubscript{3}, impaired gut absorption carriers, low concentration of vitamin D binding protein (DBP) due to impaired glycosylation of the protein
(exposing unbound 1,25(OH₂)D to increase risk of catabolism in the serum), and increased urinary excretion of the complex DBP-25(OH)D (Mailhot, 2012). To date, DBP has not been studied in CF.

Despite many reports of vitamin D insufficiency in CF patients, most studies have not found a correlation between serum 25(OH)D and BMD. Some studies have reported low BMD in CF patients despite adequate 25(OH)D. Bone histology studies have not reported osteoid features of vitamin D deficiency osteomalacia (i.e. unmineralised and “softening” of the bone). Currently, there have been no studies in patients with CF investigating the association between vitamin D status and muscle function. Studies have shown that improvements in vitamin D status may improve muscle strength, and therefore increase bone strength indirectly (Ward et al., 2009). This may also occur in CF patients.

B) Calcium - In CF, a calcium-rich diet is recommended with the aid of calcium supplements. Despite large doses of calcium via diet and supplements, CF children have been shown not to absorb calcium efficiently compared to healthy controls, where serum calcium and calcium excretion did not increase in CF children but did so in the control group (Mortensen et al., 2000). Although most studies report adequate calcium intakes in people with CF, those with poor 25(OH)D status, uncontrolled pancreatic disease (due to incorrect pancreatic enzymes replacement therapy), and poor appetite may have a poor calcium intake, due to malabsorption and increased calcium faecal loss.

C) Vitamin K - Vitamin K deficiency has been reported to be common in CF patients due to pancreatic disease and fat-malabsorption. Grey et al. reported the prevalence of vitamin K deficiency in 81 children and adolescents with CF (Grey et al., 2008). Thirty eight percent of the children had low aBMD, defined as a SD score between -1.0 and -2.0, for whole body, and 28% for lumbar spine. Nine percent of children had a SD score less than -2.0⁴ for both whole body and lumbar spine. Suboptimal vitamin K was reported in 82% of patients and was associated with low BMD. Fewtrell et al. reported that high concentrations of unOC (i.e. OC which has not been carboxylated due to vitamin K deficiency) were associated with low lumbar BMC in CF children between 8-12 years (Fewtrell et al., 2008). Conway et al. reported 65/93 (70%) children with CF to have low serum vitamin K levels (Conway et al., 2005). Serum vitamin K showed a significant negative correlation with unOC concentration but showed no correlation with any markers of bone turnover. unOC concentrations were correlated significantly with bone turnover markers, which themselves showed a significant correlation with BMD.

⁴ SD scores = standard deviation score from the mean height and weight of an age-and-gender matched control population. Osteoporosis in children is classified as having a SD score of -2.0, plus other risk factors i.e. fracture history (Bishop et al., 2014).
negative correlation with measurements of BMD and BMC. There were, however, no significant correlations between carboxylated or uncarboxylated OC levels and bone measurements.

Table 2.16 – Potential causes of vitamin D insufficiency in patients with CF.
Source: (Hall et al., 2010)

<table>
<thead>
<tr>
<th>Causes of vitamin D insufficiency</th>
<th>Reasons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor vitamin D status</td>
<td>• Low energy levels result in reduced outdoor activities.</td>
</tr>
<tr>
<td></td>
<td>• High burden of physiotherapy and medications.</td>
</tr>
<tr>
<td></td>
<td>• Photosensitivity due to antibiotics, therefore sunscreen may be used.</td>
</tr>
<tr>
<td></td>
<td>• Immobilisation and high number of hospital admissions during infection.</td>
</tr>
<tr>
<td></td>
<td>• PTH abnormally elevated.</td>
</tr>
<tr>
<td>Pancreatic exocrine insufficiency</td>
<td>• Inability to absorb fatty acids and fat-soluble vitamin D from the diet.</td>
</tr>
<tr>
<td></td>
<td>• Due to fat malabsorption, CF patients have reduced body fat therefore cannot store vitamin D.</td>
</tr>
<tr>
<td>Liver abnormalities</td>
<td>• Possible defects in the production of vitamin D carriers; albumin and DBP</td>
</tr>
<tr>
<td>Impaired renal function</td>
<td>• Possible defect in 25 hydroxylation of vitamin D.</td>
</tr>
<tr>
<td></td>
<td>• Possible accelerated excretion of vitamin D through enterohepatic dumping before exposure to 25-hydroxylase enzyme.</td>
</tr>
</tbody>
</table>

The relationship between vitamin K deficiency and CF-bone related disease is unclear. A cause-and-effect relationship between vitamin K deficiency and low BMD in CF has not been proven but subclinical vitamin K deficiency may be important in the development of CF-related low BMD. Vitamin K supplementation has shown to improve bone strength in post-menopausal women and may reduce fracture risk in later life (Feskanich et al., 1999). Little is known about vitamin K status and bone metabolism in children. Vitamin K supplementation has been shown to reduce bone turnover in children compared to controls (Kalkwarf et al., 2004, O’Connor et al., 2007). However, the effects of vitamin K status on bone phenotype and on bone measurements, quantified by pQCT, have not been carried out in a CF population.

2.7.5.2 Lung disease and inflammation
Dehydration of the lung’s epithelial lining and accumulation of thick sticky mucus leads to chronic chest infections in CF children and adults. Chest infections, especially with pseudomonas aeruginosa, become increasingly more common with age (Figure 2.48 A), which decreases lung function, as measured by volume that has been exhaled at the end of the first second of forced expiration (FEV1%) (Figure 2.48 B). CF is associated with high circulating levels of proinflammatory cytokines.
Concentrations of immunoreactive interleukin-6 (IL-6) and other cytokines are increased in CF patients with chronic pulmonary infection and this has been shown to influence bone turnover (Nixon et al., 1998, Norman et al., 1991). Ionescu et al. reported that low aBMD was related to increased levels of cytokines, IL-6 and tumour necrosis factor and reduced lung function (Ionescu et al., 2000a). Shead et al. analysed serum samples from 24 adults with CF taken before, during, and after treatment of lung infection (Shead et al., 2010). Osteoclast number and activity were increased at start of exacerbation of infection and decreased with antibiotic therapy. Other studies have also supported Ionescu et al. and Shead et al.’s findings but the mechanism linking inflammation and bone loss and/or reduced bone accrual in CF is still unclear but remains an important factor in understanding bone health in CF (Aris et al., 2000, Haworth et al., 2004, Shead et al., 2006).

Failure to maintain normal body weight and composition is associated with increased morbidity and poor quality of life. It has been suggested that host inflammatory and metabolic responses to chronic pulmonary infection may have an impact on maintaining a healthy weight and body composition (Ionescu et al., 2003). Children require a positive balance to meet energy requirements to sustain a pattern of normal growth, development, and physical activity (Trabulsli et al., 2006). However, changes in body composition suggest a chronic negative balance between energy intake and energy expenditure. Studies have reported a 25-80% greater energy requirement in moderate to severe CF patients compared to matched controls (Shepherd et al., 2001, Stallings et al., 2005, Pencharz et al., 1984). Therefore, CF children and adolescents tend to be shorter and lighter compared to their peers. Ionescu et al. reported a significant increase in urinary pseudouridine (PSU), a marker of protein breakdown, and cross-linked N-telopeptides of type I collagen (NTx), a marker of bone resorption, in forty adults with CF (Ionescu et al., 2002). The authors concluded that clinically stable adults were in a catabolic state with both cellular and connective tissue protein breakdown, which resulted in low BMC, low muscle mass and muscle weakness. The breakdown of muscle and bone was related to lung disease severity, inflammation, and body composition. However, Dufresne et al. reported that the intensity of systemic inflammation in mild to moderate CF does not account for the variance of FFM or muscle weakness and that other factors must be contributing to reduced muscle strength (Dufresne et al., 2009).
2.7.5.3 Physical exercise

Weight-bearing exercise is advocated by the WHO as an important lifelong strategy in all populations to maintain or improve bone and muscle strength through loading of the skeleton (WHO, 2010). The CF Trust recommend patients take part in regular, moderate to intense exercise to help maintain good pulmonary function, lung clearance, maintain bone and muscle strength, improve cardiovascular endurance, and for psychological well-being. Despite the positive benefits of exercise, children and adolescents with CF are not as active as their peers (Nixon et al., 2001, Hussey et al., 2002) and will become less active with increasing age and disease severity. It remains unclear whether exercise intolerance is due to decreased lung function and physical activity leading to a
reduction in muscle strength or if CF patients have an abnormal muscle phenotype which prevents them from being able to exercise (Moser et al., 2000, Wells et al., 2011).

Sahlberg et al. reported no improvements in stable CF adults after a 6 month training programme compared to controls (Sahlberg et al., 2008). Lung function was maintained but muscle strength decreased in CF patients. This may indicate a metabolic disturbance and therefore reduced exercise capacity in CF. Shah et al. also reported similar findings as aerobic and anaerobic exercise capacity in CF adults was significantly reduced compared to controls despite having good lung function (Shah et al., 1998). The authors suggested that nutritional status was a major determinant in exercise capacity in CF.

Wells et al. reported reduced exercise capacity and muscle function in CF as a result of inefficient aerobic oxidative metabolism, possible disruptions in calcium homeostasis and increased inflammation, which would reduce muscle strength (Wells et al., 2011). Dodd et al. suggested that pulmonary factors do not limit the peak exercise capacity of CF patients and that treatments such as bronchodilators which enhance lung function did not improve exercise tolerance (Dodd et al., 2005, Dodd et al., 2006). The data available suggest that people with CF have muscle abnormalities with reduced muscle function which may contribute to poorer bone strength. To date, there are very limited data on CF-related muscle function in children and adolescents, so more research is warranted.

2.7.5.4 Glucocorticoid therapy
Patients with CF are often prescribed oral and inhaled corticosteroids which have been associated with reduced aBMD and fracture risk (Conway et al., 2000, Elkin et al., 2001). Many patients with irreversible lung damage are usually given regular and stronger doses of corticosteroids. These drugs are prescribed with caution as the side effects are known to be detrimental to bone health by reducing bone accrual and/or bone loss (Elkin et al., 2001, Conway et al., 2000). These drugs inhibit gastrointestinal calcium absorption and increase renal calcium excretion, possibly leading to an increase in PTH production and bone resorption; osteoblast recruitment is decreased and apoptosis of osteoblasts increases (Weinstein et al., 1998). Long-term use of glucocorticoids can increase the development of osteoporosis and fracture risk (Weinstein et al., 1998, Elkin et al., 2000, Conway et al., 2000). Many studies have demonstrated a positive association between glucocorticoid therapy and low aBMD in patients with CF. Bhudhikanok et al. reported glucocorticoid use in CF to be associated with significantly lower aBMD SD-scores, measured by DXA, at the femoral neck and lumbar spine (Bhudhikanok et al., 1998). Conway et al. reported that patients taking steroids (oral and inhaled) had significantly reduced aBMD at the lumbar spine (p=0.017) and femoral neck.
(p=0.027) compared to a group of non-steroid treatment CF patients (98). Elkin et al. and Haworth et al. also reported similar results (Conway et al., 2000, Elkin et al., 2001, Haworth et al., 2002).

Research into the effects of glucocorticoids on muscle strength has not been examined extensively and been shown to have positive and negative effects on muscle depending on which specific glucocorticoids are used, dosage and duration of treatment. Barry et al. were the first to study the effects of steroid use on muscle strength in CF patients (Barry and Gallagher, 2003). The authors concluded that glucocorticoid therapy was negatively associated with peripheral and respiratory muscle strength, and this was independent of lung function, nutrition, and number of days spent in hospital. However, glucocorticoids have been used to prevent muscle wasting and improve walking ability in children with Duchenne muscular dystrophy (DMD) (Balaban et al., 2005, Manzur et al., 2008). Therefore, it is important for CF patients to avoid or limit their use of glucocorticoids to maintain and preserve muscle health.

2.7.5.5 Hypogonadism

Children with CF tend to have delayed puberty which can affect bone and muscle development (Ujhelyi et al., 2004). PHV has been shown to be delayed by 9 to 10 months in boys and 10-14 months in girls with CF (Patel et al., 2003). Testosterone is often associated with bone and muscle development and maintenance (Bhasin et al., 2001). Reports on testosterone levels in CF males are conflicting as some authors report CF males to have reduced serum and salivary testosterone levels compared to aged matched controls, which may explain why CF males have lower aBMD and muscle strength (Boas et al., 1996, Elkin et al., 2001). However, Hardin et al. and Barry et al. have demonstrated CF males with impaired skeletal muscle and exercise capacity to have normal testosterone levels (Hardin et al., 2001, Barry et al., 2008).

Oestradiol is associated with bone metabolism in females and males, and is associated with postmenopausal bone loss and increased bone turnover (Seeman, 2002, Eastell, 2005). Rossini et al. reported serum oestradiol levels in CF adult patients to be lower in 23% of the females and 27% of the males, and was significantly related to femur aBMD values in both females and males (Rossini et al., 2004). Significantly lower serum oestradiol and free testosterone levels were observed in males with vertebral fractures only.

2.7.6 Influence of cystic fibrosis transmembrane conductance regulator protein on bone

Bone histomorphometry characterises the alterations in bone structure and remodelling found in bone diseases. Few studies have been able to assess bone biopsies from individuals with CF, and in those that have, the bone samples have been obtained at autopsy or from patients with severe bone disease (Elkin et al., 2002, Haworth et al., 2000). To date, there are no known studies analysing bone
biopsies from children or adolescents with CF. Most bone histomorphometry studies have used animal models to help understand the aetiology of CF-related bone disease in humans.

Studies in mice have shown that the CFTR mutation may be directly involved in the CF-related muscle and bone disease. Dif et al. found that mice with the CFTR mutation had severe osteopenia and lower body mass. BMD of total body was diminished (Dif et al., 2004). These mice displayed a 50% significant reduction of cortical bone width and thinner trabeculae compared to controls. Uncoupling of osteoblast (reduced) and osteoclast (increased) activity was more pronounced in the cortical region compared to the trabecular region. X-ray images of CFTR+/+, CFTR+/-, and CFTR-/- showed that bones of mutant mice appeared osteopenic and muscle mass was significantly reduced (Figure 2.49). Decreased BMD may be a result of decrease muscle mass and physical loading on the bone, which causes alterations in bone turnover. These mice were well nourished and free from therapeutic interventions.

Paradis et al. demonstrated that mutated mice had shorter femurs with reduced BV/TV due to thinner trabeculae, compared to wild type littermates (Paradis et al., 2010). However, the osteoblast and osteoclast numbers did not differ between CF and control mice. This suggests that the CFTR does not influence bone cell numbers but may alter the cell function. Caution must be taken when using animal models to demonstrate the aetiology of diseases, as mice and humans differ in many ways and the disease may take a different form in mice. However, Sood et al. and Louis et al. both reported a reduced cortical thickness in humans (Louis et al., 2009, Sood et al., 2001).

Figure 2.49 - Radiographic images of CFTR mutant mice. Bone and muscle mass are significantly lower in CFTR/- mice compared to CFTR+/+. Source: (Dif et al., 2004).
Research into human CF bone histology is still in its infancy. However, the expression of CFTR protein has been identified by immunohistochemistry in human bone cells. Shead et al. was the first group to report the expression of CFTR in human neonatal rib bone sections, which were obtained post-mortem from six full-term infants who had no evidence of growth or skeletal abnormalities (Shead et al., 2007). Strong CFTR expression was detected in osteoblasts on forming surfaces, newly formed osteocytes, and osteoclasts. CFTR was not expressed in deeply embedded osteocytes or chondrocytes in the growth plates. This suggests that development and mineralisation of the growth plate are normal but bone modelling and remodelling on bone surfaces may be disturbed due to abnormal cell function.

Le Heron et al. reported the expression of CFTR mRNA and protein in primary human osteoblasts and showed that inhibition of CFTR-mediated Cl⁻ channel activity affected the release of osteoprotegerin (OPG) and prostaglandin E2 (PGE2), two key regulators of bone formation. OPG acts as a soluble receptor inhibiting osteoclast differentiation and resorption by binding to and neutralising Receptor Activator of Nuclear factor Kappa B Ligand (RANKL) (Le Heron et al., 2010). In CF, it has been suggested that osteoblasts cannot synthesis OPG therefore cannot inhibit osteoclasts differentiation as RANKL is not neutralised. This may result in an increase in osteoclast number and increased bone resorption. PGE2 induces differentiation and bone erosion at sites of inflammation. Therefore, this suggests that the loss of CFTR activity may result in an increased inflammation-driven bone resorption (through both reduced OPG and increased PGE2 production), and thus might contribute to bone loss in CF patients.

Haworth et al. reported analysis of autopsy bone samples from 15 patients with CF and 15 young adult controls (Haworth et al., 2000). Eleven patients with CF had received organ transplants (most were lung, but also included heart and liver) and immunosuppressive medications. Cortical and trabecular bone volume were reduced and at a cellular level there was evidence of decreased osteoblastic and increased osteoclastic activity. There was a decrease in osteoblast number and a decrease in the biosynthetic potential of osteoblasts (i.e. the osteoid seams were decreased in thickness), suggesting that osteoblast function was compromised. In addition, bone resorption increased due to an increase in osteoclast number. The uncoupling of osteoblastic and osteoclastic activity resulted in an increase in resorption surfaces. Elkin et al. also reported similar findings (Elkin et al., 2002).

These studies indicate that poor osteoblast function plays an important role in low BMD associated with CF, although the direct effect of inactive CFTR on bone activity remains unclear and further investigation is required.
2.7.7 Influence of cystic fibrosis transmembrane conductance regulator protein on muscle
Ineffective aerobic oxidation metabolism in CF patients has been reported but it remains unclear whether this is due to impaired oxygen delivery (Moser et al., 2000, Klijn et al., 2003) or muscle abnormalities (Rosenthal et al., 2009, Hussey et al., 2002). CFTR mRNA has been detected in the skeletal and diaphragm muscles of rats (Fiedler et al., 1992), mice (Divangahi et al., 2009) and in humans (Lamhonwah et al., 2010). In healthy murine and human skeletal muscle, CFTR is expressed in the sarcoplasmic reticulum in skeletal muscle and plays a role in calcium transport during contractions. Divangahi et al. reported that intrinsic alteration of muscle function was linked to the absence of CFTR protein channel from skeletal muscle, resulting in dysregulated calcium homeostasis, augmented inflammatory response and increased diaphragm weakness during pulmonary infection (Divangahi et al., 2009). Lamhonwah et al. reported similar conclusions as Divangahi et al. in human skeletal muscle, with disrupted calcium homeostasis, which is essential for excitation-contraction coupling, leading to exercise intolerance and muscle weakness in CF patients (Lamhonwah et al., 2010). These studies indicate that poor muscle function is common in CF patients but the role of CFTR and calcium transport in muscle remains unclear. Further research into the mechanism of CFTR channels in muscle is required.

2.7.8 Limitations of previous studies in cystic fibrosis-related bone disease
Prevalence of low aBMD in CF children and adults has been well documented. However, due to the nature of the disease and the patient sample, most CF studies have a number of serious limitations;

1. Most data on BMD in CF has been obtained using DXA, therefore measurements are usually presented as aBMD or SD scores, so size has not be accounted for. This is a fundamental flaw when analysing age, pubertal stage, and sex matched children with different rates of growth to normal. This technical issue is described in Chapter Strengths and Limitations of DXA.
2. The survival of CF patients has only increased recently, so studies in the past have usually observed small CF groups, which may not be representative of the whole CF population.
3. Problems may occur when comparing different studies, as the datasets are adjusted for different measures (i.e. age, gender, height, weight). This may lead to misinterpretation of the data.
4. Most CF studies do not compare CF patients with healthy control participants. Advantages of studying a control group are that; a) the control group are measured and analysed using the same protocols and scanning device as the CF group, b) the controls are usually from the same geographical region as the CF patients therefore other variables such as social
economic status are minimised, and c) differences between CF and controls can be compared and provide more reliable conclusions as confounding variables can be controlled or minimised.

5. Most studies in CF bone research are cross-sectional and there are very few longitudinal studies in CF children, which are robust and have fewer limitations mentioned than those previously. A cross-sectional study design allows the researchers to observe and compare many different variables at the same time at one time point however do not provide definite information about cause-and-effect relationships (IWH, 2009). The benefit of a longitudinal study is that researchers are able to observe the group at more than one time point and detect developments or changes in the characteristics of the CF population at both the group and the individual level (IWH, 2009).
3 Aims and objectives
The aim of this thesis was to investigate whether sex and disease status modified the relationship between: 1) puberty and bone, and 2) muscle and bone, after accounting for height, weight and pubertal stage (Molgaard et al., 1997, Ward et al., 2015, Bianchi et al., 2014, Prentice et al., 1994). The primary objective was to characterise the muscle-bone unit by measuring BMD, bone geometry, cortical and trabecular microarchitecture, and muscle function in children aged 8-16 years, using novel bone densitometry and muscle assessment techniques. This would ultimately contribute to the understanding of how sex alters the relationship between bone and muscle in healthy males and females as they transition through puberty and who, on a population level, differ in the prevalence of osteoporosis and risk of fracture in later life.

Secondly, fractures in young adults with CF are common, however the aetiology and timing of CF-related bone disease remain unclear. This study aimed to contribute to the understanding of the aetiology of CF-related bone disease and whether CF status alters the relationship between puberty and bone and body composition outcomes and the role that muscle plays in bone development.

3.1 Primary hypotheses
The hypotheses are that sex (males vs. females) and disease group (CF vs. controls), after accounting for height, weight and pubertal stage, did:

1) Modify the relationships between puberty and bone outcomes. Healthy females have lower apparent gains in bone area but have higher gains in cortical thickness and bone density, as puberty proceeds. Participants with CF have lower apparent gains in bone area and lower apparent gains in bone density, as puberty proceeds.

2) Modify the relationship between muscle function (i.e. Pmax and Fmax) and bone outcomes as the mechanostat theory states that bone adapts to changes in loading (i.e. widening the bone). Healthy females and participants with CF would have lower apparent gains in bone area, as muscle force and power increase.

3.1.1 Primary objectives
The primary objectives were to characterise the muscle-bone unit using three imaging techniques (DXA, pQCT and HR-pQCT) and jumping mechanography to investigate how sex and disease group modified the relationships between 1) puberty and bone outcomes, and 2) muscle function and bone outcomes in children with and without CF, after accounting for height, weight and pubertal stage.

There were 3 primary objectives for each hypothesis, which were to investigate whether, after accounting for height, weight and pubertal stage:
Sex modified the relationship between:

1. Puberty and anthropometric outcomes *(Chapter 5.2)*.
2. Puberty and body composition and muscle function outcomes *(Chapter 5.3)*.
3. Muscle function and bone outcomes *(Chapter 5.4)*.

Disease group modified the relationship between:

1. Puberty and anthropometric outcomes *(Chapter 6.2)*.
2. Puberty and body composition and muscle function outcomes *(Chapter 6.3)*.
3. Muscle function and bone outcomes *(Chapter 6.4)*.

### 3.1.2 Secondary hypotheses and objectives

The secondary hypothesis was:

1. There would be differences in the relationships between clinical characteristics (i.e. FEV1, 25(OH)D status, and genotype) and bone, body composition, and muscle function outcomes. CF participants with lower FEV1 and 25(OH)D status will have lower apparent gains in bone area, lower bone mineral, and lower muscle function (Fmax and Pmax) compared to CF participants with higher FEV1 and 25(OH)D status. CF participants who are homozygous for the DeltaF508 genotype will have lower apparent gains in bone area, lower bone mineral, and lower muscle function (Fmax and Pmax) compared to CF participant who have a heterozygous genotype (Fmax and Pmax) *(Chapter 6.5)*.

The secondary objectives were to investigate the relationship between clinical characteristics (i.e. FEV1, 25(OH)D status, and genotype) in CF and bone and muscle outcomes, which may contribute to disease group differences in bone and muscle outcomes.
4 Study design, methods, and recruitment

4.1 Study design and setup

The study was conducted at Medical Research Council – Human Nutrition Research (MRC-HNR) in Cambridge. Participants were recruited from East Anglia including Cambridgeshire, Bedfordshire, Norfolk, Suffolk, Hertfordshire, and Essex (Figure 4.1). Children with CF were first approached at the children’s outpatient clinic, Addenbrooke’s Hospital (ADX), Cambridge. The recruitment protocol is described in Chapters 4.1.4 and 4.1.5.

Figure 4.1 - A map showing the distribution of participants from East Anglia.
Source: Google maps.

After reviewing the literature on CF-related bone health and discussing the gaps in knowledge, Dr Kate Ward and I arranged a meeting (March 2012) with Dr Robert Ross-Russell (Paediatric consultant) to discuss a study collaboration involving his CF patients at ADX. From this meeting I was able to conclude that ADX is the main medical support centre for treating CF in East Anglia. Patients are required to attend their local hospital every three months for a medical review and attend ADX for an in-depth annual examination. At that time, approximately 138 paediatric patients (aged 0 to 16 years) with CF were resident in East Anglia and were under the medical care of ADX. Of the 138 patients, approximately 65 patients were between 8 to 16 years.

This patient group had not been approached to take part in medical research before. Therefore, I decided to carry out a preliminary assessment which involved attending the CF clinic sessions to see
if the study was feasible. Every Thursday, under the supervision of Dr Ross-Russell, I was able to observe and talk to patients and their parents/guardians during their clinic appointments, with prior consent. I was able to gain a personal understanding of how patients go about their day-to-day life with CF, to ask how they reviewed medical research, and whether they would be interested in taking part in a study. There were a number of concerns raised by patients and their parents/guardians including: how much time they would need to dedicate to the study, if needles or bloods were used/taken, burden of travelling to MRC HNR, and whether they would to take time off school/college.

This study was the first paediatric study at MRC HNR to use bone imaging techniques, and so once the protocol was designed I invited a colleague and her two children, aged 8 and 10 years, to visit MRC HNR and to go through the study protocol. The patients were asked what they thought of the study; what they liked and disliked, what they thought of their surroundings (i.e. was it child-friendly and what they thought of the scanners?). Members of the research team were able to practice positioning the children into the scanners to see if there were any potential problems for the study. It was noted that very young children would be more challenging due to their small size and immaturity (i.e. likely to move/fidget during the scanning procedure).

4.1.1 Ethical approval and research governance
After review and approval by the MRC internal Research Review Board, the protocol and all supporting documents were submitted to the Cambridge South Committee - National Research Ethics Service and to the Research and Development department at ADX for scientific and ethical approval (Appendix 10.2). The study was approved on 16th April 2013 (REC Number: 13/EE/0078).

During the study, four minor amendments and one substantial amendment were made to improve the study protocol, which were approved by the ethics committee. Two participant identification centres (PICs) were included to increase the recruitment of CF patients. Dr Amanda Equi based at West Hertfordshire Hospitals NHS Trust and Dr Chris Upton based at Norfolk and Norwich University Hospitals NHS Trust agreed to advertise this study to their patients. The study protocol and all supporting documents were submitted and approved by the relevant R&D departments.

4.1.2 Study design
The study was designed as a longitudinal (1 year), observational investigation with the aim to characterise the changes in the muscle-bone unit in children using advanced imaging and muscle measurement techniques. The data presented in this thesis refers to the baseline cross sectional measurements only. Longitudinal measurements will be analysed and published in 2015-16. The study enrolled white Caucasian children, aged 8 to 16 years old, living in East Anglia, with and
without CF. The justification for the target numbers in each group is described in Chapter 4.1.3. Participants were selected based on meeting the inclusion and exclusion criteria in (Table 4.1 and Table 4.2).

4.1.2.1 Inclusion and exclusion criteria justification

Age selection - The age range was selected to cover all pubertal stages to help understand why young adults with CF may be at increased risk of fracture and whether children with CF have compromised bone strength before puberty or during puberty. The lower age limit of 8 years was based on the maturity of the child to understand what the study was about and their ability to remain still throughout the scanning procedures. It was considered challenging to recruit children younger than 8 years of age as they are technically difficult to position within the scanner due small body size and movement during the scanning procedures, therefore reducing the quality of the image and producing inaccurate results. The upper age limit of 16 years of age was based on the research question, to characterise the changes in bone and muscle phenotype in pre-puberty through puberty to investigate why adolescents and young adults with CF develop osteoporosis and fracture.

Ethnicity - The study focused on the White Caucasian population for two main reasons. Firstly, ethnicity is an important determinant of bone health. Differences in bone mineral, shape, and size have been found between Black, Asian, and Caucasian populations which may explain differences in bone strength and fracture risk in these populations (Laskey et al., 2010, Bhudhikanok et al., 1996). For this reason all bone data in adults and in children should be compared to ethnicity matched reference data (ISCD (2013). Currently, there are no published UK reference data for DXA, pQCT or HR-pQCT measurements in children of ethnic origins other than White Caucasians. Secondly, CF predominantly affects the White Caucasian population. There are reports of CF in other ethnic groups e.g. Asians from the Indian subcontinent constitute 1 in 60 of the UK CF population and 1 in 15000 in African Americans (McCormick et al., 2002b, McCormick et al., 2005b), but the aetiology and prevalence have not been well described. For a robust comparison of CF to healthy children, a reference population of the same ethnicity is needed. Hence, why only White Caucasian children with and without CF were eligible to take part in the study.

Medical condition(s) and use of medication - The exclusion criteria for control participants included any chronic childhood disorders or use of medication affecting bone and muscle health. This included disorders which affect bone and muscle directly or indirectly such as osteogenesis imperfecta (direct - collagen defect) (Davie and Haddaway, 1994, Folkestad et al., 2012, Gatti et al., 2003) or Crohn’s disease (indirect - inflammation of the bowel causing malabsorption) (Schmidt et al., 2012, Werkstetter et al., 2011). These conditions are known to reduce bone mass and increase fracture risk. The use of medication such as glucocorticoids which are used to treat chronic disorders are also
known to affect bone mass and therefore this was also an exclusion criterion in control children. This was not an exclusion criterion for the CF group as these children were likely to have glucocorticoids for chest infections and may be one of the factors causing impaired bone accrual.

**Immobilisation** - Prolonged immobilisation following fracture or surgery can reduce BMD and cause muscle atrophy (Armbrecht et al., 2011, Buckingham and Jeffcott, 1991, Ceroni et al., 2013). Mechanical loading is essential for maintaining bone strength and, therefore children who had been immobilised for any reason in the past year prior to the study were excluded.

**Conditions making scanning difficult** - To acquire a scan of good quality the participant must remain still throughout the scanning procedure. This can be difficult for children who have bone deformities such as scoliosis (abnormal curvature of the spine) or those who have problems with their nervous system or learning difficulties. These children are likely to have an abnormal muscle-bone unit and, therefore, accurate comparisons and conclusion cannot be made. These children would also find the procedures to be uncomfortable and/or painful, therefore making scanning difficult and stressful for the child.

**Pregnancy** - To adhere to the Ionising Radiation Medical Exposure Regulations (IRMER) (IRMER, 2000) and the strict MRC HNR policies for the use of ionising radiation, pregnancy was an exclusion criterion. Parents and participants were made aware prior to the study that this sensitive question would be asked (i.e. in the study information leaflets and during the screening questionnaire).

**Previous exposure to clinical drug trials or X-ray exposure** - Children who had taken part in research involving clinical drug trials were excluded from the study. Clinical drug trials have the potential to affect bone and muscle health therefore these participants would not represent a normal control or CF population. Children who had been involved in research studies involving radiation, within one year prior to this study, were also excluded. It was considered inappropriate to further expose them to additional radiation, despite the radiation dose being minimal. Children who had been exposed to radiation for clinical reasons e.g. X-ray for fracture were included in the study.

**Organ transplant** - Children with CF who had received an organ transplant were excluded from the study. Organ transplantation is associated with low bone accrual in children and bone loss in adults that subsequently increases the risk of osteoporosis and bone fractures. Immunosuppressant drugs are essential for preventing organ rejection but are known to directly disturb bone metabolism, especially the trabecular bone of the spine which appears to be most at risk, with vertebral fractures occurring most commonly (Epstein et al., 1995). Patients with an organ transplant will be immobilised for a long period of time after surgery whilst recovering, which will induce bone loss further.
Table 4.1 - Inclusion and exclusion criteria for control participants.

<table>
<thead>
<tr>
<th>Inclusion Criteria</th>
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<tbody>
<tr>
<td>1) 8 - 16 years old, male or female</td>
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<tr>
<td>2) White Caucasian</td>
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<tr>
<td>3) Live within the East Anglia region</td>
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<table>
<thead>
<tr>
<th>Exclusion criteria</th>
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<tbody>
<tr>
<td>1) Any chronic childhood condition known to affect bone mass</td>
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<tr>
<td>2) Inherited disorders that are associated with fragile bones e.g. osteogenesis imperfecta</td>
</tr>
<tr>
<td>3) Take drugs known to affect bone density e.g. corticosteroids and anti-convulsants</td>
</tr>
<tr>
<td>4) Prolonged periods of immobilisation in the past 12 months e.g. surgery</td>
</tr>
<tr>
<td>5) Any condition making bone density measurement difficult to take e.g. scoliosis</td>
</tr>
<tr>
<td>6) Unable to give written assent/consent</td>
</tr>
<tr>
<td>7) Pregnancy</td>
</tr>
<tr>
<td>8) Have taken part in research involving clinical drug trials which may affect bone and muscle health</td>
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Table 4.2 - Inclusion and exclusion criteria for participants with CF.

<table>
<thead>
<tr>
<th>Inclusion Criteria</th>
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</thead>
<tbody>
<tr>
<td>1) Aged 8 – 16 years old, male or female</td>
</tr>
<tr>
<td>2) White Caucasian with CF</td>
</tr>
<tr>
<td>3) Live within the East Anglia region</td>
</tr>
<tr>
<td>4) Receive treatment from the NHS hospitals in East Anglia Region</td>
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<table>
<thead>
<tr>
<th>Exclusion criteria</th>
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</thead>
<tbody>
<tr>
<td>1) Have received an organ transplant</td>
</tr>
<tr>
<td>2) Prolonged periods of immobilisation in the past 12 months e.g. surgery</td>
</tr>
<tr>
<td>3) Any condition making bone density measurement difficult to take e.g. scoliosis</td>
</tr>
<tr>
<td>4) Unable to give written assent/consent</td>
</tr>
<tr>
<td>5) Pregnancy</td>
</tr>
<tr>
<td>6) Have taken part in research involving clinical drug trials which may affect bone and muscle health</td>
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4.1.2.2 Screening and consent

Eligibility for inclusion within the study was determined from completed screening questionnaires by the parent/guardian of the child if they were aged 15.99 years or younger (Appendix 10.11). Children who were 16 years old could complete their own screening questionnaire after their parent/guardian had given their assent. The completion of this questionnaire was conducted in a private setting, either over the phone or during the CF clinic appointment, to ensure privacy. Children who did not fulfil the study criteria were given a reason and parents/guardians were able to discuss this with the researcher. Children who were eligible were invited to attend MRC HNR to take part in the study. The study visit was arranged to be convenient for both parents and participants, usually during school holidays and after school. An appointment letter and the study information leaflets were sent to parents and participants (Appendix 10.12).
To satisfy research and clinical governance, participants aged between 8 and 15 years were asked to give written assent and their parent/guardian to give written informed consent (Appendix 10.13 and Appendix 10.14). Participants aged 16 years were considered to be competent and able to decide for themselves if they wish to take part in the study (Appendix 10.15). Therefore, they were asked to give written informed consent. Participants aged 16 years were asked have their parent/guardian to be present during the screening questionnaire and were encouraged to bring their parent/guardian to the study visit.

4.1.3 Sample size
There were no published data using the primary outcomes in this study to detect longitudinal change in children with CF compared to controls. Therefore, the sample size was calculated using values obtained from healthy children, aged 8-12 years (as measured by pQCT) by Ward et al. (Ward et al., 2007c). The sample size was calculated using changes in trabecular BMD values of the tibia. All other bone parameters of interest would be measured with greater precision. Therefore, this sample size provides power to detect small changes in all measurements of interest.

The mean BMD change was 7.8 mg/cc (SD 12.8) over a year. Therefore, based on an estimate of 8mg/cc change over a year (SD of 20mg/cc), with 90% power (alpha 0.05), a sample size of 68 children per group was required. (An 8 mg/cc change is approximately three-fold the precision of the pQCT instrument).

To account for potential dropouts due to illness, death, or disinterest in the study, 75 CF children and 150 healthy children (75 males and 75 females) would be recruited. This would allow for a dropout rate of 10%.

4.1.4 Recruitment of control participants
The aim of the study was to recruit 150 control children, who were aged 8 to 16 years of age, White Caucasian, and resident in East Anglia. The control group was recruited to cover the whole range of pubertal stages by gender. The study was advertised using posters and leaflets (Appendix 10.3-Appendix 10.7) placed in the local community (with permission) e.g. local hospitals, GP surgeries, libraries, local shops, university buildings and via the internet through emails and online adverts on the MRC HNR website. Primary and secondary schools and Sixth form colleges were asked to help with recruitment by placing posters around the school and to place an advert on the school’s parent webpage (Appendix 10.9). Parents and children who were interested in the study were able to contact the research team either by email or telephone to request more information about the study. Parents and children were asked to read the study information leaflets carefully and to discuss the study before agreeing to take part. If they were still happy to take part, they were asked to make
contact with the research team to go through the screening questionnaire and arrange an appointment for their study visit.

4.1.5 Recruitment of participants with cystic fibrosis
Patients with CF under the care of ADX were the first to be approached about the study. Most patients with CF in the East Anglia region are referred by their local hospital to ADX for their 3 monthly clinical reviews and for their annual in-depth review. During the CF clinic sessions, a health professional involved in the care of CF patients asked patients and their parents whether they would like to hear about the study. If yes, patients and their parents were approached about the study. Study information leaflets were given to the patients and parents which they were given to take away to consider participation. Posters and leaflets were placed around the children’s outpatient clinic in ADX. The study was advertised in the hospital newsletter which was either given or sent to patients (Appendix 10.8 and Appendix 10.9). Due to the likely low recruitment rate, the area of recruitment was extended to cover hospitals in Hertfordshire and Norfolk. Posters and leaflets were sent to the hospitals and a letter was sent to patients who had not been approached in clinic to help raise awareness of the study. Patients and their parents were able to contact the research team to go through the screening question and arrange an appointment for their study visit.

Patients with CF were given the option to invite a healthy friend or sibling to take part in the study with them, known as the buddy scheme. The aim of the scheme was to make the study visit more relaxing and enjoyable for young participants and to recruit appropriate control children.

4.1.6 The SNAP study and data sharing
Patients with CF who attended the Birmingham Children’s Hospital had been invited to take part in the “SNAP” study – a prospective fracture study in children and adolescents with chronic inflammatory and/or disabling conditions (http://public.ukcrn.org.uk/search/StudyDetail.aspx?StudyID=12292). The SNAP protocol was very similar to the BMS protocol used at MRC HNR, Cambridge. The principle investigator of the SNAP study was approached about a study collaboration to improve the recruitment of patients with CF within the current study.

4.1.7 Study visit
The study protocol was designed to include the comments and suggestions made by children and parents in the preliminary stages of the study setup. All study procedures are summarised in Table 4.3.

All participants and parents were fully informed of the procedures and were given opportunities throughout the study visit to ask any questions. The study visit took on average between one to two hours depending on the age and maturity of the participant. All participants were guided through the study and were treated as individuals. An authorisation of radiation exposure form was completed by
Dr Kate Ward (Radiation expert) prior to the visit and a data collection form was completed during the visit (Appendices 9.19-9.20) Families with more than one child had their study visits staggered. This was to ensure that participants were not pressurised to take part in the study by siblings or friends. Participants were asked to complete the consent and musculoskeletal questionnaire with their parent or by themselves. Siblings and friends were asked to wait in the volunteer suite lounge.

Table 4.3 - Summary of procedures and study outcomes.

<table>
<thead>
<tr>
<th>Study procedures</th>
<th>Study outcomes</th>
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<tbody>
<tr>
<td>Questionnaire</td>
<td>• Physical activity</td>
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<td></td>
<td>• Medical/clinical history</td>
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<td></td>
<td>• Pubertal assessment</td>
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<tr>
<td></td>
<td>• Clinical characteristics of CF - FEV1, pancreatic insufficiency, liver disease, CF-related diabetes, vitamin D and history of fracture</td>
</tr>
<tr>
<td>Anthropometry</td>
<td>• Height</td>
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<tr>
<td></td>
<td>• Weight</td>
</tr>
<tr>
<td></td>
<td>• Sitting height</td>
</tr>
<tr>
<td></td>
<td>• Tibia length</td>
</tr>
<tr>
<td>DXA - Total body (less head)</td>
<td>• Area: Bone area</td>
</tr>
<tr>
<td></td>
<td>• Density: SA-BMC-LH</td>
</tr>
<tr>
<td></td>
<td>• Body composition: LM, FM, LM:FM ratio, FFMI and FMI</td>
</tr>
<tr>
<td>pQCT (4% and 66% sites of the tibia)</td>
<td>4% site:</td>
</tr>
<tr>
<td></td>
<td>• Area: Total CSA</td>
</tr>
<tr>
<td></td>
<td>• Density: Total vBMD and trabecular vBMD</td>
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<tr>
<td></td>
<td>66% site:</td>
</tr>
<tr>
<td></td>
<td>• Area: Total CSA and cortical CSA</td>
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<tr>
<td></td>
<td>• Density: Cortex vBMD</td>
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<tr>
<td></td>
<td>• Strength: SSI</td>
</tr>
<tr>
<td>HR-pQCT (8% site of the distal tibia)</td>
<td>• Area: Tt.Ar and Ct.Ar</td>
</tr>
<tr>
<td></td>
<td>• Density: D100, BV/TV, Ct.BMD and Ct.TMD</td>
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<tr>
<td></td>
<td>• Microarchitecture: Ct.Po, Tb.N and Tb.Th</td>
</tr>
<tr>
<td>Jumping mechanography (S2LJ and M1LH)</td>
<td>• Fmax</td>
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<tr>
<td></td>
<td>• Pmax</td>
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</tbody>
</table>

Key: Cystic fibrosis (CF), maximal amount of air you can forcefully exhale in one second (FEV1), total body less head size adjusted bone mineral content (SA-BMC-LH), lean and fat mass (LM and FM), fat-free mass index (FFMI), and fat mass index (FMI), cross-sectional area (CSA), total and cortical area (Tt.Ar and Ct.Ar), volumetric bone mineral density (vBMD), stress-strain index (SSI), total vBMD (D100), cortical porosity (Ct.Po), trabecular number and thickness (TB.N and Tb.Th), muscle force and power (Fmax and Pmax).

4.2 Methods
4.2.1 Selection of methods
To assess bone and muscle development, a combination of methods including a musculoskeletal questionnaire, pubertal assessment, anthropometry, bone assessment (DXA, pQCT, and HR-pQCT) and muscle assessment (pQCT and jumping mechanography) were used and are described in this section.
4.2.2 Study team and responsibilities

The team who were involved in the recruitment, data collection and image analysis were as shown in Table 4.4.

Table 4.4 - Study staff at HNR and ADX.

<table>
<thead>
<tr>
<th>Individual</th>
<th>Role</th>
<th>Duties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amy Riddell (HNR)</td>
<td>Principal investigator</td>
<td>• Study design&lt;br&gt;• Ethical application&lt;br&gt;• Recruitment/screening of controls&lt;br&gt;• Recruitment/screening of CF participants&lt;br&gt;• Consent&lt;br&gt;• Questionnaire&lt;br&gt;• Anthropometry&lt;br&gt;• pQCT, HR-pQCT, DXA, jumping mechanography&lt;br&gt;• Image and jumping mechanography analysis</td>
</tr>
<tr>
<td>Jenny Thompson (HNR)</td>
<td>Research assistant</td>
<td>• Recruitment/screening of controls&lt;br&gt;• Consent&lt;br&gt;• Questionnaire&lt;br&gt;• Anthropometry&lt;br&gt;• pQCT, HR-pQCT, DXA, jumping mechanography</td>
</tr>
<tr>
<td>Helena Scully (HNR)</td>
<td>Research assistant</td>
<td>• Consent&lt;br&gt;• Questionnaire&lt;br&gt;• Anthropometry</td>
</tr>
<tr>
<td>Dr Kate Ward (HNR)</td>
<td>PhD supervisor and senior research scientist</td>
<td>• Study design&lt;br&gt;• Ethical application&lt;br&gt;• Radiation and imaging expert&lt;br&gt;• Paediatric bone research</td>
</tr>
<tr>
<td>Dr Robert Ross-Russell (ADX)</td>
<td>Paediatric consultant</td>
<td>• Recruitment/screening of CF participants&lt;br&gt;• Study physician</td>
</tr>
</tbody>
</table>

4.2.3 Anthropometry

Anthropometry was undertaken by a trained member of the research team (Figure 4.2). These measurements were carried out in a private room to ensure that the participants had privacy and to ensure the equipment used was not tampered with.

I) Weight - Weight was measured to the nearest 0.1 kg using an electronic digital scale (Seca, Hamburg, Germany). Participants were asked to remove shoes and heavy clothing.

II) Height- Height was measured to the nearest 0.1 cm using a permanent wall-mounted stadiometer (Seca, Hamburg, Germany) in vast majority of (>95%) of participants. Those who were very small were measured using a portable stadiometer (Leicester Height Measure Mk II, Seca, Hambrug, Germany) designed to measure children. Participants were asked to remove shoes and to stand erect with their heels and shoulders against the wall, keeping knees and back straight with the Frankfort plane (i.e. the line between the left eye and superior border of the external auditory meatus) in the
horizontal position. Female participants were asked to remove any hair accessories and to flatten their hair to ensure that height was correctly measured.

III) Sitting height - Sitting height was measured to the nearest 0.1 cm using a stadiometer (Leicester Height Measure Mk II, Seca, Hamburg, Germany) which was positioned on a table. Participants were instructed to sit on the footplate with their gluteals placed against the stadiometer and with their back straight. The participant’s feet were supported by wooden blocks to ensure that their thighs were horizontal, feet flat, and to ensure that their legs were not hanging. The measurement was taken from the level of the table to the top of the participant’s head.

IV) Body mass index - Body mass index (BMI) was calculated as the participant’s weight in kilograms divided by the square of their height in meters (kg/m²). The BMI score was plotted on the BMI-for-age growth charts (for either girls or boys) to obtain a percentile ranking. The growth charts used were produced by the Royal College of Paediatrics and Child Health (RCPCH) and are based on the 1990 UK growth data (Freeman et al., 1995). The percentile indicates the relative position of the child's BMI score among children of the same sex and age. Underweight, normal, overweight, and obese were defined as BMI percentile <2nd, 2nd–91st, 92nd–97th, >98th respectively (RCPCH, 2012). BMI results were reported to participants and their parents/guardians as well as their general practitioners (GP) (with prior consent). A BMI percentile of <2nd or >91st was reported to be clinically abnormal and parents/guardians were advised to speak to their GP for further assessment (RCPCH, 2012).

Figure 4.2 - Anthropometry measurements.
Anthropometric measurements included (A) weight, (B) height and (C) sitting height.
4.2.4 Musculoskeletal questionnaire

After the consent process, the study questionnaire was given to the participant and their parent/guardian to complete. The researcher explained that all information given in the questionnaire would be kept confidential and that some questions may be considered as very personal and/or sensitive, especially questions regarding their pubertal assessment. The questionnaire was composed of six sections relating to bone and muscle health (Appendix 10.16). These were; 1) family history of osteoporosis, 2) history of fractures, 3) medication usage/ surgery, 4) physical activity assessment, 5) smoking, and 6) pubertal assessment. The questionnaire was designed to be short and easy for participants and parents/guardians to understand and to complete. The researcher explained how to answer each section and gave examples of answers.

4.2.5 Pubertal staging

The onset of puberty can vary in children and is not dependent on age. The most widely used method for assessing pubertal staging (PS) is using the Tanner criteria, which divides pubertal development into five stages based upon the appearance and development of physical sexual characteristics (Marshall and Tanner, 1969, Marshall and Tanner, 1970).

![Figure 4.3 - Orchidometer used for assessing testicular volume.](image)

All participants were asked to complete a self-assessment of pubertal staging (Appendix 10.17-Appendix 10.18). The researcher allowed the participant with or without their parent/guardian to complete the questionnaire in a private room. The assessment was a series of drawings: for females this was breast development and pubic hair growth; for males this was pubic hair and mean testicular volume, measured by comparison to a set of beads known as an orchidometer (Prader, ESP Ltd, UK) (Figure 4.3). The mean testicular volume was graded as follows; PS 1 = ≤ 3mL, PS 2 = 4-9 mL, PS 3 = 10-15 mL, PS 4 = 16-20 mL, and PS 5 = > 20 mL (Wardhaugh, 2003). Additional questions for assessing PS included; for females, if they had started their menstrual cycles and how old they were at the time, and for males; if their voice had deepened (i.e. voice broke, and how old they were at the time) (Ong et al., 2012). Start of menstrual cycle was classed as PS = 4 (Marshall and Tanner, 1969) and voice breaking as PS = 3 (Marshall and Tanner, 1970). Participants self-assessed (with
parental guidance) their PS from the diagrams and marked the pictures which described them. Participants were assigned a PS grade of between one and five.

4.2.6 Clinical characteristics of participants with cystic fibrosis

Permission was obtained during consent from participants with CF and their parent/guardian to allow access to their medical records. Most recent medical information including; FEV1, genotype, 25(OH)D status, list of medication and other comorbidities (i.e. CF-related diabetes, pancreatic disease and liver disease) was retrieved via the CF data manager (Vinod Thoppil). All data was anonymised and stored on an encrypted memory card.
4.3 Bone densitometry

4.3.1 Dual energy X-ray absorptiometry (DXA)

DXA was performed using a Lunar Prodigy Advance DXA (Prodigy enCORE 2006 software, version 10.51.006, GE Healthcare Lunar, Belgium) according to standard procedures (Figure 4.4 A).

![Participant undergoing DXA scanning.](image)

**Figure 4.4 – Participant undergoing DXA scanning.**

(A) Participant in the correct scanning position for undergoing a DXA scan, and (B) a total body DXA scan for skeletal assessment and body composition.

Scans of the TB were obtained from which TB aBMD and body composition (LM and FM) were measured (Figure 4.4 B). The scans were performed with participants wearing light clothing and all metal objects removed such as jewellery, hair and clothing accessories, and clothing with metal buttons or zips. The TB scan was obtained with the participant lying on their back in a straight position. The researcher positioned the participant in the centre of the DXA bed with their head facing the ceiling. The arms were placed at the side of the body with arms pronated and the feet were held in a plantarflexion (feet turned into each other) and held together with a velcro strap. The DXA bed has a narrow scanning field and for participants who were wider around the trunk, they had to have their arms in a neutral position rather than pronated to ensure that both arms were completely scanned. Participants were instructed to remain still throughout the scan.

4.3.1.1 Quality assurance (QA) and quality control (QC)

During the study, daily QA checks were made using a spine phantom to monitor the performance of the DXA scanner and to assess the ability of the scanner to reproduce the BMD of the phantom (Figure 4.5). The QA and QC checks were stable throughout the study (i.e. no significant drift and no breakdowns/replacement of device parts occurred). The inter-observer coefficients of variation for...
duplicate measurements (measured by MRC-HNR research assistant) in 35 adults were; 1.65, 2.05, 1.89, and 0.10 for BMC, bone area, FM and LM respectively.

![Figure 4.5 - DXA quality assurance.](image)

(A) DXA spine phantom, (B) phantom scanned daily prior to study visits, and (C) the QA test results.

4.3.1.2 Scan acquisition and analysis

Prior to the study, all members of the study team were trained by an experienced senior research scientist (Dr Kate Ward) on how to use the scanner, subject positioning and scan analysis. All the scans were assessed and graded by the principal investigator (Amy Riddell) and reviewed by Dr Kate Ward. The quality of each scan was assessed and then graded from one to four, as is standard practice at MRC HNR (Table 4.5). Scan quality can be affected by various factors such as incorrect positioning, subject movement, and artefacts caused by jewellery and metal objects on clothing (Figure 4.6).

Whilst the grading of scans was subjective it was carried out by the same investigator to ensure consistency. All scans were reviewed by Dr Kate Ward and any abnormalities were discussed with Dr Sumantra Ray (MRC HNR clinician), Dr Robert Ross-Russell (study collaborator and consultant paediatrician at ADX) and Dr Ken Poole (consultant in rheumatology at ADX). Further action/referral was taken as required.
Table 4.5 - Grading criteria for DXA scans.

<table>
<thead>
<tr>
<th>Grade</th>
<th>Scan quality</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>High standard with no movement, correct positioning, and no artefacts, resulting in the ability to extract accurate data from the scan</td>
</tr>
<tr>
<td>2</td>
<td>Incorrect positioning of participant</td>
</tr>
<tr>
<td>3</td>
<td>Participant movement. Young subjects aged between 8-10 years were likely to move and change position despite the researcher explaining the importance of staying still and after being instructed to keep still. The most common problems were movement of the fingers and lower legs.</td>
</tr>
<tr>
<td>4</td>
<td>Significant distorted from movement, incorrect positioning and/or artefacts. These scans were excluded from the study as the data extracted from these scans would be inaccurate. In this study, no scans were excluded.</td>
</tr>
</tbody>
</table>

Figure 4.6 – Examples of image artefacts in DXA scans.
(A) Movement of total body, (B) incorrect positioning of the participant’s leg and metal zip and buttons on trousers are shown as dense white patches and will be regarded as bone, and (C) high body fat mass can affect the attenuation and resolution of the X-ray beams, producing a distorted image. These participants are often difficult to position on the scanning table as the scanning gantry is narrow and is not designed for very large body size. Source: NOS (Eastell, 2004).
4.3.2 Peripheral quantitative computed tomography (pQCT)

pQCT scans were conducted on a Stratec XCT 2000L™pQCT™ bone densitometer, software version 6.20C (Stratec, Pforzheim, Germany) according to standard procedures. Measurements of vBMD, bone geometry and muscle parameters were taken and are described in Chapter 2.5.4. Manufacturer’s instructions were followed for correct positioning. The non-dominant leg was measured from the tibial plateau to the distal end of the medial malleolus in each participant. Participants who had previously broken their non-dominant leg were measured on their dominant side. Participants were asked to remove footwear and clothing of the lower non-dominant leg prior to positioning and scanning. The length of the tibia was measured from the distal plafond to the tibial plateau using a metal one metre ruler. The participant was seated on a chair with their leg positioned through the gantry and the foot placed in the footholder (Figure 4.7). A velcro strap was placed around the foot to securely hold the foot. The red laser position indicator was placed on the middle of the inner ankle. The leg was positioned in the centre of the gantry and adjustments were made to ensure that the whole lower leg could be scanned. Once correctly positioned, the leg fixation clamp was used to secure the upper leg.

Figure 4.7 - Participant undergoing pQCT.

4.3.2.1 Scanogram

Prior to scanning, a scanogram was performed. This scan produces a 3 cm coronal view of the distal ends of the tibia and fibula, and the talus. The scanogram allows visual assessment for positioning of the reference line. The researcher placed a reference line at the endplate of the tibia. If the growth plate was visible, the reference line was repositioned to bisect the medial border of the distal metaphysis, as illustrated in Figure 4.8. Once the reference line was in the correct position, the participant was instructed to remain still until the scan was completed.
Figure 4.8 - pQCT scanogram.
A pQCT scanogram is performed to identify the end of the growth plate or most proximal region of the open growth plate (dotted lines). The plain X-ray image of the ankle and distal tibia helps to make good comparison with the scanogram. Source: Xray (Chisholm, 2014).

A pQCT scan was performed at the 4%, 14%, 38%, and 66% sites of the total bone length. Each CT slice was of 2.4 mm in thickness. Each slice was analysed using the manufacturer’s standard software. A ROI was placed around the tibia and around the whole cross-section of the leg to calculate cortical and trabecular bone parameters, bone geometry, and muscle measurements at the four sites (Figure 4.9).

Figure 4.9 - pQCT scanning sites and cross-sectional images.
X-ray of the tibia labelled with the scanning sites and a pQCT image of the site. Source: (fotosearch, 2014).
4.3.2.2  Quality assurance and quality control
During the study, a daily quality assurance check was made using the standard phantom to monitor the daily performance of the pQCT scanner. In addition to the standard phantom, a weekly cone phantom was performed. The cone phantom is used to check the linearity of the results and to confirm the precision of the repositioning of the device. During this procedure three different density ranges are measured (Figure 4.10).

4.3.2.3  Scan acquisition and analysis
The quality of each scan was assessed in accordance to standard practice at MRC HNR. The quality of the scanogram was graded zero to two for correct placement of the reference line (Table 4.6). Quality of a scanogram can be affected by various factors such as incorrect positioning of the red laser position indicator and participant movement. If the scanogram did not include the tibia end plate and all the growth plates, then the SV-scan was repeated to ensure the reference line was placed in the correct position. The four pQCT slices (4%, 14%, 38%, and 66% sites) were assessed and graded zero to three for movement (Table 4.6 and Table 4.7). The pQCT scanner is very sensitive to participant’s movement and can result in the scan being distorted, showing as movement artefact with red streaks and breaks on the scan (Figure 4.11).

Figure 4.10 - Quality assurance for pQCT scanner.
(A-B) pQCT scanning phantom, (C) standard, and (D) cone QA test results.
Table 4.6 - Grading criteria for pQCT scanogram.

<table>
<thead>
<tr>
<th>Grade</th>
<th>Scanogram quality</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Correct positioning of the reference line (i.e. Either on the endplate of the distal tibia or if the growth plate was visible, the reference line was repositioned to bisect the medial border of the distal metaphysis).</td>
</tr>
<tr>
<td>1</td>
<td>The reference line positioned either slightly too distal or proximal to where it should have been.</td>
</tr>
<tr>
<td>2</td>
<td>Incorrect positioning.</td>
</tr>
</tbody>
</table>

Table 4.7 - Grading criteria for pQCT scans.

<table>
<thead>
<tr>
<th>Grade</th>
<th>pQCT scan quality</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No movement and the bone shape was not distorted.</td>
</tr>
<tr>
<td>1</td>
<td>Slight movement with small amounts of red patches/streaks.</td>
</tr>
<tr>
<td>2</td>
<td>Thick red streaks with small patches/breaks on the scan, the bone shape was distorted.</td>
</tr>
<tr>
<td>3</td>
<td>Too distorted and were excluded from the study.</td>
</tr>
</tbody>
</table>

Figure 4.11 - Grading criteria for pQCT tibia scans.
Source: MRC-HNR pQCT grading standards.
4.3.3 High resolution peripheral quantitative computed tomography (HR pQCT)

HR-pQCT was performed using Scanco XtremeCT (Scanco Medical AG, Bassersdorf, Switzerland) and scans were assessed using the manufacturer’s image processing language software (μCT Evaluation Program v6.0; Scanco Medical AG, Brüttisellen, Switzerland). All scans were performed using the following scan settings: an X-ray potential of 60 kVp, X-ray tube current of 900 µA, integration time of 100 mins, matrix size of 1536 x 1536 and voxel size of 82 µm. This system enables the simultaneous acquisition of a stack of 110 CT slices (Slice thickness 0.08 µm), which corresponding to a 9.02 mm section along the axial direction, were used to reproduce a three dimensional (3D) image of the scanned tibia.

The participant was seated in an adjustable chair with their non-dominant leg (the same leg which was scanned using pQCT) was immobilised in a carbon fibre cast (Figure 4.12). The cast helped to position the participant’s leg in the correct position within the gantry and to reduce participant movement. The height of the chair was adjusted to ensure that the participant’s leg was in a horizontal position within the gantry. Participants were then instructed to remain still throughout the scan.

![Figure 4.12 - Participant undergoing HR-pQCT.](image)
The participant’s lower leg is placed in a carbon fibre foot cast to support the leg during the scanning procedure.

4.3.3.1 The scanogram

In this study, a HR-pQCT protocol designed by Dr Heather McKay’s group (University of British Columbia, Vancouver, Canada) was adopted (Burrows et al., 2010a, Burrows et al., 2010c, Nishiyama et al., 2012b). The distal tibia was scanned at the 8% site of the total tibia length. The participant’s tibia length was measured from the middle of the inner ankle to the tibial plateau using a metal one metre ruler. The researcher calculated 8% of the participant’s tibia length and then adjusted the scanner’s control file to ensure that the ROI was set to scan the 8% site (Appendix 10.21). The
researcher placed the reference line on the tibial plafond and the ROI selected the last slice to be at the 8% site and the start slice to be 9.02 mm distal of the 8% slice, as shown in Figure 4.13.

![Figure 4.13 - HR-pQCT scans.](image)

(A) HR-pQCT scanogram of the distal tibia. The reference line is placed on the tibial plafond (dotted line). The control file is set to measure the 8% region on the 110th slice. (B) Each CT slice is assessed and a reference line is placed around the periosteal boundary, and (C) The CT slices are reconstructed to produce a 3D image of the bone. Source: X-ray from (Burrows et al., 2010a).

### 4.3.3.2 Quality assurance and quality control
During this study, a daily quality assurance was performed using the standard phantom to monitor the performance of the scanner and to ensure there was no drift in the measurements. In addition to the standard phantom, the scanner was pre-calibrated before every subject was scanned (Figure 4.14). The precision of HR-pQCT measurements was 0.7–1.5% for total, trabecular, and cortical densities and 2.5–4.4% for trabecular architecture, <1% for cortical density, <1.5% for cortical CSA and 6.0-13.5% for cortical porosity (Burghardt et al., 2010, Boutroy et al., 2005).
4.3.3.3 Scan acquisition and analysis

HR-pQCT is highly sensitive to participant motion during image acquisition. Despite the participant’s leg being immobilised in the carbon fibre foot cast and the chair adjustments, very slight movements can be detected and the image quality can be degraded, resulting in visible blurring, streaks and discontinuities of bone structures in the reconstructed images. These artefacts can affect the accuracy of morphological parameters and bone strength estimates, however, the exact effects are unknown. The quality of the scan was graded in accordance to Pauchard et al. (Figure 4.15) (Pauchard et al., 2012). The scans were analysed using the manufacturer’s standard clinical evaluation protocol in Imaging Processing Language (v6.0). The researcher assessed each slice by identifying the periosteal perimeter by using a semi-automated edge-finding algorithm which produced a closed contour around the periosteal surface (Kazakia et al., 2008). All contours were examined manually and modified as necessary to delineate the periosteal boundary. In addition to the standard analysis, a cortical porosity script was used to assess cortical parameters. This script identified the endosteal perimeter by using a semi-automated edge-finding algorithm which produced a closed contour around the endosteal surface. All contours were examined manually and modified as necessary.
4.4 Jumping mechanography

Dynamic muscle function was assessed using a Leonardo Mechanography® Ground Reaction Force Plate (Novotec Medical, Pforzheim, Germany). The manufacturer’s software (Leonardo Mechanography GRFP version 4.2, Novotec, Pforzheim, Germany) was used to store, and calculate the data. Participants were instructed to remove heavy clothing and shoes. Before the tests were performed, the researcher demonstrated a number of stretching exercises for the participant to perform. This was to reduce any risk of injury. The researcher explained and demonstrated each test. The platform was calibrated to be zero before each test.

4.4.1 Methods
4.4.1.1 Single two-legged jump (S2LJ)

The aim of this test was to achieve the maximum jump height. The participant stood on the plate, with one foot on each side of the plate, and remained still. Before the first jump, the platform records the participant’s weight (static ground reaction force whilst standing still) of the individual to calculate body mass (body mass = body weight / acceleration of gravity). The jump was performed as

<table>
<thead>
<tr>
<th>Motion grade</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streaks</td>
<td>None</td>
<td>Small</td>
<td>Medium-Large</td>
<td>Medium-Large</td>
<td>Medium-Large</td>
</tr>
<tr>
<td>Discontinuities</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>Small</td>
<td>Medium-Large</td>
</tr>
</tbody>
</table>

Figure 4.15 - Grading criteria for HR-pQCT scans.
Source: Pauchard et al. (Pauchard et al., 2012).
a counter-movement jump with freely moving arms, and the participant was instructed to jump as high as possible. The counter-movement allows the muscles to store energy and act as spring to achieve maximum jump height. Three jumps were performed with 30 seconds break in-between jumps. The highest jump of the three recording was selected. Parameters used for analysis were maximal peak power (Pmax, Watts; W), maximal peak power per body mass (Pmax, W/kg), and maximum height (Hmax, Meters; m) (Figure 4.16).

![Figure 4.16 - Single two-legged jump.](image)

4.4.1.2 Multiple one-legged hopping (M1LH)

The aim of this test was to measure maximum ground force reaction during landing. The participant stood on the plate, with one foot on each side of the plate, and remained still. When the test started, the participant started hopping on the forefoot of their non-dominant leg (the leg which had been scanned previously), keeping their knee straight and ankle stiff. They were instructed to hop as hard and fast as possible for approximately 10 seconds (Figure 4.17). Participants were instructed to place their whole body weight through the forefoot and not to touch the platform with their heel. Parameters used for analysis were maximum force (Fmax, Newtons; N) and maximum force per body weight (Fmax/BW; N/kg).
4.4.2 Jumping mechanography acquisition and analysis
The outputs for S2LJ and M1LH are illustrated in Figure 4.18 A-C. The S2LJ test was repeated three times with the aim to achieve maximum jump height. The first jump attempt was often the lowest jump height measurement, with the second and/or third attempt being higher as a learning effect occurred. Participants learned that the counter-movement was like a ‘spring’ which enabled them to achieve a higher jump. The difference between S2LJ with and without a counter-movement is demonstrated in Figure 4.18 A-B. Maximum jump height, peak power, and peak force is significantly lower in the non-counter-movement attempt. The participant was informed of their jump height at each attempt which encouraged them to jump higher in the following jump attempt. Date from the highest jump was analysed.

The M1LH was repeated twice with the aim to achieve maximum ground reaction force. The first hopping attempt by young participants (aged 8-10 years) was often poor as their heel would touch the jumping plate. The researcher was able to show the participant the hopping results output and explain that the heel contact with the plate would not be recorded and was identified by the grey boxes (Figure 4.18 C). A learning effect improved the second hopping attempt and the M1LH results output with the highest Fmax rel/g was analysed.
Figure 4.18 - Jumping mechanography data output.

(A) S2LJ with counter-movement prior to jump enables the participant to store energy in the lower limbs, which is then released during the lump. (B) S2LJ without counter-movement prior to jump results in a significant lower jump height, force and power. A jump without counter-movement is called a heel rise test and does not measure muscle force and power of the whole lower body. Source: Leonardo Mechanography® manual.
4.4.3 Quality assurance and quality control

During the study, regular checks of the ground reaction force plate surface and all eight sensors were carried out to ensure that the plate was flat and even. The plate was calibrated at every measurement to ≤ 0.05 Kg prior to the participant standing on. The CV of muscle power measurement in adults is 3.6% and in children is 3.7%; peak jumping force (S2UJ) in children was 8.9% (Fricke et al., 2006).

4.5 Recruitment

The study was initiated in January 2012 and was completed in April 2015. The breakdown of important study phases are shown in Table 4.8.

Table 4.8 - Study timelines.

<table>
<thead>
<tr>
<th>Timing of phase</th>
<th>Study phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jan 2012 – Jul 2012</td>
<td>Study design and PhD first year transfer report</td>
</tr>
<tr>
<td>Sep 2012 – Nov 2012</td>
<td>Internal MRC-HNR study design and ethics review</td>
</tr>
<tr>
<td>Dec 2012 – Apr 2013</td>
<td>Ethics application and approval</td>
</tr>
<tr>
<td>May 2013 – Aug 2013</td>
<td>Study setup, advertisement and screening/recruitment</td>
</tr>
<tr>
<td>Jun 2013 – Mar 2014</td>
<td>Baseline screening/recruitment and study visits</td>
</tr>
<tr>
<td>Jan 2014 – Jul 2014</td>
<td>Baseline image and jumping mechanography analyses and data entry from questionnaires</td>
</tr>
</tbody>
</table>

4.5.1 Recruitment of control participants

The screening/recruitment and baseline study visits started in June 2013 and were completed by March 2014, a total of 10 months of recruitment (Table 4.9 and Figure 4.19). Recruitment of control participants involved contacting 130 educational establishments (primary/secondary schools and sixth form colleges). Sixty one schools/colleges throughout East Anglia agreed to advertise the study by distributing leaflets, placing posters around the school/college, and on the school’s parent-net website. Fourteen schools declined to help as the school/college either had strict policies about advertising, students had special needs, or the school was too short staffed to help.

There were 150 study places available, 75 each for males and for females. The aim was to recruit 8-9 participants per age-and-sex group. Potential participants were screened on a first come, first served basis. If there were no places available for the potential participant for their age-gender group, they were asked if they would agree to be placed on a reserve list. Approximately 267 potential control participants responded to the advertisement and were screened. Thirteen participants were excluded through the screening process for various reasons including: Asian/black ethnicity, hypermobility, hemiplegia, use of medication which could affect growth or inability to consent, unexplained bowel and stomach problems, and chronic pain. One participant declined to take part
due to exam and time pressure. In total, 151 participants took part in the study and completed all study procedures. The study over recruited by one participant.

4.5.2 Recruitment of participants with cystic fibrosis

Participants with CF who were registered and treated by the CF health team at ADX were the first to be approached about the study. The CF health team were responsible for 138 paediatric patients, of which 64 patients aged between 8 and 16 years were identified. Patients and their parents/guardians who attended the weekly CF clinic sessions between June 2013 and February 2014 were approached during their appointment. In total, 41 of the 64 patients were approached in person to discuss the study; 35 patients were interested and 6 patients were not interested after being introduced to the study or did not want to be approached. Patients who lived in Peterborough and Ipswich were most likely to turn down the study as the burden of time and travelling to Cambridge (approx. 45 miles each way) was considered inconvenient, especially having spent the whole day at hospital.

To increase the awareness of the study, Dr Ross-Russell sent an invitation letter in November 2013 (Appendix 10.9) to patients who had expressed an interested in the study but had not been in touch with the research team and to patients who had not been approached during the ADX CF clinic sessions. In total, 31 invitation letters were sent to patients and only 2 patients responded. Due to low recruitment of patients with CF the recruitment area was extended to include Norfolk and Norwich NHS Trust and Watford General NHS Hospital (after ethical approval). Patients were approached by healthcare professionals about the study and were given the study information leaflets. Unfortunately, no patients from these hospitals wanted to take part in the study due to time commitments and burden of travel.

Table 4.9 - Recruitment of participants between June 2013 and March 2014.

<table>
<thead>
<tr>
<th>Recruitment time 2013 - 2014</th>
<th>No of control participants</th>
<th>No of participants with CF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jun – Jul</td>
<td>48</td>
<td>2</td>
</tr>
<tr>
<td>Aug – Sept</td>
<td>76</td>
<td>10</td>
</tr>
<tr>
<td>Oct – Nov</td>
<td>19</td>
<td>8</td>
</tr>
<tr>
<td>Dec – Jan</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Feb – Mar</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>151</td>
<td>25</td>
</tr>
</tbody>
</table>
During the recruitment phase, 29 patients were screened and were eligible to take part in the study. However, four patients did not take part; three cancelled their study visit due to illness and did not make further contact and one patient who attended MRC HNR for her study visit but parents of the patient refused to give consent due to the additional radiation exposure on top of her clinical exposure. However, the patient’s non-CF siblings did take part in the study. A total of 25 patients took part in the study (Table 4.9). All participants, except one, completed all the study procedures. To boost study numbers, data collaboration with the SNAP study occurred.

4.5.3 Exclusion of participant data
All DXA, pQCT or HR-pQCT scans were included in the data analysis and were of good quality. Six participants had metal objects or intravenous chest ports which could not be removed prior to scanning and therefore needed to be painted out. Three participants had abnormal findings and were referred for further medical investigation (as per protocol). After medical follow-up, these participants were reported to be normal and were included in the final analysis. One participant with CF did not take part in jumping mechanography due to recent minor surgery prior to the study.

4.5.4 Calibration of scanners used for BMS and SNAP study
DXA scans were acquired from two different scanners. The SNAP study based in Birmingham and the BMS based in Cambridge both used the GE Lunar (Prodigy). Standard manufacturer QA and QC were used at both sites. As the inclusion of SNAP data was retrospective, cross calibrate between the DXA and the pQCT scanners were not performed. This is a limitation to the study. For DXA, there were no significant differences between 7 centres for measurements of BMC or BMD (Crabtree et al, in review 2016). For pQCT measurements studies have reported little necessity to cross-calibrate measurements (Ward et al., 2011). Clearly an in-vivo cross-calibration would be much more robust but this is not possible nor practical in children.

To calibrate differences between pQCT scanners, the European forearm phantom (EFP) was scanned 10 times on each pQCT scanner at the beginning of the study. The differences between scanners were tested for total and trabecular vBMD and total area, cortical vBMD. No cross-calibration was necessary for pQCT measurements. The manufacture’s quality assurance (QA) phantom was scanned daily (Ashby et al., 2009b).

No cross-calibration was necessary for jumping mechanography as all jumping plates were calibrated using the manufacture’s standard protocol.
4.5.5 The SNAP study and merging of datasets

In total the data of 40 patients from the SNAP study were eligible to be compared with BMS healthy controls for bone and muscle measurements performed by DXA, pQCT and jumping mechanography. Anthropometric measurements and clinical characteristics of participants with CF from the BMS and SNAP studies are presented in Table 4.10.

The mean age of participants with CF was 12 (±2.6) years. Overall, there were no significant group differences except for height SDS, which were significantly higher in BMS participants compared to SNAP participants (-0.67, p<0.05). In the BMS, two participants were classed as overweight (BMI 92\textsuperscript{nd}-98\textsuperscript{th} percentile). In the SNAP study, two participants were classed as clinically underweight (BMI <2\textsuperscript{nd} percentile), two participants as overweight and two participants were clinically obese (BMI >98\textsuperscript{th} percentile). A broad range of cystic fibrosis transmembrane conductance regulator (CFTR) genotypes were represented in the BMS and SNAP studies, although most participants had at least one copy of the most common mutation, F580del. The mean FEV1 was above 75% which indicating mild-to-moderate pulmonary impairment. The majority (90.5%) of participants had pancreatic insufficiency requiring pancreatic enzyme replacement, 14.3% had CF-related diabetes and 17.5% had liver disease. There were no significant differences between studies for any clinical characteristics or fracture history but 25(OH)D concentration was lower in the SNAP participants Neither group had 25(OH)D concentration lower than clinical cut off for bone disease. Participants with CF are prescribed regular doses of vitamin D supplements due to their lack of sun exposure and malabsorption of fats and fat-soluble vitamins. The two datasets were merged together as there were no differences.
## Table 4.10 - Summary of group differences between BMS and SNAP participants with CF for anthropometric measurements during puberty.

<table>
<thead>
<tr>
<th>Anthropometric outcome measures</th>
<th>Participants with CF Mean (SD) or n (%)</th>
<th>Overall group differences P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BMS participants with CF</td>
<td>SNAP participants with CF</td>
</tr>
<tr>
<td>n (Females)</td>
<td>25 (10)</td>
<td>40 (18)</td>
</tr>
<tr>
<td>Age (Yrs) *</td>
<td>11.68 (±2.9)</td>
<td>12.45 (±2.8)</td>
</tr>
<tr>
<td>Height (cm) **</td>
<td>146.3 (±15.4)</td>
<td>147.1 (±13.5)</td>
</tr>
<tr>
<td>Height (SDS)</td>
<td>-0.07 (±1.1)</td>
<td>-0.67 (±1.0)</td>
</tr>
<tr>
<td>Weight (cm) **</td>
<td>38.5 (±11.9)</td>
<td>41.4 (±13.4)</td>
</tr>
<tr>
<td>Weight (SDS)</td>
<td>-0.18 (±0.9)</td>
<td>-0.32 (±1.7)</td>
</tr>
<tr>
<td>BMI (kg/m²) *</td>
<td>17.5 (±2.4)</td>
<td>18.7 (±4.1)</td>
</tr>
<tr>
<td>BMI (SDS)</td>
<td>-0.15 (±1.0)</td>
<td>0.00 (1.1)</td>
</tr>
<tr>
<td>Sitting height (cm) **</td>
<td>76.9 (±7.6)</td>
<td>77.1 (±6.8)</td>
</tr>
<tr>
<td>Most recent FEV1 (%) **¹</td>
<td>85.93 (±19.0)</td>
<td>89.30 (±17.0)</td>
</tr>
<tr>
<td>Pancreatic insufficiency **²</td>
<td>22 (88%)</td>
<td>35 (92.1%)</td>
</tr>
<tr>
<td>CF-related diabetes **²</td>
<td>2 (8%)</td>
<td>7 (18.4%)</td>
</tr>
<tr>
<td>Liver disease **²</td>
<td>4 (16%)</td>
<td>7 (18.4%)</td>
</tr>
<tr>
<td>25(OH)D (nmol/L) **³</td>
<td>78.83 (±26.6)</td>
<td>59.69 (±24.6)</td>
</tr>
<tr>
<td>History of fracture ***</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>Long bone</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>Other</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Genotype ***²</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ΔF508/ΔF508</td>
<td>15 (60%)</td>
<td>24 (63.2%)</td>
</tr>
<tr>
<td>ΔF508/Other</td>
<td>10 (40%)</td>
<td>10 (26.3%)</td>
</tr>
<tr>
<td>Other/Other</td>
<td>0 (0%)</td>
<td>4 (10.5%)</td>
</tr>
</tbody>
</table>

**Key:** * Independent samples two-sided t-test for normally distributed data, ** Wilcoxon rank sum for non-normally distributed data, and *** chi-squared for categorical data. ¹ missing data for three participants in SNAP study, ² missing data from two participants in the SNAP study, ³ missing data from two participants from BMS and three from SNAP. Standard deviation score from the mean height and weight of an age-and-gender matched control population (SDS), body mass index (BMI), forced expiratory volume in 1 second i.e. lung function (FEV1), 25-hydroxy vitamin D (25(OH)D) and most common CF mutation (ΔF508). Other fractures include metatarsals, phalanges and collar bone. Statistically significant when p<0.05 and is presented in bold and highlighted in grey.
Figure 4.19 - Flowchart of the study recruitment process.

**Recruitment of control participants**

- **BMS**
  - Posters displayed in the local community.
  - n = 80
- **BMS**
  - Inquiry letters sent to educational establishments.
  - n = 130

Agree to distribute leaflets and display posters.
- n = 61 agreed
- n = 14 declined
- n = 55 no reply

Total number of potential control participants made contact with researcher
- n = 267

__Excluded or cancelled__

- Controls n = 13
- CF n = 4

__Total number of eligible participants:__

- Control n = 254
- CF n = 29

__Total number who took part:__

- Control n = 151 (76 F & 75 M)
- CF n = 65 (28 F & 37 M)

- Completed questionnaires
  - Controls n = 151
  - CF n = 65

- Completed DXA scans
  - Controls n = 151
  - CF n = 65

- Completed pQCT scans
  - Controls n = 151
  - CF n = 65

- Completed HR-pQCT scans
  - Controls n = 151
  - CF n = 25

- Completed JM
  - Controls n = 151
  - CF n = 64

**Recruitment of participants with CF**

- **BMS**
  - Patients aged 0-16, resident in East Anglia and treated by ADH: n = 138
  - CF aged 8-16 years old: n = 64

Patents approached in clinic.
- Total approached n = 41
- Interested n = 35
- Not interested n = 6

Patients approached by Dr Ross-Russell's study invitation letter if interested during clinic or not seen at clinic.
- n = 31

Patients who agreed to take part and screened.
- n = 27

Patients who agreed to take part and screened.
- n = 2

__Total number of potential control participants made contact with researcher__

- n = 80

Total number of eligible participants:
- Control n = 254
- CF n = 29

Total number who took part:
- Control n = 151 (76 F & 75 M)
- CF n = 65 (28 F & 37 M)

**Key:** Bone and muscle study in Cambridge (BMS), 'Study in children and adolescents with chronic inflammatory and/or disabling conditions' in Birmingham (SNAP), number of participants (n), participants with cystic fibrosis (CF), females (F), males (M), dual-energy X-ray absorptiometry (DXA), peripheral quantitative computed tomography (pQCT), high resolution pQCT (HR-pQCT) and jumping mechanography (JM).
5 Modifying effects of sex on anthropometric, body composition, and muscle-bone outcomes

5.1 Data management and statistical analysis
This thesis is based on the cross-sectional baseline measurements of this longitudinal study. Longitudinal measurements were obtained at the 12 month follow up visit and are not presented.

5.1.1 Data management
Demographic variables were taken from the musculoskeletal questionnaire and merged with anthropometry, DXA, pQCT, HR-pQCT and jumping mechanography data. The five Tanner stages (TS) were condensed into three stages to increase statistical power to detect differences between pubertal groups. Participants in TS 1 were classed as pre-pubertal, participants in TS 2 and TS 3 were classed as early pubertal, and participants in TS 4 and TS 5 were classed as late pubertal. Age and quadratic age were treated as continuous data and pubertal stage was treated as discrete data (i.e. categorised into pre-, early and late puberty).

5.1.2 Statistical analyses
Statistical analyses were performed in three stages (i.e. models) (Table 5.1) to determine whether sex modified the relationship between puberty and bone and muscle outcomes in healthy children and adolescents.

Anthropometric, bone, body composition, and muscle function outcomes were analysed according to the research aims. In summary, the analysis models were:

**Model 1** (sex, pubertal stage, and sex-by-pubertal stage interaction) using ANCOVA and Scheffé post hoc analyses to test whether sex modifies the relationship between puberty and anthropometric, body composition, bone and muscle outcomes,

**Model 2** (sex, pubertal stage, age, quadratic age, height and weight, and sex-by-pubertal stage interaction) using multiple linear regression to test whether sex modifies the relationship between puberty and anthropometric, body composition, bone and, muscle outcomes, after adjusting for height (Figure 5.1), and finally,

**Model 3** (sex, pubertal stage, age, quadratic age, height and weight, Pmax, Fmax, sex-by-pubertal stage interaction, sex-by-Pmax interaction, and sex-by-Fmax interaction) using multiple linear regression to test whether sex modifies the relationship between muscle function and bone outcomes (Figure 5.2).

The methodology and justification for including co-variates used in the linear regression models are described in the following section.
5.1.2.1 Multiple linear regression – Methodology and justification of covariates

Tests for collinearity between age and pubertal stage confirmed that these measurements were non-collinear and therefore both age and pubertal stage were used in the model together ($R^2 = 0.60$). Height was retained in the model throughout the analyses to adjust for differences in body size and bone length during growth (Molgaard et al., 1997, Ward et al., 2015, Bianchi et al., 2014, Prentice et al., 1994). Weight was not included in models which included lean mass and fat mass as these outcomes contribute to total body mass (i.e. weight).

Jumping mechanography was used to measure maximum muscle force ($F_{\text{max}}$) and maximum muscle power ($P_{\text{max}}$). $F_{\text{max}}$, measured by single-legged hopping, is a measure of maximum ground reaction force and $P_{\text{max}}$, measured by two-legged jump, is a measure of maximum power (i.e. power = force multiplied by velocity) of the lower limbs. Tests for collinearity between $F_{\text{max}}$ and $P_{\text{max}}$ confirmed that these measurements were independent and therefore both $F_{\text{max}}$ and $P_{\text{max}}$ were used in the model together ($R^2 = 0.13$).

Three interactions were tested; 1) sex-by-pubertal stage to test whether sex modifies the relationship between puberty and bone, 2) sex-by-$F_{\text{max}}$ to test whether sex modifies the relationship between $F_{\text{max}}$ and bone, and 3) sex-by-$P_{\text{max}}$ to test whether sex modifies the relationship between $P_{\text{max}}$ and bone.

The methodology of the analyses has been described in this section with an example of the modelling process as shown in Figure 5.1 and Figure 5.2. Firstly, all co-variates were tested in the original model (Figure 5.1A), the least significant covariate was removed from the model (i.e. sex-by-pubertal stage interaction) and then the model was tested without this covariate (Figure 5.1B). Then the next least significant covariate in the model (i.e. pubertal stage (PS) was removed (Figure 5.1C). This process was repeated until the final model contained only significant covariates and those that need to be retained in the model (i.e. height) (Figure 5.1D). Sex and height were retained throughout the analyses whether they were significant or not.

As is standard practice, covariates involved in the interactions were not removed from the model before the interaction term, even if they were not significant (NS) (e.g. If a sex-by-pubertal stage interaction was significant, sex and pubertal stage were retained separately in the model irrespective of significance). If there were no significant interactions between sex and pubertal stage or sex and muscle function, then the main effect (i.e. sex difference) was interpreted and reported when significant. Insignificant covariates were excluded from subsequent models. The adjustments of the final model were checked by placing the previously NS covariates back into the final model to test whether these covariates then became significant. If the previously NS covariate remained NS in final model, then the covariate was removed again. If this covariate became significant in the final model...
then the covariate would be kept in the final model. All previously NS covariates were tested in the final model one at a time. Data are presented as beta-coefficients (β, %) and p-value with the significance level set to p<0.05. The β value is the difference in bone outcome for a doubling of the independent variable (e.g. Pmax), which was multiplied by 100 to express as a percentage difference (Cole, 2000). Interaction plots were used to help to interpret statistically significant interactions (p<0.05). Pubertal staging was split into three groups (i.e. pre-puberty, early puberty and late puberty) and muscle Pmax and Fmax were plotted as lower and upper quantiles so that bone and muscle relationships could be identified clearly. The exploratory covariates included in these models have been listed in Table 5.1.

Table 5.1 - Summary of the exploratory covariates used in the analyses models.

<table>
<thead>
<tr>
<th>Covariates and analyses used in this study</th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Covariates in ANCOVA with Scheffé post hoc</strong></td>
<td>Sex</td>
<td>Sex</td>
<td>Sex</td>
</tr>
<tr>
<td></td>
<td>Pubertal stage</td>
<td>Height</td>
<td>Height</td>
</tr>
<tr>
<td></td>
<td>Sex-by-pubertal stage interaction*</td>
<td>Age</td>
<td>Age</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Quadratic age</td>
<td>Quadratic age</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pubertal stage</td>
<td>Pubertal stage</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Weight</td>
<td>Weight</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sex-by-pubertal stage interaction*</td>
<td>Sex-by-pubertal stage interaction*</td>
</tr>
</tbody>
</table>

Key: * As is standard practice, if an interaction was significant, the covariates were also retained separately in the model. If there was a significant interaction, then the relationship between the two covariates in the interaction was interpreted, whereas, if there were no significant interactions, then these interaction terms were omitted and the main effect (i.e. sex difference) was interpreted where significant. Covariates in bold font were ‘forced’ into all models whether significant or not, whereas covariates in regular font were only included in the final model if significant.
Figure 5.1- An example of multiple linear regression for Model 2 (i.e. Height-adjusted)

Multiple linear regression for assessing sex differences in the relationship between puberty and total body less head BMC (measured by DXA) in healthy males and females (Model 2).

(A) Initial Model 2 with all covariates included. Significant covariates are highlighted in yellow and remained in the model (with sex and height). The least significant covariate (i.e. sex*PS) was removed which is highlighted in red.

<table>
<thead>
<tr>
<th></th>
<th>β coefficient</th>
<th>Std. Error</th>
<th>t value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Intercept)</td>
<td>-3.5506275</td>
<td>0.9701010</td>
<td>-3.660</td>
<td>0.000356 ***</td>
</tr>
<tr>
<td>Sex2</td>
<td>-0.0155354</td>
<td>0.0377344</td>
<td>-0.412</td>
<td>0.681182</td>
</tr>
<tr>
<td>PS2</td>
<td>-0.0094310</td>
<td>0.0381463</td>
<td>-0.247</td>
<td>0.805089</td>
</tr>
<tr>
<td>PS3</td>
<td>0.0290624</td>
<td>0.0516222</td>
<td>0.563</td>
<td>0.573431</td>
</tr>
<tr>
<td>Age</td>
<td>0.0522548</td>
<td>0.044912</td>
<td>1.174</td>
<td>0.241767</td>
</tr>
<tr>
<td>I(Age^2)</td>
<td>-0.0009494</td>
<td>0.0016835</td>
<td>-0.564</td>
<td>0.573678</td>
</tr>
<tr>
<td>I(log(Height_cm))</td>
<td>1.5968217</td>
<td>0.2394507</td>
<td>6.669</td>
<td>5.42e-10 ***</td>
</tr>
<tr>
<td>I(log(Weight_kg))</td>
<td>0.5817971</td>
<td>0.0622160</td>
<td>9.351</td>
<td>&lt; 2e-16 ***</td>
</tr>
<tr>
<td>Sex2*PS2</td>
<td>0.0307747</td>
<td>0.0462058</td>
<td>-0.666</td>
<td>0.506477</td>
</tr>
<tr>
<td>Sex2*PS3</td>
<td>0.0135783</td>
<td>0.0502906</td>
<td>0.270</td>
<td>0.787557</td>
</tr>
</tbody>
</table>

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.102 on 141 degrees of freedom, Multiple R-squared: 0.9459, Adjusted R-squared: 0.9425, F-statistic: 274.2 on 9 and 141 DF, p-value: < 2.2e-16

B) Model 2 was retested without sex-by-pubertal stage interaction. Height and weight remain significant in the model (highlighted in yellow) and were retained in the model while quadratic age, the next least significant covariate was removed (highlighted in red).

<table>
<thead>
<tr>
<th></th>
<th>β coefficient</th>
<th>Std. Error</th>
<th>t value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Intercept)</td>
<td>-3.359298</td>
<td>0.920707</td>
<td>-3.649</td>
<td>0.000369 ***</td>
</tr>
<tr>
<td>Sex2</td>
<td>-0.025048</td>
<td>0.017606</td>
<td>-1.423</td>
<td>0.157010</td>
</tr>
<tr>
<td>PS2</td>
<td>-0.021041</td>
<td>0.028011</td>
<td>-0.751</td>
<td>0.453791</td>
</tr>
<tr>
<td>PS3</td>
<td>0.041857</td>
<td>0.037762</td>
<td>1.108</td>
<td>0.269530</td>
</tr>
<tr>
<td>Age</td>
<td>0.057107</td>
<td>0.044152</td>
<td>1.293</td>
<td>0.197949</td>
</tr>
<tr>
<td>I(Age^2)</td>
<td>-0.001093</td>
<td>0.001674</td>
<td>-0.653</td>
<td>0.514678</td>
</tr>
<tr>
<td>I(log(Height_cm))</td>
<td>1.556402</td>
<td>0.219615</td>
<td>7.258</td>
<td>2.25e-11 ***</td>
</tr>
<tr>
<td>I(log(Weight_kg))</td>
<td>0.576204</td>
<td>0.060482</td>
<td>9.527</td>
<td>&lt; 2e-16 ***</td>
</tr>
</tbody>
</table>

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.1017 on 143 degrees of freedom, Multiple R-squared: 0.9453, Adjusted R-squared: 0.9428, F-statistic: 354.3 on 7 and 143 DF, p-value: < 2.2e-16

C) Model 2 was retested without quadratic age. Height and weight remain significant in the model (highlighted in yellow) and were retained in the model while pubertal stage, the next least significant covariate was removed (highlighted in red).

<table>
<thead>
<tr>
<th></th>
<th>β coefficient</th>
<th>Std. Error</th>
<th>t value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Intercept)</td>
<td>-3.377460</td>
<td>0.918453</td>
<td>-3.677</td>
<td>0.000332 ***</td>
</tr>
<tr>
<td>Sex2</td>
<td>-0.023283</td>
<td>0.017363</td>
<td>-1.341</td>
<td>0.182047</td>
</tr>
<tr>
<td>PS2</td>
<td>-0.014523</td>
<td>0.026123</td>
<td>-0.556</td>
<td>0.570044</td>
</tr>
<tr>
<td>PS3</td>
<td>0.043330</td>
<td>0.037619</td>
<td>1.152</td>
<td>0.251314</td>
</tr>
<tr>
<td>Age</td>
<td>0.082620</td>
<td>0.068277</td>
<td>1.242</td>
<td>0.216103</td>
</tr>
<tr>
<td>I(log(Height_cm))</td>
<td>1.599992</td>
<td>0.219615</td>
<td>7.258</td>
<td>2.25e-11 ***</td>
</tr>
<tr>
<td>I(log(Weight_kg))</td>
<td>0.576857</td>
<td>0.060353</td>
<td>9.527</td>
<td>&lt; 2e-16 ***</td>
</tr>
</tbody>
</table>

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.1015 on 144 degrees of freedom, Multiple R-squared: 0.9453, Adjusted R-squared: 0.9443, F-statistic: 414.9 on 6 and 144 DF, p-value: < 2.2e-16

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D) Pubertal stage was removed from the model and the model was retested. All covariates (except sex) were significant (highlighted in yellow). This is the final model. Non-significant covariates were placed back into model one at a time to check that they were not significant in the final model. As there was no significant interaction between sex and puberty, the main effect (i.e. sex difference) was interpreted.

### Coefficients:

<table>
<thead>
<tr>
<th></th>
<th>β</th>
<th>Std. Error</th>
<th>t value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Intercept)</td>
<td>-3.267686</td>
<td>0.926865</td>
<td>-3.526</td>
<td>0.000565 ***</td>
</tr>
<tr>
<td>Sex2</td>
<td>-0.014153</td>
<td>0.016894</td>
<td>-0.838</td>
<td>0.403536</td>
</tr>
<tr>
<td>Age</td>
<td>0.034174</td>
<td>0.006194</td>
<td>5.517</td>
<td>1.53e-07 ***</td>
</tr>
<tr>
<td>I(log(Height_cm))</td>
<td>1.545054</td>
<td>0.221377</td>
<td>6.979</td>
<td>9.64e-11 ***</td>
</tr>
<tr>
<td>I(log(Weight_kg))</td>
<td>0.595519</td>
<td>0.059370</td>
<td>10.031</td>
<td>&lt; 2e-16 ***</td>
</tr>
</tbody>
</table>

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.1028 on 146 degrees of freedom, Multiple R-squared: 0.9432, Adjusted R-squared: 0.9416, F-statistic: 605.7 on 4 and 146 DF, p-value: < 2.2e-16
Figure 5.2 - An example of multiple linear regression for Model 3 (i.e. Sex*Muscle function interactions).

An example of multiple linear regression for assessing sex differences in the relationship between muscle function and total body less head BMC (measured by DXA) in healthy males and females (Model 3).

A) Initial Model 3 with all covariates included (i.e. all covariates in Model 2 and the addition of power, force, sex-by-power interaction and sex-by-force interaction). The process of selecting the less significant co-variant, removing the co-variant and re-testing the model is repeated as shown in Figure 5.1.

### Coefficients:

<table>
<thead>
<tr>
<th></th>
<th>( \beta ) coefficient</th>
<th>Std. Error</th>
<th>( t ) value</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Intercept)</td>
<td>-0.7942131</td>
<td>0.9560318</td>
<td>-0.831</td>
<td>0.407566</td>
</tr>
<tr>
<td>Sex2</td>
<td>-0.0079714</td>
<td>0.0404703</td>
<td>-0.197</td>
<td>0.844143</td>
</tr>
<tr>
<td>PS2</td>
<td>-0.0127832</td>
<td>0.0349453</td>
<td>-0.366</td>
<td>0.715074</td>
</tr>
<tr>
<td>PS3</td>
<td>-0.0200909</td>
<td>0.0503700</td>
<td>-0.399</td>
<td>0.766395</td>
</tr>
<tr>
<td>Age</td>
<td>-0.0121537</td>
<td>0.0408275</td>
<td>-0.298</td>
<td>0.766395</td>
</tr>
<tr>
<td>I(Age^2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I(log(Height_cm))</td>
<td>1.3913506</td>
<td>0.2161040</td>
<td>6.438</td>
<td>1.89e-09 ***</td>
</tr>
<tr>
<td>I(log(Weight_kg))</td>
<td>0.1990116</td>
<td>0.0812167</td>
<td>2.450</td>
<td>0.015531 *</td>
</tr>
<tr>
<td>I(log(Power))</td>
<td>0.2928761</td>
<td>0.0733088</td>
<td>3.995</td>
<td>0.000105 ***</td>
</tr>
<tr>
<td>I(log(Force))</td>
<td>0.2026694</td>
<td>0.0954258</td>
<td>2.124</td>
<td>0.035480 *</td>
</tr>
<tr>
<td>Sex2:PS2</td>
<td>0.0182685</td>
<td>0.0473733</td>
<td>0.386</td>
<td>0.700370</td>
</tr>
<tr>
<td>Sex2:PS3</td>
<td>0.1020107</td>
<td>0.0656977</td>
<td>1.533</td>
<td>0.122795</td>
</tr>
<tr>
<td>Sex2:I(log(Power))</td>
<td>-0.0898101</td>
<td>-0.0902817</td>
<td>-0.995</td>
<td>0.321599</td>
</tr>
<tr>
<td>Sex2:I(log(Force))</td>
<td>0.1530642</td>
<td>0.1152400</td>
<td>1.346</td>
<td>0.180662</td>
</tr>
</tbody>
</table>

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.0895 on 137 degrees of freedom, Multiple R-squared: 0.9596, Adjusted R-squared: 0.9557, F-statistic: 250.2 on 13 and 137 DF, \( P \)-value: < 2.2e-16

B) Model 3 assessed without quadratic age.

### Coefficients:

<table>
<thead>
<tr>
<th></th>
<th>( \beta ) coefficient</th>
<th>Std. Error</th>
<th>( t ) value</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Intercept)</td>
<td>-0.81169</td>
<td>0.95339</td>
<td>-0.851</td>
<td>0.39604</td>
</tr>
<tr>
<td>Sex2</td>
<td>-0.01034</td>
<td>0.04019</td>
<td>-0.257</td>
<td>0.79739</td>
</tr>
<tr>
<td>PS2</td>
<td>-0.01928</td>
<td>0.03317</td>
<td>-0.581</td>
<td>0.56212</td>
</tr>
<tr>
<td>PS3</td>
<td>-0.02202</td>
<td>0.05015</td>
<td>-0.439</td>
<td>0.66135</td>
</tr>
<tr>
<td>Age</td>
<td>0.01216</td>
<td>0.00684</td>
<td>1.778</td>
<td>0.07762</td>
</tr>
<tr>
<td>I(log(Height_cm))</td>
<td>1.36247</td>
<td>0.21027</td>
<td>6.480</td>
<td>1.51e-09 ***</td>
</tr>
<tr>
<td>I(log(Weight_kg))</td>
<td>0.20349</td>
<td>0.08069</td>
<td>2.522</td>
<td>0.01281 *</td>
</tr>
<tr>
<td>I(log(Power))</td>
<td>0.28620</td>
<td>0.07230</td>
<td>3.958</td>
<td>0.00012 ***</td>
</tr>
<tr>
<td>I(log(Force))</td>
<td>0.20505</td>
<td>0.09513</td>
<td>2.156</td>
<td>0.03285 *</td>
</tr>
<tr>
<td>Sex2:PS2</td>
<td>0.02069</td>
<td>0.04709</td>
<td>0.439</td>
<td>0.66112</td>
</tr>
<tr>
<td>Sex2:PS3</td>
<td>0.10438</td>
<td>0.06545</td>
<td>1.592</td>
<td>0.14374</td>
</tr>
<tr>
<td>Sex2:I(log(Power))</td>
<td>-0.08948</td>
<td>-0.09007</td>
<td>-0.993</td>
<td>0.32223</td>
</tr>
<tr>
<td>Sex2:I(log(Force))</td>
<td>0.14728</td>
<td>0.11425</td>
<td>1.289</td>
<td>0.19954</td>
</tr>
</tbody>
</table>

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.0893 on 138 degrees of freedom, Multiple R-squared: 0.9595, Adjusted R-squared: 0.9559, F-statistic: 272.3 on 12 and 138 DF, \( P \)-value: < 2.2e-16

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C) **Model 3** assessed without quadratic age and sex-by-pubertal stage interaction.

<table>
<thead>
<tr>
<th>Coefficients:</th>
<th>β coefficient</th>
<th>Std. Error</th>
<th>t value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Intercept)</td>
<td>-0.562512</td>
<td>0.945404</td>
<td>-0.595</td>
<td>0.552793</td>
</tr>
<tr>
<td>Sex2</td>
<td>-0.000827</td>
<td>0.031593</td>
<td>-0.026</td>
<td>0.979153</td>
</tr>
<tr>
<td>Age</td>
<td>0.018610</td>
<td>0.006200</td>
<td>3.002</td>
<td>0.003174**</td>
</tr>
<tr>
<td>I(log(Height_cm))</td>
<td>1.283147</td>
<td>0.209130</td>
<td>6.136</td>
<td>7.99e-09***</td>
</tr>
<tr>
<td>I(log(weight_kg))</td>
<td>0.223080</td>
<td>0.078813</td>
<td>2.830</td>
<td>0.005322***</td>
</tr>
<tr>
<td>I(log(Power))</td>
<td>0.257533</td>
<td>0.070793</td>
<td>3.638</td>
<td>0.000384***</td>
</tr>
<tr>
<td>I(log(Force))</td>
<td>0.195854</td>
<td>0.092987</td>
<td>2.106</td>
<td>0.036942*</td>
</tr>
<tr>
<td>Sex2:I(log(Power))</td>
<td>-0.031832</td>
<td>0.087466</td>
<td>-0.364</td>
<td>0.716446</td>
</tr>
<tr>
<td>Sex2:I(log(Force))</td>
<td>0.170875</td>
<td>0.112740</td>
<td>1.516</td>
<td>0.131828</td>
</tr>
</tbody>
</table>

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Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 1

Residual standard error: 0.09044 on 142 degrees of freedom, Multiple R-squared: 0.9572, Adjusted R-squared: 0.9548, F-statistic: 397.2 on 8 and 142 DF, p-value: < 2.2e-16

D) **Model 3** assessed without quadratic age, sex-by-pubertal stage interaction and sex-by-Pmax interaction. The sex-by-force interaction was interpreted

<table>
<thead>
<tr>
<th>Coefficients:</th>
<th>β coefficient</th>
<th>Std. Error</th>
<th>t value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Intercept)</td>
<td>-0.568142</td>
<td>0.942406</td>
<td>-0.603</td>
<td>0.54755</td>
</tr>
<tr>
<td>Sex2</td>
<td>-0.010052</td>
<td>0.018799</td>
<td>-0.535</td>
<td>0.59367</td>
</tr>
<tr>
<td>Age</td>
<td>0.018420</td>
<td>0.006159</td>
<td>2.991</td>
<td>0.00328**</td>
</tr>
<tr>
<td>I(log(Height_cm))</td>
<td>1.285714</td>
<td>0.208376</td>
<td>6.170</td>
<td>6.64e-09***</td>
</tr>
<tr>
<td>I(log(weight_kg))</td>
<td>0.222853</td>
<td>0.078571</td>
<td>2.836</td>
<td>0.00523**</td>
</tr>
<tr>
<td>I(log(Power))</td>
<td>0.243222</td>
<td>0.058690</td>
<td>4.144</td>
<td>5.81e-05***</td>
</tr>
<tr>
<td>I(log(Force))</td>
<td>0.215109</td>
<td>0.076236</td>
<td>2.822</td>
<td>0.00546**</td>
</tr>
<tr>
<td>Sex2:I(log(Force))</td>
<td>0.134479</td>
<td>0.051891</td>
<td>2.592</td>
<td>0.01054*</td>
</tr>
</tbody>
</table>

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Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 1

Residual standard error: 0.09016 on 143 degrees of freedom, Multiple R-squared: 0.9572, Adjusted R-squared: 0.9551, F-statistic: 456.7 on 7 and 143 DF, p-value: < 2.2e-16
5.2 The influence of sex on the relationship between puberty and anthropometric outcomes in healthy participants

### Aim

1) The aim was to investigate whether sex modified the relationship between puberty and anthropometric outcomes (i.e. height, weight, sitting height and BMI).

### Hypothesis

1) The hypothesis is that sex does modify the relationship between puberty and anthropometric outcomes. Females have lower apparent gains in weight, height, sitting height, and BMI compared to males as puberty proceeds.

#### 5.2.1 Analyses

Anthropometric measurements were assessed for normality and transformed into natural logs. Primary outcomes were age, pubertal stage, height, weight, sitting height and BMI. Standard deviation scores (SDS or Z-score) for height, weight and BMI were calculated using the 1990 UK growth reference dataset, to compare our population with current UK standards (Cole, 1990, Cole et al., 1998, Cole and Green, 1992). Analyses were performed using Data Desk® statistical package (Version 6.3.1). In summary, the analysis models were:

**Model 1** (Sex, pubertal stage, and sex-by-pubertal stage interaction) using ANCOVA and Scheffé post hoc analyses to test whether sex modifies the relationship between puberty and age, height, height SDS, weight, weight SDS, BMI, BMI SDS and sitting height.

Data are presented as mean and standard deviation. The statistical significance level was set at p<0.05. Boxplots were used to present the effects of sex on anthropometric outcomes (i.e. height, height SDS, weight, weight SDS, BMI, BMI SDS and sitting height) as puberty proceeds. Any outlying values were scrutinised.

#### 5.2.2 The results after Model 1 analyses

Anthropometric characteristics of healthy participants are presented in Table 5.2 and in Figure 5.3 and Figure 5.4. The mean age of males and females was 12.2 (±2.6) years. Sex did modify the relationship between puberty and anthropometric outcomes. With increasing maturation, females showed lower apparent gains in height (p=0.001), weight (p=0.021), and sitting height (p=0.015) compared to males.

Post hoc comparisons showed that females were taller (p<0.001) and weighed more (p<0.001) in early puberty but were shorter (p=0.003) in late puberty compared to males (Figure 5.3). Females had a shorter sitting height (p=0.047) in late puberty compared to males (Figure 5.4). There were no
significant sex-by-pubertal stage interactions for the remaining outcomes (i.e. age, height SDS, weight SDS, BMI, or BMI SDS).

Table 5.2 – The modifying effect of sex on the relationship between puberty and anthropometric outcomes in healthy participants (Model 1).

<table>
<thead>
<tr>
<th>Anthropometric outcome measures</th>
<th>Healthy participants (n = 151)</th>
<th>Model 1 Sex*PS interaction p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males Mean (SD)</td>
<td>Females Mean (SD)</td>
</tr>
<tr>
<td>Puberty (n)</td>
<td>Pre (n=12)</td>
<td>Early (n=45)</td>
</tr>
<tr>
<td>Age (Yrs)</td>
<td>9.1 (±0.9)</td>
<td>11.8 (±2.1)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>134.1 (±7.0)</td>
<td>150.5 (±13.3)</td>
</tr>
<tr>
<td>Height (SDS)</td>
<td>0.07 (±1.1)</td>
<td>0.28 (±1.1)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>32.2 (±8.9)</td>
<td>42.1 (±11.9)</td>
</tr>
<tr>
<td>Weight (SDS)</td>
<td>0.34 (±1.4)</td>
<td>0.30 (±0.9)</td>
</tr>
<tr>
<td>BMI (kg/m&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>17.6 (±3.1)</td>
<td>18.2 (±2.7)</td>
</tr>
<tr>
<td>BMI (SDS)</td>
<td>0.49 (±1.3)</td>
<td>0.21 (±1.0)</td>
</tr>
<tr>
<td>Sitting height (cm)</td>
<td>70.7 (±4.3)</td>
<td>78.3 (±7.0)</td>
</tr>
</tbody>
</table>

**Key:** All values are mean (SD) unless indicated. Letters are used to indicate significance of sex and pubertal stage differences as tested by ANCOVA/Scheffé. * Categorical data was compared using a chi-squared test. Puberty was based on Tanner staging (TS) and was categorised into three groups: pre-puberty = TS 1, early puberty = TS 2 and TS 3, and late puberty = TS 4 and TS 5. (SDS) Standard deviation score from the mean height and weight of an age-and-sex matched control population. Body mass index (BMI) = weight (kg) divided by height (m<sup>2</sup>). Statistically significant when p<0.05 and is presented in bold and highlighted in grey.

a Significant between healthy pre-pubertal males and females, P<0.05.
b Significant between healthy early pubertal males and females, P<0.05.
c Significant between healthy late pubertal males and females, P<0.05.
Figure 5.3 – The modifying effect of sex on the relationship between puberty and anthropometric (mean, SD) outcomes in healthy participants (Model 1).
Figure 5.4 - Modifying effect of sex on the relationship between puberty and sitting height (mean, SD) in healthy participants (Model 1).

5.2.3 Summary
- Females had lower apparent gains in standing and sitting height as puberty proceeded compared to males (Table 5.2, Figure 5.3 and Figure 5.4).
5.3 The influence of sex on the relationship between puberty and body composition and muscle function in healthy participants

Aims

The aims were to investigate whether, after accounting for height, weight and pubertal stage, sex modified the relationship between puberty and:

1) Body composition outcomes (i.e. total body lean mass (LM), fat mass (FM), fat-free mass index (FFMI), fat mass index (FMI), lean-to-fat mass ratio (LM:FM), muscle CSA, muscle density and subcutaneous fat CSA).

2) Muscle function outcomes (i.e. maximum power (Pmax) and force (Fmax)).

Hypotheses

The hypotheses were that sex, after accounting for height, weight and pubertal stage, would modify the relationship between puberty and:

1) Body composition, where females would have lower apparent gains in lean mass (LM), fat-free mass index (FFMI), lean-to-fat mass ratio (LM:FM), and muscle density but higher apparent gains in fat mass (FM), fat mass index (FMI), subcutaneous fat CSA compared to males, as puberty proceeds.

2) Muscle function, where females would have lower apparent gains in muscle force (Fmax) and power (Pmax) compared to males, as puberty proceeds.

5.3.1 Analyses

Body composition and muscle function measurements were assessed for normality and transformed into natural logs. Primary outcomes for the total body site were; LM, FM, LM:FM ratio, FFMI (i.e. FFM / [height^2]) and FMI (i.e. FM / [height^2]) (Williams et al., 2010), for the proximal 66% site of the tibia were: muscle CSA, muscle density and subcutaneous fat CSA, and for muscle function were: Pmax and Fmax.

Analyses were performed using Data Desk® (Version 6.3.1) and Rstudio® (Version 3.3.1) statistical package. In summary, the analysis models were: Model 1 (sex, pubertal stage, and sex-by-pubertal stage interaction) using ANCOVA and Scheffé post hoc analyses to test whether sex modifies the relationship between puberty and body composition and muscle function outcomes, and Model 2 (sex, pubertal stage, age, quadratic age, height and weight, and sex-by-pubertal stage interaction) using multiple linear regression to test whether sex modifies the relationship between puberty and body composition and muscle function outcomes, after adjusting for height.
Data are presented as mean and standard deviation. The statistical significance level was set at p<0.05. Any outlying values were scrutinised. In model 2, height was retained in the analyses for all measurements to adjust for differences in body size during growth, except for FFMI and FMI which include adjustments for height. Weight was not included in the model for lean mass and fat mass. Data are presented as beta-coefficient (%) and p-value, with the significance level set to p<0.05.

5.3.2 The results of Model 1 analyses

The modifying effects of sex on the relationship between puberty and body composition and muscle function outcomes after Model 1 analyses (i.e. sex, pubertal stage, and sex-by-pubertal stage interaction) are presented in Table 5.3.

**DXA (Total body)** – Sex did modify the relationship between puberty and body composition outcomes (Table 5.3). With increasing maturation, females showed lower apparent gains in LM (p<0.001) and FFMI (p=0.0165) but showed higher apparent gains in FM (p=0.0128), and FMI (p=0.0034) compared to males. As a result, females had an apparent reduction in LM:FM ratio (p=0.0408). Post hoc comparisons showed that females had lower LM in late puberty and lower FFMI and LM:FM ratio in early and late puberty compared to males. Females had higher FM and FMI in early and late puberty compared to males.

**pQCT (The proximal 66% site of the tibia)** – Sex did modify the relationship between puberty and pQCT body composition outcomes (Table 5.3). With increasing maturation, females showed lower apparent gains in muscle CSA (p=0.0242) and muscle density (p=0.0113) but showed higher apparent gains in subcutaneous fat CSA (p=0.0064) compared to males. Post hoc comparisons showed that females had lower muscle CSA and muscle density in late puberty and higher subcutaneous fat CSA in early and late puberty compared to males.

**Jumping mechanography (Lower body)** – Sex did modify the relationship between puberty and muscle function outcomes (Table 5.3). With increasing maturation, females showed lower apparent gains in Pmax (p<0.0001) and Fmax (p=0.0006) compared to males. Post hoc comparisons showed that females had lower Pmax and Fmax in late puberty compared to males.
Table 5.3 – Modifying effect of sex on the relationship between puberty and body composition and muscle function outcomes in healthy participants (Model 1).

<table>
<thead>
<tr>
<th>Body composition outcome measures</th>
<th>Healthy participants (n = 151)</th>
<th>Model 1</th>
<th>Sex*PS interaction p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Puberty (n)</td>
<td>Males</td>
<td>Females</td>
<td></td>
</tr>
<tr>
<td>Pre (n=12)</td>
<td>Early (n=45)</td>
<td>Late (n=18)</td>
<td></td>
</tr>
<tr>
<td>Pre (n=21)</td>
<td>Early (n=24)</td>
<td>Late (n=31)</td>
<td></td>
</tr>
<tr>
<td>DXA (Total body less head site [TBLH])</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LM (g)</td>
<td>22870.6 (±2481.7)</td>
<td>32022.7 (±9183.3)</td>
<td>49279.7 (±7479.3)</td>
</tr>
<tr>
<td>FM (g)</td>
<td>7675.2 (±6386.5)</td>
<td>8108.5 (±4920.6)</td>
<td>11319.6 (±6452.0)</td>
</tr>
<tr>
<td>LM:FM ratio</td>
<td>5.0 (±2.8)</td>
<td>5.1 (±2.8)</td>
<td>6.5 (±4.8)</td>
</tr>
<tr>
<td>FFMI (kg/m&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>13.3 (±0.5)</td>
<td>14.5 (±1.9)</td>
<td>17.2 (±1.6)</td>
</tr>
<tr>
<td>FMI (kg/m&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>4.0 (±3.0)</td>
<td>3.5 (±2.0)</td>
<td>3.8 (±2.2)</td>
</tr>
<tr>
<td>pQCT (Proximal 66% site of tibia)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Muscle CSA (mm&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>3562.4 (±390.8)</td>
<td>4841.5 (±1241.4)</td>
<td>6801.6 (±1127.3)</td>
</tr>
<tr>
<td>Muscle density (g)</td>
<td>77.4 (±1.7)</td>
<td>79.9 (±2.8)</td>
<td>84.8 (±2.1)</td>
</tr>
<tr>
<td>Subcutaneous fat CSA (mm&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>1972.1 (±979.2)</td>
<td>1876.1 (±668.3)</td>
<td>2115.6 (±946.0)</td>
</tr>
<tr>
<td>Jumping mechanography (Lower body)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pmax (W)</td>
<td>1.11 (±0.2)</td>
<td>1.82 (±0.8)</td>
<td>3.35 (±0.8)</td>
</tr>
<tr>
<td>Fmax (N)</td>
<td>0.89 (±0.2)</td>
<td>1.23 (±0.3)</td>
<td>1.95 (±0.3)</td>
</tr>
</tbody>
</table>

Key: All values are mean (SD) unless indicated. Letters are used to indicate significance of sex and pubertal stage differences as tested by ANCOVA /Scheffé. Puberty was based on Tanner staging (TS) and was categorised into three groups: pre-puberty = TS 1, early puberty = TS 2 and TS 3, and late puberty = TS 4 and TS 5. (SDS) Standard deviation score from the mean height and weight of an age- and sex-matched control population. Lean mass (LM), fat mass (FM), fat-free mass index (FFMI = FFM/[height<sup>2</sup>]), fat mass index (FMI = FM/[height<sup>2</sup>]), cross-sectional area (CSA), maximum power (Pmax) and maximum force (Fmax). Statistical significant when p<0.05 and is presented in bold and highlighted in grey. If a sex-by-pubertal stage interaction was not found, then the interaction was removed from the model and the β coefficient (i.e. sex) was interpreted alone.

a Significant difference between healthy pre-pubertal males and females, P<0.05.
b Significant difference between healthy early pubertal males and females, P<0.05.
c Significant difference between healthy late pubertal males and females, P<0.05.
5.3.3 The results of Model 2 analyses (i.e. Height-adjusted)
The modifying effects of sex on the relationship between puberty and body composition and muscle function after Model 2 analyses (i.e. sex, pubertal stage, age, quadratic age, height, weight, and sex-by-pubertal stage interaction) are presented in Table 5.4.

5.3.3.1 DXA (Total body)
As shown in Table 5.4, sex did modify the relationship between puberty and DXA body composition for FM, LM:FM ratio, and FMI. With increasing maturation (Pre-to-late puberty), females showed higher apparent gains in FM (76.8%, p=0.002) and in FMI (65.7%, p=0.006) but had lower apparent gains in LM:FM ratio (-83%, p<0.001) compared to males (Table 5.4 and Figure 5.5). These trends were similar in pre-to-early puberty. There were no significant sex-by-pubertal stage interactions for LM and FFMI but females had consistently lower LM (-9.3%, p<0.001) and FFMI (-8.9%, p<0.001) compared to males, across all stages of puberty.

Figure 5.5 – The modifying effects of sex on the relationship between puberty and total body lean-to-fat mass ratio (LM:FM) in healthy participants (Model 2 - Height-adjusted).

Key: Model 2 analyses included sex, pubertal stage, age, quadratic age, height, weight, and sex-by-pubertal stage interaction. Dual-energy X-ray absorptiometry (DXA), males (M) and females (F).

5.3.3.2 pQCT (The proximal 66% site of the tibia)
As shown in Table 5.4 and Figure 5.6, sex did modify the relationship between puberty and pQCT body composition for muscle density and subcutaneous fat CSA. With increasing maturation (pre-to-late puberty), females showed lower apparent gains in muscle density (-2.9%, pp=0.003) but showed higher apparent gains in subcutaneous fat CSA (30.7%, p=0.005) compared to males. There were no significant sex-by-pubertal stage interactions for muscle CSA, but females had consistently lower muscle CSA (-3.8%, p=0.028) compared to males, across all stages of puberty (Table 5.4).
Figure 5.6 - The modifying effects of sex on the relationship between puberty and subcutaneous fat area and muscle density at the proximal 66% site of the tibia in healthy participants (Model 2 - Height-adjusted).

![Graphs showing subcutaneous fat area and muscle density](image)

**Key:** Model 2 analyses included sex, pubertal stage, age, quadratic age, height, weight, and sex-by-pubertal stage interaction. Peripheral quantitative computed tomography (pQCT), males (M) and females (F).

5.3.3.3 Jumping mechanography (Lower body)
As shown in Table 5.4 and Figure 5.7, sex did modify the relationship between puberty and muscle function outcomes (Pmax and Fmax). With increasing maturation (pre-to-late puberty), females showed lower apparent gains in Pmax (-16.8%, p<0.001) and Fmax (-16.2%, p=0.002) compared to males. These trends were similar in pre-to-early puberty.

Figure 5.7 – The modifying effects of sex on the relationship between puberty and muscle function (Pmax and Fmax) in healthy participants (Model 2 - Height-adjusted).

![Graphs showing muscle power and muscle force](image)

**Key:** Model 2 analyses included sex, pubertal stage, age, quadratic age, height, weight, and sex-by-pubertal stage interaction. Maximum muscle power, watts (Pmax, W) and force, newton (Fmax, N), males (M) and females (F).
Table 5.4 – Modifying effect of sex on the relationship between puberty and body composition and muscle function outcomes in healthy participants (Model 2 - Height-adjusted).

<table>
<thead>
<tr>
<th>Outcome measures</th>
<th>MODEL 2</th>
<th>( \beta (%) ) of sex or interaction</th>
<th>SE</th>
<th>p value of sex</th>
<th>Sig. interaction</th>
<th>p value of interaction</th>
<th>Sig. variables</th>
<th>R²</th>
<th>Graph Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DXA (Total body)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LM (g)</td>
<td>-9.3</td>
<td></td>
<td>1.4</td>
<td>&lt;0.001</td>
<td>-</td>
<td>-</td>
<td>Age</td>
<td>0.92</td>
<td>-</td>
</tr>
<tr>
<td>FM (g)</td>
<td>56.1</td>
<td></td>
<td>22.2</td>
<td></td>
<td>Sex*PS2</td>
<td>0.013</td>
<td>Age</td>
<td>0.45</td>
<td>NP</td>
</tr>
<tr>
<td></td>
<td>76.8</td>
<td></td>
<td>24.3</td>
<td></td>
<td>Sex*PS3</td>
<td>0.002</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LM:FM ratio</td>
<td>-55.6</td>
<td></td>
<td>22.6</td>
<td></td>
<td>Sex*PS2</td>
<td>0.015</td>
<td>Age</td>
<td>0.33</td>
<td>Figure 5.5</td>
</tr>
<tr>
<td></td>
<td>-83.2</td>
<td></td>
<td>24.8</td>
<td></td>
<td>Sex*PS3</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FFMI</td>
<td>-8.9</td>
<td></td>
<td>1.4</td>
<td>&lt;0.001</td>
<td>-</td>
<td>-</td>
<td>Age</td>
<td>0.56</td>
<td>-</td>
</tr>
<tr>
<td>FMI</td>
<td>58.6</td>
<td></td>
<td>22.3</td>
<td></td>
<td>Sex*PS2</td>
<td>0.010</td>
<td>Age &amp; Age²</td>
<td>0.30</td>
<td>NP</td>
</tr>
<tr>
<td></td>
<td>65.7</td>
<td></td>
<td>23.8</td>
<td></td>
<td>Sex*PS3</td>
<td>0.006</td>
<td></td>
<td></td>
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<tr>
<td><strong>pQCT (The proximal 66% site of the tibia)</strong></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Muscle CSA (mm²)</td>
<td>-3.8</td>
<td></td>
<td>1.7</td>
<td>0.028</td>
<td>-</td>
<td>-</td>
<td>Age</td>
<td>0.86</td>
<td>-</td>
</tr>
<tr>
<td>Muscle density (g)</td>
<td>1.2</td>
<td></td>
<td>0.9</td>
<td></td>
<td>Sex*PS2</td>
<td>0.050</td>
<td>PS3</td>
<td>0.78</td>
<td>Figure 5.6</td>
</tr>
<tr>
<td></td>
<td>-2.9</td>
<td></td>
<td>1.0</td>
<td></td>
<td>Sex*PS3</td>
<td>0.003</td>
<td>Ht &amp; Wt</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subcutaneous fat CSA (mm²)</td>
<td>15.0</td>
<td></td>
<td>9.9</td>
<td></td>
<td>Sex*PS2</td>
<td>0.132</td>
<td>Age²</td>
<td>0.71</td>
<td>Figure 5.6</td>
</tr>
<tr>
<td></td>
<td>30.7</td>
<td></td>
<td>10.8</td>
<td></td>
<td>Sex*PS3</td>
<td>0.005</td>
<td>Ht &amp; Wt</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Jumping mechanography</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pmax (W)</td>
<td>-14.1</td>
<td></td>
<td>4.8</td>
<td></td>
<td>Sex*PS2</td>
<td>0.022</td>
<td>Age &amp; Age²</td>
<td>0.91</td>
<td>Figure 5.7</td>
</tr>
<tr>
<td></td>
<td>-16.8</td>
<td></td>
<td>5.2</td>
<td></td>
<td>Sex*PS3</td>
<td>&lt;0.001</td>
<td>Ht &amp; Wt</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fmax (N)</td>
<td>-10.6</td>
<td></td>
<td>4.8</td>
<td></td>
<td>Sex*PS2</td>
<td>0.029</td>
<td>PS2 &amp; PS3</td>
<td>0.90</td>
<td>Figure 5.7</td>
</tr>
<tr>
<td></td>
<td>-16.2</td>
<td></td>
<td>5.2</td>
<td></td>
<td>Sex*PS3</td>
<td>0.002</td>
<td>Wt</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Key: Model 2 analyses included sex, age, quadratic age (age²), early puberty compared to pre-puberty (PS2), late puberty compared to pre-puberty (PS3), height (Ht) and weight (Wt). \( \beta \) coefficient (Females vs males). Dual-energy X-ray absorptiometry (DXA), peripheral quantitative computed tomography (pQCT). Outcome measures: Lean-to-fat mass ratio (LM:FM), fat-free mass index (FFMI), fat mass index (FMI), maximum muscle force (Fmax) and power (Pmax). Graph not presented in thesis (NP). Statistically significant when p<0.05 and is presented in bold and highlighted in grey. If a sex-by-pubertal stage interaction was not found, then the interaction was removed from the model and the \( \beta \) coefficient (i.e. sex) was interpreted alone. * denotes a significant interaction.
5.3.4 Summary
The following summary statements are based on height-adjusted analyses.

- Females had higher apparent gains in fat mass (FM), fat mass index (FMI) and subcutaneous fat area (CSA) but lower apparent gains in lean-to-fat mass ratio (LM:FM) compared to males as puberty proceeded Figure 5.4, Figure 5.5 and Figure 5.6).

- Females had lower apparent gains in muscle density and muscle function (i.e muscle force [Fmax] and power [Pmax]) compared to males as puberty proceeded (Figure 5.6 and Figure 5.7).

- Females had consistently lower lean mass (LM), fat free mass index (FFMI), and muscle area (CSA) compared to male across all stages of puberty (Table 5.4).
The influence of sex on the relationship between puberty and the muscle-bone unit outcomes in healthy participants

**Aims**

The aims were to investigate whether, after accounting for height, weight and pubertal stage, sex modified the relationships between puberty and muscle function on:

1) DXA bone outcomes (i.e. Total body less head bone mineral content [TBLH BMC], size adjusted BMC [TBLH SA-BMC], and bone area [TBLH BA]).

2) pQCT bone outcomes (i.e. vBMD, geometry, and SSI) outcomes at the distal 4% and proximal 66% sites of the tibia.

3) HR-pQCT bone outcomes (i.e. vBMD, geometry, and microarchitecture) at the distal 8% site of the distal tibia.

**Hypotheses**

The hypotheses were that sex, after accounting for height, weight and pubertal stage, would:

1) Modify the relationship between puberty and bone outcomes, where females have lower apparent gains in bone area but have higher apparent gains in bone density, as puberty proceeds.

2) Modify the relationship between muscle function (i.e. Pmax and Fmax) and bone outcomes. With increasing muscle force and power, females would have lower apparent gains in bone area and greater increases in BMC compared to males.

**5.4.1 Analyses**

Bone and muscle measurements were assessed for normality and transformed into natural logs. Primary outcomes for bone were measured using DXA at the total body less head site (TBLH BMC, TBLH size adjusted BMC [TBLH SA-BMC] and TBLH BA), pQCT at the 4% and 66% sites of the tibia (total bone CSA, total vBMD, cortical CSA, cortical vBMD, trabecular vBMD and SSI), and HR-pQCT at the 8% site of the distal tibia (Tt.Ar, Ct.Ar, D100, Ct.BMD, Ct.TMD, Ct.Po, BV/TV, Tb.N and Tb.Th). Muscle function was measured using jumping mechanography of the lower limbs (Pmax and Fmax). Analyses were performed using Data Desk® (Version 6.3.1) and Rstudio® (Version 3.3.1) statistical package. In summary, the analysis models were: **Model 1** (sex, pubertal stage, and sex-by-pubertal stage interaction) using ANCOVA and Scheffé post hoc analyses to test whether sex modifies the relationship between puberty and bone outcomes, **Model 2** (sex, pubertal stage, age, quadratic age, height and weight, and sex-by-pubertal stage interaction) using multiple linear regression to test whether sex modifies the relationship between puberty and bone outcomes, after adjusting for height, and finally, **Model 3** (sex, pubertal stage, age, quadratic age, height and weight, Pmax, Fmax,
sex-by-pubertal stage interaction, sex-by-Pmax interaction, and sex-by-Fmax interaction) using multiple linear regression to test whether sex modifies the relationship between muscle function and bone outcomes.

To assess TBLH SA-BMC, TBLH BA was retained in the model for TBLH BMC to adjust for body size. In models 2 and 3, height was retained in the model analyses for all measurements to adjust for differences in body size during growth. Data are presented as mean, standard deviation, beta-coefficient (%) and p-value, with the significance level set to p<0.05. Interaction plots were used to help to interpret statistically significant interactions (p<0.05).

5.4.2  The muscle-bone unit - Total body using DXA

5.4.2.1  The results of Model 1 analyses

The modifying effects of sex on the relationship between puberty and DXA bone outcomes after Model 1 analyses (i.e. sex, pubertal stage, and sex-by-pubertal stage interaction) are presented in Table 5.5.

As shown in Table 5.5, sex did modify the relationship between puberty and DXA bone outcomes. With increasing maturation, females showed lower apparent gains in TBLH BMC (p<0.0001) and TBLH BA (p=0.0143) compared to males. Post hoc comparisons showed that females had lower TBLH BMC and TBLH BA in late puberty compared to males.

5.4.2.2  The results of Models 2 (Height-adjusted) and 3 (Sex*Muscle function interactions)

The modifying effects of sex on the relationships between 1) puberty and DXA bone outcomes after Model 2 (i.e. sex, pubertal stage, age, quadratic age, height, weight, and sex-by-pubertal stage interaction) and 2) muscle function and DXA bone outcomes after Model 3 (i.e. sex, age, quadratic age, height, weight, pubertal stage, Pmax, Fmax, sex-by-pubertal stage interaction, sex-by-Pmax interaction, and sex-by-Fmax interaction) analyses are presented in Table 5.6.

As shown in Table 5.6 – Model 2 - Sex did not modify the relationship between puberty and DXA bone outcomes for TBLH BA, BMC, and SA-BMC. There were no sex differences in TBLH BA (-0.64%, p=0.488), BMC (-1.40%, p=404), and SA-BMC (-0.53%, p=0.493) across all stages of puberty.

As shown in Table 5.6 – Model 3, sex did modify the relationship between Fmax and TBLH BMC and BA. With increasing muscle Fmax, females had higher apparent gains in TBLH BMC (13.4%, p=0.012) and in BA (7.8%, p=0.011) compared to males. There were no significant sex-by-Fmax interactions for TBLH SA-BMC and no significant sex-by-Pmax interactions for TBLH BMC, SA-BMC or BA. There was no sex difference in SA-BMC (1.1%, p=0.132) across all stages of puberty.
Table 5.5 - Modifying effect of sex on the relationship between puberty and DXA bone outcomes in healthy participants (Model 1).

<table>
<thead>
<tr>
<th>Outcome measures</th>
<th>Healthy participants (n = 151)</th>
<th>Model 1 Sex*PS interaction p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males</td>
<td>Females</td>
</tr>
<tr>
<td></td>
<td>Pre (n=12)</td>
<td>Early (n=45)</td>
</tr>
<tr>
<td>Total body (less head)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Puberty (n)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TBLH BMC (g/cm^2)</td>
<td>807.9 (±207.2)</td>
<td>1262.8 (±487.8)</td>
</tr>
<tr>
<td>TBLH bone area (cm^2)</td>
<td>1060.9 (±199.9)</td>
<td>1426.9 (±355.1)</td>
</tr>
</tbody>
</table>

Key: Model 1 analyses included sex, pubertal stage, and sex-by-pubertal stage interaction. All values are mean (SD) unless indicated. Letters are used to indicate significance of sex and pubertal stage differences as tested by ANCOVA/Scheffé. Puberty was based on Tanner staging (TS) and was categorised into three groups: pre-puberty = TS 1, early puberty = TS 2 and TS 3, and late puberty = TS 4 and TS 5. (SDS) Standard deviation score from the mean height and weight of an age-and-sex matched control population. Total body less head bone mineral content (TBLH BMC). Statistically significant when p<0.05 and is presented in bold and highlighted in grey.

^a Significant difference between healthy pre-pubertal males and females, P<0.05,
^b Significant difference between healthy early pubertal males and females, P<0.05,
^c Significant difference between healthy late pubertal males and females, P<0.05.
Table 5.6 - Summarising the modifying effect of sex on the relationship between puberty and DXA bone outcomes in healthy participants (Model 2 - Height-adjusted and Model 3 - Sex*Muscle function interactions).

<table>
<thead>
<tr>
<th>Outcome measures</th>
<th>MODEL 2</th>
<th></th>
<th></th>
<th></th>
<th>MODEL 3</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(\beta) (%) of sex or interaction</td>
<td>SE</td>
<td>(p) value</td>
<td>Sig. interaction</td>
<td>(p) value</td>
<td>Sig. variables</td>
<td>(R^2)</td>
<td>Graph Ref</td>
<td>(\beta) (%) of sex or interaction</td>
</tr>
<tr>
<td>TBLH BMC (g/cm(^2))</td>
<td>-1.40</td>
<td>1.7</td>
<td>0.404</td>
<td></td>
<td>Age Ht &amp; Wt</td>
<td>0.94</td>
<td></td>
<td></td>
<td>13.4</td>
</tr>
<tr>
<td>TBLH SA-BMC (g/cm(^2))</td>
<td>-0.53</td>
<td>0.8</td>
<td>0.493</td>
<td></td>
<td>Age Ht BA</td>
<td>0.99</td>
<td></td>
<td></td>
<td>1.1</td>
</tr>
<tr>
<td>TBLH BA (cm(^2))</td>
<td>-0.64</td>
<td>0.9</td>
<td>0.488</td>
<td></td>
<td>Age Ht &amp; Wt</td>
<td>0.96</td>
<td></td>
<td></td>
<td>7.8</td>
</tr>
</tbody>
</table>

Key: \(\beta\) coefficient (Females vs males). Dual-energy X-ray absorptiometry (DXA) outcome measures: Total body less head size adjusted bone mineral content (TBLH SA-BMC) = BMC adjusted for bone area (TBLH BA). Model 2 analyses included sex, age, quadratic age (age\(^2\)), early puberty compared to pre-puberty (PS2), late puberty compared to pre-puberty (PS3), height (Ht) and weight (Wt), and Model 3 included model 2 covariates, muscle force (Fmax), and muscle power (Pmax). Statistically significant when \(p<0.05\) and is presented in bold and highlighted in grey. If a sex-by-pubertal stage or sex-by-Fmax/Pmax interaction was not found, then the interaction was removed from the model and the \(\beta\) coefficient (i.e. sex, pmax and Fmax) was interpreted alone.
5.4.3 The muscle-bone unit - The distal 4% and proximal 66% sites of the tibia using pQCT.

5.4.3.1 The results of Model 1 analyses

The modifying effects of sex on the relationship between puberty and pQCT bone outcomes after Model 1 analyses (i.e. sex, pubertal stage, and sex-by-pubertal stage interaction) are presented in Table 5.7.

Table 5.7 - Modifying effect of sex on the relationship between puberty and pQCT bone outcomes (Model 1).

<table>
<thead>
<tr>
<th>Bone outcome measures</th>
<th>Healthy participants (n = 151)</th>
<th>Model 1 Sex*PS interaction p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males (n)</td>
<td>Females (n)</td>
</tr>
<tr>
<td>Puberty (n)</td>
<td>Pre (n=12)</td>
<td>Early (n=45)</td>
</tr>
<tr>
<td>pQCT (Distal 4% site of the tibia)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total CSA (mm²)</td>
<td>557.3 (±87.7)</td>
<td>742.5 (±159.9)</td>
</tr>
<tr>
<td>Total vBMD (mg/cm³)</td>
<td>308.8 (±26.3)</td>
<td>309.7 (±36.2)</td>
</tr>
<tr>
<td>Trab vBMD (mg/cm³)</td>
<td>206.6 (±17.8)</td>
<td>206.9 (±26.6)</td>
</tr>
</tbody>
</table>

| pQCT (Proximal 66% site of the tibia) | | | | |
| Total CSA (mm²)       | 368.5 (±59.3) | 510.8 (±139.3) | 723.7 (±79.8) | 397.0 (±76.2) | 487.5 (±94.3) | 562.4 (±90.1) | 0.0002<sup>c</sup> |
| Cortical CSA (mm²)    | 164.4 (±24.1) | 224.6 (±60.3) | 311.9 (±36.7) | 173.8 (±35.1) | 219.2 (±34.6) | 260.5 (±31.6) | 0.0005<sup>c</sup> |
| Cortical vBMD (mg/cm³)| 1015.2 (±31.1) | 1022.3 (±21.3) | 1052.0 (±42.5) | 1012.4 (±35.8) | 1045.1 (±29.0) | 1098.0 (±29.6) | 0.0030<sup>a,c</sup> |
| SSI (mm⁴)             | 1023.2 (±198.4) | 1618.2 (±622.4) | 2480.2 (±308.1) | 1148.0 (±322.8) | 1522.7 (±352.5) | 1890.7 (±322.0) | 0.0010<sup>c</sup> |

Key: Model 1 analyses included sex, pubertal stage, and sex-by-pubertal stage interaction. All values are mean (SD) unless indicated. Letters are used to indicate significance of sex and pubertal stage differences as tested by ANCOVA/Scheffé. Puberty was based on Tanner staging (TS) and was categorised into three groups: pre-puberty = TS 1, early puberty = TS 2 and TS 3, and late puberty = TS 4 and TS 5. (SDS) Standard deviation score from the mean height and weight of an age- and sex-matched control population. Cross-sectional area (CSA), volumetric bone mineral density (vBMD) and stress-strain index (SSI). Statistically significant when p<0.05 and is presented in bold and highlighted in grey.

a Significant difference between healthy pre-pubertal males and females, P<0.05.
b Significant difference between healthy early pubertal males and females, P<0.05.
c Significant difference between healthy late pubertal males and females, P<0.05.
pQCT - The distal 4% site of the tibia

As shown in Table 5.7, sex did modify the relationship between puberty and total CSA at the 4% site. With increasing maturation, females showed lower apparent gains in total CSA (p=0.0008) compared to males. There were no significant sex-by-puberty interactions for total vBMD (p=0.2440) or trabecular vBMD (p=0.8240). Post hoc comparisons showed that females had lower total CSA in late puberty compared to males.

pQCT - The proximal 66% site of the tibia

As shown in Table 5.7, sex did modify the relationship between puberty and pQCT bone outcomes at the 66% site. With increasing maturation, females showed lower apparent gains in total CSA (p=0.0002), cortical CSA (p=0.0005), and SSI (p=0.0010) but showed higher apparent gains in cortical vBMD (p=0.0030) compared to males. Post hoc comparisons showed that females had lower total CSA, cortical CSA, and SSI in late puberty and higher cortical vBMD in early and late puberty compared to males.

5.4.3.2 The results for Models 2 (height-adjusted) and 3 (sex*muscle function interactions)
The modifying effects of sex on the relationships between 1) puberty and pQCT bone outcomes after Model 2 (i.e. sex, pubertal stage, age, quadratic age, height, weight, and sex-by-pubertal stage interaction), and 2) muscle function and pQCT bone outcomes after Model 3 (i.e. sex, age, quadratic age, height, weight, pubertal stage, Pmax, Fmax, sex-by-pubertal stage interaction, sex-by-Pmax interaction, and sex-by-Fmax interaction) analyses are presented in Table 5.8.

pQCT - The distal 4% site of the tibia

As shown in Table 5.8 – Model 2, sex did not modify the relationship between puberty and pQCT bone outcomes at the 4% site. Females had consistently lower total CSA (-5.3%, p=0.005) and total vBMD (-4.8%, p=0.009) compared to males across all stages of puberty. No sex difference was detected for trabecular vBMD (-3.2%, p=0.126), across all stages of puberty.

As shown in Table 5.8 – Model 3, sex did modify the relationship between muscle function and pQCT bone outcomes for total vBMD and trabecular vBMD. With increasing Fmax, females showed higher apparent gains in total vBMD (12.6%, p=0.035) and trabecular vBMD (16.5%, p=0.015) compared to males (Figure 5.8). There were no significant sex-by-Fmax interactions for total CSA and no significant sex-by-Pmax interactions for total CSA, total vBMD or trabecular vBMD outcomes. Females had consistently lower total CSA across all stages of puberty, after adjusting for muscle.
Figure 5.8 - The modifying effects of sex on the relationship between Fmax and total and trabecular vBMD outcomes at the distal 4% of the tibia in healthy participants (Model 3 - Sex*Muscle function interactions).

Key: Model 3 analyses included sex, age, quadratic age, puberty, height, weight, Fmax, Pmax, and sex-by-pubertal stage interaction. Peripheral quantitative computed tomography (pQCT), volumetric BMD (vBMD, mg/cm\(^3\)), muscle force, Newtons (Fmax, N), males (M) and females (F). Fmax: Lower quantile = 0.59 – 1.29 N and upper quantile = 1.29 – 2.65 N.
Table 5.8 - Summarising the modifying effect of sex on the relationship between puberty and pQCT bone outcomes in healthy participants (Model 2 - height-adjusted and Model 3 - Sex*Muscle function interactions).

<table>
<thead>
<tr>
<th>Outcome measures</th>
<th>MODEL 2</th>
<th>MODEL 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β (%) of sex</td>
<td>SE</td>
</tr>
<tr>
<td>The distal 4% site of the tibia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total CSA (mm²)</td>
<td>-5.3</td>
<td>1.9</td>
</tr>
<tr>
<td>Total vBMD (mg/cm³)</td>
<td>-4.8</td>
<td>1.8</td>
</tr>
<tr>
<td>Trab vBMD (mg/cm³)</td>
<td>-3.2</td>
<td>2.1</td>
</tr>
<tr>
<td>The proximal 66% site of the tibia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total CSA (mm²)</td>
<td>-16.1</td>
<td>5.2</td>
</tr>
<tr>
<td>Cortical CSA (mm²)</td>
<td>-6.2</td>
<td>1.8</td>
</tr>
<tr>
<td>Cortical vBMD (mg/cm³)</td>
<td>2.6</td>
<td>5.4</td>
</tr>
<tr>
<td>SSI (mm⁴)</td>
<td>-15.7</td>
<td>7.0</td>
</tr>
</tbody>
</table>

Key: β coefficient (Females vs males). Peripheral quantitative computed tomography (pQCT) outcome measures: Cross-sectional area (CSA), volumetric bone mineral density (vBMD) and stress-strain index (SSI). Graph not presented in thesis (NP). Model 2 analyses included sex, age, quadratic age (age²), early puberty compared to pre-puberty (PS2), late puberty compared to pre-puberty (PS3), height (Ht) and weight (Wt), and Model 3 included model 2 covariates, muscle force (Fmax), and muscle power (Pmax). Statistical significant when p<0.05 and is presented in bold and highlighted in grey. If a sex-by-pubertal stage or sex-by-Fmax/Pmax interaction was not found, then the interaction was removed from the model and the β coefficient (i.e. sex, pmax and Fmax) was interpreted alone.
**pQCT - The proximal 66% tibia site**

As shown in Table 5.8 – Model 2, sex did modify the relationship between puberty and pQCT bone outcomes at the 66% site. With increasing maturation, females showed lower apparent gains in total CSA (-16.1%, p=0.003) in pre-to-early puberty and similar trends in pre-to-late puberty (-19.0%, p=0.001) but higher apparent gains in cortical vBMD in pre-to-early puberty (2.6%, p=0.021) and similar trends in pre-to-late puberty (5.4%, p<0.001) compared to males (Figure 5.9). With increasing maturation, females showed lower apparent gains in SSI in pre-to-early puberty (-15.7%, p=0.026) but there was no significant interaction in pre-to-late puberty (-13.8%, p=0.733). There was no significant sex-by-puberty interaction for cortical CSA but females had consistently lower cortical CSA (-6.2%, p=0.001) compared to males, across all stages of puberty.

As shown in Table 5.8 – Model 3, sex did modify the relationship between Pmax and cortical vBMD. With increasing levels of Pmax, females showed higher apparent gains in cortical vBMD (3.1%, p=0.055 – borderline significant) compared to males (Figure 5.10). There were no significant interactions for sex-by-Pmax for total CSA, cortical CSA or SSI and no significant interactions for sex-by-Fmax for total CSA, cortical CSA, cortical vBMD, or SSI. Females had consistently lower SSI (-6.1%, p=0.013) compared to males, across all stages of puberty (Table 5.8). There were no significant differences in cortical area (-2.7%, p=0.108), across all stages of puberty.

**Figure 5.9** - The modifying effects of sex on the relationship between puberty and total CSA and cortical vBMD at the proximal 66% site of the tibia in healthy participants (Model 2 - Height-adjusted).

---

**Key:** Model 2 analyses included sex, age, quadratic age, puberty, height, weight, and sex-by-pubertal stage interaction. Peripheral quantitative computed tomography (pQCT), cross-sectional area (CSA, cm²), volumetric BMD (vBMD, mg/cm³), males (M) and females (F).
Figure 5.10 - The modifying effects of sex on the relationship between Pmax and cortical vBMD at the proximal 66% site of the tibia in healthy participants (Model 3 - Sex*Muscle function interactions).

5.4.4 The muscle-bone unit - The 8% site of the tibia using HR-pQCT

5.4.4.1 The results of model 1 analyses

The effects of sex on the relationship between puberty and HR-pQCT bone outcomes after Model 1 analyses (i.e. sex, pubertal stage, and sex-by-pubertal stage interaction) are presented in Table 5.9.

As shown in Table 5.9, sex did modify the relationship between puberty and HR-pQCT bone outcomes. With increasing maturation, females showed lower apparent gains in Tt.Ar (p=0.0008), Ct.Ar (p=0.0334), and Ct.Po (p<0.0001) outcomes but showed higher apparent gains in Ct.BMD (p=0.009) and Ct.TMD (p=0.0062) compared to males. There were no significant sex-by-pubertal stage interactions for D100, BV/TV, Tb.N, or Tb.Th, across all stages of puberty. Post hoc comparisons showed that females had lower Tt.Ar in late puberty compared to males. Females had lower Ct.Ar and Ct.Po but higher Ct.BMD and Ct.TMD in early and late puberty compared to males.

**Key:** Model 3 analyses included sex, age, quadratic age, puberty, height, weight, Fmax, Pmax, sex-by-pubertal stage interaction, sex-by-Pmax interaction, and sex-by-Fmax interaction. Peripheral quantitative computed tomography (pQCT), volumetric BMD (vBMD, mg/cm^3), muscle power (Pmax), males (M) and females (F). Pmax: Lower quantile = 0.75 – 1.76 W and upper quantile = 1.76 – 5.04 W.
Table 5.9 - Modifying effect of sex on the relationship between puberty and HR-pQCT bone outcomes in healthy participants (Model 1).

<table>
<thead>
<tr>
<th>Bone outcome measures</th>
<th>Healthy participants (n = 151)</th>
<th>Model 1 Sex*PS interaction p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males</td>
<td>Females</td>
</tr>
<tr>
<td></td>
<td>Pre (n=12)</td>
<td>Early (n=45)</td>
</tr>
<tr>
<td>Puberty (n)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Area</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tt.Ar (cm²)</td>
<td>431.0 (±76.3)</td>
<td>575.9 (±123.8)</td>
</tr>
<tr>
<td>Ct.Ar (cm²)</td>
<td>75.7 (±10.6)</td>
<td>99.7 (±24.6)</td>
</tr>
<tr>
<td>Density</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D100 (mg/cm³)</td>
<td>268.7 (±29.5)</td>
<td>271.7 (±41.7)</td>
</tr>
<tr>
<td>BV/TV (mg/cm³)</td>
<td>0.15 (±0.02)</td>
<td>0.15 (±0.02)</td>
</tr>
<tr>
<td>Ct.BMD (mg/cm³)</td>
<td>755.8 (±33.0)</td>
<td>758.9 (±37.8)</td>
</tr>
<tr>
<td>Ct.TMD (mg/cm³)</td>
<td>818.4 (±20.8)</td>
<td>823.4 (±35.3)</td>
</tr>
<tr>
<td>Ct.Po (%)</td>
<td>49.2 (±18.6)</td>
<td>66.7 (±26.6)</td>
</tr>
<tr>
<td>Micro-architecture</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tb.N (mm-1)</td>
<td>2.17 (±0.32)</td>
<td>2.21 (±0.27)</td>
</tr>
<tr>
<td>Tb.Th (mm)</td>
<td>0.07 (±0.01)</td>
<td>0.07 (±0.01)</td>
</tr>
</tbody>
</table>

**Key:** Data adjusted for Model 1 (sex, pubertal stage, and sex-by-pubertal stage interaction). All values are mean (SD) unless indicated. Letters are used to indicate significance of sex and pubertal stage differences as tested by ANCOVA /Scheffé. Puberty was based on Tanner staging (TS) and was categorised into three groups: pre-puberty = TS 1, early puberty = TS 2 and TS 3, and late puberty = TS 4 and TS 5. (SDS) Standard deviation score from the mean height and weight of an age-and-sex matched control population. Total area (Tt.Ar), cortical area (Ct.Ar), total bone mineral density (D100), cortical bone mineral density (Ct.BMD), cortical tissue mineral density (Ct.TMD), cortical porosity (Ct.Po), and trabecular number (Tb.N) and thickness (Tb.Th). Statistically significant when p<0.05 and is presented in bold and highlighted in grey.

a Significant difference between healthy pre-pubertal males and females, P<0.05.
b Significant difference between healthy early pubertal males and females, P<0.05.
c Significant difference between healthy late pubertal males and females, P<0.05.
5.4.4.2  The results of Models 2 (i.e. Height-adjusted) and 3 (i.e. Sex*Muscle function interactions) 
The effects of sex on the relationship between puberty and HR-pQCT bone outcomes after Model 2 
(i.e. sex, pubertal stage, age, quadratic age, height, weight, and sex-by-pubertal stage interaction) 
and Model 3 (i.e. sex, age, quadratic age, height, weight, pubertal stage, Pmax, Fmax, sex-by- 
pubertal stage interaction, sex-by-Pmax interaction, and sex-by-Fmax interaction) analyses are 
presented in Table 5.10, Figure 5.11, and Figure 5.12.

As shown in Table 5.10 and Figure 5.11 – Model 2, sex did modify the relationship between puberty 
and HR-pQCT bone outcomes. With increasing maturation, females showed lower apparent gains in 
Tt.Ar (-13.9%, p=0.032) and higher apparent gains in Ct.TMD (7.3%, p<0.001) in pre-to-late puberty 
compared to males but no significant interactions in pre-to-early puberty (Figure 5.11 A and D). 
Females showed lower apparent gains in Ct.Ar (-15.2%, p=0.015) in pre-to-early puberty but no 
significant interaction in pre-to-late puberty (-9.8%, p=0.146) compared to males (Figure 5.11 B). 
Females had higher apparent gains in Ct.BMD from pre-to-early puberty (4.3%, p=0.040) and a 
similar trend from pre-to-late puberty (10.4%, p<0.001) compared to males (Figure 5.11 C). 
Females had lower apparent gains in Ct.Po from pre-to-early puberty (-4.4%, p=0.012) and a similar trend 
from pre-to-late puberty (-7.3%, p<0.001) compared to males (Figure 5.11 E). There were no 
significant sex-by-pubertal stage interactions for D100, BV/TV, Tb.N, or Tb.Th. Females had 
consistently lower BV/TV (-7.0%, p=0.003) and Tb.N (-4.0%, p=0.034) compared to males, across all 
stages of puberty. There were no sex differences for D100 (-1.9%, P=0.420) or Tb.Th (-3.1%, p=0.143), 
across all stages of puberty.

As shown in Table 5.10 – Model 3, sex did modify the relationship between Pmax and HR-pQCT 
outcomes. With increasing levels of Pmax, females had higher apparent gains in D100 (17.2%, 
P=0.005), Ct.BMD (10.8%, P<0.001), Ct.TMD (5.2%, P=0.022), and Tb.Th (16.9%, p=0.002) but lower 
apparent gains in Ct.Po (-5.6%, p<0.001) and Tb.N (-12.3, p=0.012) compared to males (Figure 5.12A-
F). There were no significant sex-by-Pmax interactions for Tt.Ar, Ct.Ar, or BV/TV and no significant 
sex-by-Fmax interactions for any HR-pQCT bone outcomes. Females had consistently lower BV/TV (- 
5.5%, p=0.012) and Ct.A (-7.9%, p=0.001) compared to males (after adjusting for muscle), across all 
stages of puberty.

Table 5.10 - Footnote 
Key: β coefficient (Females vs males). High-resolution peripheral quantitative computed tomography (HR-pQCT) outcome measures: Total and cortical area (Tt.Ar and Ct.Ar.), total and trabecular vBMD (D100 and BV/TV), cortical vBMD and tissue mineral density (Ct.BMD and Ct.TMD), cortical porosity (Ct.Po) and trabecular number and thickness (Tb.N and Tb.Th). Model 2: Adjusted for explanatory variables including sex, age, quadratic age (age²), early puberty compared to pre-puberty (PS2), late puberty compared to pre-puberty (PS3), height (Ht) and weight (Wt). Model 3: Adjusted for model 2 and muscle force, Newtons (Fmax, N) and muscle power, Watts (Fmax, W). Graph not presented in thesis (NP). Statistically significant when p<0.05 and is presented in bold and highlighted in grey. If a sex-by-pubertal stage or sex-by-Fmax/Pmax interaction was not found, then the interaction was removed from the model and the β coefficient (i.e. sex, pmax and Fmax) was interpreted alone.
Table 5.10 - Summarising the modifying effect of sex on the relationship between puberty and HR-pQCT bone outcomes at the distal 8% site of the tibia in healthy participants (Model 2 - Height-adjusted and Model 3 - Sex*Muscle function interactions).

| Outcome measures | MODEL 2 | | | MODEL 3 | | |
|------------------|---------|---------|---------|---------|---------|---------|---------|
|                  | β (% of sex or interaction) | SE | p value for sex | Sig. interaction | p value for interaction | Sig. variables | R² | Graph Ref | β (% of sex or interaction) | SE | p value for sex | Sig. interaction | p value for interaction | Sig. variables | R² | Graph Ref |
| **Area**         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |
| Tt.Ar (cm²)      | -6.4    | 6.0     |         | Sex*PS2 | 0.286   | Age & Age² | 0.69   | Figure 5.11A | -8.7    | 5.9     |         | Sex*PS2 | 0.143   | Age & Age² | 0.032   |         |         |         |         |         |         |         |         |         |         |         |         |         |
| Ct.Ar (cm²)      | -15.2   | 6.2     |         | Sex*PS2 | 0.015   | Age       | 0.77   | Figure 5.11B | -7.9    | 2.1     | 0.001   |         |         |         |         |         |         |         |         |         |         |         |         |         |
| **Density**      |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |
| D100 (mg/cm³)   | -1.9    | 2.4     | 0.420   |         |         | Age & Age² | 0.41   |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |
| BV/TV (mg/cm³)  | -7.0    | 2.3     | 0.003   |         |         | Wt       | 0.09   |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |
| Cl.BMD (mg/cm³) | 4.3     | 10.4    | 2.1     | Sex*PS2 | 0.040   | Age & Age² | 0.75   | Figure 5.11C | 10.8    | 2.1     |         | Sex*Pmax | <0.001  | Age & Age² | 0.76    | Figure 5.12B |         |         |         |         |         |         |         |         |         |         |         |         |         |         |
| Cl.TMD (mg/cm³) | 1.9     | 7.3     | 1.7     | Sex*PS2 | 0.261   | Age & Age² | 0.78   | Figure 5.11D | 5.2     | 2.2     |         | Sex*Pmax | 0.022   | Age & Age² | 0.79    | Figure 5.12C |         |         |         |         |         |         |         |         |         |         |         |         |         |         |
| Cl.Po (%)       | -4.4    | 7.3     | 1.7     | Sex*PS2 | 0.012   | Wt       | 0.46   | Figure 5.11E | -5.6    | 1.5     |         | Sex*Pmax | <0.001  | Age       | 0.53    | Figure 5.12D |         |         |         |         |         |         |         |         |         |         |         |         |         |         |
| **Micro-architecture** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Tb.N (mm-1)     | -4.0    | 1.9     | 0.034   |         |         | Age       | 0.16   |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |
| Tb.Th (mm)      | -3.1    | 2.1     | 0.143   |         |         | Age       | 0.09   |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |

Notes: Table 5.10 summarises the modifying effect of sex on the relationship between puberty and HR-pQCT bone outcomes at the distal 8% site of the tibia in healthy participants. Models 2 and 3 adjust for height and sex*muscle function interactions, respectively. Significant p-values are highlighted in bold.
Figure 5.11 – The modifying effects of sex on the relationship between puberty and HR-pQCT bone outcomes at the distal 8% site of the tibia in healthy participants (Model 2 - Height-adjusted).

Key: Model 2 analyses included sex, age, quadratic age, puberty, height, weight, Fmax, Pmax, and sex-by-pubertal stage interaction. High-resolution peripheral quantitative computed tomography (HR-pQCT), total area (Tt.Ar), cortical area (Ct.Ar), cortical volumetric bone mineral density (Ct.BMD), cortical volumetric tissue mineral density (Ct.TMD), cortical porosity (Ct.Po), males (M) and females (F).
Figure 5.12 - The modifying effects of sex on the relationship between Pmax and HR-pQCT bone measures at the 8% site of the tibia in healthy participants (Model 3 - Sex*Muscle function interactions).

Key: Model 3 analyses included sex, age, quadratic age, puberty, height, weight, Fmax, Pmax, and sex-by-pubertal stage interaction. High-resolution peripheral quantitative computed tomography (HR-pQCT), maximum muscle power, watts (Pmax, W), total bone mineral density, cortical volumetric bone mineral density (Ct.BMD), cortical volumetric tissue mineral density (Ct.TMD), cortical porosity (Ct.Po), trabecular number (Tb.N), trabecular thickness (Tb.Th), males (M) and females (F). Pmax: Lower quantile = 0.75 – 1.76 W and upper quantile = 1.76 – 5.04 W.
5.4.5 Summary

The following summary statements are based on height-adjusted analyses.

**DXA**
- As puberty proceeded, sex did not modify the relationships between puberty and bone area (TBLH-BA), bone mineral content (TBLH-BMC), or size-adjusted bone mineral content (SA-BMC) (*Table 5.6*).

- Compared to males, females showed higher apparent gains in bone mineral content (TBLH-BMC) and in bone area (TBLH-BA) with increasing muscle force (Fmax) but there was no sex difference in the relationship between muscle force (Fmax) and size-adjusted bone mineral content (SA-BMC). There were no sex difference in the relationships between muscle power (Pmax) and any DXA bone outcomes (*Table 5.6*).

**pQCT**
- At the distal 4% site of the tibia, sex did not modify the relationships between puberty and bone outcomes. However, females had consistently lower total bone area (total CSA) and total bone density (total vBMD), across all stages of puberty (*Table 5.8*). There was no sex difference in trabecular bone density (Trab vBMD), across all stages of puberty.

- At the proximal 66% site of the tibia, with increasing pubertal maturation, females had lower apparent gains in total area (total CSA) but higher apparent gains in cortical density (cortical vBMD) compared to males (*Figure 5.9*). Females had lower apparent gains in bone strength (SSI) in pre-to-early puberty but not in pre-to-late puberty compared to males (*Table 5.8*). Females had consistently lower cortical area compared to males, across all stages of puberty (*Table 5.8*).

- At the distal (4%) site of the tibia, females showed higher apparent gains in total density (Total vBMD) and trabecular density (Trab vBMD) with increasing muscle force (Fmax) and consistently lower total area (CSA) compared to males, across all stages of puberty (*Figure 5.8 and Table 5.8*).

- At the proximal (66%) site, females had higher apparent gains in cortical density (Cortical vBMD) with increasing levels of muscle power (Pmax) (*Figure 5.10*), but had consistently lower bone strength (SSI) compared to males, across all stages of puberty (*Table 5.8*).
HR-pQCT

- At the distal 8% site, with increasing pubertal maturation, females had lower apparent gains in total area (Tt.Ar), higher apparent gains in cortical density (Ct.BMD and Ct.TMD), and lower apparent gains in cortical porosity (Ct.Po) compared to males (Figure 5.11). Females had consistently lower trabecular density (BV/TV) and number (Tb.N) compared to males, across all stages of puberty (Table 5.10).

- With increasing muscle power (Pmax), females had higher apparent gains in total density (D100), cortical density (Ct.BMD and Ct.TMD), and trabecular thickness (Tb.Th) but lower apparent gains in cortical porosity (Ct.Po) and trabecular number (Tb.N) compared to males (Table 5.10 and Figure 5.12A-F).

5.5 Discussion

The work described in this chapter has sought to gain insights into how sex modifies the relationships between puberty and bone, as well as the relationship between muscle and bone (i.e. the muscle-bone unit) in healthy participants. This will help to broaden our knowledge and understanding of how sex modifies bone strength and fracture risk. It also sought to explore differences in anthropometry, body composition and muscle function outcomes. The study was designed to test several key hypotheses that were developed after scrutinising the existing literature on bone mineral, geometry, micro-architecture, muscle mass, and muscle function in children and adolescents.

5.5.1 Modifying effects of sex on the relationship between puberty and anthropometric outcomes in healthy participants

Childhood and adolescence are dynamic periods of growth and development marked by rapid changes in body size, shape, and body composition. During infancy, growth velocity is slightly slower in females compared to males; therefore, males have a longer total body length and are heavier compared to females (Ireland et al., 2014, Tanner, 1989). During pre-puberty, growth velocity and weight gain of both sexes increases at similar rates, with an average rate of 5-6 cm/year for height and 2.5 kg/year for weight (Tanner, 1989). In this study, post-hoc analysis of the sex-by-puberty interaction showed that there were no sex differences in pre-pubertal children aged approximately 8-11 years for height, weight, BMI, or sitting height (Table 5.2).

The onset of puberty is associated with the release of sex hormones; oestrogen in females and testosterone in males, which starts the several sequential stages of puberty (adrenarche, pubarche, and thelarche [only in females]) within each sex (Marshall and Tanner, 1969, Marshall and Tanner,
Females start and finish puberty before males and have a shorter pubertal growth window compared to males (i.e. in females = age 10-14 years and in males = age 11-18 years), resulting in adult males having a larger skeleton compared to adult females (Baxter-Jones et al., 2011, Theintz et al., 1992). In this study, sex did modify the relationship between puberty and anthropometric measurements, where females were taller and heavier compared to males in early puberty but this was reversed in late puberty, as females were shorter compared to males (Table 5.2 and Figure 5.3). Sitting height was significantly lower in females in late puberty compared to males, which is a consequence of having a shorter growth period and therefore females are shorter in adulthood (Table 5.2 and Figure 5.4). Male participants were still continuing to pass through puberty at age 16 years, whereas most females had finished puberty by age 12-13 years, which may explain why differences in weight and BMI in late puberty were not found in this study (Table 5.2). It is likely that these male participants would continue to grow until the age of ~18 years and the sex differences in anthropometric measurements would be greater compared to the findings in this study (i.e. post pubertal males would be taller and heavier than post pubertal females). In this study, early pubertal females had higher height SDS (Females = 0.82 ± 1.0 vs. Males = 0.28 ± 1.1) and weight SDS (Females = 0.75 ± 1.1 vs. Males = 0.30 ± 0.9) compared to males (Table 5.2), which is consistent with the phenomenon that the pubertal growth spurt occurs at an earlier stage of puberty in girls than in boys.

5.5.2 Modifying effects of sex on the relationship between puberty and body composition and muscle function outcomes in healthy participants

Sex differences in body composition during infancy and childhood are modest compared with post pubertal differences. Females have been shown to have a greater percentage body fat (1–3%) compared to males during infancy and childhood (Butte et al., 2000). Changes in adiposity and muscle mass become more striking due to the release of sex hormones. Females gain more fat, especially on the hips and thighs (gynoid fat distribution), and have lower muscle mass and muscle strength compared to males. In contrast, males tend to be more muscular and store fat in the abdominal and chest areas (android fat distribution) compared to females. Consistent with this, in this study, with increasing pubertal maturation, females showed higher apparent gains in fat mass (FM), fat mass index (FMI), and subcutaneous fat outcomes but lower apparent gains in lean-to-fat mass ratio (LM:FM), muscle density, muscle force (Fmax), and muscle power (Pmax) compared to males (Table 5.4, Figure 5.5, Figure 5.6, and Figure 5.7), which was as expected. During puberty, testosterone drives muscle development in males by increasing the number of muscle fibers (hyperplasia) and by increasing the size of existing cells/ fibers (hypertrophy), which increases muscle mass and function (Bhasin et al., 2001). Therefore, adult males have great muscle mass and are physically stronger than adult females. In this study, males had consistently higher lean mass (LM), fat-free mass index (FFMI), and muscle area (CSA) compared to females across all stages of puberty.
(Table 5.4). Increases in muscle mass, density and function would increase load on the bone and drive periosteal apposition (i.e. bone widening), as indicated by the findings of this study.

5.5.3 Modifying effects of sex on the relationship between puberty and the muscle-bone unit outcomes in healthy participants
When a muscle contracts, the force generated creates a strain on the bone, changing the shape and length of the bone (Ward, 2012). The relationship between muscle and bone is described by Frost’s mechanostat theory, which postulates that increasing maximal muscle force during growth would increase mechanical loading, resulting in bone adaptation through changes in bone mineral content, size, and length (Frost, 1996, Frost, 1987) (Figure 2.17).

5.5.3.1 The muscle-bone unit of the total body
DXA studies have shown no sex differences in bone accrual during pre-puberty (Martin et al., 1997, Ferretti et al., 1998, Theintz et al., 1992, Maynard et al., 1998). However, Specker et al. concluded that sex differences did exist in children aged 3-5 years, where males had a higher bone mineral content compared to females, after adjusting for height but the others also reported bone size to be similar in males and females (Specker et al., 2001). Data generated from this study showed that sex did not modify the relationship between puberty and DXA bone outcomes (total body less head BMC, size adjusted BMC, and bone area) after accounting for height (Table 5.6 – Model 2).

The mean age of peak height velocity is ~12 years in females and ~14 years in males, therefore females in this study may have already achieved their peak height velocity and peak bone area, and be in the phase of mineral consolidation, whereas males would have not (Javaid and Cooper*, 2002, Bailey et al., 1999). This would explain why there were no sex-by-pubertal stage interactions in bone area and mineral, after accounting for height (Table 5.6 – Model 2). The male skeleton will continue to expand and to accrue mineral until the age of ~18 years, which results in a 22% more BMC compared to females (Baxter-Jones et al., 2011, Theintz et al., 1992)

The muscle-bone unit has previously been described by correlating total body lean mass (TBLH LM) with bone mineral content (TBLH BMC) and have shown females to accrue more bone mineral per unit of lean mass compared to males, which supports the theory that females may store bone mineral as a reservoir for pregnancy and lactation in adulthood (Ashby et al., 2011, Crabtree et al., 2004, Pludowski et al., 2005, Schiessl et al., 1998, Ferretti et al., 1998). Exploratory analyses of DXA results from this study, using simple linear regression to test whether sex modified the relationship between lean mass (TBLH LM) and bone mineral (TBLH BMC), showed that with increasing lean mass, females had higher apparent gains in bone mineral (22.4%, SE 6.6, p=0.001) compared to males (Appendix 10.22 and Figure 5.13). These findings are consistent with Ashby et al. and Shiessl et al. (Schiessl et al., 1998, Zanchetta et al., 1995, Ashby et al., 2011).
Lean mass is commonly used as a proxy of muscle strength. However, lean mass does not give any insight into muscle function (i.e. muscle power [Pmax] and force [Fmax]). Muscle strength is not solely dependent on mass but also on other factors such as the number of fast and slow twitch fibres, blood flow, and metabolism. To my knowledge, this is the first study to use total body DXA measurements together with jumping mechanography to understand the relationship between muscle function and bone mineral and bone area. This study did support the theory of a bone mineral reservoir in females as sex did modify the relationship between muscle force (Fmax) and bone mineral content (TBLH BMC) and bone area (TBLH BA) (Table 5.6 — Model 3 and Figure 5.8). With increasing force (Fmax), females showed higher apparent gains in bone mineral and in bone area compared to males. Sex did not modify the relationship between muscle power (Pmax) and all DXA bone outcomes (i.e. BMC, size-adjusted BMC, or bone area). This suggests that the skeleton of females responds more effectively to muscle force by widening the bone and increasing bone mineral compared to males. However, sex did not modify the relationship between muscle force and size-adjusted BMC (TBLH SA-BMC), which suggests that this muscle-bone relationship is the same in males and females (Table 5.6 — Model 3).

Oestrogen is thought to be responsible for increasing bone mineral accrual on the endosteal surface of bone in females, therefore female have more bone mineral per muscle/lean mass compared to males. In males, the effects of increasing testosterone during puberty are thought to have direct and indirect effects on bone. The direct effects of testosterone are to influence bone accrual to occur on the periosteal surface of bone, therefore widening the bone. At the same time, testosterone is also
related to increases in muscle growth, which will increase weight, muscle force, and drives periosteal apposition (Bhasin et al., 2001, Kirmani et al., 2009, Seeman, 2001).

### 5.5.3.2 The muscle-bone unit of the tibia

This study is one of few which have combined the distal and proximal sites of the tibia using pQCT, HR-pQCT and jumping mechanography, therefore comparison can be made at different regions of the same bone (Anliker et al., 2011b, Binkley and Specker, 2008b). Sex differences in the muscle-bone unit at the distal regions (i.e. 4% site measured by pQCT and 8% site by HR-pQCT) and the proximal region (i.e. 66% site measured by pQCT) of the tibia will be discussed.

**Distal 4% and 8% sites of the tibia**

After size-adjusted analyses, results of the 4% site of the tibia showed that females had narrower bones (Total CSA) with less total bone mineral (total vBMD) but similar trabecular density (Trab vBMD) compared to males, across all stages of puberty (Table 5.8). At the 8% site, with increasing maturation, females showed lower apparent gains in total area (Tt.Ar) and cortical porosity (Ct.Po), but higher apparent gains in cortical bone and tissue mineral densities (Ct.BMD and Ct.TMD). Females had consistently lower trabecular density (BV/TV) and trabecular number (Tb.N) compared to males (Table 5.9 – Model 2 and Figure 5.11). These results are similar to Wang et al. at the distal 7% site, who showed females to have a smaller bone area with thinner, denser cortices compared to males in late puberty (Figure 2.38) (Wang et al., 2010). Individuals with a faster rate of bone growth may experience a lag between bone expansion and bone mineral accrual, therefore the cortical bone at the distal regions becomes thin and porous, reducing bone strength and increasing the risk of fracture. This lag is temporary and may explain why males have a higher incidence of fracture compared to females during growth (Nishiyama et al., 2012).

Differences in imaging techniques for example: the site of measurement, whether bone outcomes are measured directly or derived, image resolution and study power, and precision of measurement, may give rise to different results. For example, results for trabecular density differed at the distal 4% and 8% sites of the tibia show that at the 4% site, there were no sex differences in trabecular density (Trab vBMD), whereas at the 8% site, females had lower trabecular density (BV/TV), similar trabecular thickness (Tb.Th), and lower trabecular number Tb.N) compared to males, across all stages of puberty. Differences in site of measurement (i.e. a larger volume is measured at the 8% site [using HR-pQCT] compared to a single slice at the 4% site [using pQCT]) and differences in image resolution are likely reasons for why there are differences in the results. However, in this study, most of the results showed similar trends in the muscle-bone relationship (i.e. females had lower total and cortical bone areas, higher cortical density, lower cortical porosity compared to males), despite differences in methodology.
There were significant sex differences in the relationships between muscle function (i.e. power \([P_{\text{max}}]\) and force \([F_{\text{max}}]\)) and bone outcomes at the distal sites of the tibia. Similar to DXA results, with increasing muscle force \((F_{\text{max}})\), females showed higher apparent gains in total and trabecular bone densities (total and trab vBMD) at the 4% site compared to males (Table 5.8 – Model 3 and Figure 5.8). Similarly at the 8% site, with increasing muscle power \((P_{\text{max}})\), females had higher apparent gains in total \((D100)\), cortical bone mineral \((\text{Ct.BMD})\), cortical tissue mineral \((\text{Ct.TMD})\) densities and trabecular thickness \((\text{Tb.Th})\) compared to males (Table 5.10 and Figure 5.12). Whereas males showed higher apparent gains in cortical porosity \((\text{Ct.Po})\) and trabecular number \((\text{Tb.N})\) with increasing muscle power \((P_{\text{max}})\) compared to females (Table 5.10 and Figure 5.12). This suggests that as muscle force and power increases and the bone widens, trabecular number increases as a result of bone resorption on the endocortical surface and bone mineral relocation onto the periosteal surface in males. Whereas females have a narrower bone, with lower bone turnover and thicker trabeculae and denser cortices. The sex differences in bone response and adaptation to muscle force and power were unexpected findings. Bone is thought to adapt to muscle power, which is generated from muscle contractions, whereas force is the strain/load placed on bone. Therefore, these findings may suggest different mechanisms of bone adaptation in males and females which remain unclear.

**The proximal 66% site of the tibia**

Similar to the distal sites, measurements taken at the proximal 66% site of the tibia have shown that with increasing maturation, females had lower apparent gains in total area (Total CSA) but higher apparent gains in cortical bone density (vBMD) compared to males (Table 5.8 – Model 2 and Figure 5.9). Females had lower apparent gains in bone (SSI) in pre-to-early puberty but no significant interaction in pre-to-late puberty compared to males. Females had lower cortical area compared to males across all stages of puberty (Table 5.8 – Model 2).

In contrast to muscle force \((F_{\text{max}})\) at the distal 4% site of the tibia, with increasing muscle power \((P_{\text{max}})\) at the proximal 66% site of the tibia, females showed higher apparent gains in cortical bone density (vBMD) compared to males (Table 5.8 – Model 3 and Figure 5.10). Other studies have reported similar findings (Anliker et al., 2011a, Binkley and Specker, 2008a). These findings support the theory that testosterone may drive periosteal apposition in males, which causes bone to be placed further from the neutral axis, expanding the bone area and increasing the resistance to bending forces caused by greater height and muscle power compared to females. Periosteal apposition occurs at a slower rate in females due to oestrogen, which inhibits periosteal apposition and drives endosteal contraction by placing bone within the medullary cavity (i.e. closer to the neutral axis), producing a smaller, narrower bone compared to males. Endosteal contraction has less...
beneficial effect on bone strength than periosteal apposition and may be an evolutionary mechanism for women to store calcium as a reserve for pregnancy and lactation (Kontulainen et al., 2006).

In conclusion, females have a smaller, narrower bone, with denser cortices with similar or less trabecular density compared to males as puberty proceeds. Sex modified the relationships between muscle function and bone, where bone adaptation to muscle force and muscle power were different in males and females. These differences need to be explored in greater detail to help understand how bone mineral, geometry, and architecture are influenced by muscle, which may lead to interventions to improve bone strength throughout the life course and reduce fracture risk.
6 Modifying effects of disease group on anthropometric, body composition, and muscle-bone outcomes between participants with cystic fibrosis and controls

6.1 Data management and statistical analysis
This thesis is based on the cross-sectional baseline measurements of this longitudinal study. Longitudinal measurements were obtained at 12 month follow up visit and are not presented.

6.1.1 Data management
Demographic variables were taken from the musculoskeletal questionnaire and CF annual review medical notes from Addenbrooke’s hospital and merged with anthropometry, DXA, pQCT, HR-pQCT and jumping mechanography data. The five Tanner stages (TS) were condensed into three stages to increase statistical power to detect differences between pubertal groups. Participants in TS 1 were classed as pre-pubertal, participants in TS 2 and TS 3 were classed as early pubertal, and participants in TS 4 and TS 5 were classed as late pubertal. Age and quadratic age were treated as continuous data and pubertal stage was treated as discrete data (i.e. categorised into pre-, early and late puberty).

6.1.2 Statistical analyses
Statistical analyses were performed in three stages (i.e. models) (Table 6.1) to determine whether disease group modified the relationship between puberty and bone and muscle outcomes in children with cystic fibrosis and controls.

Anthropometric, bone, body composition, and muscle function outcomes were analysed according to the research aims. In summary, the analysis models were:

**Model 1** (disease group, pubertal stage, and disease group-by-pubertal stage interaction) using ANCOVA and Scheffé post hoc analyses to test whether disease group modifies the relationship between puberty and anthropometric, body composition, bone and, muscle outcomes,

**Model 2** (disease group, sex, pubertal stage, age, quadratic age, height and weight, sex-by-pubertal stage interaction, and disease group-by-pubertal stage interaction) using multiple linear regression to test whether disease group modifies the relationship between puberty and anthropometric, body composition, bone and, muscle outcomes, after adjusting for height, and finally,

**Model 3** (disease group, sex, pubertal stage, age, quadratic age, height and weight, Pmax, Fmax, sex-by-pubertal stage interaction, disease group-by-pubertal stage interaction, disease group-by-Pmax interaction, and disease group-by-Fmax interaction) using multiple linear regression to test whether disease group modifies the relationship between muscle function and bone outcomes.
The methodology and justification for including co-variates used in the linear regression models are described in the following section. The methodology has been described in Figure 5.1, Figure 5.2, and the exploratory covariates included in these models have been listed in Table 6.1.

Table 6.1 - Summary of the exploratory covariates used in the analyses models.

<table>
<thead>
<tr>
<th>Covariates and analyses used in this study</th>
<th>Model 1 Covariates in ANCOVA with Scheffé post hoc</th>
<th>Model 2 Covariates in multiple linear regression models</th>
<th>Model 3 Covariates in multiple linear regression models</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Disease group</td>
<td>1. Disease group</td>
<td>1. Disease group</td>
<td></td>
</tr>
<tr>
<td>2. Pubertal stage</td>
<td>2. Sex</td>
<td>2. Sex</td>
<td></td>
</tr>
<tr>
<td>3. Disease group-by-pubertal stage interaction*</td>
<td>3. Height</td>
<td>3. Height</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4. Age</td>
<td>4. Age</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5. Quadratic age</td>
<td>5. Quadratic age</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6. Pubertal stage</td>
<td>6. Pubertal stage</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7. Weight</td>
<td>7. Weight</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8. Muscle force (Fmax)</td>
<td>8. Muscle force (Fmax)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9. Muscle power (Pmax)</td>
<td>9. Muscle power (Pmax)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10. Sex-by-pubertal stage interaction*</td>
<td>10. Sex-by-pubertal stage interaction*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>11. Disease group-by-pubertal stage interaction*</td>
<td>11. Disease group-by-pubertal stage interaction*</td>
<td></td>
</tr>
</tbody>
</table>

Key: * As is standard practice, if an interaction was significant, the covariates were retained separately in the model. If there was a significant interaction, then the relationship between the two covariates in the interaction was interpreted, whereas, if there were no significant interactions, then these interaction terms were omitted and the main effect (i.e. disease group difference) was interpreted. Covariates in bold font were ‘forced’ into all models whether significant or not, whereas covariates in regular font were only included in the final model if significant.
6.2 The influence of disease status on the relationship between puberty and anthropometric outcomes in participants with CF and controls

**Aim**

1) The aim was to investigate whether disease group modified the relationship between puberty and anthropometric outcomes (i.e. height, weight, sitting height and BMI).

**Hypothesis**

1) The hypothesis is that disease group does modify the relationship between puberty and anthropometric outcomes. Participants with CF have lower apparent gains in height, weight, sitting height, and BMI compared to controls, as puberty proceeds.

**6.2.1 Analyses**

Anthropometric measurements were assessed for normality and transformed into natural logs. Primary outcomes were age, pubertal stage, height, weight, sitting height and BMI. Standard deviation scores (SDS or Z-score) for height, weight and BMI were calculated using the 1990 UK growth reference dataset, to compare our population with current UK standards (Cole, 1990, Cole et al., 1998, Cole and Green, 1992). Analyses were performed using Data Desk® statistical package (Version 6.3.1). In summary, the analysis models were: **Model 1** (disease group, pubertal stage, and disease group-by-pubertal stage interaction) using ANCOVA and Scheffé post hoc analyses to test whether disease group modifies the relationship between puberty and age, height, height SDS, weight, weight SDS, BMI, BMI SDS and sitting height. Data are presented as mean and standard deviation. The statistical significance level was set at p<0.05. Boxplots were used to present the effects of disease group on anthropometric outcomes (i.e. height, height SDS, weight, weight SDS, BMI, BMI SDS and sitting height) in males and females with CF and controls, as puberty proceeds. Any outlying values were scrutinised.

**6.2.2 The results after Model 1 analyses**

Anthropometric characteristics of participants with CF and controls are presented in **Table 6.2** and **Figure 6.1**. Disease group did not modify the relationships between puberty and anthropometric outcomes in participants with CF and controls (**Figure 6.1**). However, participants with CF had consistently lower height (p=0.0116), height SDS (p<0.0001), weight (p=0.0083), weight SDS (p<0.0001), and BMI SDS (p=0.0387), and sitting height (p=0.0306) across all stages of puberty (Data not presented in table). There were no disease group differences for age (p=0.2520) or BMI (p=0.1809).
Table 6.2 – The modifying effect of disease group on the relationship between puberty and anthropometric outcomes in participants with CF and controls (Model 1).

<table>
<thead>
<tr>
<th>Anthropometric outcome measures</th>
<th>Participants with CF vs. Control</th>
<th>Model 1 p value of disease grp-by-PS interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Participants with CF (n=65)</td>
<td>Control (n=151)</td>
</tr>
<tr>
<td>Puberty (n)</td>
<td>Pre (n=25)</td>
<td>Early (n=21)</td>
</tr>
<tr>
<td>Age (Yrs)</td>
<td>10.08 (±1.9)</td>
<td>11.92 (±2.6)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>135.93 (±9.7)</td>
<td>148.37 (±13.3)</td>
</tr>
<tr>
<td>Height (SDS)</td>
<td>-0.47 (±0.8)</td>
<td>-0.10 (±1.1)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>31.68 (±7.5)</td>
<td>39.58 (±12.1)</td>
</tr>
<tr>
<td>Weight (SDS)</td>
<td>-0.24 (±0.9)</td>
<td>-0.23 (±1.2)</td>
</tr>
<tr>
<td>BMI (kg/m^2)</td>
<td>16.92 (±2.2)</td>
<td>17.59 (±3.3)</td>
</tr>
<tr>
<td>BMI (SDS)</td>
<td>-0.04 (±0.9)</td>
<td>-0.26 (±1.3)</td>
</tr>
<tr>
<td>Sitting height (cm)</td>
<td>70.27 (±6.4)</td>
<td>72.50 (±8.6)</td>
</tr>
</tbody>
</table>

Key: Model 1 analyses included disease group, puberty, and disease group-by-pubertal stage interaction. All values are mean (SD) unless indicated. Puberty was based on Tanner staging (TS) and was categorised into three groups: pre-puberty = TS 1, early puberty = TS 2 and TS 3, and late puberty = TS 4 and TS 5. (SDS) Standard deviation score from the mean height and weight of an age-and-sex matched control population. Body mass index (BMI) = weight (kg) divided by height (m^2). Statistically significant when p<0.05 and is presented in bold and highlighted in grey.
Figure 6.1 - The modifying effect of disease group on the relationship between puberty and anthropometric outcomes (mean, SD) in participants with CF and controls (Model 1).
Figure 6.1 – continued; The modifying effect of disease group on the relationship between puberty and anthropometric outcomes (mean, SD) in participants with CF and controls (Model 1).

6.2.3 Summary
- Disease status did not modify the relationship between puberty and anthropometry outcomes (Table 6.2 and Figure 6.1). Participants with CF were consistently shorter and had lower body weight compared to controls, across all pubertal stages.
The influence of disease group on the relationship between puberty and body composition and muscle function in participants with CF and controls

Aims

The aim was to investigate whether, after accounting for height, weight and pubertal stage, disease group modified the relationship between puberty and:

1) Body composition outcomes (i.e. total body lean mass (LM), fat mass (FM), fat-free mass index (FFMI), fat mass index (FMI), lean-to-fat mass ratio (LM:FM), muscle CSA, muscle density, and subcutaneous fat CSA).

2) Muscle function outcomes (i.e. maximum power (Pmax) and force (Fmax)).

Hypotheses

The hypotheses were that disease group, after accounting for height, weight and pubertal stage, would modify the relationship between puberty and:

1) Body composition, where participants with CF have lower apparent gains in lean mass (LM), fat-free mass index (FFMI), lean-to-fat mass ratio (LM:FM), muscle CSA, and muscle density but higher apparent gains in fat mass (FM), fat mass index (FMI), and subcutaneous fat CSA compared to controls, as puberty proceeds.

2) Muscle function, where participants with CF have lower apparent gains in muscle force (Fmax) and muscle power (Pmax) compared to controls, as puberty proceeds.

Analyses

Body composition and muscle function measurements were transformed into natural logs and were assessed for normality. Primary outcomes for the total body site were; LM, FM, LM:FM ratio, FFMI (i.e. FFM / [height^2]) and FMI (i.e. FM / [height^2]) (Williams et al., 2010), for the proximal 66% site of the tibia were: muscle CSA, muscle density and subcutaneous fat CSA, and for muscle function were: Pmax and Fmax.

Analyses were performed using Data Desk® (Version 6.3.1) and Rstudio® (Version 3.3.1) statistical package. In summary, the analysis models were: **Model 1** (disease group, pubertal stage, and disease group-by-pubertal stage interaction) using ANCOVA and Scheffé post hoc analyses to test whether disease group modifies the relationship between puberty and body composition and muscle function outcomes, and **Model 2** (disease group, sex, pubertal stage, age, quadratic age, height and weight, sex-by-pubertal stage interaction, and disease group-by-pubertal stage interaction) using multiple linear regression to test whether disease group modifies the relationship between puberty and body composition and muscle function outcomes, after adjusting for height.
In model 1, data are presented as mean and SD. Any outlying values were scrutinised. In model 2, height was retained in the analyses for all measurements to adjust for differences in body size during growth, except for FFMI and FMI which include adjustments for height. Weight was not included in the model for lean mass and fat mass. Data are presented as beta-coefficient (%) and p-value, with the significance level set to p<0.05.

6.3.2 The results after Model 1 analyses

The modifying effects of disease group on the relationship between puberty and body composition and muscle function outcomes after Model 1 analyses (i.e. disease group, pubertal stage, and disease group-by-pubertal stage interaction) are presented in Table 6.3.

**DXA (Total body)** - Disease group did modify the relationship between puberty and LM:FM ratio. With increasing pubertal maturation, participants with CF had lower apparent gains in LM:FM ratio compared to controls (p=0.0192) (Table 6.3). Disease group did not modify the relationship between puberty and LM, FM, FFMI or FMI outcomes (Table 6.3).

**pQCT (The proximal 66% site of the tibia)** – Disease group did modify the relationship between puberty and muscle CSA and muscle density. With increasing pubertal maturation, participants with CF had lower apparent gains in muscle CSA (p=0.0177) and in muscle density (p<0.0001) compared to controls (Table 6.3). Post hoc comparisons showed that participants with CF had lower muscle CSA in early and late puberty and lower muscle density in late puberty compared to males. Disease group did not modify the relationship between puberty and subcutaneous fat CSA outcome (Table 6.3). There were no disease group differences for subcutaneous fat CSA outcome between participants with CF and controls (Table 6.3).

**Jumping mechanography (Lower body)** – Disease group did modify the relationship between puberty and muscle function. With increasing maturation, participants with CF had lower apparent gains in muscle Pmax (p=0.0034) and Fmax (p=0.0010) compared to controls. Post hoc comparisons showed that participants with CF had lower muscle Pmax and Fmax compared to controls in late puberty (Table 6.3).
Table 6.3 - Modifying effect of disease group on the relationship between puberty and body composition and muscle function outcomes in participants with CF and controls (Model 1).

<table>
<thead>
<tr>
<th>Body composition outcome measures</th>
<th>Participants with CF (n= 65)</th>
<th>Controls (n=151)</th>
<th>P value of disease grp-by-P5 interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre (n=25)</td>
<td>Early (n=21)</td>
<td>Late (n=19)</td>
</tr>
<tr>
<td>DXA (Total body less head)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LM (g)</td>
<td>22317.0 (±3942.6)</td>
<td>2910.1 (±8134.7)</td>
<td>36033.7 (±5850.4)</td>
</tr>
<tr>
<td>FM (g)</td>
<td>9586.3 (±6603.4)</td>
<td>8451.3 (±6731.5)</td>
<td>14075.8 (±7697.0)</td>
</tr>
<tr>
<td>LM:FM ratio</td>
<td>3.1 (±1.1)</td>
<td>5.6 (±4.0)</td>
<td>3.2 (±1.78)</td>
</tr>
<tr>
<td>FFMI</td>
<td>12.6 (±1.1)</td>
<td>13.6 (±1.6)</td>
<td>14.9 (±1.6)</td>
</tr>
<tr>
<td>FMI</td>
<td>5.2 (±3.7)</td>
<td>3.7 (±2.8)</td>
<td>5.7 (±3.5)</td>
</tr>
<tr>
<td>pQCT (proximal 66% site)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Muscle CSA (mm&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>1013.9 (±767.3)</td>
<td>4265.7 (±1021.8)</td>
<td>5060.0 (±726.3)</td>
</tr>
<tr>
<td>Muscle density (g)</td>
<td>77.16 (±1.3)</td>
<td>78.8 (±2.9)</td>
<td>77.7 (±2.1)</td>
</tr>
<tr>
<td>Subcutaneous fat CSA (mm&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>1688.5 (±599.3)</td>
<td>1759.0 (±965.0)</td>
<td>2396.8 (±1199.9)</td>
</tr>
<tr>
<td>Jumping Mechography (Lower body)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pmax (W)*</td>
<td>1.05 (±0.3)</td>
<td>1.56 (±1.1)</td>
<td>1.81 (±0.5)</td>
</tr>
<tr>
<td>Fmax (N)*</td>
<td>0.77 (±0.2)</td>
<td>0.60 (±0.3)</td>
<td>1.20 (±0.3)</td>
</tr>
</tbody>
</table>

Key: Model 1 analyses included disease group, puberty, and disease group-by-pubertal stage interaction. * denotes missing data for one early pubertal participant with CF. All values are mean (SD) unless indicated. Letters are used to indicate significance of group and pubertal stage differences as tested by ANCOVA with Scheffé post hoc tests. Puberty was based on Tanner staging (TS) and was categorised into three groups: pre-puberty = TS 1, early puberty = TS 2 and TS 3, and late puberty = TS 4 and TS 5. (SDS) Standard deviation score from the mean height and weight of an age-and-sex matched control population. Lean mass (LM), fat mass (FM), fat-free mass index (FFMI = FFM/[height<sup>2</sup>]), fat mass index (FMI = FM/[height<sup>2</sup>]), cross-sectional area (CSA), maximum power (Pmax) and maximum force (Fmax). Statistically significant when p<0.05 and is presented in bold and highlighted in grey. <sup>a</sup>The scheffe post hoc analyses did not indicate a significant disease group-by-puberty interaction.

a Significant difference between pre-pubertal participants with CF and controls, P<0.05.
b Significant difference between early pubertal participants with CF and controls, P<0.05.
c Significant difference between late pubertal participants with CF and controls, P<0.05.
6.3.3 The results after Model 2 analyses (i.e. Height-adjusted)
The modifying effects of disease group on the relationship between puberty and body composition and muscle function after Model 2 analyses (i.e. disease group, sex, pubertal stage, age, quadratic age, height, weight, sex-by-pubertal stage interaction, and disease group-by-pubertal stage interaction) are presented in Table 6.4.

6.3.3.1 DXA (Total body)
As shown in Table 6.4, disease group did modify the relationship between puberty and DXA body composition for FM, LM:FM ratio, and FMI. Participants with CF had lower apparent gains in FM (-53.0%, p=0.006) and FMI (-47.1%, p=0.015) in pre-to-early puberty compared to controls but no significant interactions in pre-to-late puberty in either FM (-25%, P=0.213) or FMI (-25.7%, p=0.197) (Figure 6.2). Participants with CF had higher apparent gains in LM:FM ratio (48.7%, p=0.013) in pre-to-early puberty compared to controls but no significant interactions pre-to-late puberty (23.1%, p=0.253) (Figure 6.2). There were no significant disease group-by-pubertal stage interactions for LM or FFMI but participants with CF had consistently lower LM (-4.2%, p=0.003) and FFMI (-5.2%, p<0.001) across all stages of puberty compared to controls (Table 6.4).

6.3.3.2 pQCT (The proximal 66% site of the tibia)
As shown in Table 6.4, disease group did modify the relationship between puberty and muscle density. With increasing maturation, participants with CF had lower apparent gains in muscle density (-5.9%, p<0.001) compared to controls in pre-to-late puberty (Figure 6.3). However, there were no disease group differences in apparent gains in muscle density (0.9%, p=0.328) in pre-to-early puberty.

There were no significant disease group-by-pubertal stage interactions for muscle CSA or subcutaneous fat CSA but participants with CF had consistently lower muscle CSA (-9.2%, p=0.001) across puberty stages compared to controls (Table 6.4). There was no disease group difference for subcutaneous fat (-2.4%, p=0.506) across all puberty stages.

6.3.3.3 Jumping mechanography (Lower body)
As shown in Table 6.4, disease group did modify the relationship between puberty and muscle function outcomes (Pmax and Fmax). Participants with CF showed higher apparent gains in Pmax (10.8%, p=0.045) and Fmax (9.8%, p=0.020) in pre-to-early puberty but lower apparent gains in Pmax (-13.1%, p=0.018) and Fmax (-8.9%, p=0.039) in pre-to-late puberty, compared to controls (Figure 6.4).
Figure 6.2 - The modifying effects of disease group on the relationship between puberty and DXA body composition outcomes in participants with CF and controls (Model 2 - Height-adjusted).

Key: Model 2 analyses included disease group, sex, pubertal stage, age, quadratic age, height, weight, sex-by-pubertal stage interaction, and disease group-by-pubertal stage interaction. Dual-energy X-ray absorptiometry (DXA), fat mass index (FMI, kg/m²), controls (Con) and participants with cystic fibrosis (CF).
Figure 6.3 - The modifying effects of disease group on the relationship between puberty and muscle density at the proximal 66% site of the tibia in participants with CF and controls (Model 2 - Height-adjusted).

**Key:** Model 2 analyses included disease group, sex, pubertal stage, age, quadratic age, height, weight, sex-by-pubertal stage interaction, and disease group-by-pubertal stage interaction. Peripheral quantitative computed tomography (pQCT), controls (Con) and participants with cystic fibrosis (CF).

Figure 6.4 - The modifying effects of disease group on the relationship between puberty and muscle function outcomes (Pmax and Fmax) in participants with CF and controls (Model 2 - Height-adjusted).

**Key:** Model 2 analyses included disease group, sex, pubertal stage, age, quadratic age, height, weight, sex-by-pubertal stage interaction, and disease group-by-pubertal stage interaction. Maximum muscle power (Pmax), maximum muscle force (Fmax), controls (Con) and participants with cystic fibrosis (CF).
Table 6.4 - Modifying effect of disease group on the relationship between puberty and body composition and muscle function outcomes in participants with CF and controls (Model 2 - Height-adjusted).

<table>
<thead>
<tr>
<th>Outcome measures</th>
<th>MODEL 2</th>
<th>p value of disease grp diff.</th>
<th>Sig. interaction</th>
<th>p value of interaction</th>
<th>Sig. variables</th>
<th>R²</th>
<th>Graph Ref</th>
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</thead>
<tbody>
<tr>
<td>DXA (Total body)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LM (g)</td>
<td>-4.2</td>
<td>1.4</td>
<td>0.003</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FM (g)</td>
<td>-53.0</td>
<td>-25.0</td>
<td>19.2</td>
<td>-</td>
<td>Grp*PS2</td>
<td>0.006</td>
<td>Ht</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Grp*PS3</td>
<td>0.213</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LM:FM ratio</td>
<td>48.7</td>
<td>23.1</td>
<td>19.5</td>
<td></td>
<td>Grp*PS2</td>
<td>0.013</td>
<td>Age²</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Grp*PS3</td>
<td>0.253</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FFMI</td>
<td>-5.2</td>
<td>1.3</td>
<td>&lt;0.001</td>
<td>-</td>
<td>-</td>
<td></td>
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<td></td>
<td>Sex</td>
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<td></td>
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<td>PS3</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FMI</td>
<td>-47.1</td>
<td>-25.7</td>
<td>19.3</td>
<td></td>
<td>Grp*PS2</td>
<td>0.015</td>
<td>Age &amp; age²</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Grp*PS3</td>
<td>0.197</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pQCT (The proximal 66% site of the tibia)</td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Muscle CSA (mm²)</td>
<td>-9.2</td>
<td>1.7</td>
<td>0.001</td>
<td>-</td>
<td>-</td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Muscle density (g)</td>
<td>-0.9</td>
<td>0.9</td>
<td>0.328</td>
<td></td>
<td>Grp*PS2</td>
<td>0.328</td>
<td>PS2 &amp; PS3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Grp*PS3</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subcutaneous fat CSA (mm²)</td>
<td>-2.4</td>
<td>3.6</td>
<td>0.506</td>
<td></td>
<td>-</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Age</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Ht &amp; Wt</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jumping mechanography</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pmax (W)</td>
<td>10.8</td>
<td>5.3</td>
<td>0.045</td>
<td></td>
<td>Grp*PS2</td>
<td>0.045</td>
<td>PS3</td>
</tr>
<tr>
<td></td>
<td>-13.1</td>
<td>5.5</td>
<td></td>
<td></td>
<td>Grp*PS3</td>
<td>0.018</td>
<td>Age</td>
</tr>
<tr>
<td>Fmax (N)</td>
<td>9.8</td>
<td>4.2</td>
<td>0.020</td>
<td></td>
<td>Grp*PS2</td>
<td>0.020</td>
<td>PS3 &amp; PS3</td>
</tr>
<tr>
<td></td>
<td>-8.9</td>
<td>4.3</td>
<td></td>
<td></td>
<td>Grp*PS3</td>
<td>0.039</td>
<td>Ht &amp; Wt</td>
</tr>
</tbody>
</table>
6.3.4 Summary
The following summary statements are based on height-adjusted analyses.

- With increasing pubertal maturation, participants with CF had lower apparent gains in fat mass (FM) and fat mass index (FMI) but higher apparent gains in lean-to-fat mass ratio (LM:FM) in pre-to-early puberty compared to controls but no significant interactions in pre-to-late puberty (Table 6.4 and Figure 6.2).

- Participants with CF had consistently lower lean mass (LM), fat free mass index (FFMI), and muscle CSA compared to controls (Table 6.4).

- Participants with CF had lower apparent gains in muscle density and muscle function i.e. muscle force (Fmax), and muscle power (Pmax) as puberty proceeded compared to controls (Table 6.4, Figure 6.3, and Figure 6.4).
### Aims

The aim was to investigate whether, after accounting for height, weight and pubertal stage, disease group (CF vs. controls) modified the relationships between puberty and muscle function on:

1) DXA bone outcomes (i.e. Total body less head bone mineral content [TBLH BMC], size adjusted BMC [TBLH SA-BMC], and bone area [TBLH BA]).

2) pQCT bone outcomes (i.e. vBMD, geometry, and SSI outcomes at the distal 4% and proximal 66% sites of the tibia.

3) HR-pQCT bone outcomes (i.e. vBMD, geometry, and microarchitecture) at the distal 8% site of the tibia.

### Hypotheses

The hypotheses were that disease group, after accounting for height, weight and pubertal stage, would:

1) Modify the relationship between puberty and bone outcomes, where participants with CF have lower apparent gains in bone area and bone density as puberty proceeds.

2) Modify the relationship between muscle function (i.e. Pmax and Fmax) and bone outcomes. With increasing muscle force and power, participants with CF would have lower apparent gains in bone area and bone mineral compared to controls.

### Analyses

Bone and muscle measurements were assessed for normality and transformed into natural logs. Primary outcomes for bone were measured using DXA at the total body less head site (TBLH BMC, TBLH size adjusted BMC [TBLH SA-BMC] and TBLH BA), pQCT at the distal 4% and proximal 66% sites of the tibia (total bone CSA, total vBMD, cortical CSA, cortical vBMD, trabecular vBMD and SSI), and HR-pQCT at the distal 8% site of the distal tibia (Tt.Ar, Ct.Ar, D100, Ct.BMD, Ct.TMD, Ct.Po, BV/TV, Tb.N and Tb.Th). Muscle function was measured using jumping mechanography of the lower limbs (Pmax and Fmax). Analyses were performed using Data Desk® (Version 6.3.1) and Rstudio® (Version 3.3.1) statistical package. In summary, the analysis models were: **Model 1** (disease group, pubertal stage, and disease group-by-pubertal stage interaction) using ANCOVA and Scheffé post hoc analyses to test whether disease group modifies the relationship between puberty and bone outcomes, **Model 2** (disease group, sex, pubertal stage, age, quadratic age, height and weight, sex-by-pubertal stage interaction, and disease group-by-pubertal stage interaction) using multiple linear regression to
test whether disease group modifies the relationship between puberty and bone outcomes, after adjusting for height, and finally, **Model 3** (disease group, sex, pubertal stage, age, quadratic age, height and weight, Pmax, Fmax, sex-by-pubertal stage interaction, disease group-by-pubertal stage interaction, disease group-by-Pmax interaction, and disease group-by-Fmax interaction) using multiple linear regression to test whether disease group modifies the relationship between muscle function and bone outcomes.

To assess TBLH SA-BMC, TBLH BA was retained in the model for TBLH BMC to adjust for body size. In models 2 and 3, height was retained in the model analyses for all measurements to adjust for differences in body size during growth. Data are presented as mean, standard deviation, beta-coefficient (%) and p-value, with the significance level set to p<0.05. Interaction plots were used to help to interpret statistically significant interactions (p<0.05).

### 6.4.2 The muscle-bone unit - Total body using DXA

#### 6.4.2.1 Results of Model 1 analyses

The modifying effects of disease group on the relationship between puberty and DXA bone outcomes after **Model 1** analyses (i.e. disease group, pubertal stage, and disease group-by-pubertal stage interaction) are presented in **Table 6.5**.

**Table 6.5 - Modifying effect of disease group on the relationship between puberty and DXA bone outcomes in participants with CF and controls (Model 1).**

<table>
<thead>
<tr>
<th>Body composition outcome measures</th>
<th>Participants with CF (n= 65)</th>
<th>Control (n=151)</th>
<th>P value of disease grp-by-PS interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Puberty (n)</td>
<td>Participants with CF</td>
<td>Control</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pre (n=25)</td>
<td>Pre (n=33)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Early (n=21)</td>
<td>Early (n=69)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Late (n=19)</td>
<td>Late (n=49)</td>
<td></td>
</tr>
<tr>
<td>TBLH BMC (g/cm²)</td>
<td>788.2 (±219.7)</td>
<td>842.1 (±216.8)</td>
<td>0.0017 c</td>
</tr>
<tr>
<td></td>
<td>1069.6 (±376.5)</td>
<td>1286.0 (±436.2)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1465.0 (±230.5)</td>
<td>2005.6 (±405.1)</td>
<td></td>
</tr>
<tr>
<td>TBLH bone area (cm²)</td>
<td>1186.1 (±323.8)</td>
<td>1093.8 (±202.7)</td>
<td>0.0029 c</td>
</tr>
<tr>
<td></td>
<td>1343.2 (±356.1)</td>
<td>1453.8 (±323.8)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1690.3 (±196.5)</td>
<td>1962.4 (±261.7)</td>
<td></td>
</tr>
</tbody>
</table>

**Key:** Model 1 analyses included disease group, puberty, and group-by-pubertal stage interaction. All values are mean (SD) unless indicated. Letters are used to indicate significance of group and pubertal stage differences as tested by ANCOVA with Scheffé post hoc tests. Puberty was based on Tanner staging (TS) and was categorised into three groups: pre-puberty = TS 1, early puberty = TS 2 and TS 3, and late puberty = TS 4 and TS 5. (SDS) Standard deviation score from the mean height and weight of an age-and-sex matched control population. Total body less head bone mineral content (TBLH BMC). Statistically significant when p<0.05 and is presented in bold and highlighted in grey.

- **a** Significant difference between pre-pubertal participants with CF and controls, P<0.05.
- **b** Significant difference between early pubertal participants with CF and controls, P<0.05.
- **c** Significant difference between late pubertal participants with CF and controls, P<0.05.
As shown in Table 6.5, disease group did modify the relationship between puberty and DXA bone outcomes. Participants with CF had lower apparent gains in TBLH BMC (p=0.017) and TBLH BA (p=0.0029) compared to controls. Post hoc comparisons showed that participants with CF had lower TBLH BMC and TBLH BA compared to controls in late puberty.

6.4.2.2 The results of Models 2 (i.e. Height-adjusted) and 3 (i.e. Disease group*Muscle function interactions)

The modifying effects of disease group on the relationships between 1) puberty and DXA bone outcomes after Model 2 (i.e. disease group, sex, pubertal stage, age, quadratic age, height, weight, sex-by-pubertal stage interaction, and disease group-by-pubertal stage interaction) and 2) muscle function and DXA bone outcomes after Model 3 (i.e. disease group, sex, age, quadratic age, height, weight, pubertal stage, Pmax, Fmax, sex-by-pubertal stage interaction, disease group-by-pubertal stage interaction, disease group-by-Pmax stage interaction, and disease group -by-Fmax stage interaction) analyses are presented in Table 6.6.

As shown in Table 6.6 – Model 2, disease group did modify the relationship between puberty and DXA bone outcomes for TBLH BA, BMC, and SA-BMC. Participants with CF had lower apparent gains in BA in pre-to-early puberty (-10.6%, p<0.001) and a similar trend in pre-to-late puberty (-12.6%, p<0.001) compared to controls (Figure 6.5). For TBLH BMC, there was no significant interaction in pre-to-early puberty (-2.6%, p=0.499) but participants with CF showed lower apparent gains in TBLH BMC (-9.5%, p=0.017) in pre-to-late puberty compared to controls. Participants with CF had higher apparent gains in TBLH SA-BMC (9.4%, p=0.001) in pre-to-early puberty but no significant interaction in pre-to-late puberty (4.7%, p=0.091) (Table 6.6).

As shown in Table 6.6 – Model 3, disease group did modify the relationship between muscle function and DXA bone outcomes for TBLH BMC, SA-BMC and BA. With increasing Fmax, participants with CF had lower apparent gains in TBLH BMC (-12.0%, p=0.009) and similar lower trends in BA (-8.4%, p=0.054 – borderline significant) compared to controls (Figure 6.6). There was no significant disease group-by-Fmax interaction for TBLH SA-BMC. With increasing Pmax, participants with CF showed a trend to lower apparent gains in TBLH SA-BMC (-6.1%, p=0.051 – borderline significant) compared to controls (Figure 6.7).
Figure 6.5 - The modifying effects of disease group on the relationship between puberty and DXA total body less head bone area in participants with CF and controls (Model 2 - Height-adjusted).

Key: Model 2 analyses included disease group (Grp), sex, age, quadratic age ($age^2$), height (Ht), weight (Wt), sex-by-pubertal stage interaction, and disease group-by-pubertal stage interactions. Dual-energy X-ray absorptiometry (DXA), total body less head bone area (TBLH BA, cm$^2$) and participants with cystic fibrosis (CF).

Figure 6.6 - The modifying effects of disease group on the relationship between Fmax and DXA bone outcomes in participants with CF and controls (Model 3 - Disease group*Muscle function interactions).

Key: Model 3 analyses included disease group (Grp), sex, age, quadratic age ($age^2$), height (Ht), weight (Wt), Pmax, Fmax, sex-by-pubertal stage interaction, disease group-by-pubertal stage interactions, disease group-by-Pmax interaction, and disease group-by-Fmax interaction. Dual-energy X-ray absorptiometry (DXA), total body less head unadjusted BMC less head (TBLH BMC, g), total body less head bone area (TBLH BA, cm$^2$), maximum muscle force, Newtons (Fmax, N), control participants (Con) and participants with cystic fibrosis (CF). Fmax: Lower quantile = 0.50 - 1.18 N and upper quantile = 1.18 - 2.65 N.
Figure 6.7 - The modifying effects of disease group on the relationship between Pmax and DXA bone outcomes in participants with CF and controls (Model 3 - Disease group*Muscle function interactions).

**Key:** Model 3 analyses included disease group (Grp), sex, age, quadratic age (age^2), height (Ht), weight (Wt), Pmax, Fmax, sex-by-pubertal stage interaction, disease group-by-pubertal stage interactions, disease group-by-Pmax interaction, and disease group-by-Fmax interaction). Dual-energy X-ray absorptiometry (DXA), total body less head size adjusted BMC less head (TBLH SA-BMC, g), maximum muscle power, watts (Pmax, W), control participants (Con) and participants with cystic fibrosis (CF). Pmax: Lower quantile = 0.56 – 1.66 W and upper quantile = 1.66 – 5.04 W.
Table 6.6 - Modifying effect of disease group on the relationship between puberty and DXA bone outcomes in participants with CF and controls (Model 2 - Height-adjusted and Model 3 - Disease group*Muscle function interaction).

<table>
<thead>
<tr>
<th>Outcome measures</th>
<th>MODEL 2</th>
<th>MODEL 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β (%)</td>
<td>SE</td>
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<tr>
<td></td>
<td>of Grp</td>
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</tr>
<tr>
<td></td>
<td>Sig.</td>
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<td>variables</td>
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<tr>
<td><strong>Total body (less head)</strong></td>
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<tr>
<td>TBLH BMC (g/cm²)</td>
<td>-2.6</td>
<td>3.8</td>
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<tr>
<td></td>
<td>-9.5</td>
<td>4.0</td>
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<tr>
<td>TBLH SA-BMC (g/cm²)</td>
<td>9.4</td>
<td>4.7</td>
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<tr>
<td></td>
<td>4.7</td>
<td>2.8</td>
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<tr>
<td>TBLH BA (cm²)</td>
<td>-10.6</td>
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<td>-12.6</td>
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</tbody>
</table>

Key: β coefficients for disease group differences = participants with CF or for sex differences = females. Dual-energy X-ray absorptiometry (DXA) outcome measures: Total body less head size adjusted bone mineral content (TBLH SA-BMC) = BMC adjusted for bone area (TBLH BA). Model 2: Adjusted for explanatory variables including sex, group (Grp), age, quadratic age (age²), early puberty compared to pre-puberty (PS2), late puberty compared to pre-puberty (PS3), height (Ht) and weight Model 3: Adjusted for model 2 and maximum muscle force (Fmax) and maximum muscle power (Pmax). Graph not presented in thesis (NP). Statistically significant when p<0.05 and is presented in bold and highlighted in grey. If a disease group-by-pubertal stage or disease group-by-Fmax/Pmax interaction was not found, then the interaction was removed from the model and the β coefficient (i.e. disease group, Pmax and Fmax) was interpreted alone. * denotes a significant interaction.
6.4.3  The muscle-bone unit - The distal 4% and proximal 66% sites of the tibia using pQCT.

6.4.3.1  The results of Model 1 analyses
The modifying effects of disease group on the relationship between puberty and pQCT bone outcomes after Model 1 analyses (i.e. disease group, pubertal stage, and group-by-pubertal stage interaction) are presented in Table 6.7.

pQCT – The distal 4% site of the tibia
As shown in Table 6.7, disease group did not modify the relationship between puberty and pQCT bone outcomes at the 4% site (i.e. total CSA, p=0.4728, total vBMD, p=0.1129, and trabecular vBMD, p=0.4520).

pQCT - The proximal 66% site of the tibia
As shown in Table 6.7, disease group did modify the relationship between puberty and pQCT outcomes. With increasing maturation, participants with CF had lower apparent gains in total CSA (p=0.0318) and cortical CSA (p=0.0397), and higher apparent gains in cortical vBMD (p=0.0110) compared to controls. There were no disease group-by-pubertal stage interactions for SSI but a trend towards lower apparent gains in SSI (p=0.0613). Post hoc comparisons showed that participants with CF had lower total and cortical CSAs in late puberty and higher cortical vBMD in pre-puberty and late puberty compared to controls.

6.4.3.2  The results of Model 2 (i.e. Height-adjusted) and 3 (i.e. Disease group*Muscle function interactions)
The effects of disease group on the relationships between 1) puberty and pQCT bone outcomes after Model 2 (i.e. disease group, sex, pubertal stage, age, quadratic age, height, weight, sex-by-pubertal stage interaction, and disease group-by-pubertal stage interaction), and 2) muscle function and pQCT bone outcomes after Model 3 (i.e. disease group, sex, age, quadratic age, height, weight, pubertal stage, Pmax, Fmax, sex-by-pubertal stage interaction, disease group-by-pubertal stage interaction, disease group-by-Pmax stage interaction, and disease group-by-Fmax stage interaction) analyses are presented in Table 6.8.

pQCT - The distal 4% site of the tibia
As shown in Table 6.8 – Model 2, disease group did not modify the relationship between puberty and pQCT bone outcomes at the 4% site. Participants with CF had consistently lower total vBMD (-5.9%, p=0.001) and trabecular vBMD (-7.8%, p=0.001) across all stages of puberty. No disease group difference was detected for total CSA.
As shown in Table 6.8 – Model 3, disease group did not modify the relationship between muscle function and pQCT bone outcomes for total CSA, total vBMD or trabecular vBMD. However, disease group differences in total vBMD and trabecular vBMD were attenuated when adjusted for muscle function.

**pQCT – The proximal 66% site of the tibia**

As shown in Table 6.8 – Model 2, disease group did modify the relationship between puberty and cortical vBMD. With increasing maturation, participants with CF had lower apparent gains in cortical vBMD from pre-to-early puberty (-3.9%, p<0.001) and a similar trend from pre-to-late puberty (-2.0%, p=0.060 – borderline significant) compared to controls (Figure 6.8). There were no significant disease group-by-puberty interactions for total CSA, cortical CSA, or SSI. Participants with CF had consistently lower total CSA (-9.9%, p=0.001), cortical CSA (-4.1%, p=0.019) and SSI (-7.6%, p=0.0012) compared to controls across all stages of puberty.

**Figure 6.8 - The modifying effects of disease group on the relationship between puberty and cortical vBMD outcomes at the proximal 66% site of the tibia in participants with CF and controls (Model 2 - Height-adjusted).**

Key: Model 2 analyses included disease group (Grp), sex, age, quadratic age (age^2), height (Ht), weight (Wt), sex-by-pubertal stage interaction, and disease group-by-pubertal stage interactions. Peripheral quantitative computed tomography (pQCT), volumetric BMD (vBMD, mg/cm^3) and participants with cystic fibrosis (CF).

As shown in Table 6.8 – Model 3, disease group did not modify the relationship between muscle function and pQCT bone outcomes for total CSA, cortical CSA, cortical vBMD or SSI. There were no significant interactions for disease group-by-Pmax stage, or disease group-by-Fmax stage for all outcomes at the proximal 66% site of the tibia. However, disease group differences in total CSA (-
1.6%, p=0.440), cortical CSA (2.15, p=0.229), and SSI (0.2%, p=0.920) were attenuated when adjusted for muscle function.

Table 6.7 - Modifying effect of disease group on the relationship between puberty and pQCT bone outcomes in participants with CF and controls (Model 1).

<table>
<thead>
<tr>
<th>Bone outcome measures</th>
<th>Participants with CF vs. Control</th>
<th>Model 1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Participants with CF (n=65)</td>
<td>Control (n=151)</td>
</tr>
<tr>
<td></td>
<td>Pre (n=25)</td>
<td>Early (n=21)</td>
</tr>
<tr>
<td>Puberty (n)</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>Pre</td>
<td>Early</td>
</tr>
<tr>
<td></td>
<td>(n=25)</td>
<td>(n=21)</td>
</tr>
<tr>
<td>pQCT (The distal 4% site of tibia)</td>
<td>Total CSA (mm²)</td>
<td>552.8 (±107.3)</td>
</tr>
<tr>
<td></td>
<td>Total vBMD (mg/cm³)</td>
<td>288.4 (±32.1)</td>
</tr>
<tr>
<td></td>
<td>Trab vBMD (mg/cm³)</td>
<td>181.8 (±27.9)</td>
</tr>
<tr>
<td>pQCT (The proximal 66% site of tibia)</td>
<td>Total CSA (mm²)</td>
<td>349.7 (±79.2)</td>
</tr>
<tr>
<td></td>
<td>Cortical CSA (mm²)</td>
<td>164.5 (±37.4)</td>
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<tr>
<td></td>
<td>Cortical vBMD (mg/cm³)</td>
<td>1059.4 (±40.3)</td>
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<tr>
<td></td>
<td>SSI (mm⁴)</td>
<td>1013.9 (±298.8)</td>
</tr>
</tbody>
</table>

Key: All values are mean (SD) unless indicated. Letters are used to indicate significance of group and pubertal stage differences as tested by ANCOVA/Scheffé. Puberty was based on Tanner staging (TS) and was categorised into three groups: pre-puberty = TS 1, early puberty = TS 2 and TS 3, and late puberty = TS 4 and TS 5. (SDS) Standard deviation score from the mean height and weight of an age- and sex matched control population. Cross-sectional area (CSA), volumetric bone mineral density (vBMD) and stress-strain index (SSI). Statistically significant when p<0.05 and is presented in bold and highlighted in grey.

a) Significant difference between pre-pubertal participants with CF and controls, P<0.05,
b) Significant difference between early pubertal participants with CF and controls, P<0.05,
c) Significant difference between late pubertal participants with CF and controls, P<0.05.
Table 6.8 - Modifying effect of disease group on the relationship between puberty and pQCT bone outcomes in participants with CF and controls (Model 2 - Height-adjusted and Model 3 - Disease group*Muscle function interactions).

<table>
<thead>
<tr>
<th>Outcome measure</th>
<th>MODEL 2</th>
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<tbody>
<tr>
<td></td>
<td>β (%)</td>
<td>SE</td>
<td>p value</td>
<td>Sig. interaction</td>
<td>p value</td>
<td>Sig. variables</td>
<td>R²</td>
<td>Graph Ref</td>
<td>β (%)</td>
<td>SE</td>
<td>p value</td>
<td>Sig. interaction</td>
<td>p value</td>
<td>Sig. variables</td>
<td>R²</td>
<td>Graph Ref</td>
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<td></td>
<td>of Grp</td>
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<td>of Grp</td>
<td>interaction</td>
<td>of interaction</td>
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<td></td>
<td>of Grp</td>
<td></td>
<td>of Grp</td>
<td>interaction</td>
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<tr>
<td>The distal (4%) site of the Tibia</td>
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<tr>
<td>Total CSA (mm²)</td>
<td>-3.2</td>
<td>1.9</td>
<td>0.86</td>
<td>– –</td>
<td>0.79</td>
<td>–</td>
<td></td>
<td></td>
<td>-0.8</td>
<td>2.0</td>
<td>0.672</td>
<td>– –</td>
<td></td>
<td>Age &amp; age² Ht &amp; Wt</td>
<td>0.81</td>
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<tr>
<td>Total vBMD (mg/cm³)</td>
<td>-5.9</td>
<td>1.8</td>
<td>0.001</td>
<td>– –</td>
<td>0.18</td>
<td>–</td>
<td></td>
<td></td>
<td>-1.3</td>
<td>2.0</td>
<td>0.502</td>
<td>– –</td>
<td></td>
<td>Age &amp; age² Ht Fmax &amp; Pmax</td>
<td>0.27</td>
<td>–</td>
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<tr>
<td>Trab vBMD (mg/cm³)</td>
<td>-7.8</td>
<td>2.1</td>
<td>0.001</td>
<td>– –</td>
<td>0.22</td>
<td>–</td>
<td></td>
<td></td>
<td>-0.5</td>
<td>2.3</td>
<td>0.823</td>
<td>– –</td>
<td></td>
<td>PS3 Age Ht Fmax &amp; Pmax</td>
<td>0.33</td>
<td>–</td>
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<tr>
<td>The proximal 66% site of the Tibia</td>
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<tr>
<td>Total CSA (mm²)</td>
<td>-9.9</td>
<td>1.9</td>
<td>0.001</td>
<td>– –</td>
<td>0.82</td>
<td>–</td>
<td></td>
<td></td>
<td>-1.6</td>
<td>2.0</td>
<td>0.440</td>
<td>– –</td>
<td></td>
<td>Sex Age Ht Fmax &amp; Pmax</td>
<td>0.84</td>
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<tr>
<td>Cortical CSA (mm²)</td>
<td>-4.1</td>
<td>1.7</td>
<td>0.019</td>
<td>– –</td>
<td>0.82</td>
<td>–</td>
<td></td>
<td></td>
<td>2.1</td>
<td>1.8</td>
<td>0.229</td>
<td>– –</td>
<td></td>
<td>Sex Ht Fmax &amp; Pmax</td>
<td>0.86</td>
<td>–</td>
<td></td>
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<tr>
<td>Cortical vBMD (mg/cm³)</td>
<td>-3.9</td>
<td>1.0</td>
<td>0.001</td>
<td>Grp<em>PS2 &lt;0.001 Grp</em>PS3 0.060</td>
<td>0.61</td>
<td>Figure 6.8</td>
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<td></td>
<td>-3.9</td>
<td>1.0</td>
<td>0.001</td>
<td>Grp<em>PS2 0.001 Grp</em>PS3 0.060</td>
<td></td>
<td>Age &amp; age²</td>
<td>0.61</td>
<td>–</td>
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<tr>
<td>SSI (mm)</td>
<td>-7.6</td>
<td>2.4</td>
<td>0.002</td>
<td>– –</td>
<td>0.83</td>
<td>–</td>
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<td>0.2</td>
<td>2.6</td>
<td>0.920</td>
<td>– –</td>
<td></td>
<td>Sex Ht Fmax &amp; Pmax</td>
<td>0.85</td>
<td>–</td>
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Key: β coefficients for disease group differences = participants with CF or for sex differences = females. Peripheral quantitative computed tomography (pQCT) outcome measures: Cross-sectional area (CSA), volumetric bone mineral density (vBMD) and stress-strain index (SSI). Model 2: Adjusted for explanatory variables including sex, group (Grp), age, quadratic age (age²), early puberty compared to pre-puberty (PS2), late puberty compared to pre-puberty (PS3), height (Ht) and weight (Wt). Model 3: Adjusted for model 2 and muscle force (Fmax) and muscle power (Pmax). Graph not presented in thesis (NP). Statistically significant when p<0.05 and is presented in bold and highlighted in grey. If a disease group-by-pubertal stage or disease group-by-Fmax/Pmax interaction was not found, then the interaction was removed from the model and the β coefficient (i.e. disease group, pmax and Fmax) was interpreted alone. * denotes a significant interaction.
6.4.4 The muscle-bone unit - The 8% site of the tibia using HR-pQCT

6.4.4.1 The results of model 1 analyses

The effects of disease group on the relationship between puberty and HR-pQCT bone outcomes after **Model 1** analyses (i.e. disease group, pubertal stage, and disease group-by-pubertal stage interaction) are presented in Table 6.9.

As shown in Table 6.9, disease group did modify the relationship between puberty and HR-pQCT bone outcomes. With increasing maturation, participants with CF had lower apparent gains in total vBMD (D100) (p=0.0083), trabecular BV/TV (p<0.0001), Tb.N (P=0.0468), and Tb.Th (p=0.0015) compared to controls. There were no significant disease group-by-pubertal stage interactions for Tt.Ar, Ct.Ar, Ct.BMD, Ct.TMD, and Ct.Po. Post hoc comparisons showed that participants with CF had lower total vBMD (D100), trabecular BV/TV, Tb.N, and Tb.Th in late puberty compared to controls.

6.4.4.2 The results of Models 2 (Height-adjusted) and 3 (Disease group*Muscle function interactions)

The effects of disease group on the relationship between puberty and HR-pQCT bone outcomes after **Model 2** (i.e. disease group, sex, pubertal stage, sex-by-pubertal stage interaction, and disease group-by-pubertal stage interaction) and **Model 3** (i.e. disease group, sex, pubertal stage, sex-by-pubertal stage interaction, disease group-by-pubertal stage interaction, disease group-by-Pmax stage interaction, and disease group-by-Fmax stage interaction) are presented in Table 6.10.

As shown in Table 6.10 - **Model 2**, disease group did modify the relationship between puberty and HR-pQCT bone outcomes for BV/TV, Ct.Po, and Tb.Th (Figure 6.9). Disease group did not modify the relationship between puberty and BV/TV (0.1%, p=0.431), Ct.Po (0.3%, p=0.190), and Tb.Th (9.0%, p=0.220) in pre-to-early puberty but participants with CF showed lower apparent gains in BV/TV (-0.4%, p<0.001), Ct.Po (-0.6%, p=0.020), and Tb.Th (-20.3%, p=0.016) in pre-to-late puberty compared to controls. There were no significant disease group-by-pubertal stage interactions for Tt.Ar, Ct.Ar, D100, Ct.BMD, Ct.TMD, and Tb.N outcomes. There were disease group differences in Tt.Ar, Ct.Ar, and D100, where participants with CF had consistently lower Tt.Ar (-7.7%, p=0.011), Ct.Ar (-10.2%, p=0.002) and D100 (-8.3%, p=0.012) compared to controls, across all stages of puberty. There were no significant disease group differences for Ct.BMD, Ct.TMD, and Tb.N outcomes (Table 6.10).
As shown in Table 6.10 – Model 3, disease group did modify the relationship between muscle function and HR-pQCT outcomes for D100, trabecular BV/TV, and Tb.N. With increasing Fmax, participants with CF had lower apparent gains in D100 (-25.3%, p=0.015), trabecular BV/TV (-1.1%, P<0.001), and Tb.N (-59.7%, p=0.003) compared to controls (Figure 6.10). There were no significant disease group-by-Fmax interactions for Tt.Ar, Ct.Ar, Ct.BMD, Ct.TMD, Ct.Po, or Tb.Th. With increasing Pmax, participants with CF showed higher apparent gains in trabecular BV/TV (0.6%, p=0.002) and Tb.N (32.2%, p=0.040) compared to controls. There were no significant disease group-by-Pmax interactions for Tt.Ar, Ct.Ar, D100, Ct.BMD, Ct.TMD, Ct.Po, or Tb.Th. Participants with CF had consistently lower Tt.Ar (-7.9%, p=0.009) compared to controls, across all stages of puberty (Table 6.10). There was no significant disease group differences in Ct.Ar (-3.0%, p=0.323), Ct.BMD (-0.1%, P=0.917), Ct.TMD (0.3%, p=0.742), Ct.Po (-0.05%, p=0.591), and Tb.Th (-10.2%, p=0.102) across all stages of puberty.

Table 6.9 - Modifying effect of disease group on the relationship between puberty and HR-pQCT bone outcomes in participants with CF and controls (Model 1).

<table>
<thead>
<tr>
<th>Bone outcome measures</th>
<th>Participants with CF (n=65)</th>
<th>Participants with CF vs. Control</th>
<th>Control (n=151)</th>
<th>P value of disease grp-by-PS interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre (n=5)</td>
<td>Early (n=14)</td>
<td>Late (n=6)</td>
<td>Pre (n=33)</td>
</tr>
<tr>
<td>Tt.Ar (cm²)</td>
<td>403.2 ±47.0</td>
<td>480.8 ±152.8</td>
<td>497.6 ±100.5</td>
<td>446.5 (±87.6)</td>
</tr>
<tr>
<td>Ct.Ar (cm²)</td>
<td>63.8 ±5.4</td>
<td>85.3 ±19.2</td>
<td>93.2 ±17.8</td>
<td>75.3 ±11.2</td>
</tr>
<tr>
<td>D100 (mg/cm³)</td>
<td>239.8 ±23.1</td>
<td>269.4 ±34.3</td>
<td>258.1 ±39.1</td>
<td>260.2 ±30.1</td>
</tr>
<tr>
<td>BV/TV (mg/cm³)</td>
<td>0.14 ±0.02</td>
<td>0.14 ±0.02</td>
<td>0.10 ±0.04</td>
<td>0.14 ±0.02</td>
</tr>
<tr>
<td>Ct.BMD (mg/cm³)</td>
<td>749.3 ±65.2</td>
<td>759.8 ±34.4</td>
<td>909.4 ±97.6</td>
<td>754.9 ±28.1</td>
</tr>
<tr>
<td>Ct.TMD (mg/cm³)</td>
<td>807.0 ±51.0</td>
<td>827.4 ±30.4</td>
<td>934.6 ±76.8</td>
<td>813.6 ±23.1</td>
</tr>
<tr>
<td>Ct.Po (%)</td>
<td>35.4 ±16.8</td>
<td>57.9 ±23.8</td>
<td>20.9 ±22.3</td>
<td>45.2 ±17.5</td>
</tr>
<tr>
<td>Tb.N (mm-1)</td>
<td>2.25 ±0.3</td>
<td>2.20 ±0.3</td>
<td>1.89 ±0.3</td>
<td>2.15 ±0.25</td>
</tr>
<tr>
<td>Tb.Th (mm)</td>
<td>0.06 ±0.01</td>
<td>0.07 ±0.01</td>
<td>0.05 ±0.01</td>
<td>0.07 ±0.01</td>
</tr>
</tbody>
</table>

Key: All values are mean (SD) unless indicated. Letters are used to indicate significance of group and pubertal stage differences as tested by ANCOVA/Scheffé. Puberty was based on Tanner staging (TS) and was categorised into three groups: pre-puberty = TS 1, early puberty = TS 2 and TS 3, and late puberty = TS 4 and TS 5. (SDS) Standard deviation score from the mean height and weight of an age-and-sex matched control population. Total area (Tt.Ar), cortical area (Ct.Ar), total bone mineral density (D100), cortical bone mineral density (Ct.BMD), cortical tissue mineral density (Ct.TMD), cortical porosity (Ct.Po), and trabecular number (Tb.N) and thickness (Tb.Th). Statistically significant when p<0.05 and is presented in bold and highlighted in grey.

a Significant difference between pre-pubertal participants with CF and controls, P<0.05.
b Significant difference between early pubertal participants with CF and controls, P<0.05.
c Significant difference between late pubertal participants with CF and controls, P<0.05.
Table 6.10 - Modifying effect of disease group on the relationship between puberty and HR-pQCT bone outcomes in participants with CF and controls (Model 2 - Height-adjusted and Model 3 - Disease group*Muscle function interactions).

<table>
<thead>
<tr>
<th>Outcome measures</th>
<th>MODEL 2</th>
<th>MODEL 3</th>
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<tbody>
<tr>
<td></td>
<td>β (%) of Grp interaction</td>
<td>SE</td>
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<tr>
<td><strong>Area</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tl.Ar (cm²)</td>
<td>-7.7</td>
<td>3.0</td>
</tr>
<tr>
<td>Cl.Ar (cm²)</td>
<td>-10.2</td>
<td>3.2</td>
</tr>
<tr>
<td><strong>Density</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D100 (mg/cm³)</td>
<td>-8.3</td>
<td>3.3</td>
</tr>
<tr>
<td>BV/TV (mg/cm³)</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Ct.BMD (mg/cm³)</td>
<td>-0.6</td>
<td>1.1</td>
</tr>
<tr>
<td>Ct.TMD (mg/cm³)</td>
<td>-0.2</td>
<td>0.8</td>
</tr>
<tr>
<td>Ct.Po (%)</td>
<td>0.3</td>
<td>0.2</td>
</tr>
<tr>
<td><strong>Micro-architecture</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tb.N (mm-1)</td>
<td>-0.6</td>
<td>2.6</td>
</tr>
<tr>
<td>Tb.Th (mm)</td>
<td>9.0</td>
<td>7.2</td>
</tr>
</tbody>
</table>

Key: β coefficients for disease group differences = participants with CF or for sex differences = females. High-resolution peripheral quantitative computed tomography (HR-pQCT) outcome measures: Total and cortical area (Tl.Ar and Cl.Ar), total and trabecular vBMD (D100 and BV/TV), cortical vBMD and tissue mineral density (Ct.BMD and Ct.TMD), cortical porosity (Ct.Po) and trabecular number and thickness (Tb.N and Tb.Th). Model 2: Adjusted for explanatory variables including sex, group (Grp), age, quadratic age (Age²), early puberty compared to pre-puberty (PS2), late puberty compared to pre-puberty (PS3), height (Ht) and weight (Wt). Model 3: Adjusted for model 2 and muscle force, Newtons (Fmax, N) and muscle power, Watts (Pmax, W). Graph not presented in thesis (NP). Statistically significant when p<0.05 and is presented in bold and highlighted in grey. If a disease group-by-pubertal stage or disease group-by-Fmax/Pmax interaction was not found, then the interaction was removed from the model and the β coefficient (i.e. disease group, pmax and Fmax) was interpreted alone. * denotes a significant interaction.
Figure 6.9 - The modifying effects of disease group on the relationship between puberty and HR-pQCT bone outcomes at the distal 8% site of the tibia in participants with CF and controls (Model 2 - Height-adjusted).

Key: Model 2 analyses included disease group (Grp), sex, age, quadratic age ($age^2$), height (Ht), weight (Wt), sex-by-pubertal stage interaction, and disease group-by-pubertal stage interactions. High-resolution peripheral quantitative computed tomography (HR-pQCT), trabecular bone volume to tissue volume (BV/TV), cortical porosity (Ct.Po), trabecular thickness (Tb.Th), and participants with cystic fibrosis (CF).
Figure 6.10 - The modifying effects of disease group on the relationship between Fmax and HR-pQCT bone outcomes at the distal 8% site of the tibia in participants with CF and controls (Model 3 - Disease group*Muscle function interactions).

**Key:** Model 3 analyses included disease group (Grp), sex, age, quadratic age (age²), height (Ht), weight (Wt), Pmax, Fmax, sex-by-pubertal stage interaction, disease group-by-pubertal stage interactions, disease group-by-Pmax interaction, and disease group-by-Fmax interaction. High-resolution peripheral quantitative computed tomography (HR-pQCT), maximum muscle force, Newtons (Fmax, N), total volumetric bone density (D100), cortical porosity (Ct.Po), trabecular thickness (Tb.Th) and participants with cystic fibrosis (CF). Fmax: Lower quantile = 0.59 – 1.23 N and upper quantile = 1.23 – 2.65 N.)
6.4.5 Summary

The following summary statements are based on height-adjusted analyses.

**DXA**

- With increasing pubertal maturation, participants with CF had lower apparent gains in bone area (BA) and bone mineral content (BMC) compared to controls *(Table 6.6 and Figure 6.5)*.

- With increasing muscle force (Fmax), participants with CF showed lower apparent gains in bone mineral content (BMC) and bone area (BA), and trends towards lower apparent gains in area-adjusted bone mineral content (SA-BMC) with increasing muscle power (Pmax) *(Table 6.6, Figure 6.6, and Figure 6.7)*.

- The difference in DXA measurements between participants with CF and controls are illustrated in *Figure 6.11*.

**pQCT**

- At the distal 4% site of the tibia, disease group did not affect the relationship between puberty and pQCT bone outcomes. Participants with CF had consistently lower total bone density (total vBMD) and trabecular bone density (trab vBMD), across all stages of puberty *(Table 6.8)*.

- At the proximal 66% site of the tibia, with increasing pubertal maturation, participants with CF had lower apparent gains in cortical bone density (vBMD) compared to controls *(Table 6.8 and Figure 6.8)* and also had consistently lower total CSA, cortical CSA and bone strength (SSI) compared to controls, across all puberty stages *(Table 6.8)*.

- Disease group did not modify the relationship between muscle function and bone outcomes at the distal 4% or proximal 66% sites of the tibia *(Table 6.8)*.

- The difference in pQCT measurements between participants with CF and controls illustrated in *Figure 6.12*.

**HR-pQCT**

- At the distal 8% site of the tibia, with increasing pubertal maturation, participants with CF had lower apparent gains in cortical porosity (Ct.Po), trabecular density (BV/TV), and
trabecular thickness (Tb.Th) compared to controls. Participants with CF also had consistently lower total bone area (Tt.Ar), cortical area (Ct.Ar), and density (D100) compared to controls, across all stages of puberty (Table 6.10).

- Disease group did modify the relationship between muscle function (i.e. Fmax and Pmax) and HR-pQCT bone outcomes. With increasing muscle force (Fmax), participants with CF had lower apparent gains in total density (D100), trabecular density (BV/TV), and trabecular number (Tb.N) compared to controls (Figure 6.10). With increasing muscle power (Pmax), participants with CF had higher apparent gains in trabecular density (BV/TV), and trabecular number (Tb.N) compared to controls (Table 6.10).

- The difference in HR-pQCT measurements between participants with CF and controls are illustrated in Figure 6.13.
Figure 6.11 - DXA scans of participants with CF compared to controls.
Males are taller, heavier, have a larger skeleton (higher BA) compared to females. Participants with CF are generally shorter, have a smaller skeleton (low BA) and less BMC compared to controls. Artefacts have been painted out in male participant with CF.
Figure 6.12 - Scout view and pQCT scans (distal 4% and proximal 66% sites of the tibia) of participants with CF compared to controls.
Participants with CF have smaller bones and less vBMD compared to controls.
Figure 6.13 - Group differences at the distal 8% site of the tibia in participants with CF compared to controls using HR-pQCT.
Participants with CF have less trabecular BV/TV compared to controls. These scans have not been size adjusted.
6.5 The effects of lung function, 25(OH) vitamin D status, and genotype on bone, and muscle outcomes in participants with cystic fibrosis

**Aim**

The aim was to explore the relationship between clinical characteristics (i.e. forced expiratory volume-one second [FEV1], 25(OH) vitamin D status, and genotype) and bone and muscle outcomes in participants with CF.

**Hypotheses**

The hypotheses are that there will be:

1) A positive association between FEV1 and bone and muscle outcomes. Participants with higher FEV1 status have wider bones, with more bone mineral, and higher muscle function (Fmax and Pmax) compared to participants with lower FEV1 status.

2) A positive association between 25(OH)D and bone and muscle outcomes. Participants with higher 25(OH)D status have wider bones, with more bone mineral, and higher muscle function (Fmax and Pmax) compared to participants with lower 25(OH)D status.

3) A negative association between homozygous genotype and bone and muscle outcomes. Participants who are homozygous for the DeltaF508 genotype will have narrower bones, with less mineral, and lower muscle function (Fmax and Pmax) compared to participants who have a heterozygous genotype.

6.5.1 Analyses

The secondary objectives of this study were to explore the relationships between clinical characteristics and bone, muscle, body composition, and muscle function outcomes in participants with CF. Clinical covariates, which include lung function (FEV1), vitamin D [25(OH)D] status, and CF genotype, were selected based on scrutiny of the current literature. The justification of these covariates is briefly described below:

**FEV1** – In CF, FEV1 deteriorates as a result of chronic pulmonary infection and is used as a clinical indicator of disease severity. Inflammation has been shown to increase bone turnover and cause sarcopenia as a result of reduced physical activity and long periods of bedrest. Therefore, FEV1 may disrupt the muscle-bone unit in CF.

**25(OH)D** – Vitamin D deficiency is common in CF and is known to disrupt calcium absorption and bone mineralisation. Reduced mineralisation will result in bone softening and reduced bone strength.
**Genotype** - The most common CF genotype is delta F508, where mutations are translated into full-length nascent polypeptide chains but are defective in folding. Therefore, these chains are targeted for degradation rather than trafficked to the plasma membrane. Human and animal studies have shown that the CFTR mutation is present in all bone cells and is likely to be present in muscle cells.

FEV1 and 25(OH)D status are shown to be associated with CF-related bone disease in adults but whether these clinical covariates effect bone and muscle outcomes in children and adolescents with CF are unclear. The effects of genotype in bone and muscle also remain unclear. This study will help to understand whether these covariates influence the muscle-bone unit in children and adolescents with CF.

Clinical characteristics, bone and muscle outcomes were transformed into natural logs and were assessed for normality. Primary outcomes for clinical characteristics were extracted, with permission, from patient’s medical notes and included: lung function [FEV1], vitamin D status [25(OH)D], and CF genotype. Primary bone outcomes were selected after main study analyses in Chapter 6 and were measured using DXA at the total body less head site (TBLH size adjusted BMC [TBLH SA-BMC] and TBLH BA), pQCT at the distal 4% and proximal 66% sites of the tibia (total bone CSA, total vBMD, cortical CSA, cortical vBMD, trabecular vBMD and SSI), and HR-pQCT at the distal 8% site of the distal tibia (Tt.Ar, Ct.Ar, D100, Ct.BMD, Ct.TMD, Ct.Po, BV/TV, Tb.N and Tb.Th). Primary outcomes for muscle mass and function were measured using DXA (lean-to-fat mass ratio), pQCT muscle density and using jumping mechanography of the lower limbs (Pmax and Fmax).

Analyses were performed using Rstudio® (Version 3.3.1) statistical package. In summary, the analysis models were: **Model 1** (clinical characteristic, sex, and pubertal stage) using multiple linear regression to test whether the clinical characteristic had a relationship with bone and muscle outcomes, and then **Model 2** (clinical characteristic, sex, pubertal stage, age, quadratic age, height, and weight) using multiple linear regression to test whether the clinical characteristic had a relationship with bone and muscle outcomes after adjusting for height. The exploratory covariates included in these models have been listed in Table 6.11. Genotype was categorised into two groups; 1) homozygous for delta F508, and 2) heterozygous for delta F508. Four patients with other genotypes were excluded. To assess TBLH SA-BMC, TBLH BA was retained in the model for TBLH BMC to adjust for body size. In model 2, height was retained in the model analyses for all measurements to adjust for differences in body size during growth. Weight was not included in the model for lean-to-fat mass ratio. Data are presented as mean, standard deviation, beta-coefficient (%) and p-value, with the significance level set to p<0.05.
Table 6.11 - Summary of the exploratory covariates used in the multiple linear regression

<table>
<thead>
<tr>
<th>Model 1</th>
<th>Covariates in multiple linear regression models</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical characteristic</td>
<td>Sex</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Model 2</th>
<th>Covariates in multiple linear regression models</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical characteristic</td>
<td>Sex</td>
</tr>
</tbody>
</table>

Key: Clinical covariates (FEV1, 25(OH)D status, and genotype) were tested individually i.e. only one clinical covariate in the model at one time. Covariates in bold font were ‘forced’ into all models whether significant or not, whereas covariates in regular font were only included in the final model if significant.

6.5.2 The relationship between FEV1 and bone and muscle outcomes

6.5.2.1 The results of Model 1 analyses

The relationship between FEV1 and bone, body composition and muscle function outcomes after Model 1 analyses (i.e. FEV1, sex, and pubertal stage) are presented in Table 6.12.

DXA (Total body) – There were no relationships between FEV1 and any of the DXA bone outcomes.

pQCT (Distal 4% and proximal 66% sites of the tibia) – At the distal 4% site, the data showed that there were positive relationships between FEV1 and total vBMD (16.2%, p=0.005) and trabecular vBMD (22.4%, p=0.010). There were no relationships between FEV1 and 4% total CSA, 66% total CSA, 66% cortical CSA, and 66% SSI.

HR-pQCT (Distal 8% site of the tibia) – The data showed that there was a positive relationship between FEV1 and trabecular BV/TV (38.6%, p=0.021). There were no relationships between FEV1 and total area, total density (D100), Tb.N or Tb.Th.

Jumping mechanography (Lower body) – There were no significant relationships between FEV1 and muscle function (i.e. Fmax and Pmax).

Body composition (Total body and the proximal 66% site of the tibia) - There were no significant relationships between FEV1 and body composition (i.e. lean-to-fat mass and muscle density).
6.5.2.2 The results of Model 2 analyses (i.e. Height-adjusted)

The relationship between FEV1 and bone, body composition and muscle function outcomes after Model 2 analyses (i.e. 25(OH)D status, sex, pubertal stage, age, quadratic age, height, and weight) are presented in Table 6.12.

DXA (Total body) – There were no relationships between FEV1 and all DXA bone outcomes.

pQCT (Distal 4% and proximal 66% sites of the tibia) – At the distal 4% site, similarly to model 1, there were positive relationships between FEV1 and total vBMD (16.2%, p=0.009) and trabecular vBMD (18.2%, p=0.03) after accounting for height. There were no relationships between FEV1 and 4% total CSA, 66% total CSA, 66% cortical CSA, and 66% SSI.

HR-pQCT (Distal 8% site of the tibia) – The relationship between FEV1 and trabecular BV/TV was attenuated after accounting for body size. There were no relationships between FEV1 and total area, total density (D100), Tb.N or Tb.Th.

Jumping mechanography (Lower body) – There were no significant relationships between FEV1 and muscle function (i.e. Fmax and Pmax).

Body composition (Total body and the proximal 66% site of the tibia) - There were no significant relationships between FEV1 and body composition (i.e. lean-to-fat mass and muscle density).

6.5.3 The relationship between 25(OH)D status and bone and muscle outcomes

6.5.3.1 Results of Model 1 analyses

The relationship between 25(OH)D status and bone, body composition and muscle function outcomes after Model 1 analyses (i.e. 25(OH)D status, sex, and pubertal stage) are presented in Table 6.13.

DXA (Total body) – At the total body site, there was a positive relationship between 25(OH)D status and SA-BMC (5.4%, p=0.046) but negative relationships between 25(OH)D status and BMC (-14.4%, p=0.033) and BA (-15.8%, p=0.002).

pQCT (Distal 4% and proximal 66% sites of the tibia) – At the distal 4% site, the data showed that were positive relationships between 25(OH)D status and total vBMD (5.9%, p=0.032) and trabecular vBMD (10.5%, p=0.013) but a negative relationship between 25(OH)D status and total CSA (-11%, P=0.035) existed. At the proximal 66% site, the data showed that there were negative relationships
between 25(OH)D status and total CSA (-11.5%, p=0.019) and SSI (-19.5%, p=0.003). There was no relationship between 25(OH)D status and cortical CSA.

**HR-pQCT (Distal 8% site of the tibia)** – The data showed that was a negative relationship between 25(OH)D status and total area (-21.9%, p=0.023). There were no relationships between 25(OH)D status and total density (D100), trabecular BV/TV, Tb.N or Tb.Th.

**Jumping mechanography (Lower body)** – There were no significant relationships between 25(OH)D status and muscle function (i.e. Fmax and Pmax).

**Body composition (Total body and the proximal 66% site of the tibia)** - There were no significant relationships between 25(OH)D status and body composition (i.e. lean-to-fat mass and muscle density).

### 6.5.3.2 Results of Model 2 analyses (i.e. Height-adjusted)

The relationship between 25(OH)D status and bone, body composition and muscle function outcomes after Model 2 analyses (i.e. 25(OH)D status, sex, pubertal stage, age, quadratic age, height, and weight) are presented in Table 6.13.

**DXA (Total body)** – At the total body site, the positive relationship between 25(OH)D status and SA-BMC (4.1%, p=0.04) remained after accounting for height. However, the negative relationships between 25(OH)D status and BMC and BA were attenuated.

**pQCT (Distal 4% and proximal 66% sites of the tibia)** – At the distal 4% site, the data showed that the positive relationships between 25(OH)D status and total vBMD (5.8%, p=0.045) and trabecular vBMD (8.4%, p=0.047) remained, after accounting for height. However, the negative relationship between 25(OH)D status and total CSA was attenuated after model 2 analyses. At the proximal 66% site, the data showed that all negative relationships between 25(OH)D status and total CSA and SSI were attenuated after model 2 analyses. There was no relationship between 5(OH)D status and cortical CSA.

**HR-pQCT (Distal 8% site of the tibia)** – The data the negative relationship between 25(OH)D status and total area was attenuated after accounting for height. There were no relationships between 25(OH)D status and total density (D100), trabecular BV/TV, Tb.N or Tb.Th.

**Jumping mechanography (Lower body)** – A significant relationship between 25(OH)D status and Fmax, where Fmax was positively associated with 25(OH)D status (8.1%, p=0.021) emerged after model 2 analyses. There was no significant relationship between 25(OH)D status and Pmax.
Body composition (Total body and the proximal 66% site of the tibia) – There were no significant relationships between 25(OH)D status and body composition (i.e. lean-to-fat mass and muscle density).

6.5.4  The relationship between genotype and bone and muscle outcomes

6.5.4.1  Results of Model 1 and 2 (i.e. Height-adjusted) analyses

The relationship between CF genotype and bone, body composition and muscle function outcomes after Model 1 analyses (i.e. genotype, sex, and pubertal stage) and Model 2 analyses (i.e. Genotype, sex, pubertal stage, age, quadratic age, height, and weight are presented in Table 6.14.

DXA (Total body) – At the total body site, there were no significant relationships between genotype and all DXA bone outcomes, after adjusting for models 1 and 2.

pQCT (Distal 4% and proximal 66% sites of the tibia) – There were no significant relationships between genotype and all pQCT bone outcomes, after adjusting for models 1 and 2.

HR-pQCT (Distal 8% site of the tibia) – There were no significant relationships between genotype and all HR-pQCT bone outcomes, after adjusting for models 1 and 2.

Jumping mechanography (Lower body) – There were no significant relationships between genotype and muscle function (i.e. Fmax and Pmax), after adjusting for models 1 and 2.

Body composition (Total body and the proximal 66% site of the tibia) – Participants with a heterozygous genotype had a higher muscle density after adjusting for model 1 (1.7%, p=0.020) and model 2 (1.7%, p=0.023) compared to those with homozygous genotype. There were no relationships between genotype and all other bone, body composition and muscle function outcomes. There was no significant relationship between genotype and lean-to-fat mass ratio, after adjusting for models 1 and 2.
Table 6.12 - Relationships between lung function (FEV1) and bone and muscle outcomes in participants with CF (Model 1 and Model 2 – Height-adjusted).

<table>
<thead>
<tr>
<th>Outcome measures</th>
<th>Forced Expiratory Volume 1 (FEV1)</th>
<th></th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Model 1</td>
<td>Model 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>n= 65 participants with CF</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>β (%)</td>
<td>SE</td>
<td>p value</td>
<td>Sig. variables</td>
<td>R²</td>
<td>β (%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>DXA (Total body less head)</strong></td>
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<td></td>
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<tr>
<td>BMC</td>
<td>-9.3</td>
<td>16.3</td>
<td>0.571</td>
<td>PS2 &amp; PS3</td>
<td>0.51</td>
<td>6.6</td>
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<tr>
<td>SA-BMC</td>
<td>2.8</td>
<td>5.9</td>
<td>0.640</td>
<td>PS2 &amp; PS3</td>
<td>0.94</td>
<td>2.5</td>
</tr>
<tr>
<td>BA</td>
<td>-9.9</td>
<td>12.5</td>
<td>0.432</td>
<td>PS3</td>
<td>0.39</td>
<td>3.4</td>
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<tr>
<td><strong>pQCT (The distal 4% and proximal 66% sites of the tibia)</strong></td>
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<td>Total CSA (4%)</td>
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<td>PS2 &amp; PS3</td>
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<td>0.3</td>
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<td>Total BVMD (4%)</td>
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<td>5.6</td>
<td>0.005</td>
<td>-</td>
<td>0.16</td>
<td>16.2</td>
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<tr>
<td>Trab vBMD (4%)</td>
<td>22.4</td>
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<td>PS3</td>
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<td>18.2</td>
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<tr>
<td>Total CSA (66%)</td>
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<td>12.3</td>
<td>0.860</td>
<td>Sex</td>
<td>PS2 &amp; PS3</td>
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<td>Cortical CSA (66%)</td>
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<td>12.3</td>
<td>0.904</td>
<td>Sex</td>
<td>PS2 &amp; PS3</td>
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<td>SSI (66%)</td>
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<td>0.761</td>
<td>Sex</td>
<td>PS2 &amp; PS3</td>
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<td><strong>HR-pQCT (The distal 8% of the tibia) n= 25</strong></td>
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<tr>
<td>Total area</td>
<td>-34.2</td>
<td>19.6</td>
<td>0.095</td>
<td>Sex</td>
<td>0.21</td>
<td>-1.0</td>
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<tr>
<td>D100</td>
<td>9.7</td>
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<td>0.413</td>
<td>-</td>
<td>0.03</td>
<td>9.9</td>
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<tr>
<td>BV/TV</td>
<td>38.6</td>
<td>15.5</td>
<td>0.021</td>
<td>Sex</td>
<td>0.48</td>
<td>22.6</td>
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<td>Tb.N</td>
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<td>11.7</td>
<td>0.084</td>
<td>-</td>
<td>0.19</td>
<td>7.7</td>
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<tr>
<td>Tb.Th</td>
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<td>11.9</td>
<td>0.166</td>
<td>Sex</td>
<td>0.39</td>
<td>14.0</td>
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<tr>
<td><strong>Jumping mechanography n= 65</strong></td>
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<tr>
<td>Pmax</td>
<td>-7.2</td>
<td>17.9</td>
<td>0.688</td>
<td>Sex</td>
<td>PS2 &amp; PS3</td>
<td>0.43</td>
</tr>
<tr>
<td>Fmax</td>
<td>-13.8</td>
<td>14.7</td>
<td>0.353</td>
<td>PS2 &amp; PS3</td>
<td>0.39</td>
<td>-5.4</td>
</tr>
<tr>
<td><strong>Body composition n= 65</strong></td>
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<tr>
<td>Lean-to-fat ratio</td>
<td>-17.0</td>
<td>33.6</td>
<td>0.614</td>
<td>Sex</td>
<td>PS2</td>
<td>0.22</td>
</tr>
<tr>
<td>Muscle density</td>
<td>-1.3</td>
<td>1.5</td>
<td>0.397</td>
<td>PS2</td>
<td>0.11</td>
<td>-2.1</td>
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</tbody>
</table>

Key: β coefficient = FEV1. Model 1 analyses included FEV1, sex, and pubertal stage. Model 2 analyses included FEV1, sex, age, quadratic age (age2), pubertal stage, height (Ht) and weight (Wt). Dual-energy X-ray absorptiometry (DXA), peripheral quantitative computed tomography (pQCT) and high resolution QCT (HR-pQCT). Outcome measures: Total body less head size adjusted BMC (TBLH SA-BMC), Total body less head bone area (TBLH BA), cross-sectional area (CSA), Stress-strain index (SSI), total and trabecular vBMD (D100, mg HA/cm³ and BV/TV), trabecular number and thickness (Tb.N, mm-1 and Tb.Th, mm) and maximum muscle force (Fmax) and power (Pmax). Statistically significant when p<0.05 and is presented in bold and highlighted in grey.
Table 6.13 - Relationships between 25(OH)D status and bone and muscle outcomes in participants with CF (Model 1 and Model 2 – Height-adjusted).

<table>
<thead>
<tr>
<th>Outcome measures</th>
<th>25(OH)D Status</th>
<th>Model 1</th>
<th>Model 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n= 60 participants with CF</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>β (%)</td>
<td>SE</td>
<td>p value</td>
</tr>
<tr>
<td>DXA (Total body less head)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMC</td>
<td>-14.4</td>
<td>6.6</td>
<td>0.033</td>
</tr>
<tr>
<td>SA-BMC</td>
<td>5.4</td>
<td>2.7</td>
<td>0.046</td>
</tr>
<tr>
<td>BA</td>
<td>-15.8</td>
<td>4.9</td>
<td>0.002</td>
</tr>
<tr>
<td>pQCT (The distal 4% and proximal 66% sites of the tibia)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total CSA (4%)</td>
<td>-11.0</td>
<td>5.1</td>
<td>0.035</td>
</tr>
<tr>
<td>Total vBMD (4%)</td>
<td>5.9</td>
<td>2.7</td>
<td>0.032</td>
</tr>
<tr>
<td>Trab vBMD (4%)</td>
<td>10.5</td>
<td>4.1</td>
<td>0.013</td>
</tr>
<tr>
<td>Total CSA (66%)</td>
<td>-11.5</td>
<td>4.8</td>
<td>0.019</td>
</tr>
<tr>
<td>Cortical CSA (66%)</td>
<td>-9.4</td>
<td>5.0</td>
<td>0.065</td>
</tr>
<tr>
<td>SSI (66%)</td>
<td>-19.5</td>
<td>6.4</td>
<td>0.003</td>
</tr>
<tr>
<td>HR-pQCT (The distal 8% of the tibia)</td>
<td>n= 25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total area</td>
<td>-21.9</td>
<td>8.9</td>
<td>0.023</td>
</tr>
<tr>
<td>D100</td>
<td>2.9</td>
<td>5.5</td>
<td>0.599</td>
</tr>
<tr>
<td>BV/TV</td>
<td>8.8</td>
<td>8.3</td>
<td>0.298</td>
</tr>
<tr>
<td>Tb.N</td>
<td>0.3</td>
<td>6.0</td>
<td>0.966</td>
</tr>
<tr>
<td>Tb.Th</td>
<td>8.9</td>
<td>5.5</td>
<td>0.125</td>
</tr>
<tr>
<td>Jumping mechanography</td>
<td>n= 65</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pmax</td>
<td>-10.5</td>
<td>7.4</td>
<td>0.163</td>
</tr>
<tr>
<td>Fmax</td>
<td>-3.8</td>
<td>6.3</td>
<td>0.546</td>
</tr>
<tr>
<td>Body composition</td>
<td>n= 65</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lean-to-fat ratio</td>
<td>1.6</td>
<td>13.5</td>
<td>0.908</td>
</tr>
<tr>
<td>Muscle density</td>
<td>0.8</td>
<td>0.7</td>
<td>0.285</td>
</tr>
</tbody>
</table>

Key: β coefficient = 25(OH)D status. Model 1 analyses included 25(OH)D status, sex, and pubertal stage. Model 2 analyses included 25(OH)D status, sex, age, quadratic age (age²), pubertal stage, height (HT) and weight (WT). Dual-energy X-ray absorptiometry (DXA), peripheral quantitative computed tomography (pQCT) and high resolution pQCT (HR-pQCT). Outcome measures: Total body less head size adjusted BMC (TBLH SA-BMC), Total body less head bone area (TBLH BA), cross-sectional area (CSA), Stress-strain index (SSI), total and trabecular vBMD (D100, mg HA/cm³ and BV/TV), trabecular number and thickness (Tb.N, mm-1 and Tb.Th, mm) and maximum muscle force (Fmax) and power (Pmax). Statistically significant when p<0.05 and is presented in bold and highlighted in grey.
Table 6.14 - Relationships between genotype and bone and muscle outcomes in participants with CF (Model 1 and Model 2 – Height-adjusted).

<table>
<thead>
<tr>
<th>Outcome measures</th>
<th>Genotype</th>
<th>Model 1</th>
<th>Model 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n= 65 participants with CF</td>
<td>β (%)</td>
<td>SE</td>
</tr>
<tr>
<td><strong>DXA (Total body less head)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMC</td>
<td>4.0</td>
<td>8.3</td>
<td>0.628</td>
</tr>
<tr>
<td>SA-BMC</td>
<td>-0.3</td>
<td>3.0</td>
<td>0.909</td>
</tr>
<tr>
<td>BA</td>
<td>3.5</td>
<td>6.2</td>
<td>0.572</td>
</tr>
<tr>
<td><strong>pQCT (The distal 4% and proximal 66% sites of the tibia)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total CSA (4%)</td>
<td>0.7</td>
<td>6.3</td>
<td>0.914</td>
</tr>
<tr>
<td>Total vBMD (4%)</td>
<td>-2.5</td>
<td>3.3</td>
<td>0.443</td>
</tr>
<tr>
<td>Trab vBMD (4%)</td>
<td>-0.8</td>
<td>4.8</td>
<td>0.861</td>
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<tr>
<td>Total CSA (66%)</td>
<td>2.1</td>
<td>6.3</td>
<td>0.741</td>
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<tr>
<td>Cortical CSA (66%)</td>
<td>0.2</td>
<td>6.3</td>
<td>0.969</td>
</tr>
<tr>
<td>SSI (66%)</td>
<td>4.8</td>
<td>8.5</td>
<td>0.576</td>
</tr>
<tr>
<td><strong>HR-pQCT (The distal 8% of the tibia) n= 25</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total area</td>
<td>14.7</td>
<td>11.2</td>
<td>0.203</td>
</tr>
<tr>
<td>D100</td>
<td>-1.0</td>
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<td>0.875</td>
</tr>
<tr>
<td>BV/TV</td>
<td>6.6</td>
<td>8.7</td>
<td>0.460</td>
</tr>
<tr>
<td>Tb.N</td>
<td>9.9</td>
<td>6.5</td>
<td>0.144</td>
</tr>
<tr>
<td>Tb.Th</td>
<td>-4.5</td>
<td>6.8</td>
<td>0.521</td>
</tr>
<tr>
<td><strong>Jumping mechanography n= 65</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pmax</td>
<td>6.7</td>
<td>9.2</td>
<td>0.471</td>
</tr>
<tr>
<td>Fmax</td>
<td>1.7</td>
<td>7.7</td>
<td>0.826</td>
</tr>
<tr>
<td><strong>Body composition n= 65</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Lean-to-fat ratio</td>
<td>-4.9</td>
<td>16.6</td>
<td>0.768</td>
</tr>
<tr>
<td>Muscle density</td>
<td>1.7</td>
<td>0.7</td>
<td>0.020</td>
</tr>
</tbody>
</table>

Key: β coefficient is genotype = heterozygous delta508. Model 1 analyses included genotype, sex, and pubertal stage. Model 2 analyses included genotype, sex, age, quadratic age (age2), pubertal stage, height (Ht) and weight (Wt). Dual-energy X-ray absorptiometry (DXA), peripheral quantitative computed tomography (pQCT) and high resolution pQCT (HR-pQCT). Outcome measures: Total body less head size adjusted BMC (TBH SA-BMC), Total body less head bone area (TBH BA), cross-sectional area (CSA), Stress-strain index (SSI), total and trabecular vBMD (D100, mg HA/cm3 and BV/TV), trabecular number and thickness (Tb.N, mm-1 and Tb.Th, mm) and maximum muscle force (Fmax) and power (Pmax). Statistically significant when p<0.05 and is presented in bold and highlighted in grey.
6.5.5 Summary

The following summary statements are based on height-adjusted analyses.

- There were positive relationships between lung function (FEV1) and bone outcomes, where CF participants with higher lung function (FEV1) had higher total and trabecular densities (total and trab vBMD) at the distal 4% site of the tibia compared to CF participants with lower lung function (FEV1). There were no relationships between lung function (FEV1) and any other bone, body composition and muscle function (i.e. muscle force [Fmax] and power [Pmax]) outcomes (Table 6.12).

- There were positive relationships between 25(OH)D status and bone and muscle outcomes, where CF participants with higher 25(OH)D status had higher total body less head size-adjusted bone mineral content (TBLH SA-BMC) and higher total and trabecular densities (total and trab vBMD) at the distal (4%) site of tibia (Table 6.13).

- There was a positive relationship between 25(OH)D status and muscle force (Fmax), where CF participants with higher 25(OH)D status had a higher muscle force (Fmax) compared to CF participants with lower 25(OH)D status (Table 6.13).

- CF participants with a heterozygous genotype (deltaF508/other) had a higher muscle density compared to those with homozygous genotype (deltaF508/deltaF508). There were no relationships between genotype and all other bone, body composition and muscle function outcomes (i.e. muscle force [Fmax] and power [Pmax]) (Table 6.14).
6.6 Discussion
Survival rates in the CF population have improved significantly over the last 50 years due to advances in medicines and interventions. However, the emergence of osteoporosis and increased fracture risk during adolescence and young adulthood in CF patients has become more common (Aris et al., 1998, Conway et al., 2008, Elkin et al., 2001, Rossini et al., 2004, Stephenson et al., 2006). The work described in this chapter has sought to gain insights into whether disease group effects the relationships between puberty and bone, as well as the relationship between muscle and bone (i.e. the muscle-bone unit) in participants with CF and controls. The results are discussed in this section.

6.6.1 Modifying effects of disease group on the relationship between puberty and anthropometric outcomes in participants with CF and controls (Model 1)

Despite the advances in treatments and management of CF, growth disturbances are still commonplace in 2016 (Bai et al., 2016, Brookes et al., 2015). The UK CF Trust’s annual data report for 2012 present the most recent dataset for median height and weight of 4256 children and adolescents with CF relative to percentiles of the healthy population (Figure 2.46) (CF_Trust, 2013). Pre-pubertal children tracked between the 30-40th percentile for median height and 30-50th percentile for weight. However, as puberty begins, there is a period of ‘catch-up’ in height and weight for females aged 10-12 years and for males aged 11-13 years. This is followed by rapid decline and the phase of rapid pubertal growth is inadequate and cannot compensate for the slow growth during pre-puberty.

Data generated from this study showed that disease group did not modify the relationships between puberty and anthropometric outcomes (Table 6.2 and Figure 6.1). Participants with CF were consistently shorter and weighed less compared to controls across all puberty stages, which suggest that there are growth disturbances in the CF population (Data not presented).

6.6.2 Modifying effects of disease group on the relationship between puberty and body composition and muscle function outcomes in participants with CF and controls

Poor muscle growth, sarcopenia, and exercise intolerance are common in children and adults with CF as a result of poor pulmonary function, malnutrition, delayed puberty, chronic inflammation, and use of glucocorticoid steroids (Sheikh et al., 2014, Stallings et al., 2005, Reix et al., 2010, Mughal et al., 2006, Bianchi et al., 2006, Sood et al., 2003, Ionescu et al., 2003, King et al., 2014). However, the loss of lean mass has been shown to be a significant negative predictor of survival, independently of pulmonary function and gas exchange (Sharma et al., 2001). Animal studies in CF mutant mice, which were free from malnourishment, chronic infection, and glucocorticoid steroids, have also been shown to have significantly lower muscle mass compared to controls, which may suggest a direct effect via the CFTR channel in CF muscle (Dif et al., 2004).
In this study, disease group did modify the relationship between puberty and body composition outcomes. DXA results showed that participants with CF had lower apparent gains in fat mass (FM) and fat mass index (FMI) in pre-to-early puberty and a similar (but not significant) trend in pre-to-late puberty, after adjusting for height (Table 6.4 - Model 2). In contrast, participants with CF had higher apparent gains in lean-to-fat mass ratio (LM:FM) in pre-to-early puberty and a similar (but not significant) trend in pre-to-late puberty, after adjusting for height (Table 6.4 - Model 2). Disease group did not modify the relationships between puberty and body composition in pre-to-late puberty, which may suggest that participants with CF are experiencing a phase of ‘catch-up’ growth in fat mass (FM). However, the graphs for fat mass (FM), fat mass index (FMI), and lean-to-fat mass ratio (LM:FM) seem skewed, especially in early puberty (Figure 6.2). Lean mass (LM) and fat free mass index (FFMI) were significantly lower in participants with CF across all stages of puberty (Table 6.4 - Model 2), which may suggest that participants with CF may have smaller, weaker muscles compared to controls.

In contrast, pQCT and jumping mechanography results showed that with increasing maturation, participants with CF had lower apparent gains in muscle density, muscle force (Fmax), and muscle power (Pmax) compared to controls in pre-to-late puberty (Table 6.4 - Model 2, Figure 6.3, and Figure 6.4). Participants with CF had consistently lower muscle area (muscle CSA) at the proximal 66% site of the tibia across all stages of puberty (Table 6.4 - Model 2). These results suggest that participants with CF do not experience a ‘catch-up’ phase in muscle mass or function. Other studies have reported similar findings (Troosters et al., 2009, Elkin et al., 2000, Pinet et al., 2003, Lands et al., 1993, De Meer et al., 1999, Jong et al., 2001).

6.6.3 Modifying effects of disease group in the relationship between puberty and the muscle-bone unit outcomes in participants with CF and controls

The relationship between muscle and bone is described by Frost’s mechanostat theory, which postulates that increasing maximal muscle force during growth increases mechanical loading which will elicit a response in the bone tissue mechanostat to maintain strength either by increasing/decreasing bone mass, size, and length (Figure 2.17) (Frost, 1996, Frost, 1987). When a muscle contracts, the force generated creates a strain on the bone, changing the shape and length of the bone (Ward, 2012). To date, research into musculoskeletal growth and development in CF has mainly focused on bone and as described previously, many studies into CF-related bone disease have many limitations including: the use of aBMD, small sample sizes, data analyses do not account for differences in height or pubertal stage, and either no or inappropriate controls used to make comparisons. In paediatric bone research, it is essential to adjust for height and pubertal stage, especially in patients with growth disturbances, as a risk of under or over estimating BMD is likely to occur.
The aim of this part of the thesis was to investigate whether disease group modified the relationship between puberty and bone outcomes (model 2), as well as the relationship between muscle and bone outcomes (model 3). This study will contribute to our understanding of the muscle-bone unit in children and adolescents with CF and the aetiology of CF-related bone disease. The muscle-bone unit of the total body, distal regions of the tibia, and proximal region of the tibia are discussed in this section.

6.6.3.1 The muscle-bone unit of the total body site
The majority of published studies demonstrate low aBMD to be associated with CF, although some do not support these observations and have shown no difference between well-nourished CF patients and their healthy peers (Louis et al., 2009). In this study, disease group did modify the relationship between puberty and DXA bone outcomes after accounting for height (model 2). With increasing pubertal maturation, participants with CF had lower apparent gains in bone area (TBLH BA) and bone mineral content (TBLH BMC) compared to controls (Table 6.6 – Model 2 and Figure 6.5). These results suggest that participants with CF are unable to widen the bone and accrue bone mineral compared to their healthy peers. However, for size adjusted BMC (which accounts for bone area), participants with CF had higher apparent gains in pre-to-early puberty but no significant interaction in pre-to-late puberty (Table 6.6 – Model 2). This suggests that participants with CF may have appropriate bone mineral for their bone size. Therefore, adults with CF are likely to have narrow bones with less or appropriate amount of bone mineral for bone size, and reduced bone strength compared to their healthy peers.

Disease group did modify the relationship between muscle function and DXA bone outcomes (Table 6.6 – Model 3). With increasing muscle force (Fmax), participants with CF showed lower apparent gains in bone area (TBLH BA) and bone mineral content (TBLH BMC), and trends towards lower apparent gains in area-adjusted bone mineral content (TBLH SA-BMC) with increasing muscle power (Pmax) (Table 6.6 – Model 3, Figure 6.6, and Figure 6.7). These results suggest that the adaptation of the skeleton in response to strains is different between CF and controls as puberty proceeds. Therefore, participants with CF have less bone expansion and accrue less mineral for the same amount of muscle force (Fmax), and less area-adjusted bone mineral content (TBLH SA-BMC) for the same amount of muscle power (Pmax) compared to controls.

6.6.3.2 The muscle-bone unit of the tibia
To date, there have been five studies using pQCT and no studies using HR-pQCT to assess bone in children and adolescents with CF and only three studies have been carried out in adults with CF (Louis et al., 2009, Putman et al., 2014, De Schepper et al., 2012, Roth et al., 2008, Kelly et al., 2016, Bai et al., 2016, Sood et al., 2001, Brookes et al., 2015).
The distal 4% and 8% sites of the tibia

In this study, pQCT results at the distal 4% site of tibia showed that disease group did not modify the relationship between puberty and pQCT bone outcomes (Table 6.8 – Model 2). Participants with CF had consistently lower total and trabecular densities (vBMDs) across all stages of puberty but no disease group difference was shown for total area (total CSA). After adjusting for Model 3, disease group differences in total and trabecular densities (vBMD) were attenuated by muscle function (Table 6.8 – Model 3). These results suggest that participants with CF had similar total bone CSA with lower bone mineral densities compared to controls. The attenuation of disease group differences in bone densities suggests that muscle loading has a role in bone development in CF at the distal tibia.

Kelly et al. reported different results, where females with CF (aged 8-21 years) had lower total and trabecular vBMDs and lower cortical CSA after adjusting for BMI and muscle CSA compared to controls, which suggests that other factors other than muscle influence bone density in CF. Similar results were reported for CF males but trabecular bone density (trab vBMD) was barely significant (Kelly et al., 2016). The mechanisms underlying the sex differences in bone outcomes remain unclear but could be associated with lower lung function. The contrasting results might highlight differences in using muscle function compared to muscle cross-sectional area.

In this study, HR-pQCT results at the distal 8% site of the tibia showed that disease group did modify the relationship between puberty and HR-pQCT outcomes (Table 6.10). With increasing maturation, participants with CF had lower apparent gains in trabecular density (BV/TV), trabecular thickness (Tb.Th), and cortical porosity (Ct.Po) compared to controls in pre-to-late puberty. Participants with CF had narrower bones (Tt.Ar), with lower cortical area (Ct.Ar) and similar cortical bone and tissue mineral densities (Ct.BMD and Ct.TMD) and trabecular number (Tb.N) compared to controls, across all stages of puberty and after adjustment for height (Table 6.10 - Model 2). These results may suggest that children with CF have a lower bone turnover, and together with poorer growth rates which has resulted in narrower, less total and trabecular densities, and less porous bones, which may be less resistant to bending forces. This may explain why patients with CF have a higher risk of fracture in young adulthood. Chest infection and malabsorption of vital nutrients (i.e. vitamin D and calcium) may increase bone turnover of the trabecular compartment, causing trabecular thinning and lowering trabecular density.

Only one study has measured bone using HR-pQCT in young adults with CF (Putman et al., 2014). The authors concluded that CF patients had narrower bones (Tt.Ar) and lower total and trabecular densities (D100 and BV/TV). However, it should be noted that this study positioned the reference line at a fixed distance from the tibia plafond despite reporting patients with CF to be significantly smaller compared to controls. Therefore, the ROI in patients with CF would be situated more proximal, where the bone is narrower and has less trabecular bone, compared to controls. It
would be more appropriate to position the reference line at a relative percent position, which would account for bone length as was done in the protocol for this study (Ward et al., 2015). Nevertheless, my findings are similar to those of Putman et al., (2014).

Disease group did modify the relationship between muscle function and HR-pQCT bone outcomes (Table 6.10 - Model 3). With increasing muscle force (Fmax), participants with CF had significantly lower apparent gains in total bone density (D100), trabecular density (BV/TV) and trabecular number (Tb.N) compared to controls (Figure 6.10). However with increasing muscle power, participants with CF had higher apparent gains in trabecular density (BV/TV) and trabecular number (Tb.N) compared to controls (Table 6.10 - Model 3). These results may suggest that there is an impaired muscle-bone unit in CF as muscle power and force (Pmax and Fmax) are significantly lower in CF but may also reflect a maturation delay as participants with CF have higher apparent gains in bone outcomes with increasing muscle power (Pmax) but not with muscle force (Fmax). Therefore, children with CF have smaller, narrower and weaker bones, which might be partly explained by having smaller, weaker muscles but other factors such as nutrition, exercise or impaired bone responses to muscle in CF will also contribute to the differences shown in bone and muscle outcomes. Disease group differences in total bone area (Tt.Ar) did not attenuate after accounting for muscle function (Table 6.10 - Model 3), which suggests that other factors such as nutrition and/or genetics may affect total bone area in CF. The group differences in findings for total bone area between the distal 4% (i.e. total CSA) and 8% (i.e. Tt.Ar) sites of the tibia may be due to differences in the imaging methodology but also the difference in scanning region of interest (i.e. the 4% site is closer to the joint and is wider, whereas the 8% in closer to the narrow diaphysis).

The proximal 66% site of the tibia

At the proximal 66% site of the tibia, disease group did modify the relationship between puberty and cortical density (vBMD), where participants with CF had lower apparent gains in cortical density (cortical vBMD) compared to controls, as puberty proceeded. This may indicate lower bone turnover and slower growth in CF (Table 6.8 – Model 2 and Figure 6.8). Participants with CF had consistently smaller total bone area, cortical area, and bone strength (SSI) compared to controls, across all stages of puberty and after accounting for height (Table 6.8 – Model 2). These results suggest that growth is occurring at a slower rate in children with CF compared to controls, which may result in bones with lower resistance to bending forces (i.e. lower SSI), therefore, are more likely to fracture compared to healthy peers. Other studies have reported similar findings. Louis et al reported results at the 66% site of the radius in well-nourished CF patients and showed that cortical thickness was significantly thinner but total bone area and density were normal (Louis et al., 2009). This indicates that bone is expanding normally but the cortex is thin with normal BMD. Bai et al. have shown children with CF, aged 7-12 years, to have smaller, narrower bones, with thicker, denser cortices, and lower bone
strength compared to control (Bai et al., 2016). It should be noted that Bai et al., did not adjust data for pubertal stage, therefore children with slower growth and/or delayed puberty were compared to children who are likely to be more developed. Cortical bone provides ~80% of overall bone strength but having thicker cortex is unlikely to compensate for smaller bone area, therefore bone strength is compromised in CF.

Disease group did not modify the relationship between muscle and bone outcomes at the proximal 66% site. Disease group differences in bone measurements in Table 6.8 – Model 2 were attenuated by muscle power and force (Pmax and Fmax), which suggests that muscle function does play a key role in bone development at the diaphysis in CF. Therefore, treatment interventions improving muscle function may help improve bone strength and reduce the risk of fracture in young adulthood.

6.6.4 The relationships between clinical characteristics and bone and muscle outcomes
The severity and progression of CF can vary depending on whether the individual is pancreatic sufficient, has liver disease, the number and type of pulmonary infections, compliance and response to treatments and physiotherapy, and general physical and mental well-being. In this study, the secondary objectives were to explore the relationships between clinical characteristics, the following were chosen: 1) lung function measured as forced expiratory volume in 1 second (FEV1), 2) vitamin D status measured as 25(OH)D serum concentrations, and 3) genotype, which was classed into hetero- and homozygous for the delta F508 mutation, with muscle-bone unit outcomes in children and adolescents with CF to help understand whether certain clinical characteristics effect bone and muscle strength. The relationships between these clinical characteristics and muscle-bone unit outcomes are discussed in this section.

Lung Function (FEV1) - Pulmonary infections in CF are caused by the mutations of the chloride channels, which disrupt the transportation of water, in the epithelial lining of the lungs and causes severe dehydration and accumulation of thick sticky mucus. With age, pulmonary infections become increasingly more common and lung function deteriorates (Figure 2.48). High circulating levels of pro-inflammatory cytokines from chronic pulmonary infections have been shown to increase bone turnover by increasing osteoclast number and activity, and bone loss in CF (Aris et al., 2000, Haworth et al., 2004, Shead et al., 2006, Nixon et al., 1998, Norman et al., 1991, Ionescu et al., 2000b).

In this study, participants with CF had moderate to good lung function but there is a small decline in lung function (FEV1) with age (not formally tested) (Figure 6.14). In Table 6.12, the data showed that relationships between lung function (FEV1) and bone outcomes did exist, where participants with higher lung function (FEV1) had higher total and trabecular densities (vBMD) at the distal 4% site of the tibia before and after adjusting for height. A positive relationship between lung function (FEV1) and trabecular bone density (BV/TV) at the distal 8% site of the tibia, measured by HR-pQCT, was also
shown but the relationship was attenuated after model 2 analyses. These results suggest that children with CF with lower FEV1 are likely to have an increased bone turnover at the distal 4% site of the tibia which could affect bone accrual. Ionescu et al. reported that low aBMD was related to lower lung function and increased levels of cytokines (IL-6 and tumour necrosis factor) in young CF adults (Ionescu et al., 2000a). To date, there are no studies into inflammation on bone outcomes in children with CF.

![Figure 6.14 - The association between lung function and age in participants with CF.](image)

Many studies have been reported muscle weakness in CF to be related to reduced lung function (FEV1) and inflammation. Pulmonary infections and reduced lung function (FEV1) are likely to lead to reduced physical activity, increased turnover of muscle, reduced oxygen supply to muscles, and weaker muscles. Ionescu et al. reported patients with CF to be an induced catabolic state and had lower fat-free mass and lower muscle strength (Ionescu et al., 2002). This would disrupt the muscle-bone unit in CF patients as muscle contractions and strains placed on bone would be reduced. In this study, there were no relationships between lung function (FEV1) and muscle outcomes as participants with CF were young, less infectious with moderate lung functions compared to the young CF adults in previous studies.

**Vitamin D (25[OH]D) status** - Pancreatic insufficiency is common in CF (i.e. 80% of the CF population), which causes malabsorption of vital nutrients and may contribute to the slower growth, lower peak muscle mass and abnormal low muscle strength in children with CF compared to their
healthy peers. Vitamin D deficiency has been associated with low aBMD and poor muscle function in children and adolescents (Ward et al., 2009, Hussey et al., 2002, Hall et al., 2010). In this study, there were positive relationships between 25(OH)D status and bone and muscle outcomes (Table 6.13). CF participants with higher 25(OH)D status had higher total body SA-BMC and higher total and trabecular vBMDs at the distal 4% site of the tibia, in model 1 and model 2 analyses. Participants with vitamin D deficiency are likely to have a disrupted bone metabolism, lower bone mineralisation, and weaker bones. There were negative relationships between 25(OH)D status with DXA total body less head BMC and BA, pQCT 4% and 66% total CSAs, SSI, and HR-pQCT total CSA at the distal (8%) site of tibia after model 1 analyses. This may suggest that younger CF participants have a higher vitamin D status compared to older CF participants as seen in Figure 6.15 (not formally tested). These negative relationships were attenuated after accounting for height (model 2).

Currently, there have been no studies in patients with CF investigating the association between vitamin D status and muscle function. Studies have shown that improvements in vitamin D status may improve muscle strength, and therefore increase bone strength indirectly (Ward et al., 2009). In this study, a positive relationship between 25(OH)D status and muscle force (Fmax) emerged after model 2 analyses. As suggested in the mechanostat theory, better muscle function should be related to increased bone strength by exposing the bone to high strains. Therefore, CF patients with better vitamin D status are less likely to have a disrupted muscle-bone unit as patients would have adequate calcium absorption for bone mineralisation and muscle contractions. Patients with CF are prescribed vitamin D₃ to prevent deficiency and to ensure normal bone accrual in children and adolescents with CF. However, despite supplementation patients with CF still have lower bone mineral and weaker muscles compared to their peers, especially in adulthood.

5 The Global Consensus panel recommended serum 25(OH)D levels should be >30 nmol/L for the prevention of osteoporosis and rickets (Munns et al., 2016).
The association between 25(OH)D status and age in participants with CF

**Genotype** - The relationship between CF genotype and disease severity remains unclear due to the complexity of CF and the number of various mutations (i.e. over 1,900 different mutations of CF gene) (Bobadilla et al., 2002, Lap-Chee, 2011). Approximately 70% of individuals with CF are homozygous for ΔF508 mutation, and almost 90% of all CF patients have at least one ΔF508 allele (Ameen et al., 2007). The ΔF508 mutation has been classified as a class 2 mutation and is related to more severe outcomes in CF. The ΔF508 mutation causes the CFTR protein to fold incorrectly and therefore is recognised by the endoplasmic reticulum as abnormal and becomes targeted for proteasomal degradation before reaching the Golgi and cell surface to achieve chloride ion transport. Studies have shown ΔF508 to be associated with lower lung function, pancreatic insufficiency and lower bone mineral content compared to other CF genotypes (King et al., 2005, Kerem et al., 1990).

To date, there is one study which has explored the differences in muscle-bone unit outcomes between patients who are homozygous and heterozygous for ΔF508. Kelly et al. reported higher cortical vBMD to be associated with homozygosity for ΔF508 in children with CF (Kelly et al., 2016). This may indicate that homozygous patients have a lower rate of periosteal apposition and bone is placed within the endosteal envelope resulting in a denser cortex. In this study, there were no relationships between genotype and all bone and body composition outcomes but analysis was limited (Table 6.14). In contrast, the current study shows a positive relationship between genotype and muscle density existed after model 1 and 2 analyses. Heterozygous participants (ΔF508/other) had higher muscle density compared to homozygous (ΔF508/ΔF508) (Table 6.14). Muscle density is measured by pQCT at the proximal 66% site of the tibia and can be used as an indicator for the amount of fat infiltration into and between muscle groups of the lower leg and may reflect the
compactness of muscle fibres and the amount of protein within muscle (Wong et al., 2014). Therefore, this novel finding may suggest that heterozygous patients may have less fat infiltration between muscle fibres which would preserve muscle function. However, there was no evidence that compactness of muscle fibres improved muscle strength as there were no relationships between genotype and muscle force or power.

7 Study evaluation
The main focus of this section is to critically evaluate the imaging techniques (i.e. pQCT and HR-pQCT), jumping mechanography and study design.

7.1 Main achievements, strengths and limitations
Research into CF-related bone disease has been plagued by a number of limitations such as the use of DXA to assess aBMD, lack of assessment of bone geometry or microarchitecture (important components of bone strength), either no control group or comparisons with controls were not suitable, and small sample size. Differences in statistical analyses, especially whether analyses have accounted for body size/height differences, can make comparisons between studies difficult. Previous studies in CF-related bone disease have formed the foundations of this study and have given an opportunity to improve study design. This study has been able to address some of the limitations reported in previous studies.

This is the first study to characterise the muscle-bone unit at different skeletal sites including; total body (less head), distal tibia and proximal tibia in a single cohort of children with and with CF using novel techniques to assess bone and muscle outcomes (i.e. pQCT, HR-pQCT and jumping mechanography).

7.1.1 Study strengths and weaknesses
The strengths of this study include:

1. **Data analyses account for differences in body size/height** – Despite advances in medication, nutrition status and physiotherapy, the results of this study show participants with CF to be shorter compared to their healthy peers which may be caused by slow growth and delayed puberty. Slow growth is known to have detrimental effects on skeletal development and bone strength (Kuh et al., 2014). Therefore, it is essential for the data to be adjusted for puberty and height.

2. **Image acquisition** – All scanning procedures were performed by a trained and experienced operator. Movement and metal (e.g. orthodontic braces and jewellery) artefacts did occur in
a few cases, especially in young participants who had pQCT and HR-pQCT. However, all images were of good quality and were all included in the data analyses.

The weaknesses of this study include:

1. **Self-assessment of pubertal staging** – The Tanner criteria for pubertal assessment is the most widely used in clinical and research settings. While some studies have shown a good correlation between the method used in this study and assessment carried out by clinicians (Duke et al., 1980), there are some who have reported self-assessment to be unreliable (Desmangles et al., 2006). Self-assessment may lead to under or over estimation of pubertal staging. It is important for participants to self-assess correctly so accurate comparisons can be made with appropriate groups/controls, especially when delayed puberty is a concern (i.e. in patients with CF).

2. **Small sample size for HR-pQCT** – HR-pQCT measurements were obtained from BMS participants only, which means only 25 participants with CF had measurements taken. Therefore, HR-pQCT data may be underpowered and differences should be confirmed. However, this is the first study using HR-pQCT in children and adolescents with CF and the HR-pQCT data does agree with the pQCT data. Further research is needed with a larger reference population.

3. **Inclusion of additional cases from a separate study** – Due to the small sample size of patients with CF, data from another study (i.e. the SNAP) was merged with the BMS data. Although the protocols between the two studies were the same, the use of different scanners and operators may introduce technical and operator bias.

4. **Data analyses** – Some of the results were surprising and were inconsistent with our hypotheses. The reason for this may be that high correlations among the predictors, e.g. height, weight, power, force, may inflate the standard error of the coefficients, which increases the probability of false negatives (i.e. Tb.N, BVTV, and SA-BMC with Pmax).
7.2 Methodological evaluation

7.2.1 Differences between imaging techniques

Unlike DXA, pQCT and HR-pQCT have the advantage of measuring vBMD, which is size-independent. However, the methodology of how bone outcomes are measured or derived, location of the reference line, precision, image resolution, and ROI differ between these techniques. These differences are described in this section.

1) Cortical and trabecular separation

pQCT segregates the bone compartments by using various modes (i.e. Contmode, Peelmode and Cortmode - as described in Chapter 2.5.4) to separate soft-tissue, cortical and trabecular bone. The manufacturer recommends using the circular-ring mode to assume the bone is circular. However, bone shape can vary depending on the measurement site, ethnicity and time of life. Therefore, the proportion of cortical and trabecular bone will vary and will make comparisons difficult between different populations.

HR-pQCT methodology to separate the bone compartments uses an automated contouring script to identify the periosteal surface. The image is binarised and then the outer edge of the cortex is selected. This method assumes that the outer cortical edge is a complete, circular shape. Therefore, the bone images of individuals with thin, porous cortices are difficult to analyse as the automated contouring will enter into the large cortical pores and incorrectly select internal structures. The operator will have to amend the software’s contouring to correctly select the outer cortex only, which can introduce operator bias. This was an issue for some older participants with CF as cortical porosity was much higher compared to controls, which may mean that the operator was more selective when analysing the images of participants with CF.

Another issue with HR-pQCT automated contouring script is the method in which the endosteal surface is selected and how trabecular microarchitecture is measured. Thin trabeculae with a thickness of less than 2 voxels thick are removed and then a dilation step occurs to smooth the endosteal surface (Burghardt et al., 2010). Therefore, not all trabecular microstructures are assessed. This may lead to an underestimation of trabecular BV/TV and microarchitecture measurements, especially in participants with CF as they have smaller narrower bones with less trabecular bone.

Cortical porosity is measured by identifying the background voxels (i.e. voxels which contain no bone), which may represent Haversian canals and other voids, within the cortical shell. An algorithm is used to identify the connectivity between the background voxels. Voids with a volume smaller than 5 mutually connected voxels are discarded and filled in as these voids are too small to be Haversian canals and may represent noise (Burghardt et al., 2010). This may result in an underestimation of cortical porosity.
2) Precision
Both techniques are able to measure bone outcomes with good precision (Boutroy et al., 2005, Burghardt et al., 2010, Burghardt et al., 2011, Burrows et al., 2010a, Adams et al., 2014). However, HR-pQCT is able to measure trabecular microarchitecture and cortical porosity due to the scanner’s high resolution of 82 µm, whereas pQCT can only measure trabecular density as the resolution is between 0.2 mm and 0.8 mm, which cannot measure microarchitecture (Burrows et al., 2010c). The direct measurement of trabecular number is used to derive trabecular BV/TV and thickness by using algorithms. These complex algorithms are difficult for non-mathematicians to understand how these measurements are calculated. The larger volume of interest in HR-pQCT (i.e. 110 CT slices) will also increase precision, whereas the pQCT can only measure a single CT slice. In this study, HR-pQCT measurements agreed with pQCT measurements at the distal site and provided more in-depth assessment of trabecular microarchitecture and cortical porosity, which has not been assessed in children and adolescents with CF.

3) Image artefacts
The most common image artefact is movement, especially in children aged <8 years, the elderly aged >75 years, and those with muscular, neurological problems. HR-pQCT is particularly sensitive to movement due to the high resolution, therefore it is essential that participants remain still throughout the scanning procedure and are positioned comfortabably within the scanner to prevent movement. Some participants with CF had difficulty in remaining still due to poor lung function, which increased coughing and heavy breathing. Ring artefacts are common in HR-pQCT images as multiple detectors are used to generate the image. If one of the detectors is out of calibration, the detector will give a consistently inaccurate reading at each angular position, creating a ring artefact (Barrett and Keat, 2004). The effects of ring artefacts on data are unknown.

PVE is an image artefact which underestimates vBMD when voxels are only partially filled with bone, the effect is caused by the limited spatial resolution of techniques, as described in Chapter 2.5.4.3, can occur in pQCT and HR-pQCT images of participants with thin cortices and trabeculae as there is a higher proportion of voxels close to edge of bone (Prevrhal et al., 1999). This may result in underestimation of bone, especially in children with chronic illness like CF who have smaller, narrower bones. In HR-pQCT, PVEs are likely to occur in the trabecular measurements as the average thickness is 100 to 150 µm (i.e. approximately 1-2 voxels) (Boutroy et al., 2005). Using appropriate and consistent thresholds values with a CT voxel size of 0.4 mm can minimise error in pQCT (Ward et al., 2005a).
4) Region of interest

pQCT has the advantage of being able to measure bone and muscle at different sites along the length of the bone, whereas HR-pQCT can only measure bone at the most distal regions. The scanning protocol for pQCT has been standardised for both children and adults. However, for HR-pQCT, the standard protocol is based on the location of the reference line from a fixed distance from the tibia endplate. This can be an issue in short individuals, especially those with chronic illness like patients with CF as the ROI is not comparative to that of controls. Research groups have identified this as an issue and have developed methods to make the ROI as a relative distance from endplate. Therefore, accounting for differences in bone length (Burrows et al., 2010a, Burrows et al., 2010c, Nishiyama et al., 2012b). In this study, a relative distance approach was performed for all pQCT and HR-pQCT scans, therefore ensuring that differences in bone length were accounted for.

7.2.2 Differences between imaging techniques and jumping mechanography

DXA and pQCT measure lean mass and muscle CSA which have been used as markers of muscle strength. However, these measurements do not give an insight into muscle function (i.e. power and force). Jumping mechanography measures muscle function of the lower body with good precision in healthy children and adolescents (Veilleux and Rauch, 2010). However, the jumping and hopping tests must be performed correctly. This can be difficult in participants who are aged <6 years, elderly aged >75 years and participants with muscle weakness (e.g. participants with CF) as they are unable to generate muscle power to jump and have difficulties with balancing. To overcome these problems, the operator must explain and demonstrate the tests to participants. The reference dataset for jumping mechanography is based on healthy German children who have higher muscle power and force compared to other populations, especially those with chronic illness.

8 Study conclusions and future work

8.1 Conclusions

This is the first study to use a combination of novel imaging and muscle assessment techniques to help characterise the muscle-bone unit in healthy children and in children with chronic disease. The data has confirmed that sex and disease status did modify the relationship between puberty and bone as well as the relationship between muscle and bone outcomes and does support the mechanostat theory.

After height adjusted analyses, among healthy participants, females had smaller bones and lower bone density compared to males. With pubertal maturation, females had lower apparent gains in the distal and proximal total area (Tt.Ar and CSA), distal cortical porosity (Ct.Po) and proximal bone
strength (SSI) but higher apparent gains in distal and proximal cortical bone density (Ct.BMD, Ct.TMD, vBMD). Females had consistently lower distal total area (total CSA) and density (total vBMD), distal trabecular density (BV/TV) and number (Tb.N), and proximal cortical area (CSA) compared to males, across all stages of puberty. With increasing muscle force (Fmax), females had higher apparent gains in total body less head bone mineral (TBLH BMC) and bone area (BA), distal total and trabecular density (total and trab vBMD) compared to males. In contrast, with increasing muscle power (Pmax), females had higher apparent gains in distal total and cortical densities (D100, Ct.BMD and Ct.TMD), and distal trabecular thickness (Tb.Th), and proximal cortical density (cortical vBMD) but lower apparent gains in distal cortical porosity (Ct.Po) and trabecular number (Tb.N) compared to males.

After height adjusted analyses, participants with CF had smaller bones and lower bone density compared to controls. With increasing pubertal maturation, participants with CF had lower apparent gains in total body less head bone mineral (TBLH BMC) and bone area (TBLH BA), and in distal trabecular density (BV/TV), cortical porosity (Ct.Po), and trabecular thickness (Tb.Th) compared to controls. Participants with CF had consistently lower distal total and cortical area (total CSA, Tt.Ar, and Ct.Ar), distal total and trabecular densities (total and trab vBMD and D100) and proximal bone strength (SSI) compared to controls, across all stages of puberty. With increasing muscle force (Fmax), participants with CF had lower apparent gains in total body less head bone mineral (TBLH BMC) and bone area (BA), distal total density (D100), trabecular density (BV/TV), and trabecular number (Tb.N). In contrast, with increasing muscle power (Pmax), participants with CF had higher apparent gains in distal trabecular density (BV/TV) and trabecular number (Tb.N) compared to controls.

The relationship between clinical characteristics and bone-muscle unit outcomes were explored in children and adolescents with CF. Participants with higher lung function (FEV1) and vitamin D status (25(OH)D) have higher bone outcomes (TBLH size-adjusted BMC, and distal total and trabecular densities). A relationship between 25(OH)D status and muscle force (Fmax) emerged after adjusting for height. A positive relationship between genotype and muscle density existed, heterozygous participants (deltaF508/other) had a higher muscle density compared to homozygous (deltaF508/deltaF508).

Skeletal adaptation to muscle differs between sexes and in populations with chronic disease, which may explain sex and disease group differences in risks of osteoporosis and fracture. Bone adaptation to muscle in children with CF may be altered, which may lead to narrow, under-mineralised bones, with lower bone strength in later life. Understanding better impairments in muscle functions may provide targets for intervention to improve skeletal health in later life.
8.2 Future work

Future new investigations include the following:

- To increase the upper-limit of recruitment age from 16 years to 21 years therefore the whole pubertal growth phase can be observed and characterised, especially in healthy males and in those with delayed puberty.

- To analyse longitudinal measurements in bone and muscle development in children with and without CF. Approximately 80% of participants have taken part in the 12-month follow-up study (August 2014 to April 2015). This will be the first study to investigate longitudinal measurements using HR-pQCT and jumping mechanography in children with and without CF.

- Measurements can be used to produce LMS charts for HR-pQCT and could potentially be used for clinical assessment.

- Vertebral fractures are common in young adults with CF. Future studies involving children and adolescents with CF should include vertebral fracture assessment. This will contribute to our understanding of bone microarchitecture and timing of fracture of vertebrae in patients with CF.

- Assess the muscle-bone unit in children of different ethnicity. This study has focused on the White Caucasian population as previously explained in Chapter 2.6. Currently, there are no published UK reference data for bone measurements in children of ethnic origins other than White Caucasians. Therefore, there is a need to understand how the bone-muscle unit develops in these populations to help broaden our understanding of bone health.

- Assess the bone-muscle unit in different patient groups (e.g. children with cerebral palsy and Duchenne muscular dystrophy). As patients with chronic disease live longer, other complications such as osteoporosis are emerging. Therefore, bone and muscle development in children with chronic disease needs to be assessed.
References


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We read with interest the article by Putman et al (1) describing differences in bone microarchitecture and strength in adults with cystic fibrosis (CF) compared to controls. These are novel data using state-of-the-art technology to understand bone pathology in chronic disease. Although these are important, we are concerned that, potentially, the reported differences may be an artifact of the scanning protocol, which was not detailed in the paper. In addition, the authors have used a statistical correction for body mass index, whereas an adjustment for height or leg length would have been the more appropriate adjustment (2).

The references cited in the methodology used standard adult protocols (3–5). Adult scan protocols measure a fixed distance from the tibial endplate. There were marked differences in the heights of the adults in the CF group vs the control group. If a fixed distance was chosen for measurement, this would have scanned a different bone site depending on the height (or leg length) of the individual subject (6). This is a common problem when scanning children of different sizes, but it can also be an issue when comparing adults who differ in size. In a shorter individual, the measurement site will be more proximal than in a taller individual, resulting in a more proximal measurement site in the shorter subject. Bone structure changes from a predominantly trabecular compartment at the metaphysis to a cortical site at about 15–20% of the leg length. The transition involves remodeling of the bone to a more tubular structure from that at the “flared” ends of the bone at the joints. Looking at Figure 1A and comparing it to Figure 1B (1), we believe this may be the case in this study because visually it would appear that a more proximal site (more circular with less trabecular bone) has been measured in the shorter CF group compared to a more distal site in the control group. By eye, the shape (and cross-sectional area) looks markedly different.

We believe that it is important to raise this issue not only to clarify the results of the study by Putman et al (1) but also to inform others of this potential pitfall in interpreting high-resolution peripheral quantitative computed tomography data in both adults and children. It is important that protocols that take account of differences in limb length or height are used (2, 6, 7), such as those that measure the bone at a fixed percentage of the limb length, ensuring that the same relative site is measured in each individual (8). In studies such as this comparing a group of adults who have had lifelong growth and nutrition deficits with a group of age-matched healthy controls, adopting a data interpretation protocol similar to that developed by Mølgaard et al (9) would provide greater clarity about whether the CF group had compromised bone growth compared to healthy controls of the same skeletal size and body weight.

We thank the authors for this important study and hope they are able to address our concerns.

Disclosure Summary: The authors have nothing to disclose.

Ward KA, Riddell A, Prentice A
Nutrition and Bone Health, Medical Research Council Human Nutrition Research
Cambridge, UK
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5. Nishiyama KK, Macdonald HM, Buie HR, Hanley DA, Boyd SK. Postmenopausal women with osteopenia have higher cortical porosity and thinner cortices at the distal radius and tibia than women with normal aBMD: an in vivo HR-pQCT study. J Bone Miner Res. 2010;25(4):882–890. , Google Scholar Medline
Appendix 10.2 - Research ethics committee letter of approval

Page 1 of 4.

Health Research Authority

NRES Committee East of England - Cambridge South
The Old Chapel
Royal Standard Place
Nottingham
NG7 6FS

Telephone: 0115 8839308 (Direct Line)

16 April 2013

Miss Amy Riddell
PhD student
MRC Human Nutrition Research
Elsie Widdowson Laboratory
120 Fulbourn Road
Cambridge
CB1 9NL

Dear Miss Riddell

Study title: Musculoskeletal phenotype in children: A comparison of cystic fibrosis and controls
REC reference: 13/EE/0078
IRAS project ID: 113194

The Research Ethics Committee reviewed the above application at the meeting held on 28 March 2013. Thank you for attending with Dr Kate Ward to discuss the application.

We plan to publish your research summary wording for the above study on the NRES website, together with your contact details, unless you expressly withhold permission to do so. Publication will be no earlier than three months from the date of this favourable opinion letter. Should you wish to provide a substitute contact point, require further information, or wish to withhold permission to publish, please contact the Co-ordinator Ms Trish Wheat, nrescommittee.eastofengland-cambridgesouth@nhs.net.

Ethical opinion

Discussion

- The Committee were impressed with application and thanked you for the high standard of the application.

- The Committee noted that the terms 'you/my' and 'your child/my child' had become muddled in the different PIS and CF. For example, in the CF for Participant's Parents/Guardian it states 'I understand my participation is voluntary' when it should say 'I understand my child's participation is voluntary'. All the Participant Information Sheets (PISs) and Consent Forms (CF) should be reviewed in this respect.

- The Committee had no further questions regarding the application.
The members of the Committee present gave a favourable ethical opinion of the above research on the basis described in the application form, protocol and supporting documentation, subject to the conditions specified below.

**Ethical review of research sites**

**NHS Sites**

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see “Conditions of the favourable opinion” below).

**Conditions of the favourable opinion**

The favourable opinion is subject to the following conditions being met prior to the start of the study.

**Additional condition:**

1. You are asked to proof read the Participant Information Sheets and Consent forms to ensure the terms ‘you/my’ and ‘your child/my child’ are not muddled. For example in the Consent Form for Participant’s Parents/Guardian it states ‘I understand my participation is voluntary’ when it should say ‘I understand my child’s participation is voluntary’.

You should notify the REC in writing once all conditions have been met (except for site approvals from host organisations) and provide copies of any revised documentation with updated version numbers. The REC will acknowledge receipt and provide a final list of the approved documentation for the study, which can be made available to host organisations to facilitate their permission for the study. Failure to provide the final versions to the REC may cause delay in obtaining permissions.

**Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.**

Management permission (“R&D approval”) should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements.

Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at [http://www.rdforum.nhs.uk](http://www.rdforum.nhs.uk).

Where a NHS organisation’s role in the study is limited to identifying and referring potential participants to research sites (“participant identification centre”), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

**For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.**

**Sponsors are not required to notify the Committee of approvals from host organisations**

**It is responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).**
Approved documents

The documents reviewed and approved at the meeting were:

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Membership of the Committee

The members of the Ethics Committee who were present at the meeting are listed on the attached sheet.
Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document “After ethical review – guidance for researchers” gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

Feedback

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.

Further information is available at National Research Ethics Service website > After Review

Please quote this number on all correspondence

13/EE/0078

We are pleased to welcome researchers and R & D staff at our NRES committee members’ training days – see details at http://www.hra.nhs.uk/hra-training/

With the Committee’s best wishes for the success of this project.

Yours sincerely

Dr Leslie Gelling
Chair

Email: nrescommittee.eastofengland-cambridgeeast@nhs.net

Enclosures: List of names and professions of members who were present at the meeting and those who submitted written comments “After ethical review – guidance for researchers” [SL-AR2]

Copy to: Polly Page, MRC Human Nutrition Research
Mr Stephen Kelkeher, Cambridge University Hospitals NHS Foundation Trust
Appendix 10.3 - Poster advert for study recruitment

PARTICIPANTS REQUIRED

Bone & Muscle Health in Children Study

We are looking for children who are willing to take part in a study about bone and muscle health. This study will investigate how bones and muscles grow during childhood and adolescence using advanced measurement techniques. We will use scanners which are able to give very detailed images and measurements of bone and muscle. This will allow us to understand what factors make a bone ‘a healthy’ bone. By comparing children with and without cystic fibrosis (CF) we will be able to understand how diseases such as CF affect growth.

If your child:

- Is aged 8 to 16 years old
- Is White Caucasian
- Has cystic fibrosis or does not have cystic fibrosis
- Has no bone related illnesses
- Has not taken treatment which may affect bone health
- Has not been immobilised for long periods of time e.g. due to operation.

We would like to invite you to attend MRC Human Nutrition Research unit in Cambridge to take part in the study. Bone x-ray scans and a muscle jumping assessment will be carried out. You will be asked to attend the unit on two occasions (one year apart) for a maximum of two hours. In recognition of your time commitment, you will receive an honorarium of a £20 gift voucher for each session and a certificate with a picture of your skeleton. Reasonable travel expenses will also be reimbursed.

Please contact: Amy Riddell (Chief Investigator)
Telephone: 01223 437 539 or Email amy.riddell@mrc-hnr.cam.ac.uk

Version 1: 12/06/2013
REC No: 13/EE/0078
Appendix 10.4 - Participant information leaflet for parents/guardians

Front and back of leaflet.

Inside the leaflet.

What is the purpose of this study?
During childhood and adolescence, adequate bone and muscle growth is essential for long term health and prevention of fractures in later life. Growth disturbances caused by lifelong illnesses and their treatment, can result in shorter, narrower, or lighter bones which can be a risk factor for increased fracture risk in later life. Cystic Fibrosis (CF) is one such condition where bone and muscle growth can be compromised and this has led to concerns about bone health and fracture risk during adolescence and adult life. In this study we aim to use special measurement techniques to help us understand how bones and muscles develop in 8-16 year old children with and without CF.

What will be included in the study?
We will use specialist techniques to measure: 1) the size and amount of mineral in your child’s bones and 2) the size and how well your child’s muscles work. If you are interested in the study, you can contact the research team who will go through the study information sheet and ask you some screening questions about your child to see if they are suitable to take part in the study. If your child is suitable to take part, you will be invited to the MRC Human Nutrition Research Unit (HNRU) in Cambridge, which will involve two visits (one your child) to HNRU, and both visits will last 2 hours. These visits will be arranged to suit you and your child. Your child will also have the option of inviting a friend or family member, for example, who is 8-16 years old and who has not been included in the study. It is important for your child to complete the measurement techniques to help us understand how bones and muscles develop in 8-16 year old children with and without CF.

At HNRU, we will ask you to sign a consent form and a small health questionnaire about your child. This will help us to understand how bones and muscles grow. Questions about the use of certain medications and types of illnesses which may affect bone measurements will be asked. Your child’s height and weight will then be measured. Your child will be asked to complete a self-assessment of their physical activity. This involves looking at simple diagrams and will be done in a private room. If you have any questions about these questions, remember not to answer them. At HNRU, we use machines that measure bone mineral in the skeleton. The DXA scanner is shown in photo A. Your child will be on an open bed and the scanner will move over their body. The DXA scans the whole body. This procedure takes about 15 minutes (the actual scan will take 5 minutes). Children are asked to wear loose clothes e.g. school P.E kit, with no metal zips or hooks. Jogging pants or skirt would be ideal.

2. Peripheral Quantitative Computed Tomography (pQCT) – The pQCT scanner (photo B) measures the bone mineral, size and shape of the lower leg. Your child will sit in front of the scanner on a chair with their leg placed in a special holder. This is used to make your child comfortable during the scan and helps to position their leg correctly within the scanner. This procedure takes about 10 minutes (the actual scan will take 5 minutes).

3. High Resolution pQCT (HR-pQCT) – The HR-pQCT (photo C) is similar to the pQCT scanner although it uses a different machine and produces more detailed pictures. This time only the ankle region will be scanned. Your child will sit in front of the scanner with their leg placed in a special holder. This is used to make your child comfortable during the scan and helps to position their leg correctly within the scanner. This procedure takes about 15 minutes (the actual scan will take 3 minutes).

4. Jumping Mecchanography (JM) – JM (photo D) measures how well muscles work. Your child will be asked to do some stretching exercises before they start to reduce any risk of injury. Your child will then be asked to stand on a platform and carry out two activities. The first activity is to jump as high as they can. The second activity is to hop on one leg as fast and hard as possible. The jumping activity will be repeated 3 times with a one minute break in between jumps. This test should take 15 minutes.

Are X-ray scans dangerous? These scans are very safe, not painful and easy to perform. The radiation dose from the scans is extremely small (0.008 µSv) and is low compared to the daily background radiation of Cambridge (i.e. 6 µSv) or a return transatlantic flight (80 µSv). In fact, the dose from the scans is so small that staff in the department do not need to wear any protective clothing.

Follow up visits
One year after your child’s first visit, we will invite you and your child to come back for follow-up measurements, to see how your child’s bones and muscles have grown. All tests and measurements will be repeated.
Appendix 10.5 - Participant information leaflet for participants aged 8-11 years old

Front and back of leaflet.

Does it hurt when the pictures are taken?
No. The scans are safe and do not hurt. You will not feel the pictures being taken. Having lots of X-rays can be dangerous because radiation is used to take the pictures. The special scanners we use only use a tiny bit of radiation. There are small amounts of radiation in rocks and buildings and when we go on aeroplanes. The amount of radiation in this study is very low and is safe.

What else will I have to do?
When you come for your X-ray pictures, we will measure how tall you are and how much you weigh. We will also ask some questions about your health and how much exercise you do, as this has an effect on your bones and muscles. We will also need to know what stage of puberty you are at. Girls and boys will need to look at some drawings that help decide your stage of puberty. If you want, your mum, dad or guardian can help you with this. All information collected about you will be kept safe and only the research team will be able to see it.

What if I change my mind?
You can change your mind at any time. You can leave the study at any time, even if you have started to take part. If you are not happy about anything in the study please tell your parents/guardians or one of the research team.

What should I do now?
Talk to your mum, dad or guardians. They have got an information leaflet too. If you have any questions about the study that your parents/guardian cannot answer, then ask them to telephone me and I will be able to answer your questions. If you and your mum, dad/guardians decide to take part, your parents will contact me to let me know that you would like to take part. We will ask you to come back 1 year later to have more bone pictures taken to see how much your bones have grown.

Thank you for reading this leaflet.

Inside the leaflet.

Hello. My name is Amy. I am a research student at the Medical Research Council. Some doctors and I are doing a study to find out how children’s bones and muscles grow. This study will help other children who are ill and whose bones are not strong.

Cystic fibrosis is an illness which causes chest infections and stops food from being absorbed in the gut. This illness may affect how bones and muscles grow.

We would like to invite children who have cystic fibrosis and children who don’t have cystic fibrosis to take part in the study to see if there are any differences in their bones and muscles. If you would like to take part, you will also be able to invite a friend or family member like your brother, sister, or cousin to join you in the study. This may make your visit to the research unit more fun and relaxing but only if you want to invite them. Your buddy will need to have their mum or dad with them.

This leaflet tells you about this study. You can talk to your parents/guardians about it and decide if you would like to take part. If you do take part, we will take special X-ray pictures of your bones and muscles. To take the pictures we use special machines called scanners.

How will the X-ray pictures be taken?
Here is a picture of the first scanner. It is called a DXA.

You will have to lie on the bed and the scanner will move over you and take a picture of your body. This will take 15 minutes (the scan will take 5 minutes). Your mum, dad or guardian can stay with you. Afterwards you can have a picture of your skeleton to take home and show your friends.

This scanner is called a pQCT. It takes pictures of the bones in your leg. You will need to sit on a chair in front of the machine and put your leg in the scanner. This will take 20 minutes (the scan will take 5 minutes).

This scanner is called a HR-pQCT. It is very similar to the pQCT scanner but it produces more detailed pictures. You will need to sit on a chair in front of the machine and put your leg in the scanner. This cast does not hurt and is used to make you comfortable during the scan. This will take 15 minutes (the scan will take 3 minutes).

This is called jumping mechanography. This activity will test how strong your muscles are. You will be asked to do some stretching exercises before you start. You will need to stand on a square platform which will be placed on the floor. The first activity will be to jump as high as you can. The second activity is to hop on one leg as fast and hard as possible. This activity will take 15 minutes.
Appendix 10.6 - Participant information leaflet for participants aged 12-16 years old.

Front and back of leaflet.

Inside the leaflet.

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2. Peripheral Quantitative Computed Tomography (pQCT) – The pQCT scanner (photo B) measures the bone mineral of the lower leg. You will be asked to step on a platform with your leg extended through the centre of the scanner, and the scanner will read your bone density.

3. High Resolution pQCT (HR-pQCT) – The HR-pQCT scanner (photo C) is similar to the pQCT scanner, but it uses a different technique to measure bone density at a higher resolution.

4. Jumping Magnometry (JM) – The last test is an activity (D) which measures muscle function. You will be asked to do some stretching exercises before you start. You will stand on a platform and carry out two activities. The first activity is to jump as high as you can and then the second activity is to hop on one leg as fast and hard as possible. The jumping activity will be repeated 3 times with a one minute break in between jumps. This test should take 15 minutes.

---

Are the X-ray scans dangerous?

These scans are very safe*, not painful and only done if you agree to do. The radiation dose from the scans is very small (0.001 mSv) and is considered to be low compared to the background radiation of a return transatlantic flight (80 mSv). Radiation is measured in mSv and is produced by natural sources, such as rocks, buildings, outer space as well as these X-ray machines. In fact, the dose from the sun is so small that after 6 months in space, each astronaut would need to wear a protective hat and a suit.

---

Follow up visits

One year after your first visit, we will invite you back for follow-up measurements to see how your bones have grown. We will repeat all assessments and measurements at this visit.

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Appendix 10.7 - Participant information sheet part 2

Page 1 of 2.

PROTECT PRIVATE
Bone & Muscle Development in Growing Children Study
A Comparison of Cystic Fibrosis and Controls

Participant Information Sheet 2

This sheet gives information on factors such as confidentiality and data protection, communication with your GP, indemnity and compensation, publication etc. It is important that you read and are happy with this along with 'Volunteer Information Sheet 1' before agreeing to take part.

What will happen if I don't want to carry on with the study?
If you decide to withdraw from the study, with your consent, data obtained may be kept and used to contribute to study results or, with your consent, for future studies. However, should you request your scans and data to be destroyed along with any other information relating to you, we will ensure that this takes place.

What if I am asked to leave the study?
Part of volunteering will involve following instructions for the study. You will be fully informed at the start of any study what will be expected of you. If you give fully informed consent and then do not follow the study protocol you may not be able to continue with the study.

What if there is a problem?
In the unlikely event that something should go wrong during the study, procedures will be stopped and a clinician may see you. Your involvement in the rest of the study may be stopped. Standard procedures are in place at MRC Human Nutrition Research (MRC HNR) and for dealing with serious adverse events should they occur.

If you have any other problems, illnesses or concerns during the study you should discuss these with the chief investigator or a member of the study team at MRC HNR or if you are a participant with cystic fibrosis, a member of the cystic fibrosis health team at Addenbrooke’s Hospital (e.g. Dr Robert Roess-Russell).

Complaints:
Any complaints you have about this study will be fully investigated. If you have a concern about any aspect of this study, you should speak with the chief investigator who will do their best to answer your questions (Amy Riddell: Tel 01223 437539 email: amy.riddell@mrc-hnr.cam.ac.uk).
If you remain unhappy and wish to complain formally, you can contact Polly Page, MRC HNR Head of Operations: 01223 426356, polly.page@mrc-hnr.cam.ac.uk.

Harm:
In the event that something does go wrong and you are harmed during the research study there are no special compensation arrangements. If you are harmed and this is due to someone’s negligence then you may have grounds for legal action for compensation against MRC or collaborator but you may have to pay your legal costs. For research carried out at MRC HNR participants would be in the same position as if public liability insurance had been taken out.

MRC undertakes to give sympathetic consideration, on a case by case basis, to claims of non-negligent harm arising from research carried out at MRC HNR provided the claim does not relate to standard treatment.

Will my taking part in this study be kept confidential?
Any information that is collected about you during the course of the research will be kept strictly confidential and MRC HNR will be the custodian of the data. Any information about you that leaves MRC HNR will have your name and address removed so that you cannot be recognised from it.
HNR has a standard confidentiality procedure for participants involved in research. This stipulates how personal information is collected, used, stored and disposed of during and following completion of research projects.

Any information that is collected about you during the course of the study will be kept strictly confidential and secure in locked filing cabinets and/or electronic files on computers that have restricted access. Each participant is assigned a unique, linked anonymising code number that is used on all data collected during the research. This code number is used to identify data in place of personal information.

Only the specified research team will have access to personal identifying data information. However, with your agreement, your GP will be notified of your study results and copies of these letters will also be provided to you. HNR maintains a central record of all research projects but this does not include personal information on participants. With your agreement we will store data for 20 years. With your consent, and with the appropriate research ethics approval, retained data may be used for future studies.

**Involvement of your GP/clinician**

With your permission, your GP will be notified that you are participating in this study and your study results.

**What will happen to any scans I give?**

Any scans that are collected during the course of the project will be processed and kept in accordance with MRC HNR standard operating procedures. Each participant is assigned a unique, linked anonymising code to be used to label all samples collected during the research. This code number is used to identify stored scans in place of personal information. Only the specified research team will have access your scans. With your agreement we may store scans for up to 20 years and then they will be destroyed. With your consent, and with the appropriate research ethics approval, retained scans may be used for future studies.

**What will happen to the study results?**

This study is part of an educational qualification and supervisors at the MRC HNR will view data collected. The overall study results may be presented at scientific meetings or published in a scientific journal. You will not be identified in these presentations and publications. A summary of the study results will be available on the MRC HNR website and we will be happy to discuss the results with you at the end of the study.

**Who is organising and funding the study?**

This study is being organised by the ’Nutrition and Bone Health’ group at MRC HNR with Dr Kate Ward and Dr Robert Ross-Russell (based at Addenbrooke’s Hospital). The study is funded by the Medical Research Council.

**Who has reviewed the study?**

This study has been reviewed by the Research Review Board of MRC HNR and by the Cambridge South Research Ethics Committee.
Appendix 10.8 - Hospital newsletter for recruiting participants with CF

PARTICIPANTS REQUIRED

Bone & Muscle Health in Children Study

Dear Patients and Parents/Guardians,

We would like to invite you to take part in a study titled ‘Bone & Muscle Development in Growing Children: A Comparison of Cystic Fibrosis and Controls’ at MRC Human Nutrition Research, Cambridge. The study will start in June 2013 and will continue to run for a year.

This study will investigate how bone and muscle grow during childhood and adolescence using advanced measurement techniques. We will use scanners which are able to give very detailed images and measurements of bone and muscle which will allow us to understand what factors make a bone ‘a healthy’ bone. By comparing children with and without cystic fibrosis (CF) we will be able to understand how diseases such as CF affect growth.

We are looking for a total of 225 volunteers, aged 8 to 16 years old, White Caucasian, with and without cystic fibrosis for this study. Each volunteer will be required to attend MRC Human Nutrition Research on two visits which will be one year apart. Each visit will take a maximum of 2 hours. The study is designed to measure how much your bones have grown over a year.

We are also giving patients with CF an option to invite a friend or family member to take part in the study with them. This is known as the ‘Buddying Scheme’ which is optional and not a requirement. By inviting a friend to join you in the study, we hope it will make the experience more enjoyable. Your friend does not have to know you have CF. This will be kept private. Buddies will need their parent/guardian present to take part in the study.

As a sign of our appreciation, your child will be offered an honorarium of £20 (in gift vouchers) for the completion of all procedures at each visit i.e. a total of £40 in gift vouchers for the completion of both visits. Your child will also receive a certificate for their willingness and contribution to the study. Reasonable travel expenses will also be paid for each visit.

I have enclosed a study information leaflet with more details. More study leaflets can be found in the reception area of the Children’s Outpatients Clinic 6, Addenbrooke’s Hospital or you can ask the CF health team for a leaflet.

If you are interested in taking part, or if you have any questions, please contact me (Amy Riddell: The chief investigator) on 01223 437539 or by email: amy.riddell@mrc-hnr.cam.ac.uk. I will also be at Addenbrooke’s Hospital, Children’s Outpatients Clinic 6 every Tuesday and Thursday if you would like to speak to me in person about the study.

Thank you for taking the time to read this letter.

Kind regards
Amy Riddell
(Chief Investigator at MRC HNR)

Dr Robert Ross-Russell
(Collaborator at Addenbrooke’s Hospital)
Appendix 10.9 - Invitation letter from Dr Robert Ross-Russell inviting patients with CF

Department of Paediatrics
Box 45

DATE

Dear Parent/Guardian Name,

Patient name: Patient name
Patient D.O.B: Date of Birth

You may be aware that for the last year we have been running a Medical Research Council funded study into bone health in Cystic Fibrosis (CF). CF is known to cause growth disturbances which may increase a patient’s risk to developing osteoporosis (brittle bones) and fracture in adolescence and adult life. This study will help us to understand how CF affects bone and muscle development in children and to develop ways to reduce CF-related bone disorders in the future. This has been a hugely successful study and has recruited over 165 children, of whom 22 have been children with Cystic Fibrosis.

Amy Riddell, who is running the study in conjunction with our CF clinical health team, has asked that I let families know that this study will be finishing recruitment in February 2014 (after school half-term holidays). We are keen for more CF patients to take part which will help us understand more about how CF affects bone development and health. Therefore, I am writing to let you know about the study to see if you and your child would be interested in taking part.

I have enclosed a copy of the participant information sheets for parents and children. These explain clearly what the study is about and what would be involved if your child took part. If you are interested in finding out more or would like to take part please contact Amy as soon as possible. The study will involve a visit to the MRC Human Nutrition Research Centre in south Cambridge and the research team are happy to pay for travel expenses and will find a time that suits you for the visit to the research unit. The study visit can be made to coincide with your hospital appointments.

There is absolutely no obligation on any of our patients to take part in this study or any other research. As a specialist CF Team, clinical research is a very important component of our work and all studies are subject to scientific review and approval by a National Research Ethics Committee. On that basis I hope you will understand me writing to you directly to bring this study to your attention.

Please feel free to contact Amy Riddell on 01223 437 539 (during day-time) or 07814222198 (evenings) and via email: amy.riddell@mrc-hnr.cam.ac.uk.

Many thanks for your time and interest.

Yours sincerely

Dr R Ross Russell
Consultant in Respiratory & General Paediatrics
PROTECT PRIVATE

Head Teacher’s Name ..........  
School Address

Date

MRC Human Nutrition Research  
Elsie Widdowson Laboratory  
120 Fulbourn Road  
Cambridge CB1 9NL

Dear (Head Teacher/Principal Name),

RE: Bone & Muscle Development in Growing Children Study: A Comparison of Cystic Fibrosis and Controls

We are undertaking a research study entitled Bone & Muscle Development in Growing Children Study: A Comparison of Cystic Fibrosis and Controls’ (REC No: 13/EE/0078) at Medical Research Council – Human Nutrition Research (HNR) in Cambridge. This study involves taking bone mineral measurements of children and young adolescents aged between 8-16 years with and without cystic fibrosis. We are writing to ask if you and the school would be willing to help us in advertising this study.

This study is being conducted to find out how bones and muscles develop during growth. This information will be used to create a reference database of bone density and muscle function values. This data will be compared to those from children with cystic fibrosis from the East Anglia region to help us understand why they may fracture later in life.

We are aware of the heavy workloads carried by you, the teachers and administrators of the school. We would appreciate your help if we could place the enclosed posters around the school and circulate information about the study in school letters to parents/guardians (via students/school website/online newsletters).

We have enclosed copies of the study poster and examples of the study information leaflets. If you are interested in finding out more about this study and are willing to also distribute leaflets, please contact Amy Riddell (Chief Investigator) on 01223 437539, or email: amy.riddell@mrc-hnr.cam.ac.uk. I will be in contact by telephone soon to see if you are interested in helping us with this study.

Thank you for your time.

Yours sincerely,

Amy Riddell (Chief Investigator)

Tel (direct): 01223 437539  
Tel (Switchboard): 01223 426356  
Email: amy.riddell@mrc-hnr.cam.ac.uk
# SCREENING QUESTIONNAIRE

**Name of Chief Investigator:** Amy Riddell  

**Name of researcher conducting the questionnaire:** ______________________

Screening questionnaire to be conducted by the chief investigator or by a trained member of the research team. Potential participants should answer the questionnaire in a private setting i.e. during CF patient’s clinic appointment or by telephone in potential participant’s home.

## 1. PERSONAL DETAILS

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**How did you hear about the study? e.g. Posters, leaflets, newsletter etc.**

**Are you/your child currently taking part in any other research studies or have done in the last year? (Please circle)**

*Provide details if yes:*

**YES** | **NO**

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Version 2: 12/06/2013  
REC No: 13/EE/0078  
Screening Questionnaire  
Page 1 of 3
### 2. STUDY CRITERIA

Can you confirm that your child is/ has: | YES | NO |
---|---|---|
1. Aged 8 - 16 years old | | |
2. White Caucasian | | |
3. Lives within East Anglia | | |
4. Not been exposed to x-ray recently for research purposes | | |
5. Not taken part in any clinical drug trials for research purposes | | |
6. Not pregnant | | |
7. Able to give informed & written consent | | |
8. Willing to answer questions about their pubertal staging | | |
9. Cystic fibrosis | | |
If so, which hospital(s) do you attend? | | |

### 3. ANTHROPOMETRIC MEASUREMENTS

Do you know your child’s: (give approximate measures)

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<th>Weight (Kg)</th>
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**BMI**

### 4. GENERAL HEALTH

Does your child have or has had any of the following conditions? | YES | NO |
---|---|---|
1. Diabetes | | |
2. Stomach or bowel problems | | |
3. Asthma/Eczema/Mayfever | | |
4. High cholesterol | | |
5. Anaemia | | |
6. Food/Drug allergies | | |
7. Eating Disorder | | |
8. Chronic medical conditions known to affect bone mass |
   (i) Rickets |
   (ii) Osteomalacia |
   (iii) Osteoporosis |
   (iv) Osteogenesis Imperfecta |
   (v) Other |
9. Prolonged periods of immobilisation in the last year e.g. surgery |
10. Condition making scanning difficult e.g. scoliosis |
11. Take drugs known to affect bone mass e.g. steroids, bisphosphates |
12. Received or awaiting an organ transplant |
13. Growth problems e.g. due to premature birth or hypothyroidism |
If you have answered yes, please give details here:
Please list any medication that your child is taking, either prescribed by your doctor or purchased over the counter e.g. contraception pill/ injection, inhalers, vitamins etc.

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<tr>
<th>5. AVAILABILITY AND TRANSPORT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Would you have any difficulty:</td>
</tr>
<tr>
<td>(i) In attending MRC HNR on two occasions (one year apart)?</td>
</tr>
<tr>
<td>(ii) With your transport arrangements?</td>
</tr>
<tr>
<td>Do you have any preferred days to attend the unit?</td>
</tr>
<tr>
<td>If yes, please give days.</td>
</tr>
</tbody>
</table>

**TO BE COMPLETED BY RESEARCHER**

<table>
<thead>
<tr>
<th>Eligible for this study?</th>
<th>YES</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>YES</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Formal invitation and Information sheets have been given/ sent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NO</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Would you like to receive information about other studies at HNR-MRC?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Would you like this questionnaire to be sent to you?</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Name of Investigator | |
| Signature | |
| Date | |
PROTECT PRIVATE

Participant parent’s name

Participant’s Address

Date ..............

Dear Participant’s parent name,

Thank you and your child for agreeing to take part in the following study Bone & Muscle Development in Growing Children: A Comparison of Cystic Fibrosis and Controls.

Your appointment to visit the Medical Research Council - Human Nutrition Research unit will be on:

Date, Time.

Before attending your appointment, we advise that your child should wear loose clothes e.g. school P.E kit, with no metal zips or hooks. Jogging bottoms or skirt would be ideal.

The address of the research unit is:

MRC Human Nutrition Research
Elsie Widdowson Laboratory
Peterhouse Technology Park
120 Fulbourn Road
Cambridge
CB1 9NL

Directions and maps are attached to this appointment letter.

Please contact Amy Riddell (chief investigator) on Tel: 01223 437539 or Email: amy.riddell@mrc-hnr.cam.ac.uk if you cannot make your appointment or if you have any questions about the study.

We look forward to meeting you.

Yours sincerely,

Amy Riddell
Tel (Direct): 01223 437539
Tel (Switchboard): 01223 426356
MAP AND DIRECTIONS

Travelling expenses
If you travel to the department by bus, train or taxi you will need to produce a ticket or receipt in order to claim your travelling expenses. If travelling by car, you will need to record your mileage.

Car Park
If you are travelling by car to MRC HNR unit, you will be able to park your car for free in the spaces located in front of the research unit.

Visiting MRC HNR Unit
The MRC HNR unit is situated at on the left hand side of Peterhouse Technology Park. The unit is named clearly on the outside as ‘MRC Human Nutrition Research, Elsie Widdowson Laboratory’. As you enter the building, the reception area is in front of you. Please ask the receptionist for Amy Riddell. She or another member of the study team will come to greet you and take you through to the HNR Volunteer Suite.

Directions to MRC Human Nutrition Research, Elsie Widdowson Laboratory, Cambridge, CB1 9NL, UK

From Addenbrooke’s Hospital to HNR

By Bus
The bus station is located at the front entrance of the hospital on Hills Rd. The Citi 1 (Fulbourn) service operated by Stagecoach is the best option for getting to HNR. Please see Stagecoach bus time table for more information.

(http://www.stagecoachbus.com/timetables.aspx?serviceId=8&locationId=32&from=8&to=8&local=81).

The Citi 1 bus stops at Cherry Hinton High Street. HNR is a 10 minute walk from where the buses stop.

By Car
From the main hospital entrance i.e. where the bus station is located, take the second exit on the roundabout onto Fendon Rd

At the next roundabout, take the 3rd exit onto Queen Edith’s Way

Turn right onto Fulbourn Rd, continue straight ahead and take the second turning on the right which is the Peterhouse Technology Park entrance.

Turn left at the small roundabout.

MRC HNR (Elsie Widdowson Laboratory) is on the left, down the ramp.

By Rail
Trains run every half an hour from London. Trains from London Kings Cross take about 50 minutes while the less direct trains from Liverpool Street station can take about 1h 20 minutes.

HNR is a 10-15 minute drive from the station and it is advisable to take a taxi. The Citi 1 bus stops at Cherry Hinton High Street. HNR is a 10 minute walk from where the buses stop.

If travelling from the north of the country it is worth noting that all trains to Cambridge pass through Ely and often changes have to be made here.

By Bus
Citi 1 (Fulbourn) and Citi 3 (Cherry Hinton) services operated by Stagecoach are the best options for getting to HNR.

The Citi 1 and Citi 3 buses stop at Cherry Hinton High Street. HNR is a 10 minute walk from where the buses stop. Please see Stagecoach bus time table for more information.

(http://www.stagecoachbus.com/timetables.aspx?serviceId=8&locationId=32&from=8&to=8&local=81).

By Car From the South
Leave the M11 at junction 9 for the A11 to Newmarket.

Continue along the A11 until the Fulbourn / Balsham exit.

Turn left and continue through Fulbourn.

At the roundabout, continue straight ahead and take the first turning on the left which is the Peterhouse Technology Park entrance.

Turn left at the small roundabout, Elsie Widdowson Laboratory (HNR) is on the left, down the ramp.
By Car From the North
Travel south on the A14, just before it becomes the M11 turn left and continue to bear left until you 'rejoin' the A14.
Pass three further exits and then turn right at the fourth exit on to the A1303 (signposted for Newmarket, Bottisham and Cambridge).
Turn left at the first roundabout (into Airport Way).
Turn left at the next roundabout (into Gazelle Way; signposted to Trumpington and Addenbrooke's).
Carry straight on at the next two roundabouts (the second of which is for Tesco), going over a level crossing between them.
Turn right at the next roundabout, then take the first turning on the left which is the Peterhouse Technology Park entrance. Turn left at the small roundabout.
Elsie Widdowson Laboratory (HNR) is on the left, down the ramp.

Maps
From Addenbrooke's Hospital to MRC-HNR Unit

From Cambridge Train Station to MRC-HNR Unit
Appendix 10.13 - Informed consent form for parents/guardians

CONSENT FORM

(Participant’s parent/guardian if child is <16 yrs old)

Name of Chief Investigator: Amy Riddell

Parent/legal guardian’s written consent:

I __________________ being the parent/guardian of (name of child) __________________ hereby give permission fully and freely for my child to participate in the Bone & Muscle Development in Growing Children Study. I have read the parent/guardian information leaflet and understand that I can withdraw this consent at any stage.

Name of Parent/Guardian (Please print) __________________ Date __________________ Signature __________________

GENERAL STATEMENTS

1. I confirm that I have read and understand the information sheet entitled "Bone & Muscle Development in Growing Children: A Comparison of Cystic Fibrosis and Controls, 12/06/2013 (version 3.0) for the above study and have had the opportunity to ask questions.

2. I understand that my child’s participation is voluntary and that my child is free to withdraw at any time, without giving any reason, and without their medical care or legal rights being affected.

3. I consent to my child’s doctor being notified of my child’s participation in this research and to being informed of their results.

4. I understand that my child cannot take part in this study if they are pregnant. My child is not pregnant. I will inform the research team if my child becomes pregnant during the study.

5. Participants with cystic fibrosis ONLY: I understand that sections of any of my child’s medical notes may be looked at by responsible individuals from MRC HNR where it is relevant to my child’s taking part in research. I give permission for these individuals to have access to my child’s records.

6. I am willing to be contacted again in the future about the present study and any potential follow-up from it. I understand that my child is under no obligation to undergo any future additional tests and can withdraw this consent at any time by notifying the study team.

7. I agree to my child to taking part in the above study.

Section to be completed by the researcher:

Name of researcher (Please print) __________________ Date: __________________ Signature: __________________
Appendix 10.14 - Informed consent form for participants aged 16 years old and older

\[\text{CONSENT FORM} \]  
\[\text{(Participants aged 16 years or older)}\]

\textbf{Name of Chief Investigator:} Amy Riddell

\textbf{Participant's written consent:}

I (name of participant) ____________________________ hereby give permission fully and freely to participate in the Bone & Muscle Development in Growing Children Study. I have read the study information leaflets and understand that I can withdraw this consent at any stage.

\textbf{Name of Participant (Please print)} Date Signature

\textbf{GENERAL STATEMENTS}

1. I confirm that I have read and understand the information sheet entitled ‘Bone & Muscle Development in Growing Children: A Comparison of Cystic Fibrosis and Controls, 12/06/2013 (version 3.0) for the above study and have had the opportunity to ask questions.

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, and without my medical care or legal rights being affected.

3. I consent to my doctor being notified of my participation in this research and to being informed of my results.

4. I understand that I cannot take part in this study if I am pregnant. I am not pregnant and will inform the research team if I become pregnant during the study.

5. Participants with cystic fibrosis ONLY: I understand that sections of any of my medical notes may be looked at by responsible individuals from MRC HNR where it is relevant to my taking part in research. I give permission for these individuals to have access to my records.

6. I am willing to be contacted again in the future about the present study and any potential follow-up from it. I understand that I am under no obligation to undergo any future additional tests and can withdraw this consent at any time by notifying the study team.

7. I agree to take part in the above study.

\textbf{Section to be completed by the researcher:}

\textbf{Name of researcher (Please print)} Date Signature

Version 3: 12/06/2013 Consent Form (16 years old) Page 1 of 1
REC No: 13/EE/0078
Appendix 10.15 - Assent form for participants aged between 8-15 years old

PROTECT PRIVATE
Bone & Muscle Development in Growing Children Study
A Comparison of Cystic Fibrosis and Controls

ASSENT FORM
(Participants aged 8 to 15 years old)

Name of Chief Investigator: Amy Riddell

GENERAL STATEMENTS

Has somebody else explained this study to you? Yes/ No
Do you understand what this study is about? Yes/ No
Have you asked all the questions you want? Yes/ No
Have you had your questions answered in a way you understand? Yes/ No
Do you understand it's OK to stop taking part at any time? Yes/ No
Are you happy to take part? Yes/ No

If any answers are 'no' or you don’t want to take part, don’t sign your name!
If you do want to take part, you can write your name below

Participant’s written assent:
Your name ___________________________ Date ___________________________

Thank you for helping us!

Section to be completed by the researcher:

Name of researcher (Please print) ___________________________ Date ____________ Signature ___________________________
Appendix 10.16 - Musculoskeletal questionnaire

Page 1 of 4.

Date of Questionnaire: ____/____/_____

Baseline Measurement

PROTECT PRIVATE
Bone & Muscle Development in Growing Children Study
A Comparison of Cystic Fibrosis and Controls

MUSCULOSKELETAL QUESTIONNAIRE

Name of Chief Investigator: Amy Riddell

Name of researcher conducting the questionnaire: ___________________________

All personal details on this form will be kept confidential. Please answer the questions below. You may find some questions to be not relevant to you/your child and some questions may be embarrassing or very personal. Please ask the researcher for help or for any questions you may have. Once completed, place the form into the envelope provided.

1. PERSONAL DETAILS

<table>
<thead>
<tr>
<th>Participant's ID No:</th>
<th>BMS 001</th>
<th>Gender (Please circle)</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date of Birth (DD/MM/YYYY)</td>
<td></td>
<td>Age</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2. ANTHROPOMETRIC MEASUREMENTS (to be completed by researcher)

<table>
<thead>
<tr>
<th>Standing height cm</th>
<th>Weight Kg</th>
<th>Sitting height cm</th>
<th>BMI Kg/m²</th>
</tr>
</thead>
</table>

3. DETAILS OF GENERAL PRACTITIONER

<table>
<thead>
<tr>
<th>Name of Doctor:</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Doctor's Surgery Address:</td>
<td></td>
</tr>
<tr>
<td>Telephone No:</td>
<td></td>
</tr>
</tbody>
</table>

4. FAMILY HISTORY

<table>
<thead>
<tr>
<th>Is there a history of osteoporosis or broken bones in your family?</th>
<th>YES</th>
<th>NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>If yes, what is their relation to you?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>At what age were they diagnosed?</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### 5. FRACTURES

<table>
<thead>
<tr>
<th>Have you ever suffered a broken bone?</th>
<th>YES</th>
<th>NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>If yes, please tick which bone(s)?</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Wrist</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shoulder</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Foot</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other (Please state which)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>What age were you?</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### 6. MEDICATION

<table>
<thead>
<tr>
<th>Have you ever taken any of the following</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name of drug</td>
</tr>
<tr>
<td>Steroids (tablets or inhalers):</td>
</tr>
<tr>
<td>Contraceptive pill:</td>
</tr>
<tr>
<td>Calcium / Vitamin D supplements:</td>
</tr>
</tbody>
</table>

Please list all medications you have taken in the past, or which you take at present, including all prescribed drugs and 'over the counter' drugs. Also include vitamin supplements.

<table>
<thead>
<tr>
<th>Name</th>
<th>Age when taken</th>
<th>Age when stopped</th>
<th>Tick if still taking</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Have you ever had any operations or surgery?  

<table>
<thead>
<tr>
<th>YES</th>
<th>NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>If yes, what operation did you have?</td>
<td>Age at time of operation?</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### 7. EXERCISE

How many hours per week do you spend on the following activities? Do not include school PE lessons.

<table>
<thead>
<tr>
<th>Activity</th>
<th>No of hours per week</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cycling</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Swimming</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Walking</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Running</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dancing* (ballet/ tap)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basketball</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hockey **</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Football</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tennis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gymnastics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trampolining</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight training</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aerobic exercise</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other***</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Please state what type of dancing. ** Please state whether this is field hockey, roller hockey or ice hockey. *** Please state what sport or exercise it is.

** How many hours of P.E per week?**

** How many hours per day do you spend watching TV & DVDs?**

<table>
<thead>
<tr>
<th>Activity</th>
<th>No of hours per week</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

** How many hours per day do you spend on the computer/mobile phone playing computer games?**

<table>
<thead>
<tr>
<th>Activity</th>
<th>No of hours per week</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

8. SMOKING

Do you smoke cigarettes or tobacco?  

- YES
- NO

If yes, how many per day?  

- 1 - 10
- 11 - 20
- More than 20

What age did you start smoking?
9. PUBERTAL STAGE i.e. Tanner Staging

<table>
<thead>
<tr>
<th>FOR MALE PARTICIPANTS ONLY</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Has your voice broken?</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>Age of voice breaking?</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>FOR FEMALE PARTICIPANTS ONLY</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Have you started your periods?</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>If yes, what age did they start?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Are your periods regular?</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>Is there any chance you could be pregnant?</td>
<td>YES</td>
<td>NO</td>
</tr>
</tbody>
</table>

Please fill in the self-assessment form about puberty provided by the researcher. If you are having difficulties filling in this form please ask the researcher for help.

| FOR OFFICAL USE. To be completed by the researcher |
|---------------------------------|----|----|----|----|----|
| FOR MALE PARTICIPANT            | 1  | 2  | 3  | 4  | 5  |
| Pubic hair                      |    |    |    |    |    |
| Testes volume                   |    |    |    |    |    |
| Left side:                      |    |    |    |    |    |
| Right side:                     |    |    |    |    |    |
| Total:                          |    |    |    |    |    |

<table>
<thead>
<tr>
<th>FOR FEMALE PARTICIPANT</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pubic Hair</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast development</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Appendix 10.17 - Pubertal self-assessment form for female participants

Page 1 of 2.

PROTECT PRIVATE

Bone & Muscle Development in Growing Children Study
A Comparison of Cystic Fibrosis and Controls

SELF-ASSESSMENT OF
FEMALE PUBERTAL STAGING

Participant ID Number: BMS ___ ___
Name of Chief Investigator: Amy Riddell

This is a self-assessment form to help us understand your/your child’s current pubertal development.

You may find some of the pictures in this form to be embarrassing and personal. All information you give will be kept private.

The researcher will ask you to go into a private room to fill in this form. You can ask your parent/guardian to help you with this form or you can do this by yourself. The researcher will not be in the private room with you but will be outside the room if you have any questions about the form.

Please follow the instructions below. Once you have finished, place the form into the envelope provided and hand it to the researcher.

Pubic Hair

First you need to look at the area between your tummy and the top of your legs. This is the pubic area. The pictures below show you how your pubic hair will grow in this area.

Look at the pictures and put a tick underneath the picture that looks most like your own pubic area.
Breasts

The pictures below show the growth of the breasts.

Look at your own breasts and put a tick in the box under the picture which looks like your own breasts.
Appendix 10.18 - Pubertal self-assessment form for male participants

Page 1 of 2.

PROTECT PRIVATE
Bone & Muscle Development in Growing Children Study
A Comparison of Cystic Fibrosis and Controls

SELF-ASSESSMENT OF
MALE PUBERTAL STAGING

Participant ID Number:  
BMS _ _ _

Name of Chief Investigator:
Amy Riddell

This is a self-assessment form to help us understand your/your child’s current pubertal development.

You may find some of the pictures in this form to be embarrassing and personal. All information you give will be kept private.

The researcher will ask you to go into a private room to fill in this form. You can ask your parent/guardian to help you with this form or you can do this by yourself. The researcher will not be in the private room with you but will be outside the room if you have any questions about the form.

Please follow the instructions below. Once you have finished, place the form into the envelope provided and hand it to the researcher.

Pubic Hair

First you need to look at the area between your tummy and the top of your legs. This is the pubic area. The pictures below show you how your pubic hair will grow in this area.

Look at the pictures and put a tick underneath the picture that looks most like your own pubic area.
Testes

The pictures below represent the growth of the testes. You need to feel your own testes, one at a time, and compare the size of each testicle to the beads you have been given.

Decide which bead feels most like your testicle in size. On each bead there is a number. The numbers are the same as those shown on the beads in the picture.

Put a tick on the picture of the bead with the same number as the one which feels most like your own testicle. Do this first for your left testicle and then again for your right testicle. The number may or may not be the same for both sides.
Appendix 10.19 - Authorisation of radiation exposure and check list

Page 1 of 2.

PROTECT PERSONAL

Bone & Muscle Development in Growing Children Study
A Comparison of Cystic Fibrosis and Controls

Authorisation of Radiation Exposure & Check List

Name of Chief Investigator:  Amy Riddell

AUTHORISATION AND JUSTIFICATION OF RADIATION EXPOSURE

I have examined the inclusion and exclusion criteria for the following study titled 'Bone & Muscle Development in Growing Children Study: A comparison of Cystic Fibrosis and Controls'.

I take responsibility for this participant’s radiation exposure and consider the below named participant to be suitable for this study

<table>
<thead>
<tr>
<th>Name of participant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>D.O.B of participant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Name of Scanning Expert</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dr Kate Ward</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Signature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Date:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
</tbody>
</table>

When all above sections are complete proceed to Scan/X-ray
### Legal Requirements

Print name of researcher responsible: Amy Riddell

<table>
<thead>
<tr>
<th>Confirm the following</th>
<th>Signature of researcher</th>
<th>Date Day /Month/Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Consent form completed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Authorisation and justification of radiation exposure section completed</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>ONLY females</strong> Participant has confirmed that she is not pregnant nor likely to be pregnant</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Techniques involving ionising radiation

<table>
<thead>
<tr>
<th>Type of Scan</th>
<th>Location of Scan</th>
<th>Signature of researcher</th>
<th>Date Day /Month/Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>DXA (GE-Lunar Prodigy)</td>
<td>Whole-Body</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pQCT (Stratec 2000)</td>
<td>Tibia</td>
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<tr>
<td>HR-pQCT (X-Treme)</td>
<td>Tibia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Participant Information</td>
<td>Standing Height (cm):</td>
<td>Visit date:</td>
<td></td>
</tr>
<tr>
<td>--------------------------</td>
<td>-----------------------</td>
<td>-------------</td>
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<tr>
<td>Participant initials:</td>
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</tr>
<tr>
<td>Date of birth:</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Gender: M / F</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sitting Height (cm):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (kg):</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Sitting Leg length (cm):</td>
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<td></td>
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</tr>
<tr>
<td>Scanning Leg Length (mm):</td>
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<table>
<thead>
<tr>
<th>pQCT and HR pQCT</th>
<th>DXA</th>
<th>Jumping Mechanography</th>
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</thead>
<tbody>
<tr>
<td>Tibia scanned previously:</td>
<td>any implants (IV or g...</td>
<td>complete</td>
</tr>
<tr>
<td>Side to be scanned at MNR:</td>
<td>Yes / No</td>
<td></td>
</tr>
<tr>
<td>Right / Left</td>
<td>if yes, give details:</td>
<td></td>
</tr>
<tr>
<td>pQCT:</td>
<td>Whole body</td>
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<tr>
<td>Distal tibia</td>
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<tr>
<td>HR pQCT</td>
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<td></td>
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<tr>
<td>Distal tibia</td>
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<tr>
<td>Comments:</td>
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</tbody>
</table>

| PAT NO: | |

Consent completed: |
Justification form completed: |
Appendix 10.21 - HR-pQCT control files for scanning right and left tibia

Left: Standard control files for scanning adults, and right: adopted control files for scanning children.
Appendix 10.22 - Simple linear regression model testing whether sex modifies the relationship between total body BMC and lean body mass in healthy participants

```
Call:
  lm(formula = I(log(Total.LM.BMC)) ~ Sex * I(log(WB_Lean_g)),
     data = mydata)

Residuals:
     Min      1Q  Median      3Q     Max
-0.28344 -0.07421 -0.01598  0.07213  0.44097

Coefficients: 
                Estimate Std. Error t value Pr(>|t|)
 (Intercept)    -6.68965    0.40807  -16.394  < 2e-16 ***
   Sex          -2.18032    0.68596    -3.178  0.001805 **
 I(log(WB_Lean_g))  1.33152    0.03922    33.949  < 2e-16 ***
Sex2:I(log(WB_Lean_g))  0.22409    0.06625     3.382  0.000921 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 1

Residual standard error: 0.1123 on 147 degrees of freedom
Multiple R-squared:  0.9317,  Adjusted R-squared:  0.9303
F-statistic: 665.2 on 3 and 147 DF,  p-value: < 2.2e-16
```