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Parental and offspring bone mass: associations and mechanisms

by

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Thesis for the degree of Doctor of Philosophy

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ABSTRACT

FACULTY OFMEDICINE

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Parental and offspring bone mass: associations and mechanisms

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Introduction: Although there is evidence that measures of bone size, mineralisation and density are partly inherited, there are scant data available from which to elucidate independent associations of mother and father, and the mechanisms underlying any relationships. The aim of this work was to characterise the independent bone relationships between mother-child and father-child, as differences between the two may point towards an intrauterine effect in early life. As the placenta is the conduit for all maternal intrauterine effects, the role of placental size in offspring bone mass was also explored.

Methods: Using two large prospective population-based cohorts, The Southampton Women's Survey (SWS) and The Avon Longitudinal Study of Parents and Children (ALSPAC), relationships between offspring bone mass at birth through to 17.7 years, and placental size were assessed. Bone mass measurements were obtained using dual-energy X-ray absorptiometry (DXA) and peripheral quantitative computed tomography (pQCT); placental measurements were either obtained in mid-pregnancy (SWS) using ultrasound or at delivery (ALSPAC). Subsequently, correlation and regression methods were used to assess the relationships between DXA and pQCT-derived measurements of parental and offspring bone indices.

Results: Parent and offspring bone mass were positively associated, with a greater magnitude of relationship observed for measures of bone size, than bone density. Parent-child bone associations were significantly stronger for mother-child than father-child for several variables, again predominantly those associated with bone size. Placental volume was positively associated with offspring bone mass at birth, with associations remaining during puberty into late childhood. These parent-child relationships were not influenced by placental size or other environmental factors previously shown to affect offspring bone mass.

Conclusions: We observed strong relationships between offspring bone mass and both placental size and parental size. Mother-child bone associations were stronger than those for father-child, and were independent of placental size. Whilst direct genetic inheritance offers one mechanistic explanation, increasing understanding of epigenetic mechanisms and the disparity between maternal and paternal associations suggest that such relationships could be in part underpinned by gene-environment interactions in early life, and an effect on placental function.

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DECLARATION OF AUTHORSHIP

I, Christopher Holroyd declare that the thesis and the work presented in the thesis are both my own, and have been generated by me as the result of my own original research.

"The relationships between parental and offspring bone mass: associations and mechanisms"

I confirm that:

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- where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work;
- I have acknowledged all main sources of help;
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Project outputs

Findings presented in this work:

Chapter 4:

Holroyd CR, Osmond C, Barker D, Ring S, Lawlor DA, Tobias JH, Davey Smith G, Cooper C, Harvey NC. Placental size is associated differentially with postnatal bone size and density. J Bone Miner Res 2016 31(10):1855-1864

Chapter 5:

Holroyd C, Harvey NC, Crozier SR, Winder N, Mahon PA, Godfrey KM, Inskip HM, Cooper C and the SWS Study Group. Placental size at 19 weeks predicts offspring bone mass at birth: Findings from the Southampton Women's Survey. Placenta 2012; 33(8):623-9

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Definitions and Abbreviations

25(OH)D:	25 hydroxyvitamin D; calcidiol
aBMD:	Areal bone mineral density
ALSPAC:	Avon Longitudinal Study of Parents and Children
AMI:	Axial moment of inertia
BA:	Bone area
BMAD:	Bone mineral apparent density
BMC:	Bone mineral content
BMD:	Bone mineral density
CSA:	Cross-sectional area
CV:	Coefficient of variation
DNMT:	DNA methyl transferase
DXA:	Dual-energy X-ray absorptiometry
EC:	Endosteal circumference
FFQ:	Food frequency questionnaire
FRAX®:	Fracture Risk Assessment Tool
GP:	General Practice
GPC6:	Glypican 6
GWAS:	Genome wide association study
IOV:	Inter-observer variability
IU:	International units

LCPUFA: Long chain polyunsaturated fatty acid

LRP4: Low-density lipoprotein receptor-related protein 4

LRP5: Low-density lipoprotein receptor-related protein 5

μSv Microsievert

M-CSF: Macrophage colony stimulating factor

mRNA: messenger RNA

MVPA: Moderate to very vigorous physical activity

OPG: Osteoprotegerin

OPN: Osteopontin

PBM: Peak bone mass

PC: Periosteal circumference

PMCA: Placental membrane Ca^{2+} ATPase

pQCT: peripheral quantitative computed tomography

PTH: Parathyroid hormone

PTHrP: Parathyroid hormone related peptide

RANK: Receptor activator of the nuclear factor κB

RANKL: Receptor activator of the nuclear factor κB ligand

RXRA: Retinoid X receptor alpha

scBMC: Size-corrected bone mineral content

SD: Standard deviation

SNP: Single nucleotide polymorphism

SPA: Single photon absorptiometry

SSI: Stress-strain Index

SWS: Southampton Women's Survey

TNFRSF: Tumour necrosis factor receptor super family

vBMD: Volumetric bone mineral density

WHO: World Health Organisation

1. Background

1.1 Introduction

Osteoporosis is an increasing public health problem worldwide which has a massive impact both at an individual level and on society as a whole, due to its association with low trauma fractures (1). Bone strength in later life is dependent upon the peak bone mass achieved in early adult life, and the rate of bone loss with advancing age. Peak bone mass has been shown to be a major contributor to the risk of osteoporotic fracture in later life (2), and thus potential strategies to optimise peak bone mass may be important in reducing the burden of osteoporosis in later life.

There is a strong evidence base that poor growth in early life leads to a reduction in peak bone mass attained in early adulthood and an increased risk of fracture in later life (3;4). It is unclear however, how much of this relationship is due to inherited factors, and if so how much is due to contribution from the mother, and how much is paternal in origin. In addition there is the possibility that some inheritance may be a result of the shared environment between parents and their offspring (for example dietary habits and physical activity may be similar among parents and their children) –so called environmental inheritance.

In addition to direct genetic inheritance, it has been observed that certain environmental stimuli during intra-uterine and early life are associated with later bone accrual (5). Studies have found that the maternal environment, such as maternal serum 25 hydroxyvitamin D (25(OH)D), during pregnancy influences offspring bone accrual, however the mechanisms underlying this remain poorly understood (5;6). It has been hypothesised that environmental stimuli at critical periods during development lead to persisting changes in structure and function which in turn may influence the magnitude of peak bone mass achieved; a phenomenon known as programming (7). The proportional contribution of maternal genetics, inherited environment and direct effects mediated via the placenta in pregnancy on offspring bone mass remain unclear.

The paternal influence on offspring bone mass has also been investigated, albeit to a lesser extent, and again significant relationships between paternal factors and offspring bone mass have been observed (8). Again the mechanisms underlying this are unclear and the relative contribution of paternal genetic and environmental factors undetermined. No studies to date have examined the combined maternal and paternal influences, in addition to offspring environmental influences, on offspring bone mass in the Western World.

It is clear therefore that understanding the early life origins of osteoporosis should be a priority. The aims of this research are to increase our understanding of the possible parental influences on childhood bone development, and the potential mechanisms which might regulate and thus influence bone growth in early life, using parent-offspring data from two large well-phenotyped population-based cohorts.

1.2 Osteoporosis

1.2.1 **Definition**

Osteoporosis (literally "thinning of the bones") is a systematic skeletal disorder characterized by low bone mass and microarchitechtural deterioration of bone tissue which increases the fragility of bone and hence susceptibility to fracture (1). It is a major public health concern, affecting millions of individuals worldwide. The definition of osteoporosis remains difficult. Dual-energy X-ray absorptiometry (DXA) is currently the gold standard tool for measuring bone mass; and from this, bone mineral density (BMD) can be obtained. There is a strong correlation between BMD and the strength at which bones break in vitro; however, BMD does not completely explain all the changes in bone that lead to skeletal fragility. Nonetheless it is recognized that BMD is strongly predictive of fracture risk (9).

In 1994, an expert panel convened by the World Health Organization (WHO) established the most widely used definition for osteoporosis. They defined osteoporosis when BMD measurements in women fall more than 2.5 standard deviations (SD) below the young adult mean (9). This definition, however, only

takes into account reduction in bone mineralization and does not separate out changes in bone microarchitecture or distinguish any BMD independent effects that may weaken bone. More recently, there has been a move toward assessing an individual's absolute risk of osteoporotic fracture over a set period of time, an example of which is the Fracture Risk Assessment Tool (FRAX®). FRAX®, developed in the United Kingdom by the WHO collaborating Centre for Metabolic Bone Diseases in Sheffield, UK, uses an individual's clinical data to compute the 10-year probability of hip and major osteoporotic fracture (which include forearm, hip, spine, and humerus) (10). This estimate can be used alone or with BMD to enhance fracture risk prediction.

1.2.2 **Epidemiology of osteoporosis**

Based on the WHO diagnostic criteria, osteoporosis is present in approximately 20% of all Caucasian postmenopausal women, and 50% of those aged over 80 years. The prevalence of osteoporosis in the European Union in 2010 was estimated to be 27.6 million (11). Furthermore, it has been estimated that 10 million Americans older than 50 years have osteoporosis and that a further 34 million are at risk for the disease (12). This figure is likely to increase to more than 14 million in 2020. Men are less commonly affected; the prevalence of osteoporosis in men aged over 50 years is 3-times less than in women (13).

1.2.1 **Epidemiology of fragility fractures**

It is estimated that 3.5 million fragility fractures occur every year in the European Union (14). In 2013, the total cost burden, including pharmacological prevention, was estimated at €37 Billion, with the vast majority of spend on direct fracture treatment and long-term fracture care (14). A report by Strom et al highlighted that approximately 34,000 deaths as a result of fracture occur every year in the EU countries included; just under half of which were due to hip fractures (11). In the UK alone there were approximately 343,000 new fractures in 2010 (57,000 hip, 40,000 clinical vertebral, 54,000 forearm and 192,000 "other", with a total fracture burden cost of €5.5 Billion (11).

The risk of fracture depends on a) the mechanical strength of bone, and b) the force applied to that bone. Fracture incidence is bimodal, with peaks in the young and elderly. In young people, fractures are usually associated with substantial trauma, occur in the long bones, and are seen more frequently in males than females. In this group the question of bone strength rarely arises. Osteoporotic fractures characteristically occur in those areas of the skeleton with high amounts of trabecular bone after low or moderate trauma. The "classic" osteoporotic fragility fractures are hip, vertebral, and wrist fractures, but many other fractures after the age of 50 years are also related to reduced bone strength and should be also considered as osteoporotic (15). These include rib, humerus, pelvic and other femoral fractures. The frequency of fracture increases with age in both sexes, reflecting a combination of lower bone density and the increased tendency to fall in the elderly (Figure 1.1).

The prevalence of osteoporotic fractures has been studied in several epidemiological studies from North America that have estimated that the remaining lifetime risk for an osteoporotic fracture is 40% in white women and 13.1% in men at 50 years of age (16). Recent data from the General Practice Research Database in the UK, which includes 11.3 million people, have indicated a similar risk (17)

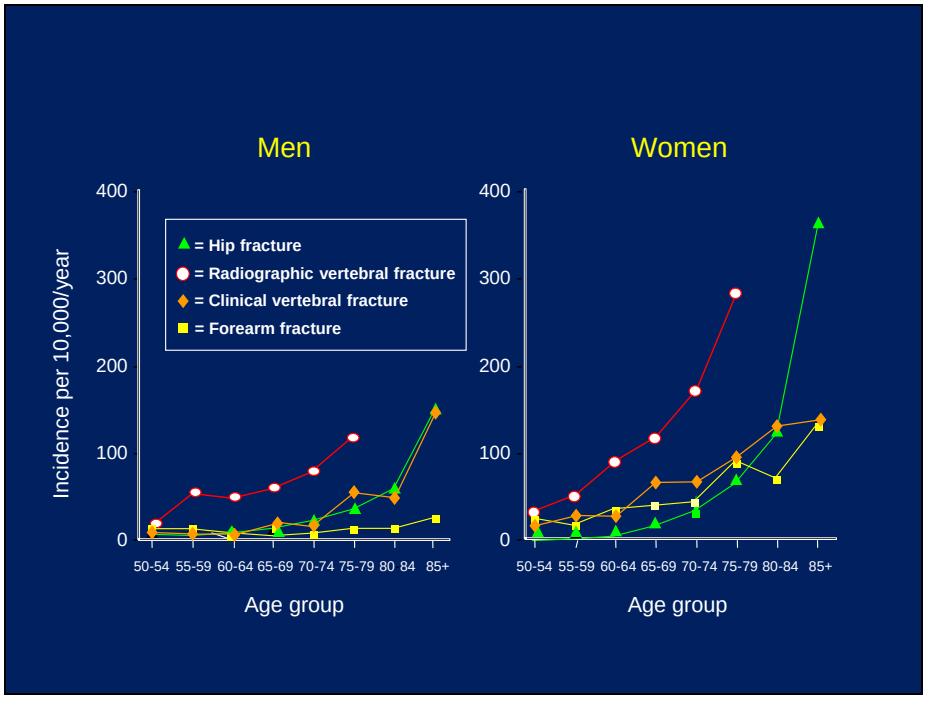


Figure 1.1: Incidence of osteoporotic fractures (Adapted with permission (18;19))

1.2.1.1 Vertebral fracture

The most frequent site for osteoporotic fracture is the thoraco-lumbar spine, however only a third of these come to clinical attention acutely. This is partly because vertebral fractures commonly occur from routine activities such as bending and lifting, rather than resulting from a fall. Data from the European Vertebral Osteoporosis Study suggests that one in eight women and men aged 50 years and older have evidence of vertebral deformity (20). Prevalence of vertebral deformity increases steadily with age in both sexes, although the gradient is steeper for women (21).

1.2.1.2 Hip fracture

Hip fractures represent the most devastating consequence of osteoporosis; they almost always necessitate hospital admission, and are associated with the highest financial burden and the highest mortality rates of all osteoporotic fractures. Hip fractures generally occur as a result of a fall from standing height. 90% of hip fractures occur among people older than 50 years of age and 80% occur in women (partly because there are more elderly women than men) (22). In the UK, the remaining life-time risk of hip fracture for a 50 year old woman is 11.4% (3.1% for men). Incidence rates rise steeply in the elderly population, such that at 50 years of age, a female's 10 year risk of hip fracture is only 0.3%; this rises to 8.7% at aged 80 years (18).

1.2.1.3 Forearm fracture

Distal forearm fracture almost always results as a consequence of a fall from standing height onto an outstretched hand. Incidence rates tend to peak in winter, but unlike hip fractures, this probably is due to falls outside on icy surfaces. These fractures show a steep rise in incidence during the perimenopausal period among women but tend to plateau thereafter. This plateau may be due to mode of fall, as later in life a woman is more likely to fall onto a hip than an outstretched hand (23). In men, the incidence remains constant between 20 and 80 years. A much stronger sex ratio exists for this

fracture than for most others, and this has been estimated to be 4:1 in favour of women. (24)

1.2.2 **Secular trends in fracture**

The frequency of osteoporotic fracture is rising in many parts of the world for several reasons including increased longevity of the population. It is estimated that in Europe the population of elderly individuals will increase by 33% over the next 25 years; in the developing world, the general population as well as life expectancy will increase by more than 2-fold over the same period (25). Over and above the ageing population, changes in age-specific fracture rates have also been observed (Figure 1.2). In many parts of the Western World, such rates were seen to increase until the 1980s, before levelling off and now appear to be falling. This is possibly in part due to the implementation of osteoporosis screening and treatment programs, but these factors do not by any means completely explain this trend. Age-specific fracture rates continue to rise in other parts of the world, including Southern Europe and parts of Asia indicating that osteoporotic fractures will lead to an ever higher disease burden in the future (25).

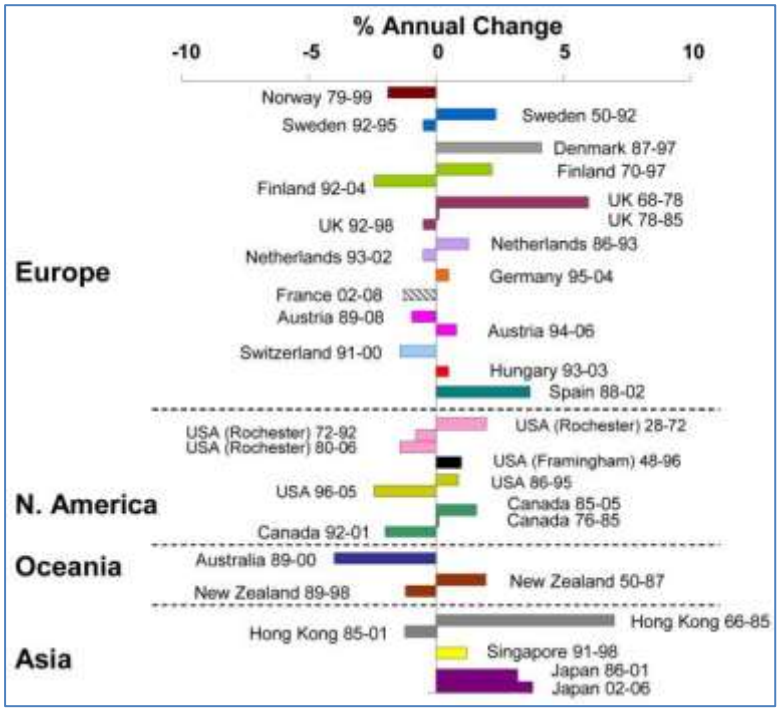


Figure 1.2: The secular trends in hip fracture (reproduced with permission (25))

1.2.3 **Bone mineral density and osteoporosis**

Limitations aside, BMD is a strong predictor of fracture independent of age. The risk of osteoporotic fracture increases continuously as bone mineral density declines, with a 1.5- to 3-fold increase in risk of fracture for each standard deviation fall in BMD (9). There is no convincing evidence of a threshold in the relationship between BMD and fracture (26). In addition, there is also some evidence to suggest a definite inverse correlation between BMD and the severity of fracture, with higher rates of early instability, malunion, and malalignment after fracture in patients with lower BMD (27).

It is becoming increasingly accepted that an individual's BMD is likely to be influenced by a combination of both genetic predisposition and environmental exposures which may act both in utero and subsequently and there is increasing evidence that the interaction between the two may play a major role. For example, a twin study looking at intra-pair differences in birth weight, found a positive, significant association between intra-pair differences in birthweight and intra-pair differences in adult BMD at the spine and hip, with stronger associations in monozygotic twins than dizygotic twins (28) . Twin studies have suggested that between 50% and 85% of the variance in BMD is genetically determined (29), however only 12% of the phenotypic variance in BMD has been accounted for by genes identified so far from genome wide association studies (GWAS) (30). Although next generation sequencing may reveal further signals, it is possible that environmental factors and their interaction with the genome may account for a greater proportion of BMD than previously suspected.

Numerous factors have been identified that influence BMD, either through inadequate peak bone mass development or an excessive rate of bone loss (or a combination of the two). Examples include age, female sex, late age at menarche, early age at menopause, physical inactivity, low calcium intake and a history of previous low trauma fracture. Protective factors include greater weight and strength, increased dietary calcium, greater physical activity, later age at menopause, and estrogen use (31).

The relationship between BMD and osteoporosis is comparable to with that between blood pressure and stroke. Although hypertension is a risk factor for

stroke, stroke can occur in individuals with normal blood pressure. Likewise, fractures can occur in the absence of osteoporosis. Of note in the Rotterdam study, only 44% of non-vertebral fractures occurred in women with a T-score of less than -2.5 (the WHO definition of osteoporosis); in men this percentage was even lower at 21% (32). This highlights the presence of other risk factors for fracture that act independently of BMD. Examples include: family history of fracture (having a mother who fractured her hip, doubles the offspring's risk of hip fracture) (33), cigarette smoking (34), excess alcohol consumption, glucocorticoid use and low body weight (every 20% decline in weight after the age of 25 increases the risk of hip fracture by 70%) (33). Thus, BMD alone cannot reliably discriminate between individuals who will sustain a fracture and those who will not.

1.3 Pathophysiology

1.3.1 **Bone turnover**

Bone consists of several key cells that play a role in bone turnover. Osteoblasts are responsible for bone formation and mineralisation, whereas osteoclasts are the only cells that are able to resorb mineralised bone; osteocytes are more numerous and are thought to be important in guarding the integrity of the strength of bone by signalling the need for adaptive remodelling in response to mechanical strain. An imbalance of osteoclast activity over osteoblast activity will lead to net bone loss and potential osteoporosis (35).

Several molecules are known to affect bone turnover, acting predominantly through one of two known signalling pathways: RANK-RANKL-OPG or Wnt signalling. Macrophage colony-stimulating factor (M-CSF) produced by osteoblasts, and RANKL (receptor activator of the nuclear factor κB ligand), which is expressed on the cell surface of osteoblasts, are both important for osteoclastogenesis. RANKL binds to its receptor RANK (receptor activator of the nuclear factor κB), which is expressed on osteoclast precursors and on mature osteoclasts, and promotes osteoclast differentiation, activation and consequent bone resorption. Osteoprotegerin (OPG) is mainly secreted by osteoblasts and acts as decoy receptor for RANKL, thus blocking binding to

RANK and acts as a physiological regulator of bone resorption (36). Several cytokines and hormones such as oestrogens, androgens and 1,25-(OH)-vitamin D are known to influence the RANK-RANKL -OPG signalling pathway. Cytokines such as TNF-α, IL-6 and IL-1β, and hormones such as parathyroid hormone (PTH) and glucocorticoids upregulate RANKL, ultimately leading to a net loss of bone. Conversely, oestrogens and tumour growth factor (TGF) enhance OPG production and thus act to inhibit osteoclast activation.

A more recent discovery in bone metabolism is the role of the Wnt signalling pathway in osteoblast differentiation and function. Wnt molecules in conjunction with low-density lipoprotein receptor-related protein 5 (LRP5; required as a co-receptor) stimulate osteoblasts (37). Sclerostin, a protein produced by osteocytes binds to LRP5 and inhibits the Wnt signalling pathway.

1.3.2 **Oestrogen and bone loss**

Oestrogen loss after the menopause is a common cause of osteoporosis and is associated with an increase in bone turnover and consequent net loss of bone through multiple mechanisms. Oestrogen loss is associated with an increase in the production of cytokines (Il-1, IL-6 and TNFα). This in turn increases the production of M-CSF, thereby increasing the lifespan and production of osteoclasts. In addition, the balance between RANKL and OPG is changed, so that RANKL is upregulated and OPG is down-regulated. This leads to a net loss of bone as the increase in bone resorption is faster than the increase in bone formation (35).

1.3.3 **Peak bone mass**

A recent theoretical analysis has suggested that an individual's peak bone mass (PBM) is one of the most important determinants for the development of osteoporosis in later life (2). 80% of a fetus' required calcium is accrued during the last trimester of pregnancy (38). Bone mass then typically increases throughout childhood, largely due to increasing bone size as a result of longitudinal growth. In adolescence there is a growth spurt, where a further

25% of PBM is achieved and typically by the age of 18 years, more than 90% of PBM has been achieved (39). Bone mass typically peaks in the mid-20s, although the exact timing will depend on sex and skeletal size. It then plateaus for around 10 years before falling at a rate of 0.3% to 0.5% each year. At menopause, due to the loss of the protective effect of oestrogen, the rate of bone loss in women accelerates to between 3% and 5% per year for 5 to 7 years before returning to the previous rate of decline (Figure 1.3).

Adult bone mass is thus equal to the peak bone mass achieved minus the amount of bone lost afterwards. A 10 % increase in peak BMD is predicted to delay the development of osteoporosis by thirteen years, while a 10 % change in the age of menopause or the rate of non-menopausal bone loss is predicted to only delay the disease by two years (2). Thus, one way to reduce an individual's risk of osteoporotic fracture is to ensure adequate bone mineral accrual in early life, childhood and early adult life, to optimize peak bone mass.

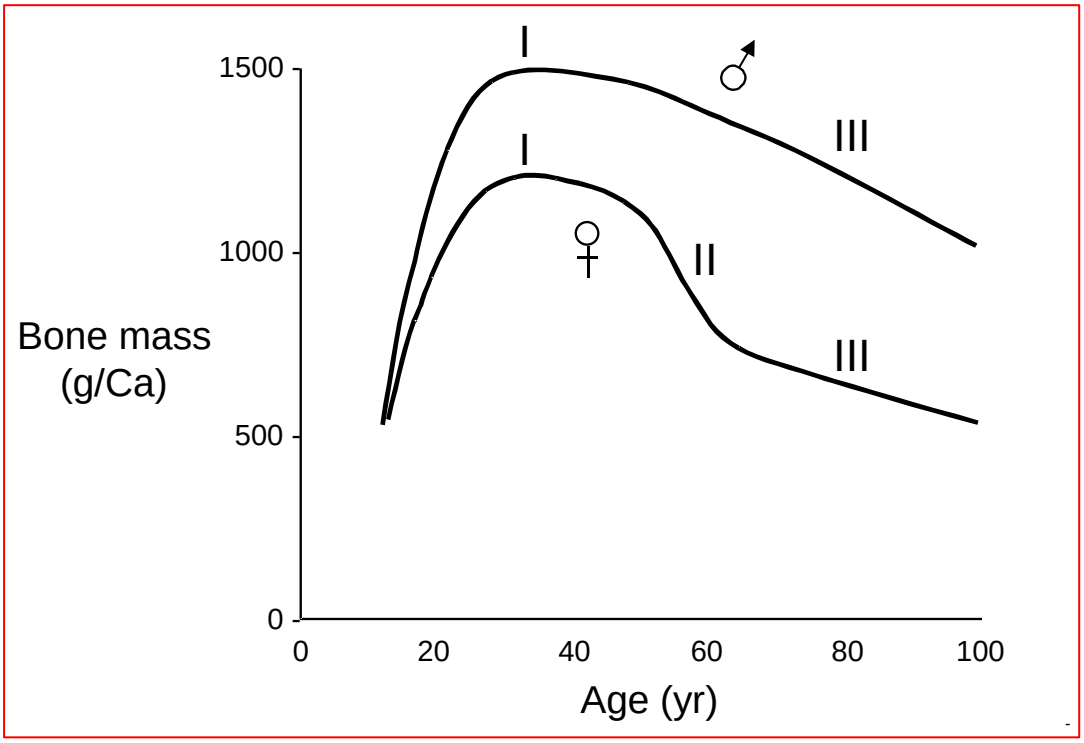


Figure 1.3: Changes in bone mass over time in men and women

1.4 Normal skeletal development

The fetal skeleton develops from embryonic mesenchymal tissue in two main processes: endochondral ossification and intramembranous ossification.

1.4.1 Endochondral ossification

The long bones of the skeleton develop mainly by the process of endochondral ossification. During development, the long skeletal bones can be divided into three portions: a long mid-section called the diaphysis, the rounded ends of the bone, the epiphysis, and the metaphysis, which is the narrow portion between the epiphysis and diaphysis that contains the growth plate. Endochondral ossification begins with mesenchymal cells condensing together and differentiating into chondroblasts to form a cartilage template; this acts as a scaffold on which the new bone is formed. In the centre of the cartilaginous template, the primary ossification centre, chondrocytes differentiate and hypertrophy, before depositing an extracellular matrix rich in cytokines which facilitates vascular invasion. Mesenchymal cells in the surrounding connective tissue, termed the perichondrium, differentiate into osteoblasts and if sufficient quantities of mineral are present, form a cuff of bone adjacent to the metaphysis. Blood vessels then invade this newly formed bone area allowing osteoclasts to invade and resorb the underlying cartilage. Additional areas of ossification, termed secondary ossification centres, form at the epiphyseal ends of the cartilage template, and by a similar process trabecular bone and a bone marrow space are formed at these ends. Epiphyseal cartilage remains between the primary and secondary ossification centres until adulthood. Continual differentiation of chondrocytes, cartilage mineralisation and subsequent remodelling at the epiphysis allows longitudinal bone growth to occur and is essential in determining the shape of bone to its final proportions. In adulthood, the growth plate closes and the epiphyseal cartilage between the primary and secondary ossification centres disappears, preventing any further longitudinal bone growth (40).

1.4.2 **Intramembranous ossification**

Intramembranous ossification is the process by which development and growth of the axial skeleton and flat bones of the skull occurs. Here, unlike in endochondral ossification, there is no cartilage template. Instead bone is laid down by direct apposition within stromal connective tissue. Mesenchymal cells proliferate and differentiate into osteoblasts, which then secrete osteoid, an unmineralised organic material predominantly composed of collagen. This is then progressively mineralised. Further bony trabeculae are added by direct apposition, and eventually cortical bone is formed.

Intramembranous ossification is also the process by which growth in diameter of the long bones occurs (40).

1.4.3 **Fetal skeletal mineralization**

Although primary ossification centres begin to form in the long bones and vertebrae between the 8th and 12th week of gestation, it is in the third trimester that the bulk of mineralisation occurs (41). The main determinant of fetal skeletal mineralization appears to be fetal blood calcium concentration (41). During the period of a normal human pregnancy the fetus accumulates approximately 30g of calcium, at a rate of up to 140mg/kg per day in the third trimester (38). This fetal demand is met through placental calcium transport, which results in a higher calcium concentration in fetal than maternal blood (42); a process that occurs as early as 20 weeks (43).

1.4.4 **Placental development and function**

The main role of the placenta is to provide oxygen and nutrients to the growing fetus and remove waste products from the fetus' blood. In humans, the placenta comprises two parts- the fetal placenta, which develops from the same blastocyst as the fetus, and the maternal placenta, which develops from the maternal uterus. The placenta contains both a maternal circulation and a feto-placental circulation. Vessels branch out over the surface of the placenta

to from a network of vessels covered by a thin cell layer, resulting in the formation of villous tree structures; on the maternal side, these structures are grouped into lobules termed cotyledons. Whilst maternal and fetal blood are brought extremely close to each other, enabling gas and nutrient exchange, there is no intermingling of blood between the two circulations, this is termed the placental barrier. Nutrient transfer across the placenta can occur via active or passive transport (44), and placental nutrient metabolism can play a role in limiting the transfer of some nutrients (45) . Adverse pregnancy situations, such as pre-eclampsia, maternal diabetes and obesity can increase or decrease levels of nutrient transporters in the placenta leading to fetal growth changes (46). Approximately 70% of human genes are expressed in the placenta; around 350 of these are more specifically expressed in the placenta and less than 100 are highly placenta specific (47).

The underlying placental mechanisms regulating fetal mineralization remain poorly understood, but parathyroid hormone (PTH) and parathyroid hormone-related peptide (PTHrP) are thought to play important roles (41). PTH primarily increases fetal renal calcium absorption thereby increasing fetal serum calcium concentrations. Animal models have shown that lack of fetal parathyroid glands results in low fetal serum calcium concentrations and reduced mineralization of the skeleton (48). Fetal PTH does not seem to influence placental calcium transfer however, in contrast to PTHrP (49). Maternal PTH does not cross the placenta, however can exert an influence by altering the calcium load presented to the fetal circulation in the placenta. Maternal hyperparathyroidism in pregnancy is associated with neonatal hypocalcaemia (41).

PTHrP is thought to be produced mainly by fetal parathyroid glands, but also by placental trophoblasts. Alongside its other roles in fetal development, PTHrP appears to be the major determinant of placental calcium transport, possibly through its interaction with the calcium sensing receptors.

The exact mechanisms underlying the transport of calcium transport across the placenta are poorly understood. Placental calcium transport occurs in the synctiotrophoblast (50); calcium crosses the placenta bound to calcium transport proteins including calbindin-D9K and calnexin before being actively extruded from the basal plasma membrane of the trophoblast layer to the fetal

circulation via a number of pumps and exchangers, such as Na^{+}/Ca^{2+} exchanger and plasma membrane Ca^{2+} ATPase (PMCA). This last group of transport proteins includes four individual isoforms (PMCA 1-4) (50). It has previously been demonstrated in animal models that a 2-3 fold increase in PMCA gene expression is associated with a 72-fold increase in calcium transport across the placenta in late gestation (51). Regulation of this process remains unknown, but it is possible that maternal influences such as maternal 25(OH)D may act on this mechanism. A study by Martin et al found that the messenger RNA (mRNA) expression of the placental calcium transporter, PMCA3, predicted neonatal whole body bone mineral content (52), suggesting a possible mechanism for the influence of maternal vitamin D on placental calcium transport and intrauterine bone mineral accrual.

1.5 Determinants of postnatal bone growth

It is increasingly accepted that many complex phenotypes and chronic diseases result from both genetic predisposition and environmental exposures, and there is evidence that the interaction between the two play a major role. Much attention has focused primarily on identifying possible genetic associations with bone mass, and although this has provided clues regarding disease pathogenesis, large GWAS studies have not identified single nucleotide polymorphisms (SNPs) to account for all the heritability. Whilst next generation sequencing may help fill the gap, environmental factors and their interaction with the genome, may also contribute to the magnitude of bone mass accrual.

In this section both the genetic determinants and environmental influences on bone mass will be discussed alongside the evidence for a possible interaction between the two.

1.5.1 **Genetic determinants of bone mass and osteoporosis**

Genetic factors are an important determinant of bone mass. Studies from the 1980s found that bone mass is lower in daughters of women with osteoporosis (53) and in young and middle-aged men and women with a family history of

osteoporosis (54). The magnitude of this relationship may have been over-estimated, due to shared environmental factors between parent and offspring. Heritability is generally understood to be the proportion of the variance in a given trait explained by genes. It has been estimated from twin and family studies that between 50% and 85% of the variance in peak BMD is heritable (55-57). Twin studies have generally yielded higher heritability estimates than family-based studies comparing individuals across generations (58;59), possibly due to non-genetic influences on rates of bone loss in family studies. In most, but not all, studies, BMD heritability appears higher at axial sites (such as the spine and hip) than at the forearm (60) .

Several studies have demonstrated that the risk of osteoporotic fracture also has a heritable component, although this appears largely independent of BMD. A family history of fracture has been shown to be a predictor for fracture independent of BMD (33;61). Heritability of wrist fracture has been estimated at between 25-54% from studies in the US and UK respectively (62;63). Again this appeared largely independent of BMD suggesting that genetic influences on other factors such as bone turnover, bone geometry or non-skeletal factors such as cognition may be the main mediator. Heritability of hip fracture in individuals under the age of 65 has been estimated at 68% (62;63). The magnitude of this effect appears to decrease substantially with advancing age, falling to almost zero by the eighth decade, suggesting that with increasing age, environmental factors may become more important.

The genetic regulation of BMD is thought to be polygenic, and determined by single nucleotide polymorphisms (SNPs; common genetic variants) in multiple genes, each with a relatively minor effect. There are around 20 million known polymorphisms in the human genome and it is hypothesized, in parallel to several common conditions, that osteoporosis and low bone mass result from the combined effects of many hundreds of polymorphisms (64). Only very rare forms of low bone mass are inherited as a result of mutations in single genes (e.g. osteogenesis imperfect (COLIA1 mutation); osteoporosis pseudoglioma syndrome (LRP5 mutation) (64).

Three main types of study have been used to understand the genetic background for bone mass; linkage studies, candidate gene analysis and GWAS. Several different loci for BMD have been identified in numerous linkage studies (65;66) , however the findings have not been replicated between

studies, and a 2007 meta-analysis incorporating nine of these studies, including over 11,000 subjects, found no loci associated with BMD (67). This may reflect that genes controlling BMD each have only a modest effect and will be difficult to detect with conventional linkage studies

Candidate gene association studies, which involve analysing polymorphic variants in select genes known to have a role in bone biology and relating carriage of a specific allele to the trait of interest, have also been widely used in the field of osteoporosis. Using such an approach, polymorphisms in certain genes have been associated with BMD and other bone characteristics including fracture risk; around 150 candidate genes relating to bone mass have been identified (68). For example, polymorphisms in the vitamin D receptor (VDR) have been shown to account for differences in BMD in pubertal and adolescent girls (69). In a large scale meta-analysis of five candidate-gene studies, only nine of the 150 candidate genes identified in individual studies were significantly associated with regulation of BMD (70); four were also significantly associated with fracture risk. These were genes encoding estrogen receptor (ESR), lipoprotein receptor-related proteins 4 and 5 (LRP4, LRP5), ITGA (integrin), Osteopontin (OPN), sclerostin (SOST) and TNF receptor super family members 11A (Rank) and B (osteoprotegerin; TNFRSF11A and TNFRSF11B). Most of the SNPs identified from individual studies failed to show a consistent relationship with BMD and the effect size was small from those identified (0.04 SD- 0.18 SD change in BMD per allele).

More recently advances in genotyping technologies, such as the introduction of the polymerase chain reaction and the completion of the Human Genome project, have made it possible to perform association studies on a genome-wide basis by analysing large numbers of SNPs spread at close intervals across the genome, rather than focusing in on selected genes one at a time. These GWAS have enjoyed considerable success in identifying replicated loci that are associated with bone density and osteoporosis. To date, there have been over 20 GWAS for BMD, with sample sizes ranging from 2,198 to 142,487. The most recent and largest GWAS to date recruited 142,487 individuals from the UK Biobank to identify loci associated with BMD estimated by quantitative ultrasound of the heel (eBMD) (30). 307 conditionally independent SNPs attaining genome-wide significance at 203 loci were found, explaining approximately 12% of the phenotypic variance. These included 153 novel loci,

and several rare variants with large effect sizes. Associations for BMD have been confirmed in or near genes that encode proteins known to influence bone mass, such as ESR, glypican 6 (GPC6), LRP5, TNFRSF11, TNFRSF11A, TNFRSF11BB and SP7 transcription factor (30;71;72).

Despite numerous loci being identified by GWAS, large scale meta-analyses of the studies have found that only 5.8% of the variance in femoral neck BMD and 2.9% of the variance in lumbar spine BMD has been explained by loci identified by GWAS (71;72); however, notably these meta-analyses were published prior to the largest and most recent UK Biobank study (30). In addition, at least half of the genes identified by GWAS have no known connection with bone mass (examples include microtubule-regulating kinase 3 (MARK3) and the major histocompatability complex (MHC)), or are not correlated with coding an obviously functional variant, and therefore do not conclusively implicate a unique gene.

Many studies have concluded that the remaining genetic component for BMD may be explained by a combination of a large number of unidentified common low-penetrance SNPs or a few high-penetrance rare SNPs; next generation sequencing may help fill in the missing heritability. It remains unclear which of these models best underlies the genetics of osteoporosis or indeed whether the two models are mutually exclusive. Although GWAS is a powerful tool for identifying new genetic associations with a phenotype or disease trait, in the case of rare variants it may have insufficient power to detect the causal loci, or they may be excluded from the initial association analysis because of their low allele frequency. It has been suggested that exploration of the loci identified by GWAS require refined deep-sequencing to try to identify these rare or common variants; a process that could be incorporated into a current GWAS design (73).

In addition to the pure genetic determinants, it is likely that other factors are of significant importance in an individual's bone mass. These include environmental factors (both in-utero and post-natally), gene-gene interactions, gene-environment interactions and epigenetic modifications, and are discussed in the next sections.

1.5.2 Postnatal influences on offspring bone mass

1.5.2.1 Nutrition in childhood

Early nutrition is likely to have an important impact on later childhood bone health. The most important nutrients for bone health are calcium and vitamin D, and hence the majority of studies have tended to focus on these key factors.

1.5.2.1.1 Calcium intake in childhood

The earliest data suggesting a positive influence of dietary calcium on peak bone mass comes from a study in the 1970s of two Croatian populations with substantially different calcium intakes (74). At age 30, bone mass was lower in the group with lower calcium intake, suggesting that the effects of dietary calcium are likely to occur during growth rather than adulthood.

Several studies have focused on the difference between breast feeding and formula milk feeding on bone mass. Most studies have had a relatively short duration of follow-up and have presented often inconsistent and conflicting findings; bone mineral in breast-fed children has been shown to be both higher and lower than in those who were formula fed. For example, whole-body bone mineral content (BMC) was lower in breast-fed babies at 12 months in one study of forty infants (75). Conversely, another study of 330 eight year old children found higher bone mineral density among those who had been breast fed in infancy, compared to those who had been formula fed (76) It remains possible that unmeasured environmental factors may explain the observed relationships. More recently, a study of 599 mother-child pairs from the Southampton Women's Survey, with wide variations in infant feeding practice, found no association between the duration of breast-feeding in the first year of life and bone size or density at 4 years (77). Few studies have investigated the long-term influence of breast-feeding on adult bone mass. In a 20 year follow-up of 202 subjects who had been born prematurely and randomised to a diet of either pre-term formula milk or banked breast milk for an average of 4 weeks, higher whole body BMC and bone area (BA) were observed in the group randomised to breast milk (78). This study was underpowered however, due to the low number of subjects in each arm of the trial at follow-up (approximately 25). In addition, the association was lost after adjusting BMC for BA suggesting

that the effect of breast milk was primarily on increased bone size with only a proportionate increase in mineral mass.

Calcium intake (either as calcium salts, milk or other dairy produce) has been shown in some, but not all, randomised controlled trials (RCT) to positively influence childhood bone mass. Many of these studies have been performed on prepubertal or adolescent girls supplemented for between 1 and 3 years. A Swiss study of prepubertal girls randomised to receive either calcium enriched foods (850mg calcium per day) or placebo for 1 year found higher BMD in the supplemented group compared to control group at 1 year (79). The response to calcium varied with skeletal site and pre-treatment calcium consumption, with greater BMD gains at cortical skeletal sites (radius and hip) and in girls with habitual dietary calcium intake less than 850mg per day. Significant differences in BMD between the two groups were still observed 3.5 years after supplement discontinuation, suggesting that the pre-pubertal intervention may have modified the trajectory of bone mass growth, resulting in long-term gains in bone mass accrual (80). Zhu et al found that milk supplementation in Chinese girls aged between 10 and 12 years had positive effects on periosteal and endosteal apposition of cortical bone, leading to a significant increase in cortical thickness (81). In contrast to the Swiss study, these effects were not long-lasting and differences between the supplemented and un-supplemented groups were no longer seen in a follow-up study, 3 years after supplementation withdrawal (82).

A meta-analysis by Winzenberg et al included 19 randomised controlled trials of childhood calcium supplementation (including 2859 children aged between 3-18 years) (83), and concluded that supplementation significantly increased total body BMC and upper limb BMD, but failed to find an association with lumbar spine or femoral neck BMD. More recently, a meta-analysis of RCTs examining the effects of dairy consumption on childhood body composition (84), found that 8 of the 11 RCTs that assessed bone, demonstrated significant effects of dairy consumption on BMC and BMD, with an average 8% increase in BMD after 16 months of dairy consumption. None of the included studies in either meta-analysis incorporated fracture as an outcome, thus it remains unknown whether calcium supplementation in childhood reduces fracture risk.

One of the key issues with dairy supplementation studies is that participants generally return to their pre-supplementation dietary intake within a year (85). Studies looking at habitual milk intake among adult women, have found reduced BMC and higher risk of fracture in those women with low childhood milk intake (86). This suggests that habitual childhood milk intake may have persisting effects on the adult skeleton.

1.5.2.1.2 Vitamin D intake in childhood

Vitamin D is a key hormone for the regulation of bone growth and mineralisation during life; insufficiency may result in rickets or osteomalacia. Currently the UK Department of Health recommends that all babies and infants from birth to 1 year should be given a supplement of 8.5-10 mcg daily (except for those receiving more than 500mls per day of infant formula fee, as this is already supplemented). Children aged 1-4 years should be given a daily supplement containing 10mcg of vitamin D (87). This however, is not based on robust data. The association between 25-hydroxyvitamin D (25(OH)D) concentration and BMC in infants has been examined in four prospective interventional studies; three of which failed to find a difference in whole body or lumbar spine BMC or BMD in the supplemented group compared to placebo (88-90). In a fourth study, distal radius BMC was significantly higher at 3 months of age in infants who received 400 IU/day vitamin D compared to placebo. However this difference was not observed at 6 months of age, despite a persistent difference in serum 25(OH)D between the two groups. (91). The low number of participants in this study (n=13) meant that it had low power to detect a difference.

Several other randomised controlled trials have investigated the effects of vitamin D supplementation in later childhood. A 2011 systematic review and meta-analysis included six placebo-controlled studies investigating the effect of vitamin D supplementation on bone mass (92). The dose and duration of vitamin D supplemented varied from 132 IU/day to 14,000 IU/week, over one to two years; five of the six studies included only females with an age range of 10-17 years. Although no statistically significant effects on whole body BMC or BMD at the hip or forearm were observed, there was a trend towards higher lumbar spine BMD in the supplemented groups. In addition, there was a trend

towards a larger positive effect on whole body BMC and lumbar spine BMD in supplemented individuals with low baseline serum 25(OH)-vitamin D (defined by the authors as <35 nmol/l); This suggests the possibility that although supplementation with vitamin D is unlikely to be of benefit to children and adolescents with normal vitamin D levels, supplementation of those deficient in vitamin D could result in clinically useful improvements. None of the included studies reported fracture as an outcome, thus the effect of childhood vitamin D supplementation on fracture risk is not clear.

1.5.2.1.3 Fruit and vegetable intake in childhood

Although most studies have focused on the effect of calcium and vitamin D on bone accrual, there is some evidence to suggest a role for dietary fruit and vegetable intake. One study of girls aged 8-13 years old found a positive association between BMD and consumption of fruit and vegetables (93;94). Similarly, a positive association has been observed with whole body BMC in boys aged 8-20 years (95). It remains possible that the observed relationships may be influenced by confounding factors such as socio-economic class or smoking status. In addition both studies used self-reporting of dietary intake which may be an additional source of bias. Nevertheless, It has been suggested that the possible mechanism underlying this relationship is that the organic potassium and magnesium salts found in these foods buffer against the high acid load typically found in Western-type diets, which is believed to lead to bone loss (96).

1.5.2.2 Physical activity in childhood

Bone can adapt to increased loading by increasing its size, changing geometry and increasing the amount of mass within the periosteal envelope. These changes help to maintain efficiency in providing structural support to the skeleton. The influence of physical activity in childhood on bone mass is thus of interest.

Cross-sectional studies of pre-pubertal gymnasts have demonstrated larger forearm cross-sectional area, cortical area and thickness, as well as increased

lumbar BMC and bone mineral apparent density (BMAD; an estimate of volumetric BMD) compared to non-gymnasts (97;98). Similarly BMD in retired gymnasts has been seen to be significantly higher than the predicted mean for controls at most sites, with no diminution across the 20 years after retirement (98). Furthermore, a prospective Australian cohort study found that childhood fitness at age 9 years was significantly associated with greater bone mass as measured by calcaneal ultrasound densitometry 20 years later, independent of adult fitness (99). Together these studies suggest that increased skeletal loading in childhood and adolescence may result in higher peak bone mass with residual benefits maintained into later adulthood. It is suggested that exercise before puberty may reduce fracture risk after menopause.

The effects of various exercise interventions on childhood bone mass have been examined in several randomised trials, generally with only short term results. Exercise interventions have ranged in duration from 3 months to 2 years, and have included games (100;101), dance (100;101), resistance training(102;103) and jumping (104). Overall, weight bearing exercise appeared to enhance bone mineral accrual. Of the 14 interventional trials included in a systematic review by Tan et al, 3 (out of 5) of the studies graded as high quality reported significant gains in bone strength for the intervention group (3%-4%) (105). Whilst there was significant heterogeneity between studies, changes in bone structure (e.g. bone cross sectional area and cortical thickness) rather than mass, most often accompanied significant bone strength. Prepuberty and peri-puberty appeared to be the most opportune time for boys and girls to enhance bone strength through physical activity, although the finding was tempered by the few studies available in more mature groups.

Despite the relative wealth of evidence regarding exercise interventions on childhood bone mass, there is a paucity of data examining physical activity in free-living young children. In the Iowa Bone Development Study, a cross-sectional study of 368 preschool children, accelerometry measures of physical activity were positively associated with BMC and BMD at age 5 years, accounting for 1.5%-9% of the variance in bone mass measures (106). At subsequent follow-up, and after the analysis was adjusted to control for BMC at age 5 years, moderate to very vigorous physical activity (MVPA) at age 5 years remained a significant predictor of BMC at ages 8 and 11 years for boys but not girls (107). This may be a result of greater physical activity in boys at 5

years compared with girls, but might also be consistent with the theory of a sex-specific sensitivity of bone to mechanical loading that favours males (108). Concurrent MVPA at ages 9 or 11 years was not significantly associated with BMD, suggesting that early childhood may represent a "window of opportunity" when the skeleton is most sensitive to mechanical loading (109). Similarly, a study of 422 British children found that daily mean time spent in moderate to very vigorous physical activity (MVPA; measured using a combined accelerometer and heart rate monitor) was positively related to hip BMC, areal BMD and estimated volumetric BMD (vBMD) (110). The relationships between MVPA and bone indices were stronger in children with calcium intake above the median. This finding is consistent with the findings from a recent meta-analysis of experimental and cross-sectional studies investigating the combined effects of physical activity and calcium intake on bone health (111), supporting the notion that adequate calcium intake may be required for optimal action of physical activity on bone development.

It remains unclear what constitutes the optimal type of exercise, intensity and duration to stimulate peak bone mineral accretion. Furthermore, there is concern that the higher bone mass associated with increased physical activity may not compensate for the risk caused by increased exposure to injuries. For example, in the Avon Longitudinal Study of Parents and Children (ALSPAC) cohort, children who reported daily or more episodes of vigorous exercise had double the fracture risk compared to those who report less than four episodes of exercise per week despite their higher bone mass (112).

1.5.2.3 **Childhood obesity**

Childhood obesity is becoming an increasing public health concern, with a well-documented rise in prevalence over the last 20 years. Between the years 1989-1998, the proportion of obese children almost doubled in those aged 2-4 years (from 5% to 9%) and trebled in those aged 6-15 year old (from 5% to 16%). Based on this trend, current estimates predict that by 2020 at least one fifth of boys and one third of girls will be obese.

In adults, high BMI or obesity has long been thought to be protective against osteoporosis and related fractures (113), however there is conflicting evidence

regarding the relationship between obesity and bone mass in children, and whilst it is clear that body mass is a significant determinant of bone mass and bone quality in children, the influence of fat on bone during critical stages of bone strength development remains uncertain. Several authors have reported a positive association between fat mass, bone size and density (114;115). If crude values of bone mass are examined, obese children seem to have denser bones consistent with the notion that higher body weight increases the mechanical loading on weight bearing bones, resulting in increased bone mineralization (116).

Concerns that obesity may have a detrimental effect on bone originated from observational studies of fracture incidence in children, showing that obese children had higher rates of fracture compared to normal weight children (117;118). This relationship has been reinforced by cross-sectional studies using DXA, which have demonstrated that increased body fat is associated with higher bone area but reduced whole body BMC in children aged 3-5 years (119), and reduced BMC, BMC corrected for bone area and BMD in girls aged 10-19 years (120). This suggests that children with higher body fat have larger bones which are undermineralized.

Further DXA-based studies have found that the association between bone and fat appears to vary according to whether the bone is weight-bearing, and the age and sex of the child. Cross-sectional analysis of the ALSPAC cohort demonstrated a strong positive relationship between total body fat mass and total body-less-head bone mass in children aged 9.9 years (121). However, when the cohort was followed up 2 years later, this positive relationship was attenuated and subsequently reversed in girls who had entered puberty suggesting that fat mass may have a positive effect on bone in pre-pubertal children but a negative effect during and immediately post-puberty. The same relationship was not observed for boys (the relationship remained positive), however only a small number of boys in the cohort had progressed far enough into puberty at the 2 year follow-up to adequately assess the effect of adiposity on bone in pubertal boys.

All of the aforementioned studies have used DXA to measure bone and fat, and there are concerns that body size and fat tissue thickness may result in inaccuracies in DXA analysis. Relatively fewer studies have used alternative

imaging techniques. Wetzsteon et al, using peripheral quantitative computed tomography (pQCT), found higher bone strength, total area and cortical area (but not density) at the distal and midshaft of the tibia in overweight children aged 10 years (122). A more recent study by Cole et al, again using pQCT in 172 children aged 6 years, found that fat mass (adjusted for lean mass) was also positively associated with bone size, but negatively associated with both trabecular and cortical density at the tibia (123).

A possible explanation for these conflicting results is that the relationship between fat and bone mass is subject to confounding factors, which are variably adjusted for in different studies; for example, obese children tend to have less dietary calcium intake (124), perform less physical activity and are generally further advanced in maturation (125). A direct biological effect is possible, although as of yet poorly understood. There are several mechanisms whereby obesity may influence bone size and density: firstly by applying a greater direct load to the skeleton; secondly via an increase in compensatory muscle mass and thirdly via physiological and biochemical modulation. The first two of these mechanisms would explain the positive association between bone and fat, but do not explain the negative associations with volumetric density. This may be explained by the fact that fat is not an inert tissue, but a highly active endocrine organ.

Adipocytes produce leptin, a hormone involved in the regulation of fat metabolism and appetite through hypothalamic mechanisms (126). In animals, the primary effect of leptin on bone formation appears to be negative via hypothalamic action on the sympathetic nervous system (127). In obese children, higher leptin levels have been associated with a reduction in OPG, resulting in reduced inhibition of RANKL, which in turn leads to increased osteoclastogenesis and increased bone resorption (128). Conversely, leptin may positively stimulate bone formation; leptin receptors have been found on osteoblasts, chondrocytes and bone marrow stromal cells (129). Additionally, Leptin has been shown to shift mesenchymal stem cells towards differentiation into osteoblasts rather than adipocytes (130). Thus it is possible that leptin may explain some of the relationship between fat mass and bone, both positive and negative.

Adiponectin is another hormone released by adipocytes. However, in contrast to leptin is negatively related to fat mass. A recent UK study found that at age 9 years, total fat mass was negatively related to adiponectin concentration, which in turn negatively predicted volumetric density at age 15.5 years (131). It seems unlikely, therefore, that adiponectin could explain negative relationships between fat mass and volumetric bone density.

Other hormones may play a role in the relationship between adiposity and bone mass. Insulin has been shown to have positive effects on bone in animal studies (132), with insulin resistance and higher levels of insulin (as might be found in obesity) associated with increased BMD (133) and reduced fracture risk in humans (134). However, elevated glucose concentrations have been shown in vitro to inhibit bone mass accrual (135) and have been associated with lower bone mass in children (136), implying that abnormal glucose regulation has a negative effect on the growing skeleton. In addition, adipose tissue is known to produce aromatase enzymes which convert steroid precursors to oestrogen, which has been shown to both stimulate (137) and suppress(138) periosteal bone growth in children.

The long-term effect of childhood obesity on adult bone mass is not known; likewise, it is unclear whether there is a persistently increased risk of fracture in adults who have been obese since early childhood.

1.5.3 **Early environmental influences on bone mass**

1.5.3.1 **Developmental Plasticity and programming of bone mass**

In addition to the previously discussed evidence highlighting the important role for environmental influences during childhood and puberty on bone mineral accrual, there is an increasing body of evidence suggesting that the early environment in-utero may play a major role. Experimentalists have repeatedly demonstrated that alterations to the diet of pregnant animals can produce lasting changes in the offspring's physiology and metabolism (139). This is one example of developmental plasticity: the ability of a single genotype to give rise to several different phenotypes depending on the prevailing environmental conditions (140). This is thought to ultimately

improve the survival of a species, as the organism can adapt in future generations with phenotypic characteristics better suited to the environment than would be possible if the same phenotype was consistently produced for a specific genotype. The varied phenotypes triggered by environmental events are thought to be induced during sensitive but brief periods in development. Outside such periods, an environmental influence that sets the characteristics of an individual may have little or no effect (141).

There are many examples of developmental plasticity in the natural world. The freshwater crustacean Daphnia yields a classic example: offspring born to mothers who have been exposed to traces of a predator are born with a defensive 'helmet' that protects them from predators. A problem arises however if the developing organism is exposed to a mismatch between the expected and actual environment and is born with a 'helmet' in a predator-free environment. This helmet reduces its reproductive competitive success relative to non-helmeted individuals (142).

The mechanism whereby environmental influences at a critical stage of early development lead to persisting changes in structure and function has been termed "programming". Programming of adult disease is a consequence of strategies by the developing fetus and infant in response to the early environment, leading to permanent changes in structure or physiology. Such adaptations, although appropriate in early life, may be inappropriate or harmful in later life, and increase the likelihood of adult disease. In humans the importance of the intrauterine environment and the concept of programming was initially hypothesized by Barker et al, who described the associations between low birth weight (suggesting poor early intrauterine environment) and elevated blood pressure, serum lipid levels, and diabetes in later adult life (140).

There is epidemiological evidence that the risk of osteoporosis may be modified by the intrauterine environment. During early life there are specific periods of rapid cell division termed 'critical periods', the timing of which vary according to the tissue type. For example, long bone growth is most rapid during the second trimester, whereas mineralization of bone occurs much later in pregnancy. In response to a lack of nutrients during such critical periods, the main response by the fetus is to slow the rate of cell division either

hormonally or via growth factors. It is suggested that some of the differences in bone mineral accrual during subsequent childhood can be explained by the programming of bone growth during these critical periods.

An early study suggesting that peak bone mass and thus osteoporosis risk may be “programmed”, traced 153 British women with detailed childhood growth records (3). There were statistically significant positive associations between weight at 1 year and childhood height, and BMC at the lumbar spine and femoral neck at age 21 years. These associations remained significant after adjusting for current weight. Similar relationships have been observed elsewhere including in the larger Hertfordshire Cohort (143) and other cohorts from several countries across the world including Sweden (144), Finland (145), Australia, the Netherlands and United States (146). Generally the associations have been stronger between birth/early childhood growth measurements and adult BMC rather than BMD. Further evidence for the intrauterine programming of skeletal development and tracking of skeletal size into adulthood comes from a recent systematic review and meta-analysis (147). This included 14 studies assessing the association between early size and adult bone mass and concluded that higher birthweight is associated with greater adult BMC at the lumbar spine and hip. Each 1kg increase in birthweight was associated with a 1.49g increase in lumbar spine BMC and a 1.4g increase in hip BMC. Most of the included studies found that birthweight was not a significant predictor of adult BMD at either of these sites.

In addition to a deficit in BMC, there is evidence to suggest that poor growth in utero and early life is also associated with alterations in bone architecture, geometry, strength (148;149) and fracture (154). Javaid et al found significant relationships between weight at one year and measures of proximal femoral width as well as intertrochanteric and cross-sectional moment of inertia, in later adult life; these associations appeared independent of BMC (148). A recent study using a variety of data including birth weight, childhood growth data and adult fracture data from the Helsinki Birth Cohort (n=8,345) found that the risk of male adult hip fracture was higher in those with low increases in height and BMI between ages 2 and 7 years (150); in women, the rate of childhood height gain was not associated with risk of hip fracture, but greater weight gain and BMI gain between ages 2 and 7 years were associated with a decreased risk of therapy for osteoporosis in later adult life (150).

1.5.4 **Epigenetic mechanisms**

Epigenetic mechanisms may in part explain how environmental factors can alter an individual's phenotype and may underlie the early environmental effects on offspring bone mass. Epigenetics refers to an alteration in gene expression caused by mechanisms other than changes in the underlying DNA sequence and are integral in determining when and where genes are expressed. Epigenetic changes are stable and potentially heritable, and may last through multiple generations (151). The two most studied forms of epigenetic mechanisms are DNA methylation and histone modification; most studies have focused on DNA methylation. DNA methylation involves the addition of a methyl group to cytosine residues at the carbon-5 position of CpG dinucleotides, and is generally associated with gene repression, either by decreased binding of transcription factors or by attracting methyl-CpG-binding proteins that act as transcriptional repressors (152;153). There is usually an inverse relationship between the extent of DNA methylation of regulatory CpGs and gene expression. Histone modification refers to post-translational modification of histone tails. Histones are small proteins involved in packaging of DNA into chromatin; if the way that DNA is wrapped around the histones changes, gene expression can also change. Histone modification can occur either by methylation or acetylation. These two types of epigenetic modification are mechanistically linked and work together to affect chromatin packaging, which in turn determines which gene or gene set is transcribed. The enzymes controlling these processes have recently been identified and include DNA methyltransferases (154).

DNA methylation patterns differ through the phases of development. After conception, and with the exception of imprinted genes, gamete methylation patterns are erased during early blastocyst formation. During the implantation stage, methylation patterns become established via *de novo* methylation by the activities of DNA methyltransferases (Dnmt) 3a and 3b. Patterns of DNA methylation are maintained through mitosis by Dnmt1 activity (155). In adulthood, there are variations in the amount and pattern of methylation depending upon cell and tissue type. During embryonic and fetal development, maternal or environmental factors can disrupt these patterns of DNA methylation; examples of this process have been shown in animal models and will be discussed in further detail. This disregulation of developmental

programming via abnormal DNA methylation may permit specific genes to undergo inappropriate expression during adult life, resulting in disease development (154). Emerging evidence strongly suggests that epigenetic mechanisms underlie the processes of developmental plasticity.

Epigenetic mechanisms are now well established in the development and progression of a variety of cancer types including prostate, lymphoma, head and neck, breast and ovarian cancer (154). Data in other human diseases are limited, particularly in relation to developmental plasticity. The first example of an association between a periconceptional exposure and DNA methylation in humans was shown in Dutch subjects prenatally exposed to famine during the Dutch Hunger Winter in 1944-1945 (156). Exposed subjects showed persistent epigenetic differences in a variety of genes compared to their unexposed, same sex siblings.

1.5.5 **Epigenetics in osteoporosis**

The calcium and vitamin D axis provides a model for investigating the epigenetic regulation of bone mass. The mechanism underlying the association between maternal vitamin D, umbilical cord calcium concentration and offspring bone mass is unclear but is an area of on-going research. 1,25(OH) vitamin D (the active form of vitamin D) mediates its effects by first binding to the vitamin D receptor, then by binding to the retinoid X receptor alpha (RXRA) forming a heterodimer. This heterodimer then acts upon vitamin D response elements in promoter target genes and initiates gene transcription by either up-regulating or down- regulating gene products (157).

One study has demonstrated that the expression of a placental calcium transporter (PMCA3) gene predicted neonatal whole body BMC (52). Modified expression of the genes encoding placental calcium transporters, by epigenetic regulation, might represent the means whereby maternal vitamin D status could influence bone mineral accrual in the neonate. Since the effects of maternal nutrition and behaviour seem to target the promoter region of specific genes rather than being associated with global changes in DNA methylation, investigating CpGs located within the promoter region of these genes, particularly those within or located near to vitamin D response

elements, may provide further clues regarding the epigenetic regulation of bone mass. In addition, if validated, these epigenetic markers might provide risk assessment tools with which to target early lifestyle interventions to individuals at greatest future risk.

Recently, in a subset of 4 year old children from the SWS, higher percentage methylation at 4 out of 6 RXRA CpG sites measured was correlated with lower offspring BMC corrected for body size, suggesting that perinatal epigenetic marking at the RXRA promoter region in umbilical cord was inversely associated with offspring size-corrected bone mineral content (scBMC) in childhood (158).

Epigenetic modifications may also underlie the inverse association between birth weight and adult fasting plasma cortisol. Animal studies have confirmed that protein restriction during mid and late pregnancy is associated with reduced methylation in the promoter region of the glucocorticoid receptor gene, which results in elevated glucocorticoid receptor expression, and features of hypercortisolism in the offspring (159). Further work in rats, and subsequent replication of the work using human umbilical cords, has shown that induction in the offspring of altered epigenetic regulation of the hepatic glucocorticoid receptor promoter may be due to reduced Dnmt1 expression (160). Epigenetic modulation of the hypothalamus-pituitary axis may represent a second mechanism for transduction between a poor maternal environment and impaired bone mineral accrual in the offspring.

1.6 Parental associations with offspring bone

1.6.1 Maternal determinants of childhood bone

Certain maternal factors appear to influence offspring bone, however it is not clear how much of this is genetic, and thus not modifiable, and how much is environmental and thus potentially modifiable.

1.6.1.1 Maternal birth weight and body build

Two cohort studies from Southampton have examined the relationships between maternal size and offspring bone mass. The first, The Birthright Cohort, used DXA to measure BMC and BMD in 145 infants born at term (6). Maternal birth weight and triceps skinfold thickness (reflecting fat stores) were positively associated with offspring whole body BMC and BMD adjusted for gestational age. Maternal height was positively associated with neonatal lumbar spine BMC and BMD, however no significant association was seen between maternal height and neonatal whole body bone variables. There is no clear explanation for this disparity and may be a reflection of the small number of participants in this study.

A second cohort, The Southampton Women's Survey, included 841 mother-infant pairs, and again found a positive association between maternal triceps skinfold thickness and neonatal whole body BMC, in addition to bone area (161). Maternal height was also a positive predictor of offspring BMC and bone area. No relationship however, was observed between any maternal measure and offspring BMD or size corrected BMC (scBMC; BMC adjusted for BA, infant height and weight).

A third prospective mother-offspring cohort, ALSPAC, found that maternal pre-pregnancy BMI was positively associated with total body-less-head and spine BMC and BMD in 7121 offspring at age 9 years (162). This significant relationship disappeared after adjusting for the child's height and weight. The authors suggest that the influence of maternal height and BMI is likely to be largely genetic, although taller mothers are likely to have a larger pelvis which may have greater capacity to nourish the fetus and thereby directly influence fetal growth.

1.6.1.2 Maternal physical activity in pregnancy

A negative association between vigorous maternal physical activity and offspring bone mass has been found in both the Birthright and SWS Cohorts (6;163). Both cohorts asked women to categorize their walking speed into one of five groups; women who described their walking speed as very slow/ easy pace in late pregnancy had offspring with higher whole body BMC compared to

those with fairly brisk/ fast walking speed. The mechanism underlying this remains unclear and raises the possibility of competition between the maternal and fetal skeleton for finite resources. The relationship was independent of the relationship between skinfold thickness and bone mass, suggesting that it was not mediated by more active women having lower fat stores.

1.6.1.3 **Maternal Vitamin D status in pregnancy**

One of the strongest risk factors for poor bone mineral accrual documented in the aforementioned mother-offspring cohort studies has been maternal vitamin D insufficiency. In the Princess Anne Study Cohort, 198 healthy, term children were followed up at age 9 years (164). Reduced maternal concentration of 25(OH)D in late pregnancy was associated with lower whole body and lumbar spine BMC and BMD in the children at age 9 years. Both the estimated exposure of ultraviolet B radiation during late pregnancy and the maternal use of vitamin D supplements predicted maternal 25(OH)-vitamin D concentration and childhood bone mass. Similar findings were observed in an analysis of the larger ALSPAC cohort, using maternal exposure to UV-B in pregnancy as a surrogate for vitamin D status (165).

We conducted a systematic review and meta-analysis of the effect of maternal Vitamin D in pregnancy on offspring bone mass (166). Eight observational studies were identified (five of these were cohort studies, three cross-sectional). All studies were assessed as being of medium to low risk of bias. The age at which offspring were assessed ranged from within 24 hours of birth to 9.9 years. Bone outcome measures also varied widely across the studies and included whole body, lumbar spine, radial mid-shaft, tibial and femoral bone BMC, whole body and lumbar spine BA, whole body and tibial BMD, tibial cross-sectional area (CSA) and whole body BMC adjusted for bone area (aBMC). Most studies (six of eight) used DXA to assess bone mass; two studies used pQCT and one study used single photon absorptiometry (SPA) in addition to DXA. Seven studies measured maternal serum 25(OH)-vitamin D concentration during pregnancy or at delivery, one study used UVB exposure in the third trimester of pregnancy as a surrogate measure of maternal vitamin D status. Five studies demonstrated a positive relationship between maternal vitamin D

status and offspring bone health; three studies showed no relationship (164;167-170).

Weiler et al found that neonates born to mothers with adequate maternal 25(OH)-vitamin D at delivery (defined by the authors as >37.5 nmol/l) had significantly higher whole body and femoral BMC per unit body weight compared to those with insufficient maternal vitamin D concentration (<37.5 nmol/l) even after adjustment for multiple confounders (168). Viljakainen et al measured neonatal bone mass, in a Finnish cohort of 125 primiparous Caucasian women and their offspring (170). Tibial bone mass was assessed by pQCT and those with maternal 25(OH)-vitamin D above the median (42.6 nmol/l) had significantly higher tibial BMC and CSA than those below the median, even after adjusting for confounders including maternal height and birth weight. A subsample of 55 children was also assessed again at 14 months (169). Tibial CSA remained significantly lower in those with maternal 25(OH)-vitamin D below the median, however tibial BMC was no longer significantly different suggesting that BMC gain was greater over the 14 month period in those with low maternal 25(OH)-vitamin D. This is possibly the result of a greater increase in maternal serum 25(OH)-vitamin D in the low vitamin D group over the 14 month period, which has only partly eliminated the differences in bone variables induced by maternal vitamin D status during the fetal period. No relationship was seen between maternal 25(OH)-vitamin D and tibial BMD at either time-point.

Sayers et al found that maternal UVB exposure in late pregnancy was positively associated with offspring BA, BMC and BMD in 6955 children at mean age 9.9 years. No relationship was seen when BMC was adjusted for offspring size, suggesting an effect on bone size rather than true volumetric density (167). More recently, the same authors analysed a subset of this group (n=3960) who had undergone maternal serum 25(OH)-vitamin D assessment in pregnancy; in contrast to the earlier study, no association was found between maternal vitamin D status in pregnancy and offspring BMC or other bone outcomes (171). The authors suggest that the conflicting results may be due to the unexpected strong collinearity between maternal UVB exposure and child's age at DXA; adjusting for child's age at DXA removed the positive relationship that the investigators identified earlier.

Three further studies have found no associations between maternal 25(OH)-vitamin D and offspring bone mass (172-174). Two studies, both cross-sectional in design, and with a similar number of participants, measured maternal 25(OH)-vitamin D at delivery and used DXA to assess offspring bone mass up to the first month of life (172;173). A third study measured mid and late pregnancy 25(OH)-vitamin D in a cohort of 125 pregnant Gambian women taking part in a randomised clinical trial of calcium supplementation (174). Offspring underwent assessment of bone mineral content and bone area using single photon absorptiometry of the midshaft radius; a subset also underwent whole body DXA at ages 2, 13 and 52 weeks. Again, no statistically significant relationship between maternal 25(OH)-vitamin D and offspring BMC at any time-point was observed even after adjusting for whether the mother had received calcium supplementation or not. It is difficult to extrapolate this study to the Western World as baseline dietary calcium intake in this cohort was low and mean maternal 25(OH)-vitamin D levels much higher than any other study with an average at 103 nmol/l for mid-pregnancy and 111 nmol/l for late pregnancy and none of the women in the study were considered vitamin D deficient.

To date, there have been four published interventional studies of gestational vitamin D supplementation. In the first trial, undertaken in the early 1980s, Congdon et al randomised 64 Asian women living in the UK to either no supplement or 1000 IU vitamin D plus calcium daily in the third trimester of pregnancy (175). Offspring had their forearm BMC measured within 5 days of birth, although the type of equipment used to measure this was not recorded. No difference in offspring radial BMC was observed between the two groups. This study was assessed to have a high risk of bias and maternal serum vitamin D concentration in pregnancy was not recorded at any time-point.

Two small intervention studies from India and Iran have also assessed bone outcomes in infants born to mothers randomised to vitamin D supplementation or placebo. Sahoo et al found that offspring BMC and BMD in the maternal groups randomised to either 60,000 IU cholecalciferol every 4 weeks or every 8 weeks, was not significantly greater than those who had received “placebo (400 IU/day cholecalciferol) (176). Similarly, Vaziri et al observed no significant differences in whole body BMC, BMD or BA amongst infants whose mothers have been randomised to receive either placebo or

2,000 IU/day cholecalciferol from 26-28 weeks gestation until delivery (177). In this latter study, out of 153 women randomised, only 25 infants underwent DXA. The small numbers included in both studies are unlikely to have sufficient power to detect a significant difference in outcomes studied.

The largest interventional study of gestational vitamin D supplementation to date is the Maternal Vitamin D Osteoporosis Study (MAVIDOS) (178). 1134 women, with a baseline 25(OH)D between 25 and 100 nmol/l were randomised to receive either 1,000 IU/day cholecalciferol or placebo, with neonatal bone mass as the primary outcome measure. In parallel to the findings from the three aforementioned interventional studies, neonatal whole body BMC did not significantly differ between the two groups (n=736) (179). Supplementation of 1000 IU/ day cholecalciferol was however demonstrated to be safe and sufficient to ensure that most pregnant women were vitamin D replete,

Evidence from observational studies does therefore suggest that higher levels of 25(OH)D in pregnancy may be beneficial to offspring bone development, but to date, interventional studies have not demonstrated significant effects of gestational supplementation with cholecalciferol on offspring bone mass; further, high quality, RCTs are needed to fully assess this.

1.6.1.4 **Other maternal nutrients in pregnancy**

Although the majority of studies investigating the effects of maternal nutrition on offspring bone mass have focused mainly on maternal vitamin D status in pregnancy, some authors have investigated the role of other nutrients. Data from the ALSPAC cohort suggested that maternal magnesium intake in late pregnancy was positively associated with whole body BMC and BMD in 4,451 children aged 9 years (180). However, this relationship was no longer observed after adjusting for the height of the child. Similarly the positive association observed between maternal potassium intake and spinal BMC disappeared after adjusting for weight of the child, to which potassium intake was also related. A significant association was observed between maternal dietary folate intake and spinal BMC adjusted for bone area, which persisted after adjusting for height and weight of the child.

The relationship between maternal dietary pattern and offspring bone mass has been examined in the Princess Anne Cohort (181). Using principal component analysis from a validated food frequency questionnaire, a maternal prudent diet score was calculated; a high prudent diet score was characterised by elevated intakes of fruit, vegetables and wholemeal bread, rice and pasta and low intakes of processed foods. The authors found that a higher prudent diet score in late pregnancy was associated with greater offspring whole body and lumbar spine BMC and BMD at age 9 years, even after adjustment for sex, socioeconomic status, height, arm circumference, maternal smoking and vitamin D status. The associations in early pregnancy were weaker and non-significant. In the SWS, positive associations between maternal long chain polyunsaturated fatty acid (LCPUFA) status during pregnancy and offspring bone mass and lean mass at age 4 years have also been found (182;183).

1.6.1.5 **Maternal smoking in pregnancy**

Several studies have identified maternal smoking as a negative predictor of offspring bone mass. Using data from a Tasmanian cohort of 8-year old children and their mothers, Jones et al found that offspring bone mass was lower in those whose mothers had smoked in pregnancy, even after adjustment for child's height and weight(184). Similarly, studies from both the SWS and Birthright cohorts have found significant negative associations between offspring bone mass and maternal smoking in pregnancy (6;163). In the Birthright Cohort, after adjustment for gestation at birth, whole body BMC of infants whose mothers smoked during pregnancy averaged 7.1g (11%) lower than those whose mothers did not smoke. Maternal smoking had no significant effect however, on offspring spinal BMC, BMD and bone mineral apparent density (BMAD) (6). Similarly in the SWS cohort, maternal smoking in late pregnancy was found to be an independent negative predictor of neonatal whole body BMC in both boys and girls (161).

Conversely, in a study of 7,121 children aged 9 years, from the ALSPAC cohort, maternal smoking in any trimester was associated with increased total body-less-head and spinal BMC, BA and BMD in girls; (185). Weight at aged 9 years was higher in those whose mothers had smoked in pregnancy and the positive relationship attenuated to the null when the child's height and weight were

included in regression models. Likewise no association was seen between maternal smoking and BMC adjusted for BA (a reflection of volumetric BMD). This suggests that the associations were driven mainly by offspring size and concurs with the evidence that maternal smoking in pregnancy is associated with an increased BMI and risk of overweight in childhood (186). No relationship between maternal smoking and any of the childhood bone measures were observed in boys, a finding possibly explained by the greater association between fat mass and bone mineral accrual in girls than boys in puberty (187).

The exact mechanism by which maternal smoking may act on offspring bone mass is not clear. Maternal smoking has been shown to impair placental calcium transport and impair placental vascular function, which may potentially reduce offspring size and bone mineral accrual (188). As mothers who smoke during pregnancy are likely to have smoked before pregnancy and will continue to smoke post-natally, it is difficult to dissect whether smoking has an in-utero effect on bone or whether pre-natal and post-natal smoking also plays a role. In addition, there is the possibility of confounding by other factors which have a strong collinearity with cigarette smoking; for example those who smoke are likely to have a poorer diet.

1.6.2 **Paternal determinants of offspring bone mass**

Despite considerable work investigating the maternal influences on childhood bone accrual, there are relatively few data on the contribution of the father to childhood bone mass. Despite the father transmitting half of the heritable information to the fetus, the focus of preconception health has been the mother. Paternal effect have been linked to complex diseases such as diabetes cancer and obesity, and are unlikely to be explained by genetics alone and highlight the potential for non-genetic inheritance through epigenetic mechanisms (189) . A previously described study from the ALSPAC cohort examining the effects of parental smoking on childhood bone mass at age 10 years (190), found significant positive relationships between paternal smoking in pregnancy and offspring bone mass in girls, with a similar effect size to maternal smoking. This would support epigenetic transmission, but may

suggest an important role of unmeasured shared family environment such as diet or physical activity, rather than a pure intra-uterine effect.

The mechanisms underlying paternal epigenetic transmission are unclear, however as DNA methylation in gene promoters within sperm is uncommon (191), it is likely to be the result of histone modification and/or changes in sperm RNA (192;193). This concept is supported by a recent animal study, which found that disruption of histone methylation in developing mouse sperm resulted in severely impaired development and survivability in the offspring (189).

In the SWS cohort, 278 fathers and their offspring underwent whole body DXA within two weeks of birth (194) . Among female neonates, significant positive associations were found between whole body BA, BMC and BMD, and the corresponding indices in the father. Associations between paternal and neonatal BA and BMC were stronger than those for BMD and vBMD. Interestingly, associations between male neonate-father pairs did not achieve statistical significance. The reason for this sex disparity is unclear, with little other existing evidence to support a differential association between father and offspring bone mass in male and female offspring. A possible explanation may be a gender/ imprinting interaction, such that the paternal allele of a gene influencing skeletal growth is expressed in girls but not boys. Alternatively, other sex-dependent factors, such as oestrogen/ androgen balance may modify genetic relationships.

1.6.3 **The combined parental influence on childhood bone mass**

Two studies to date have evaluated the relative influences of both maternal and paternal bone mass on childhood bone mass, however both studies used a non-Caucasian population. The Pune Maternal Nutrition Study assessed anthropometry, diet, physical activity and circulating micronutrients at 18 and 28 weeks gestation in 797 pregnant women from rural villages near the city of Pune, India (195). Six years post-natally, whole body and total spine BMC, BMD were measured using DXA in the children (n=698 of 762 live births) and both parents. Parental DXA measurements positively correlated with the equivalent measurements in the children with a similar strength of correlation for fathers

and mothers. From this study it is difficult to tease out the exact mechanisms underlying these relationships, however the results suggest that genetic factors or shared inherited environment may play the major role, rather than the maternal intra-uterine environment. Several potential confounding factors, such as childhood diet and activity were not measured and may have biased the observed results.

A second study, cross-sectional in design, used data from the Korea National Health and Nutrition Examination Surveys (KNHANES), to investigate the familial association of BMD between parents and offspring (aged over 10 years) in a Korean population (196). Among 1228 family trios, BMD measured at the lumbar spine, femoral neck, total hip and whole body showed significant positive associations between both parents and offspring, with whole-body BMD having the strongest relationship between offspring and parent. Independent parental association was seen in a multiple linear regression model after adjusting for co-variates such as calcium intake, serum 25(OH)D and physical activity.

1.7 Measurement of bone mass

1.7.1 **Dual-energy X-ray absorptiometry (DXA)**

DXA is generally considered to be the gold standard tool for measuring BMD, and has been validated for use in the adult population. It is important to remember, as discussed earlier, that although BMD explains a high proportion of bone strength, other factors such as shape, architecture and overall size, which partly contribute to DXA BMD but cannot be fully characterised by it, will also contribute to bone strength and risk of fracture.

The fundamental principle of DXA is to measure the transmission of X-rays through the body at high and low energies. DXA assumes the body is made of two compartments, bone and non-bone (fat and lean mass) and the use of two energies allows the discrimination between the two compartments. X-ray attenuation values are then converted into BMC (in grams). Software algorithms can detect the edges of bone, and using this, BA (in cm^2) can be calculated by adding the pixels within the bone edges. ‘Areal’ bone mineral density (aBMD,

in g/cm^2) is then calculated by dividing BMC by BA. In addition to the bone variables obtained, DXA can also obtain information on other aspects of body composition, primarily fat mass, lean mass, percentage fat mass and percentage lean mass. DXA may be used for whole body measurements or skeletal sites of interest, most often the lumbar spine and hip (197).

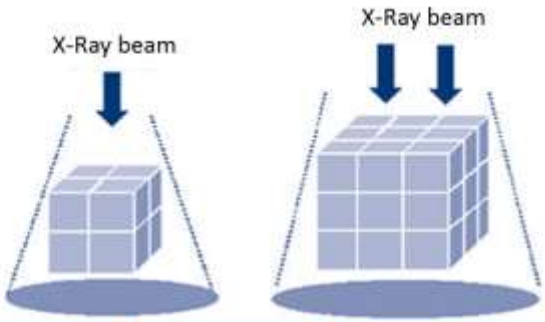
1.7.1.1 Strengths of DXA

DXA has several strengths: firstly, it subjects the patient to a very low dose of radiation. The radiation dose is machine and manufacturer specific but is appreciably less than what an individual is exposed to from the natural environment. The time taken to perform the scan is relatively short. Again, this depends upon machine and can range from 15 minutes at the whole body site with older pencil-beam scanners, to 2 to 3 minutes with newer fan-beam scanners. Thirdly, the precision of DXA measurements (i.e. the repeatability of scans) is good, with the coefficient of variation (cv) ranging from 1 to 3% depending on machine and site of scan (197); in the ALSPAC cohort, the cv for total body BMD was 0.8% based on the results of 122 children age 9 years who had two DXA scans on the same day (198). Finally, DXA has the largest normal database of all the bone density techniques, ensuring that interpretation of results is accurate against a wide range of normal populations (199).

1.7.1.2 Limitations of DXA

One of the major limitations of DXA, particularly in children, is the size dependence of the measurement. The aBMD calculation derived from DXA is based on the two-dimensional projection of a three-dimensional structure. This does not take into account the depth of the bone being measured and results in the BMD of small bones being underestimated and the BMD of large bones overestimated compared with true volumetric BMD (vBMD). An example of the impact bone size can have on DXA-derived BMD results is illustrated in Figure 1.4. This is not such a concern in adults as the volume of bone remains stable over time, however in growing children this approach is less suitable and may cause inaccuracies as aBMD is so influenced by bone size. Thus, an increase in a child's aBMD might reflect an increase in bone size or vBMD, or both. It is

therefore imperative that the size dependence of the technique is accounted for when interpreting results.



Mineral weight (g)	16	54
Volume (cm^3)	8	27
Projected area (cm^2)	4	9
Volumetric BMD (g/cm^3)	2	2
Areal BMD (g/cm^2)	4	6

Both bones have identical vBMD, however the smaller bone will have an apparently lower aBMD because DXA BMD does not take into account the depth of the bone (adapted from Carter et al (200) with permission)

Figure 1.4: Size dependence of DXA

There are several methods in the literature to try to reduce the influence that bone size has over aBMD measurements. An example of one such method is that suggested by Carter et al, which adjusts BMC for apparent bone volume (derived from the projected bone area) to give bone mineral apparent density (BMAD), an estimate of true vBMD (200). It is important to remember that this is only an estimate and makes certain assumptions about the shape of bone which may not always be correct. For example, this method assumes that lumbar vertebrae are perfectly cubic in shape.

An alternative method to reduce the influence of bone size on DXA measurements, is the method developed by Prentice et al which adjusts BMC for BA, height and weight (surrogates for bone size) to give size corrected BMC (scBMC) (201). It should be considered however, that body height and weight might not completely control for all the relevant differences in size and shape of the skeletal site of interest, and that this approach is not an estimate of vBMD.

A final approach, described by Hogler et al, is to interpret BMC in relation to lean mass, which is a major predictor of BMC (202). There is no clear "best" method to try and reduce the influence that bone size has over DXA measurements, and many studies use several of the mentioned methods to try and address this. Recently, techniques that directly measure vBMD have been used in research studies, but they are still not commonly used in clinical practice; an example of this is pQCT, which is discussed in section 1.7.2.

The machine algorithms used to separate bone from soft tissue have been designed to optimise measurements in adults. In small children, with low mineralization of bone, this may cause problems with bone-edge detection and can affect results. Specific paediatric software has been developed to try and overcome this, however, this software does significantly alter the results obtained and cannot be automatically interchanged with adult software. This is important to consider when following up children into adulthood (203). Lastly, the measurement of bone mineral density by DXA is a composite of trabecular and cortical bone, and thus it is not possible to differentiate between the two types of bone and dissect whether difference in bone density are due to changes in trabecular or cortical bone (or both).

1.7.2 **Peripheral quantitative computed tomography (pQCT)**

pQCT has been commercially available since the early 1990s and has the advantage of being able to directly measure true size-independent vBMD, unlike DXA. The method uses traditional CT technology to obtain multiple cross-sectional slices, 1-2mm thick, through a site of interest in the peripheral skeleton (radius, tibia or femur). Unlike DXA, due to the size of the apparatus, pQCT cannot measure whole body, hip and spine bone mass. In children, the most commonly used site is the distal 4% site at the radius (which equates to the distance 4% of forearm length proximal to the growth plate); the reference database for this site is from Germany and consists of 371 children aged 6 to 18 years (204). There is no robust reference dataset for tibial sites. Although several scanners are available, the most commonly used is the Stratec XCT-2000 (Stratec Inc, Pforzheim, Germany). As observed with DXA, the coefficient of variation for this technique is good, ranging from 0.8 to 1.5% in the adult population (205). pQCT has been successfully validated for use in children as

young as 3 years of age; in a study of children aged 3-5 years the CV was 3.1% for total area, 4.5% for cortical area, and 6.8% for cortical thickness. As each slice takes around 2-3 minutes, the technique is better suited to older children who are able to sit still, and thus pQCT results tend to be more reliable as a child gets older due to less movement artefact.

pQCT has several advantages over DXA for assessing bone mass. Firstly, the radiation dose is even smaller at around 0.43 microsieverts (μSV) per slice, and secondly density measurements are not affected by bone size; volumetric bone mineral density is directly measured, without having to correct for size or height, or rely on mathematically derived estimates. pQCT is also able to differentiate between cortical and trabecular bone structure and can give measurements of cortical and trabecular thickness in addition to cortical and trabecular vBMD. For these measurements scanning sites are optimised: the 4% site measures total and trabecular vBMD at the distal end of the radius and tibia, whilst the mid-diaphysis of the bone is used to assess cortical vBMD, bone area, cortical thickness, periosteal circumference, endosteal circumference and muscle cross-sectional area. Mechanical strength parameters can also be obtained at the mid-diaphyseal site and include axial moment of inertia (AMI) and the stress-strain index (SSI). The AMI is a measure of the distribution of bone material around the centre of a bone, whereas the SSI is a combination of AMI and cortical vBMD and provides information on the bending and torsional strength of bone; both relate well to fracture load (206).

1.7.3 **Limitations of pQCT**

There are several disadvantages of pQCT. One problem is the potential underestimation of cortical vBMD due to the spatial resolution of the machine. This phenomenon is called the partial volume effect and occurs when a voxel in the image represents more than one tissue type (207). The cortical rim of a bone has a considerable number of voxels with mixed tissue and therefore is more often affected by the partial volume effect, especially when the cortical bone shell is less than 2mm. Trabecular bone sites are less affected by this problem as the trabecular core area has more homogeneous voxels (208). To ameliorate this problem, algorithms that adjust for the partial volume effect have been published (209). Further disadvantages of pQCT include a paucity of reference data (compared to DXA) and an inability to obtain repeated

measurements at the same bone site in paediatric longitudinal studies due to variations in longitudinal bone growth rates. Similar to DXA, pQCT can be challenging when used in young children. Movement can cause errors in locating the measurement site, especially if it occurs between the scout view and slice imaging. Paediatric positioning devices, which have been available since the 1990s, can reduce movement and increase the percentage of valid scans (207).

1.8 Summary

In summary, osteoporosis is a public health concern due to its association with fragility fractures. Bone mass gains during childhood and adolescence may reduce an individual's risk of osteoporosis in later life. Whilst direct genetic inheritance accounts for a significant proportion of bone size and density, there is a growing body of evidence to suggest that environmental factors, possibly acting through epigenetic changes in utero, may also play a role. To explore this latter concept further, it is important to characterise the independent bone relationships between mother-child and father-child, as differences between the two may point towards intrauterine effects. As the placenta is the conduit for all maternal intrauterine effects, the relationships between childhood bone and placental size will also be explored.

2. Objectives

The overall objective of this thesis is to elucidate the following questions, using two cohorts: SWS and ALSPAC:

1) Are there relationships between placental size and offspring body composition, bone size and density at birth?

2) Do any relationships placental size and offspring bone persist into later childhood and adolescence?

3) Does placental size have differential effects on offspring bone size and volumetric bone density?

4) Are there differences in the bone relationships between mother-child versus father-child?

5) Are any parent-child bone differences related to possible maternal intra-uterine effects?

6) Are any parent-child bone differences mediated by placenta-bone relationships

3. Methods

The objectives of this study have been addressed using two unique longitudinal mother-offspring cohorts: The Southampton Women's Survey (SWS) and the Avon Longitudinal Study of Parents and Children (ALSPAC).

3.1 Overview of the Southampton Women's Survey (SWS)

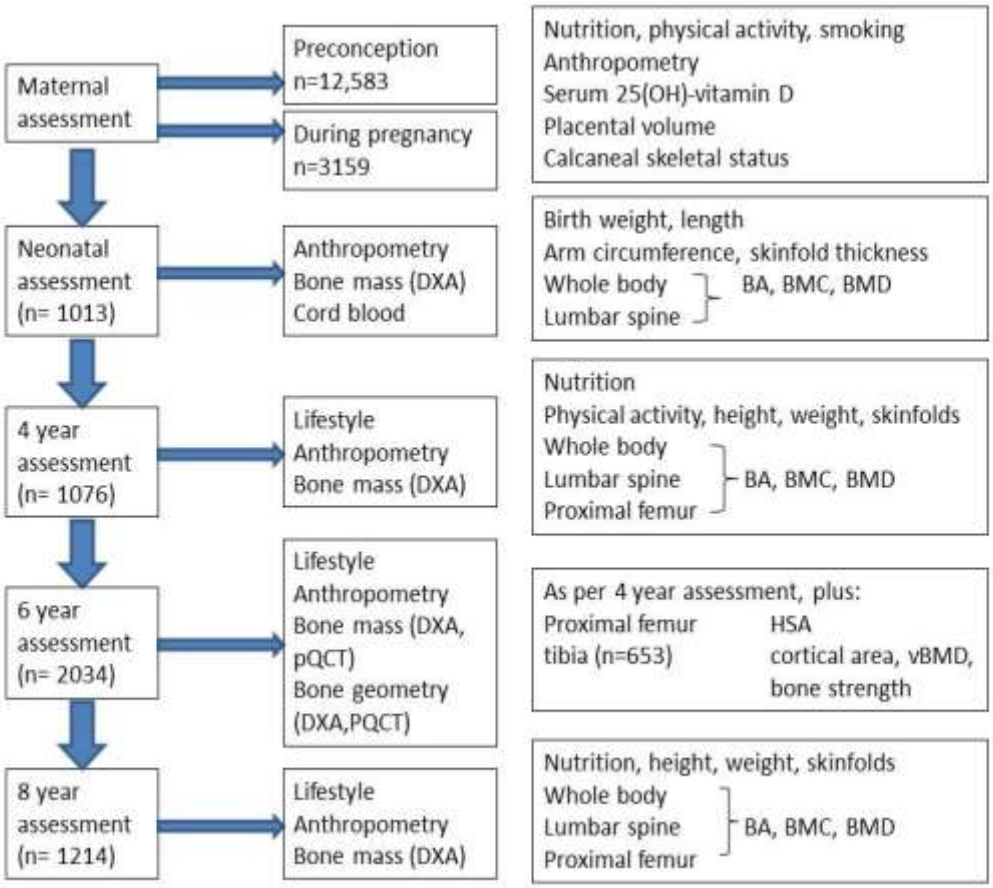


Figure 3.1: Outline of the SWS bone study, from pre-conception to 8 years

3.1.1 SWS Pre-conception phase

The Southampton Women's Survey (SWS) is a large, unique prospective cohort that recruited 12,583 women aged 20-34 years living in the City of

Southampton (210). The aim of the cohort was to assess the body composition, diet, physical activity and hormone levels of a large group of non-pregnant women. Subsequent follow-up phases of the study have focused on those women who became pregnant and on their offspring, with the purpose of investigating maternal influences on childhood development in early life (Figure 3.1).

During initial recruitment, women were sent an invitation letter from their general practice (GP) surgery, which was later followed up with a telephone call. In addition, a local advertising campaign took place with the hope of encouraging women to self-refer and to help recruit women who were not registered with GP practices, or whose contact details were out of date. Approximately 75% of those women approached agreed to participate in the study.

After agreeing to take part in the study, participants were visited at home by a trained research nurse. At the initial visit a questionnaire was administered to assess lifestyle factors such as diet (using a validated 100 item food frequency questionnaire (FFQ) (211), physical activity, general health, smoking history, menstrual and obstetric history, education, ethnicity, housing, benefits, social class and own and partner's occupation. Anthropometric assessments included height (measured by stadiometer; Seca, Birmingham, UK), weight (measured by digital scales; Seca, Birmingham, UK), waist and hip circumference, and skinfold thickness measured at four sites (triceps, biceps, subscapular and supra-iliac) using Harpenden callipers (Baty International, Sussex, UK). The research nurses were carefully trained and regular inter-observer variability studies were performed to ensure accurate measurements. Venous blood was taken via venepuncture and stored at -80^{o}C for later analysis.

3.1.2 **SWS Pregnancy follow-up**

Women enrolled in the study were asked to immediately inform the study team if they fell pregnant, and gave consent for their GP or hospital clinician to also communicate this information. 3,159 singleton pregnancies were followed. Pregnant women were invited to attend research clinics for interviews at 11 weeks (early pregnancy) and 34 weeks (late pregnancy). At these visits, a

lifestyle questionnaire was again completed along with repeat anthropometric measurements (as described for the initial visit). Venous blood was again collected and stored at -80^{0}C.

3.1.2.1 **Placental measurement**

Pregnant participants underwent a high resolution ultrasound scan at 11, 19 and 34 weeks gestation using either a Kretz Voluson 730 (GE Healthcare, GE Corporation, Madison, Wisconsin, USA) or Acuson Sequoia 512 (Siemens, Malvern, PA, USA) machine which was cross calibrated. After establishing correct positioning according to standard anatomical landmarks, fetal measurements (including femoral length and abdominal circumference) were obtained from the frozen images using electronic callipers by an experienced ultrasonographer. Each measurement was performed in triplicate and the mean value used. At the 19 week scan, additional placental measurements (circumference, length of attachment to the uterine wall and cross-sectional area) were obtained using the same technique (Figure 6). Placental volume was later estimated from the two-dimensional ultrasound measurements as follows: to estimate the volume of the placenta it was assumed to be ellipsoid in shape and the two measured circumferences and two areas were expressed as functions of the three ellipsoid radii. Estimates of the radii, obtained by least squares, were then combined to estimate the volume. This method demonstrated good correlation with placental volume measured by 3D ultrasound ($r=0.64$, $p<0.0001$)) in a subset of 28 pregnancies at mean (SD) 19.9 (0.4) weeks gestation.

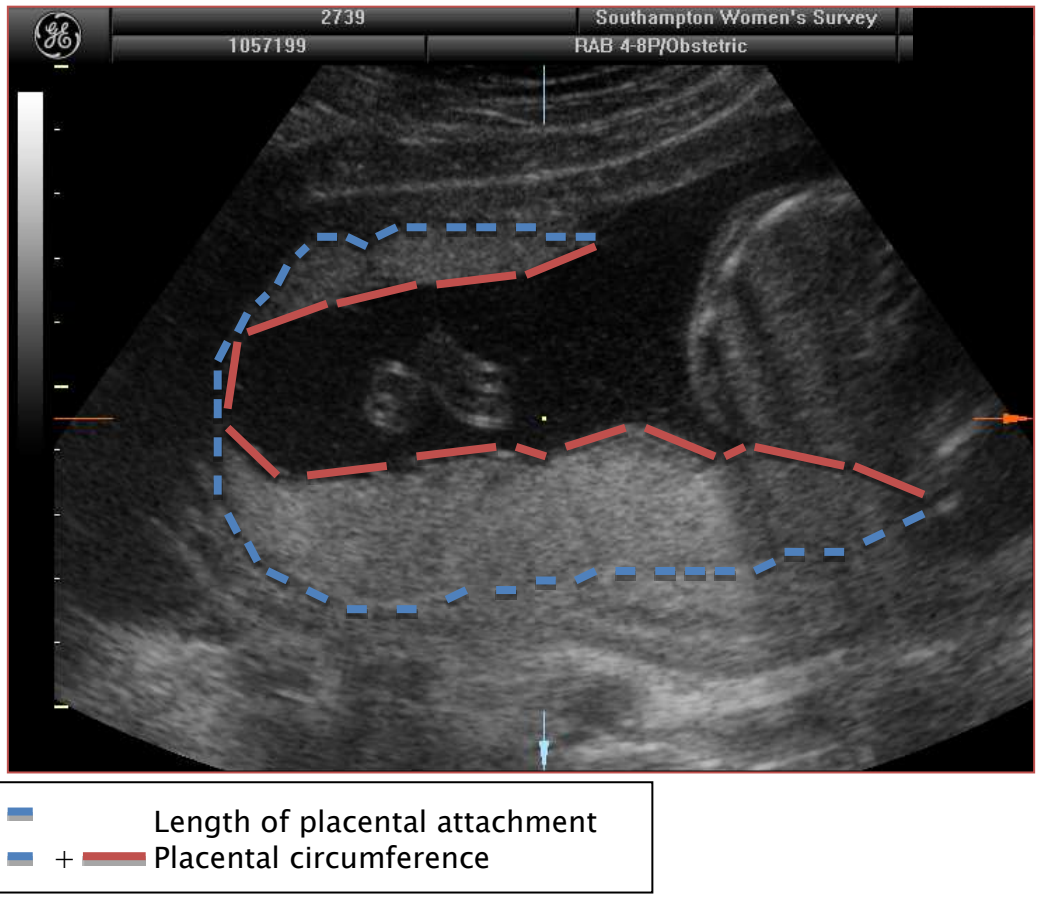


Figure 3.2: A 19 week ultrasound scan showing placental size measurements in the longitudinal plane

3.1.3 SWS Childhood follow-up

The children born in the SWS cohort have been followed-up and assessed from birth to the present phase at age 11-13 years.

3.1.3.1 Birth follow-up

Mothers registered with specific general practices were invited to participate in the bone health component of the SWS. These practices were selected to avoid the mothers participating in more than one sub-study, and were representative of the population of Southampton as a whole. At birth, the babies were measured (length, head and abdominal circumference), weighed on calibrated digital scales (Seca, Birmingham, UK) and skinfold thickness measured (triceps, sub-scapular and thigh) using Harpenden callipers (Baty International, Sussex, UK). Cord blood was collected and stored for later analysis. The mother was asked to give written informed consent for her baby

to undergo assessment of bone mass and body composition within 2 weeks of birth, using a DXA scanner with specific paediatric software (Lunar DPX-L paediatric small scan mode v 4.7c, GE Corporation, Madison, Wisconsin, USA) at Southampton General Hospital. The instrument underwent daily quality assessment and was calibrated against a water phantom on a weekly basis.

At the visit to the scan room, the baby was pacified, undressed completely, and then swaddled in a standard towel. Measurements of whole body BA, BMC, aBMD and body composition (total and proportionate fat and lean) were performed. The short-term and long-term coefficients of variation (CV) for adult whole body BMD for the DXA scanner were 0.8% and 1.4% respectively. It was not possible to repeatedly scan neonates to establish precision values in the study group; however, the ability of DXA to measure bone mass in small subjects has been previously demonstrated using miniature piglets, where correlation between DXA-derived BMC and ashed calcium content was 0.90 (P<0.001) (212). The radiation exposure to the baby was estimated as a maximum of 8.9 microsieverts for the whole body measurement, which is equivalent to 3 days' exposure to normal background radiation. All DXA scans were reviewed and those with movement artefact (n=41) were excluded from the study. In total 1013 infant DXA scans were obtained.

3.1.3.2 **6 months – 2 years follow-up**

Permission to contact the women by telephone for further follow-up studies was obtained when their baby was born. Mothers of the children were contacted when their child reached 6 months, 12 months, 2 years and 3 years of age. At each stage, the mother and child were visited in their own home by a trained research nurse who administered a questionnaire, detailing the child's feeding patterns, diet, activity and overall health, in addition to undertaking repeat anthropometric measurements (including weight, crown-heel length or height, head circumference, abdominal circumference and skinfold thickness). Periodic assessment of inter-observer variability was undertaken.

3.1.3.3 **4 year and 6 year follow up**

Mothers were invited by post to bring their child to further phases of the SWS follow-up, when their child became 4 and 6 years old. If the mother was willing for her child to participate, they were invited to a research clinic at the Osteoporosis Centre, Southampton General Hospital. Here, the child's height and weight was measured as before, in addition to their left mid-upper arm circumference. A nurse-administered questionnaire was completed detailing diet, exercise and medical history. The children then underwent DXA scanning, where measurements of whole body, lumbar spine and left hip bone mass and body composition were taken using a Hologic Discovery machine (Hologic Inc, Bedford, MA, USA). To help reduce movement artefact a suitable DVD was shown on a TV near the DXA machine. The total radiation dose for the scans were 35.3 μSv (whole body (paediatric mode) 10.5 μSv, lumbar spine (L1-L4) 13.7 μSv , hip 11.1 μSv). This is equivalent to around 5 days background radiation (based on local background radiation of 6.6 μSv). At the end of the visit, grip strength of the child was measured in each hand using a handheld dynamometer (three times in each hand, alternating between sides), with the child's arm in a standard position.

In a subset of participants at both the 4 and 6 year old follow-up clinics, an Actiheart combined accelerometer and heart rate monitor (Cambridge Neurotechnology Ltd, Cambridge, UK) was fitted to both mother and child to measure physical activity levels. These were worn continuously for 7 days and then returned in pre-paid envelopes.

At the end of the 6-year visit, a subset of parents and children were invited to attend an additional research clinic at Southampton General Hospital. If the parent consented for their child to take part, a pQCT scan (Stratec XCT-2000, Stratec Inc., Pforzheim, Germany) of the child's non-dominant lower leg was performed. Firstly, the lower leg was measured from the medial malleolus to the tibial tuberosity. The child then placed their leg into the pQCT machine, which was positioned using a laser beam at the distal end of the medial malleolus and secured in place to reduce movement artefact. A suitable DVD was shown to occupy the child whilst the scan took place, with the hope of reducing movement artefact. A scout view was first obtained to locate the distal end of the tibia and a reference line positioned to bisect the medial

border of the articular surface (Figure 3.3). Four sites of the tibia were scanned (4%, 14%, 38% and 66% of the total tibial length from the reference line to the tibial tuberosity) during the 5 minute scan time. The 4% and 14% sites give information on trabecular content and density, the 38% site cortical content, density and bending strength whereas the 66% site was used to study muscle, fat and bone ratios. The total radiation dose associated with pQCT is less than DXA at 1.72 μSv for all 4 slices; around a quarter of daily background radiation.

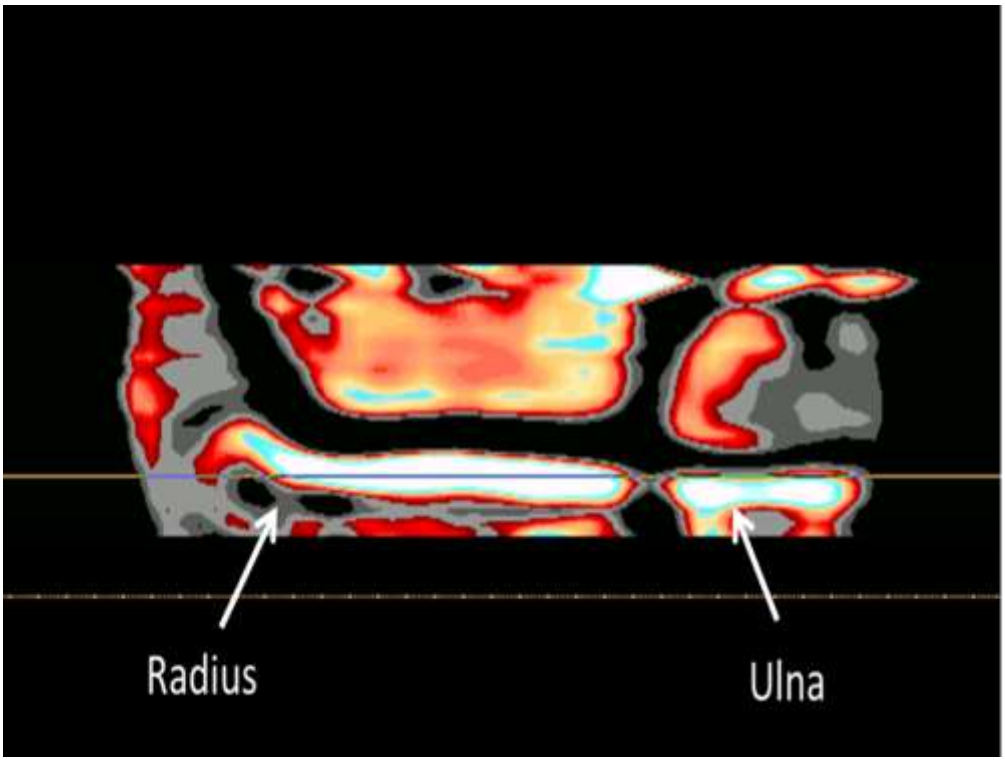


Figure 3.3; Scout view of distal tibia with reference line placement

1076 and 2034 children underwent DXA at age 4 years and 6 years respectively; 653 6 year olds additionally underwent pQCT of the lower leg.

3.1.3.4 **8 Year childhood follow-up**

When children turned 8 years of age, mothers were sent an invitation letter and information sheet regarding this phase of the study. They were then contacted by telephone asking if they are willing to participate. An appointment was then

made for willing mothers and their children to attend a research clinic at the Princess Anne Hospital, Southampton. A confirmation letter was posted to the mother.

The 8 year clinic visit consisted of a number of examination stations primarily focused at investigating childhood cardiovascular structure and function and was supported by a grant from the British Heart Foundation. At the clinic visit, after written consent had been obtained from the mother or father, a research nurse-administered questionnaire was completed, detailing aspects of the child's lifestyle including diet, physical activity and medical history. Anthropometric measurements were made, including height, weight (measured as before), and occipito-frontal, left mid-upper arm and waist circumferences. Skinfold thickness was measured using Harpenden callipers at the triceps and subscapular areas. Grip strength in both hands was measured as described previously.

Cardiovascular structure and function assessments included an echocardiogram, pulse wave velocity and an arterial ultrasound scan. Each child also underwent DXA scanning using a Hologic Discovery A machine (Hologic Inc., Bedford, MA, USA). Whole body, lumbar spine and left hip scans were taken. Total radiation doses for the scan was 29.1μSv [whole body (paediatric mode) 9.6μSv, lumbar spine (L1-L4) 10.6μSv , hip 8.9μSv]. As the child progresses through each station they were invited to complete an activity book of their visit and all children were given a copy of the DXA scan as a memento of the visit.

1214 children underwent DXA measurement in the 8 year follow-up clinic.

3.1.4 **SWS Parent follow-up**

All mothers of children who had undergone DXA at age 8 years were invited to a further research clinic to assess parental bone mass and body composition. Mothers were given an invitation letter and information sheet either by post or at the end of their 8-year childhood visit (Appendix 1,2). Included with the information sheet was a reply slip, prepaid envelope and identical materials (invitation letter, information sheet, reply slip and prepaid envelope) for the

mother to pass on to the child's father. Upon receiving a positive reply slip, the parent was telephoned and invited to arrange a 45 minute research clinic appointment at Southampton General Hospital. Appointments were offered between 9am and 5.15pm weekdays. As appointments could be booked several months in advance, nearly all parents who volunteered were able to arrange a weekday appointment. Parents could either attend together or separately. In cases where parents had separated and were no longer living together, the father was contacted directly by post if the mother was able to supply contact details. Mothers who had no contact with the father were not recruited as both sets of parents were needed for this particular sub-study. Non-paternity was not tested and it was accepted that those claiming to be the father were indeed the child's biological father.

At the clinic visit, the parent completed a written consent form (Appendix 3) before having their height and weight measured (as described in other phases of the SWS study). A questionnaire was then administered by a doctor or research nurse, detailing information on dietary and milk intake, medication and supplement use, past medical history, physical activity, contraceptive and obstetric history (for women), alcohol and smoking history and ethnicity (Appendix 4). Parents then underwent DXA of their whole body, non-dominant hip and lumbar spine using a Hologic Discovery instrument (Hologic, Inc., Bedford, MA, USA). The radiation dose for this scan was as follows: whole body 8.4μSv, lumbar spine 6.7μSv, hip 4.7 μSv, total radiation exposure 19.8 μSv. A pQCT scan of their non-dominant lower leg was also performed using the Stratec XCT-2000 machine (Stratec Inc., Pforzheim, Germany) using an identical protocol to the childhood pQCT at age 6 years (Figure 3.4; Section 3.1.3.3 describes the pQCT methodology). The radiation dose for this was 1.72μSv. Finally grip strength was measured in both hands using a handheld dynamometer (described in Section 3.1.3.3)

Figure 3.4: pQCT of the lower leg

After the scans, the parent was thanked and their DXA result explained by either a doctor or research nurse; they were also given a copy of their DXA result for their records (Appendix 5). Parents with abnormal DXA results were offered an appointment in a Metabolic Bone Disease clinic at University Hospital Southampton for further assessment.

Full ethics and NHS Research and Development approval was granted for this study by the Southampton and South West Hampshire Local Research Ethics Committee (B) (Appendix 6).

3.2 Overview of the ALSPAC cohort

The Avon Longitudinal Study of Parents and Children (ALSPAC) is one of the prospective birth cohorts within the European Longitudinal Study of Pregnancy and Childhood (ELSPAC). Its aim is to investigate the genetic, epigenetic, biological, psychological, social and other environmental influences on childhood health and development (Figure 3.5).

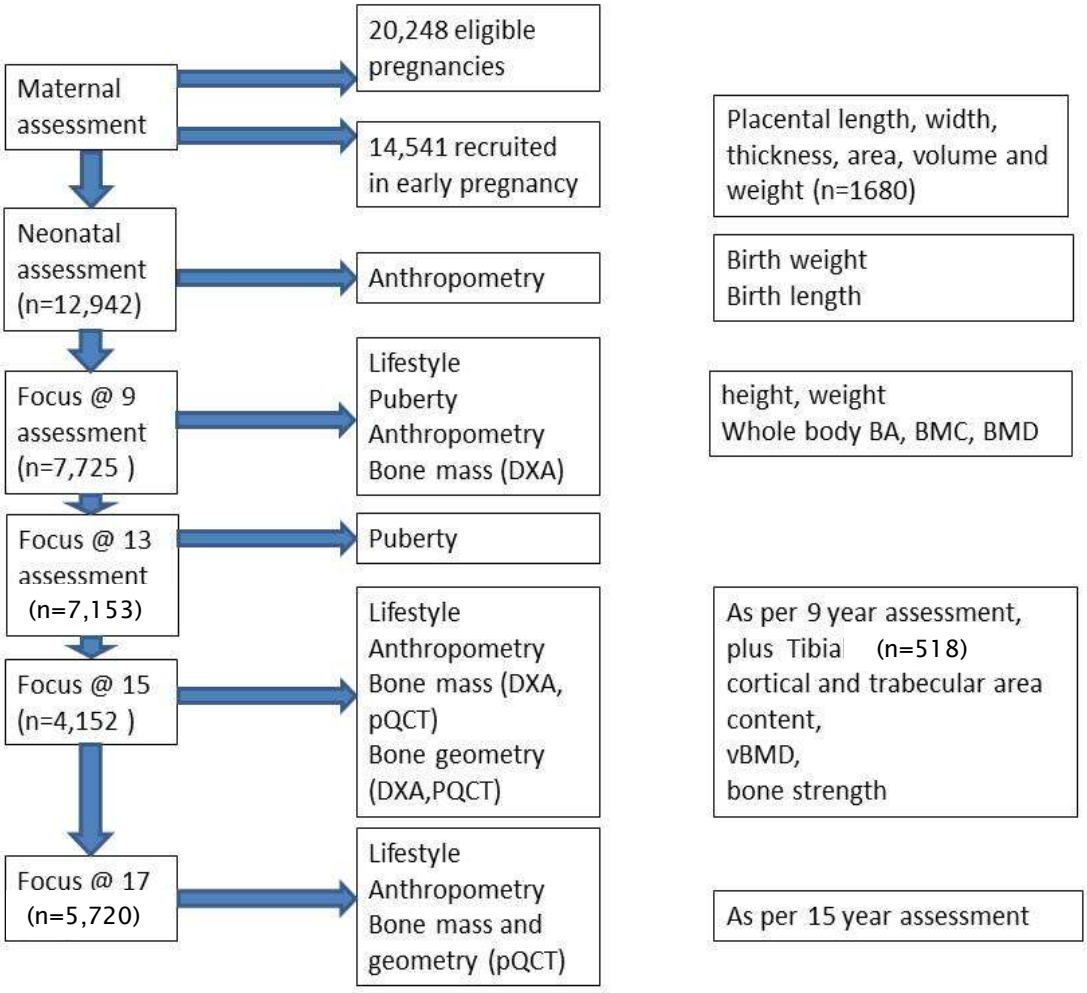


Figure 3.5: Outline of the ALSPAC bone study

3.2.1 **Recruitment of participants**

All pregnant women living in the former county of Avon, UK (total population 0.9 million) with an expected delivery date between the 1st April 1991 and 31st December 1992 were eligible to take part. Recruitment was opportunistic and aimed to recruit women as early in pregnancy as possible. The study was promoted in a numbers of ways through routine antenatal visits, maternity health services, media information, and via recruitment staff visiting community locations. An "expression of interest" card was given, allowing women to request further information or to decline participation. Women requesting further information were sent a study information booklet followed by an initial questionnaire 1 week later.

Out of 20,248 eligible pregnancies, 16,734 women are known to have been invited, of which 14,541 were recruited during early pregnancy; 1301 women opted out of the study via the expression of interest card. Two further recruitment campaigns were undertaken post-natally; the "Focus @ 7" clinical assessment of children aged 7 recruited a further 456 children and a Phase III campaign of children aged 8-18 years added a further 257 children, giving an overall total of 15,247.

3.2.2 **ALSPAC Follow-up**

Information from early pregnancy onwards was collected from a variety of sources during frequent assessments. Between birth and 18 years of age there were 68 data collection time points including 34 child-completed questionnaires, 9 "focus" clinical assessments and 25 questionnaires about the child completed by the mother or other main caregiver. Since early pregnancy, mothers and children have also provided biological samples including blood, urine, hair, toenails, teeth, saliva and placenta, which have been stored to ensure long-term preservation. Retrospectively the data collection time points have been divided into six phases; infancy (>4 weeks and <2 years), early childhood (>2 years and <7 years), childhood (7 years of age), late childhood (>7 and <13 years), adolescence (>13 and <16 years) and transition to adulthood (>16 and <18 years).

3.2.2.1 **Birth and placental assessment**

12,942 singleton infants were born at term (≥37 completed weeks). The length of gestation was estimated from the date of the mother's last menstrual period. Birth weights were extracted from hospital records and birth length (crown to heel) measured using a Harpenden neonatometer (Holtain Ltd, Crymlych Wales) by ALSPAC staff who visited all study participants within a day after birth. At delivery, the placenta was collected and stored in 10% formaldehyde for later assessment.

In 2010 a sample of 1,680 placentas, all from one maternity hospital and taken in the order in which they were stored, were removed from their containers, trimmed as per a standard protocol and measured (213). Direct measurements were made of placental thickness, volume and weight. Both sides of the placenta (maternal and fetal) were then photographed using a digital camera (Figure 3.6). Each photograph included a ruler to measure the length and breadth of the surface. Length was defined as the maximal diameter, and breadth was measured at 90 degrees to the midpoint of the length. To calculate placental area, the placenta was assumed to be elliptical in shape, and area was defined as the product of length and breadth, multiplied by π/4. Maximum thickness was measured using a calibrated needle and volume was estimated as the product of area and maximum thickness.

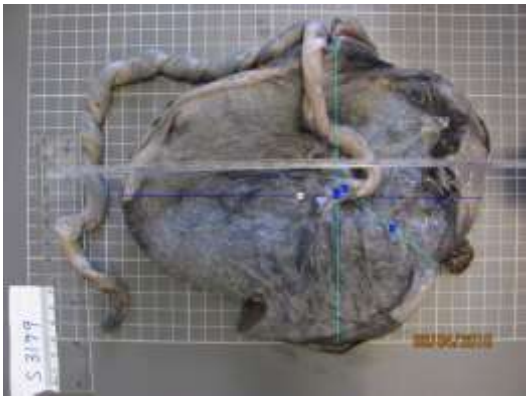


Figure 3.6: Image of the fetal side of a placenta and umbilical cord. Lines illustrate measurements of length (blue line) and width (green line)

3.2.2.2 **Childhood skeletal assessment**

The children enrolled in ALSPAC underwent skeletal assessment at 3 time points: ~9 years, ~15 years and ~17 years.

3.2.2.2.1 Focus @ 9 skeletal assessment

At age 9 years, all ALSPAC children were invited to a "Focus @ 9" research clinic which was held between January 2001 and January 2003. During this clinic, height and weight were measured using a Harpenden Stadiometer and a Tanita Body Fat Analyser respectively. Children then underwent whole body DXA scanning using a Lunar Prodigy with paediatric scanning software (GE Corporation, Wisconsin, USA). DXA scans with significant movement artefacts were excluded.

3.2.2.2.2 Focus @ 15 skeletal assessment

At approximately 15.5 years of age, all children within the ALSPAC cohort were invited to attend a research clinic. Height and weight were again measured as detailed previously, and whole body DXA scanning was repeated. The children also underwent pQCT assessment of their mid (50% of the tibial length proximal to the growth plate) right tibia using a Stratec XCT2000L instrument (Stratec, Pforzheim, Germany). Cortical BMD (BMD_C) and cortical BMC (BMC_C) were obtained. Periosteal circumference (PC), endosteal circumference (EC) and cortical thickness (CT) were derived using a circular ring model. Cortical bone was defined using a threshold above 650 mg/cm^3. Within subject coefficient of variation (CV) for pQCT measurements are displayed in parentheses: tibial length (4.04%), BMC_C (2.71%), BMD_C (1.29%), PC (1.58%), EC (4.03%). All scans were reviewed and those with artefact were excluded from analysis.

3.2.2.2.3 Focus @ 17 skeletal assessment

pQCT assessment of the tibia was repeated in the cohort at approximately 17.7 years of age using an identical protocol described in section 3.2.2.2.2.

3.2.2.3 **Pubertal assessment**

Questionnaires that addressed maturation were mailed to, and completed by participants at various time points, including within 6 weeks of the "Focus @ 9" clinic DXA scan and at age 13.5 years. The puberty questionnaire, known to participants as the Growing and Changing Questionnaire, could be answered by the child, either parent, a guardian, or any combination of these individuals; the participants recorded who completed the questionnaire. The respondent was asked to examine line drawings representing the five Tanner stages for pubic hair and to record which drawing most closely represented the child's current stage of development (Figure 3.7).

Full ethics and NHS Research and Development approval for was granted for the various ALSPAC studies by the ALSPAC Law and Ethics committee and the South West-Central Bristol Ethics committee

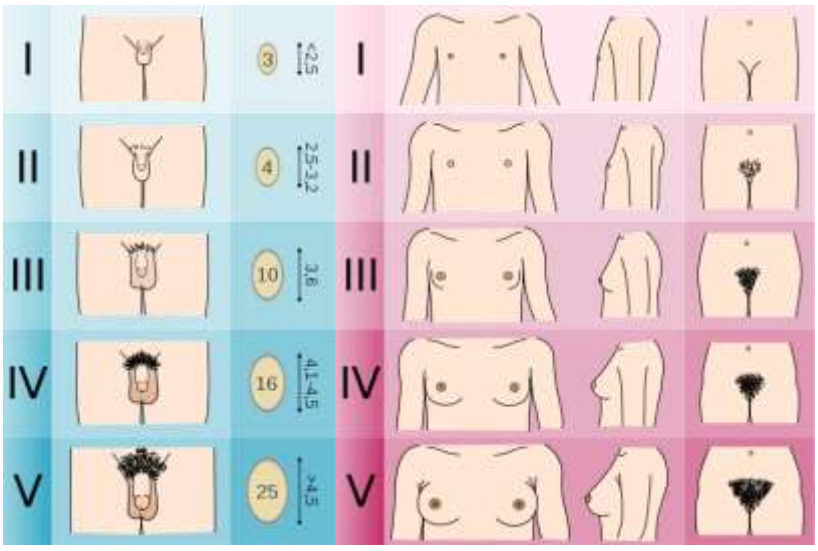


Figure 3.7: Illustration of the Tanner scales for males and females

3.3 Analysis

All data from questionnaires were anonymised, coded and double-punched onto a computer. Parental and offspring DXA results were transferred to secure servers at the MRC Lifecourse Epidemiology Unit using encrypted software. DXA and pQCT scans were analysed by trained technicians using automated software. All scans were reviewed for movement and other artefacts; those with significant artefact excluded from analysis. Childhood and parental data collected at various time points were amalgamated.

All data were analysed using Stata SE Version 14.2 (StataCorp, Texas, USA). A p-value of <0.05 was considered statistically significant. Data were checked for normality using visual inspection. Comparisons between groups were performed using t-test (for continuous parametric variables), Wilcoxon signed-rank test (for continuous non-parametric variables) and McNemar's test (for categorical variables). Correlations were assessed using Pearson's correlation coefficient and linear and multivariate regression. Differences in the magnitude of regression coefficients were compared using the Hausman test. Sex interactions were examined between parent and offspring using linear regression and a sex interaction term.

Further details of statistical methods relevant to individual analyses performed are provided in each of the results chapters.

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3.4 Role of candidate

All hypotheses and analysis ideas include in this study are my own. Both SWS and ALSPAC cohorts were established before I started my research at the MRC Lifecourse Epidemiology Unit, Southampton; the maternal and children's data collection in ALSPAC had been completed as had the SWS maternal pre-pregnancy and pregnancy data. SWS offspring data up to, and including the 6-year visit had also been collected.

My role in the SWS 8 year study included the design and writing of protocols for the parental visit, the writing of parent invitation letters and information sheets and obtaining regional ethics approval and local research and development approval for the parental component of the study. In addition, I was responsible for contacting all parents who volunteered and organising a convenient research clinic appointment. I have attended the vast majority of these clinics, where I obtained consent, administered questionnaires and measured grip strength. DXA and pQCT measurements were performed by trained technicians. I explained the DXA results to the parent and gave them a copy of their scan. When necessary, I organised specialist referral for onward investigation.

I reviewed all the DXA and pQCT data from the 8 year child and parent visit, looking for outliers, movement artefact and foreign bodies. All statistical analyses were performed by me using STATA V14.2, with supervision by a trained statistician.

The interpretation of the data in this thesis is all my own work. The two journal papers that have been published including the results included in this thesis were primarily written by me, with additional comments on the drafts from my supervisors and co-authors.

4. The relationship between placental size and offspring bone mass at birth: Southampton Women's survey findings

4.1 Background and aims

Maternal factors such as smoking, body build, physical activity, diet and circulating 25(OH)D status during pregnancy have been associated with offspring bone mineral accrual (6;164). During the period of a normal human pregnancy the fetus accumulates approximately 30g of calcium (38). This fetal demand is met through placental calcium transport, which results in a higher calcium concentration in fetal than maternal blood (42). It has been demonstrated that the expression of a placental calcium transporter (PMCA3) gene predicts neonatal bone mineral content (52), but it remains unclear whether the maternal influences described act on fetal bone development via placental size or function.

The aim of this analysis was to investigate the relationships between placental dimensions and offspring body composition, bone size and density; and investigate the maternal determinants of placental size, using an observational cohort study

4.2 Methods

This analysis used observational data collected in the SWS, including maternal assessment of lifestyle and anthropometry in early (11 weeks gestation) and late pregnancy (34 weeks gestation), placental assessment using high-resolution ultrasound at 19 weeks gestation, and assessment of neonatal bone mass and body composition within 2 weeks of birth, using DXA. The methodology is described in detail in sections 3.1.2 and 3.1.3.

4.3 Statistical analysis

Gestational age was determined using an algorithm combining last menstrual period and early ultrasound data. All variables were checked for normality. Neonatal total fat mass, percent lean mass and percent fat mass were not normally distributed and were transformed using a Fisher-Yates transformation (214). This is an alternative approach to log transformation and maps ranked data to corresponding normal scores with mean o and SD 1. The new variables are thus "forced" to be normally distributed. An advantage of this method is that there is no interpretation on the original scale of measurement; instead the new variable is in SD units. Unpaired t-tests were used to compare unstandardized neonatal characteristics by sex. A Mann-Whitney U test was used if assumptions of normality were not met.

Pearson correlation and linear regression were used to relate placental measurements to neonatal body composition and bone size and density. Multivariate linear regression was then used to explore whether previously identified maternal determinants of neonatal bone mass might be mediated via placental measurements. Bone outcomes used included whole body BA, BMC and aBMD. To adjust for body size, size-corrected BMC [BMC adjusted for BA, and the baby's length and weight (scBMC)] was used. DXA measurements were associated with the square of offspring age at the scan, consistent with the known tendency of infants to transiently lose weight over the first week of postnatal life. Thus, all neonatal outcomes were adjusted for gestational age, sex and the square of the age at DXA; birthweight was adjusted for gestational age. Placental measures were also adjusted for gestational age at which the measurement was taken using the method of Royston and subsequently standardized to z-scores, with a mean of 0 and SD of 1 (215).

4.4 Results

4.4.1 Maternal characteristics

914 mother-infant pairs had complete 19 week ultrasound and neonatal DXA data, and delivered after 37 weeks gestation. Baseline characteristics of the women are shown in Table 4.1. The median (IQR) age of the mothers at the birth of their babies was 31.1 (28.0-33.8) years. Their mean (SD) height was

163.4 (6.3) cm and median (IQR) BMI pre-pregnancy was 24.2 (22.0-27.5) kg/m^2.

Compared with mothers of children born to the SWS cohort during the same time frame but who did not have placental measurements at 19 weeks or a neonatal DXA scan, mothers in this study tended to be more highly educated (24.5% versus 21.2% achieving a higher degree, p=0.10) and were less likely to smoke in pregnancy, although neither achieved statistical significance (17.6% vs. 21.7%, p=0.06). There were no differences in maternal age at child's birth, maternal height, BMI or smoking before pregnancy between the two groups.

Table 4.1: Characteristics of the mothers

Maternal Characteristics n= 914	
Age at child's birth (yr)	31.1 (28.0-33.8)
Height (cm)	163.4 (6.3)
BMI pre-pregnancy (kg/m^2)	24.2 (22.0-27.5)
Triceps skin fold at 34 weeks (mm)	20.6 (16.7-25.6)
Parity	
0	480 (52.5%)
1 or more	434 (47.5%)
Smoking before pregnancy	
No	665 (72.8%)
Yes	249 (27.2%)
Smoking at 34 weeks	
No	760 (86.6%)
Yes	118 (13.4%)
Walking speed at 34 weeks	
Very slow	139 (16.2%)
Stroll	433 (50.5%)
Normal speed	230 (26.5%)
Fairly brisk	52 (6.1%)
Fast	3 (0.4%)
Serum 25(OH)D at 34 weeks (nmol/l)	63.9 (44.0-87.0)
Placental circumference[1] at 19 weeks (cm)	29.5 (27.2-32.2)
Placental circumference[2] at 19 weeks (cm)	29.2 (26.7-38.9)
Placental length of attachment[1] at 19 weeks (cm)	15.6 (14.1-17.4)
Placental length of attachment[2] at 19 weeks (cm)	15.6 (14.0-22.4
Placental cross-sectional area[1] at 19 weeks (cm^2)	24.4 (21.0-28.5)
Placental cross-sectional area[2] at 19 weeks (cm^2)	24.3 (20.6-28.9)
Placental volume at 19 weeks (cm^3)	230.1 (192.7-277.9)

Data are mean (SD), median (IQR) or number (%)

[1] Measured along the longest edge of attachment to the uterine wall (length)

[2] Placenta measured perpendicular to the longest edge of attachment to the uterine wall (breadth)

4.4.2 Offspring characteristics

The baseline characteristics of the 914 (474 male) neonates are shown in Table 4.2. The boys tended to be heavier at birth (p=0.002), with significantly higher BA, BMC and aBMD (including head; all p <0.001). All outcome measures were therefore adjusted for infant's sex.

Table 4.2: Characteristics of the neonates

	Boys n=474	Girls n=440	P value
Birth weight (kg)	3.59 (0.5)	3.49 (0.5)	0.002
Gestational age (weeks)	40.1 (1.2)	40.3 (1.2)	0.01
Gestational age at time of scan (weeks)	19.6 (0.6)	19.6 (0.5)	0.7
Birth crown-heel length (cm)	50.5 (1.9)	49.7 (1.9)	<0.001
Age at DXA (days)	6.4 (2-11)	6.5 (2-12)	0.69
Whole body bone area (cm^2)	121.4 (25.3)	118.0 (24.9)	0.001
Whole body BMC (g)	65.0 (15.6)	61.3 (15.1)	<0.001
Whole body aBMD (g/cm^2)	0.5 (0.03)	0.5 (0.3)	<0.001
Size corrected BMC (g)	62.4 (2.9)	61.8 (2.9)	0.003
Total lean mass (g)	3026.6 (358.8)	2884.9 (323.7)	<0.001
Total fat mass (g)	507.7 (382.4-655.6)	533.3 (403.5-694.6)	0.01
%lean mass (%)	84.2 (81.2-87.3)	83.0 (79.6-85.6)	<0.001
% fat mass (%)	13.9 (11-16.9)	15.3 (12.7-18.5)	<0.001

Data are mean (SD) or median (IQR)

4.4.3 Placental ultrasound measurements and neonatal body composition

Table 4.3 summarises the relationships between placental measurements and offspring body composition and bone size and density.

Table 4.3: Relationship between placental size and neonatal bone indices and body composition

	BA (z)	BMC (z)	aBMD (z)	scBMC (z)	Total lean (z)	Total fat (z)	%lean (z)	%fat (z)
	r	r	r	r	r	r	r	r
Placental measurements at 19 weeks								
Circumference[1](z)	0.15***	0.13***	0.04	-0.03	0.14***	0.12***	-0.10***	0.09**
Length of attachment[1] (z)	0.12***	0.11***	0.04	-0.03	0.11**	0.09**	-0.08*	0.07*
Cross sectional area[1] (z)	0.18***	0.17***	0.05	-0.01	0.18***	0.18***	-0.15***	0.15***
Circumference[2] (z)	0.20***	0.19***	0.11**	0.006	0.16***	0.16***	-0.14***	0.14***
Length of attachement[2] (z)	0.16***	0.16***	0.09**	0.008	0.10**	0.13***	-0.13***	0.13***
Cross sectional area[2] (z)	0.22***	0.21***	0.11***	0.02	0.18***	0.19***	-0.18***	0.17***
Volume (z)	0.26***	0.25***	0.11**	-0.001	0.23***	0.23***	-0.20***	0.19***

Table shows Pearson's correlation coefficients (r); scBMC = size corrected BMC
[1]measured along the longest edge of attachment to the uterine wall (length)
[2]measured perpendicular to the longest edge of attachment to the uterine wall (breadth)
*p <0.05 **p<0.01 ***p<0.001

Strong positive relationships between each of placental section perimeter, length of attachment to the uterine wall and cross-sectional area at 19 weeks and neonatal BA and BMC were observed (all $p<0.001$). However, there was some disparity in the relationship between neonatal aBMD and these placental measurements depending on the plane of placental measurement; a positive association was seen between placental measurements and aBMD when the placenta was measured along its breadth (p all<0.01), but no significant association was seen when the placenta measured along its longest axis (length).

Placental volume correlated positively with neonatal BA, BMC and aBMD (p all <0.01). Thus, for every 1 SD increase in placental volume, BA increased by 6.2cm^2, BMC increased by 3.6g and aBMD increased by 0.0029g/cm^2 (Figure 4.1). No significant association was observed between placental size and neonatal size-corrected BMC (all P>0.36).

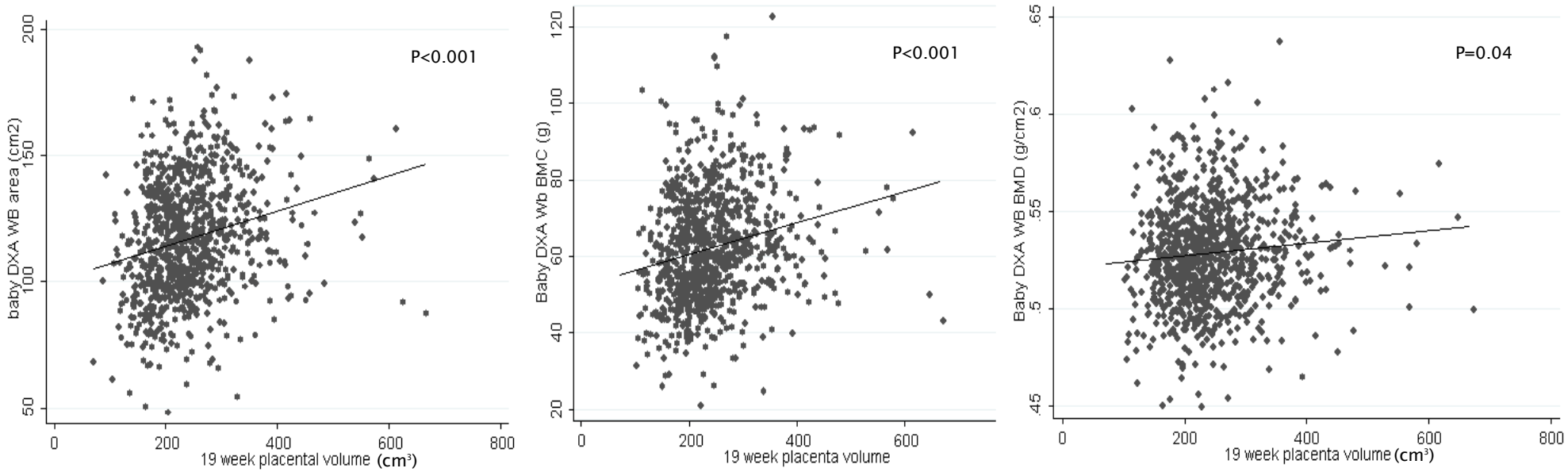


Figure 4.1: Scatterplots illustrating the relationship between placental volume at 19 weeks and neonatal bone indices

Adjusted variables for scatterplots were created using linear regression models and deriving predicted values
Placental volume adjusted for gestational age
BA, BMC, BMD adjusted for sex, gestational age and square of age at scan
WB: whole body

Placental volume at 19 weeks was positively associated with neonatal total lean mass (r=0.23, p<0.0001) and fat mass (r=0.23, p<0.0001). There was a different pattern with proportionate body composition. Thus, placental volume was positively related to percent fat (r=0.19, p<0.0001) but negatively to percent lean (r=-0.20, p<0.0001), indicating that as placental volume increased, total neonatal size increased, but with an increase in percentage fat and a reduction in percentage lean within the overall size envelope (Figure 4.2).

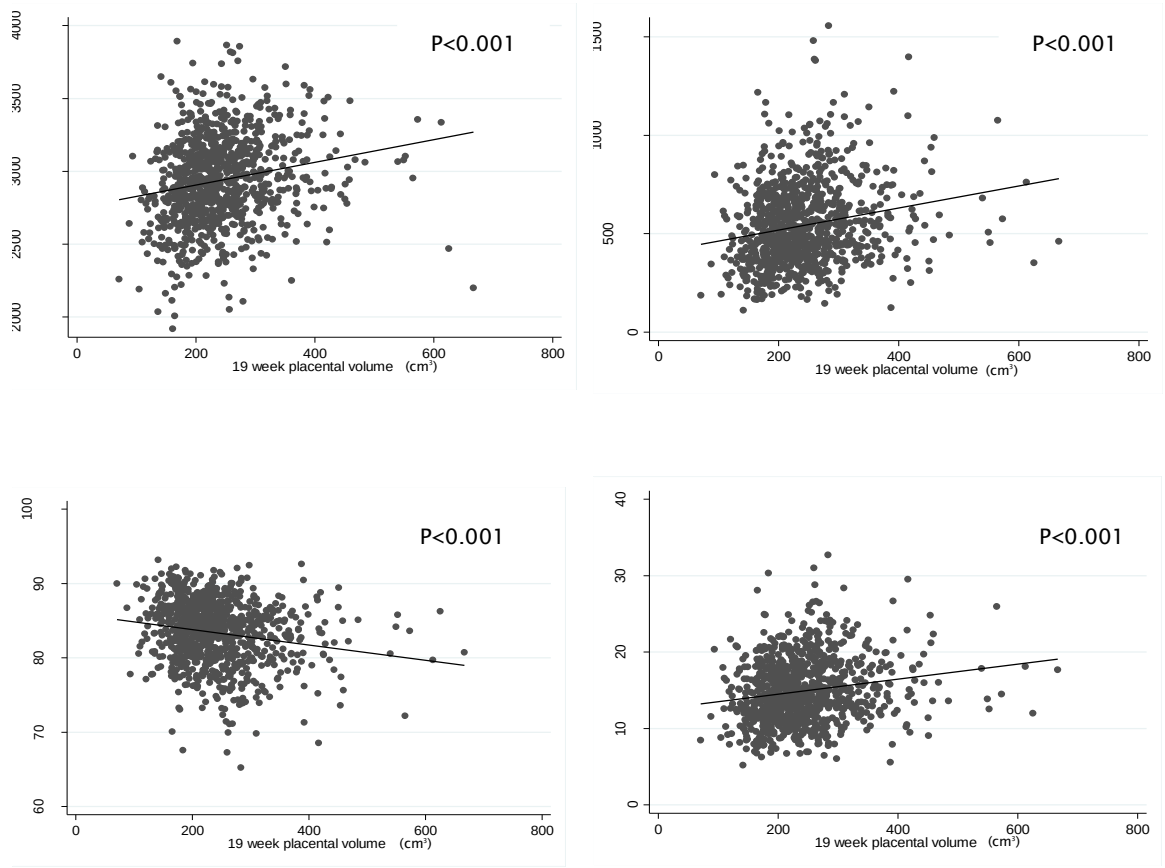


Figure 4.2: Scatterplots illustrating the relationship between placental volume at19 weeks and neonatal body composition

Adjusted variables for scatterplots were created using linear regression models and deriving predicted values

Placental volume adjusted for gestational age; Lean mass and fat mass adjusted for sex, gestational age and square of age at scan

4.4.4 Relationships after adjustment for maternal factors

All associations remained after adjustment for maternal factors previously shown to affect neonatal bone mineral accrual (parity, smoking, walking speed, maternal serum 25(OH) vitamin D and maternal triceps skinfold thickness in pregnancy). In addition, the relationship between placental size and offspring bone mass was adjusted for maternal height, as maternal body build tends to be collinear with placental size, and although this attenuated some results, the relationships remained statistically significant (Table 4.4).

Relationships between placental and DXA measurements were similar in boys and girls, with all placental measurement /sex interactions on bone outcomes failing to achieve statistical significance ($p>0.05$).

4.4.5 Placental "efficiency"

The ratio of placental volume: birth weight was calculated as a marker of placental "efficiency". This was positively associated with neonatal BA and BMC ($p<0.01$), however after adjustment for maternal factors known to affect neonatal bone mineral accrual, significant associations were no longer seen.

Table 4.4: Relationship between placental size and neonatal bone and body composition, adjusting for potentially confounding maternal influences

	BA (z)	**BMC (z)**	**aBMD (z)**	**scBMC (z)**	**Total lean (g)**	**Total fat (z)**	**%lean (z)**	**%fat (z)**
	r	r	r	r	r	r	r	r
Circumference[1](z)	0.15 ***	0.13 ***	0.03	-0.05	0.14 ***	0.14 ***	-0.12 ***	0.12 **
Length of attachment[1] (z)	0.15 ***	0.13 ***	0.04	-0.04	0.11 **	0.12 **	-0.10**	0.09**
Cross sectional area[1] (z)	0.15 ***	0.14 ***	0.03	-0.01	0.15 ***	0.16 ***	-0.14 ***	0.14 ***
Circumference[2] (z)	0.22 ***	0.22 ***	0.12***	0.02	0.19 ***	0.20 ***	-0.17***	0.17 ***
Length of attachement[2] (z)	0.20 ***	0.20 ***	0.12***	0.03	0.16 ***	0.18 ***	-0.16 ***	0.16 ***
Cross sectional area[2] (z)	0.18 ***	0.18 ***	0.10 **	0.04	0.18 ***	0.16 ***	-0.15 ***	0.14 ***
Volume (z)	0.24 ***	0.23 ***	0.10 **	0.003	0.22 ***	0.23 ***	-0.20 ***	0.19 ***

Table shows Pearson's correlation coefficients (r) from multiple regression analyses taking account of maternal height, smoking in late pregnancy, walking speed in late pregnancy, triceps skinfold thickness in late pregnancy and serum 25(OH)D in late pregnancy as confounders.
scBMC = size corrected BMC
[1]measured along the longest edge of attachment to the uterine wall (length)
[2]measured perpendicular to the longest edge of attachment to the uterine wall (breadth)
*P <0.05 **P<0.01 ***P<0.001

4.4.6 Parental characteristics and placental size

Several maternal factors were positively correlated with placental volume at 19 weeks (Table 4.5). Placental volume was positively associated with maternal height, body fat pre-pregnancy and age at child's birth. Smoking, 25(OH)D status, parity, social class and walking speed pre-pregnancy were not statistically significantly associated with placental volume. There was no association between paternal height and placental volume.

Table 4.5: Relationship between maternal characteristics and placental volume

	Placental Volume adjusted for gestation (z)			
	β (95% CI)	P	Mutually adjusted β (95% CI)	P
Age (SD)	**0.07 (0.008-0.13)**	**0.03**	0.01 (-0.008-0.12)	0.09
Height (SD)	**0.09 (0.02-0.15)**	**0.01**	**0.08 (0.01-0.14)**	**0.02**
Parity, 2 groups	0.12 (-0.01-0.24)	0.07	0.10 (-0.03-0.23)	0.14
Walking speed pre-pregnancy (5 groups)	-0.003 (-0.09-0.08)	0.95	-0.03 (-0.12-0.06)	0.50
Body fat pre-pregnancy (SD)	**0.11 (0.05-0.17)**	**0.001**	**0.09 (0.03-0.16)**	**0.004**
Smoking in pregnancy (Yes/No)	0.11 (-0.07-0.29)	0.23	0.12 (-0.06-0.30)	0.19
Social class (6 groups)	0.01 (-0.02-0.05)	0.45	0.03 (-0.03-0.09)	0.29

Table shows regression coefficient (95% confidence interval) and mutually adjusted regression coefficient from univariate and multiple regression analyses respectively. Bold text indicates statistical significance ($p<0.05$)
Parity: 2 groups- nulliparous (reference), multiparous; Smoking reference value: "No"
Walking speed: 5 groups- very slow, easy paced stroll, normal speed, fairly brisk, fast
Social class: 6 groups- unskilled (v), partly skilled (IV), skilled manual (IIIM), skilled non-manual (IIIN), management and technical (II), professional (I)

4.5 Summary of findings

Placental volume at 19 weeks gestation was positively associated with maternal height and body fat, and neonatal bone size and mineral content. There was no significant association between placental volume and paternal height. Neonatal relationships appeared independent of those maternal factors known to be associated with neonatal bone mass. This is consistent with the notion that such maternal environmental influences might act through modulation of aspects of placental function, e.g. utero-placental blood flow or maternal nutrient concentrations, rather than placental size itself. Low placental volume in pregnancy may be a marker of a reduced postnatal skeletal size and increased risk of later fracture.

5. Differential relationships between placental size and postnatal bone size and density: ALSPAC findings

5.1 Background and aims

Birthweight is positively associated with BMC in adulthood (216), and is determined by placental transfer of nutrition from mother to fetus during pregnancy (217). Analysis of the data from the SWS cohort demonstrated that placental volume, measured by high resolution ultrasound in mid-pregnancy, was positively associated with neonatal bone size and content measured by DXA (Chapter 4). It remains unclear however, whether these associations might persist into later childhood and whether placental size may have differential relationships with offspring bone size and volumetric density.

This analysis had two main aims:

1) To investigate whether the positive relationship between placental size and bone size persists into later childhood and adolescence.

2) To investigate whether placental size has differential effects on offspring bone size and volumetric bone density.

5.2 Methods

This analysis used observational data collected in the ALSPAC cohort. Placental measurements included placental area, thickness, volume and weight. Bone mass was measured at 15.5 years using whole body DXA and tibial pQCT (using the 50% tibial site). Additionally, bone mass had been previously measured by DXA at 9 years and subsequently at 17.7 years of age using both tibial pQCT and DXA. Pubertal assessment was assessed at age 13.5 years via a questionnaire. The methodology is described in detail in section 3.2.

5.3 Statistical analysis

All variables were checked for normality. Sex differences between baseline characteristics were compared using unpaired t-tests and chi-squared tests. Pubertal stage information was missing in 100 individuals and in these cases data were imputed: Individuals who did not have pubertal stage information were assigned a value of 4.5, which was close to the mean value (4.46) and stands midway between the two most commonly observed tanner stages- 4 and 5. A sensitivity analysis was undertaken using the complete case data. Univariate and multivariate linear regression were used to relate placental measurements to offspring DXA and pQCT measurements. For the investigation of associations between placental area or volume and indices of bone size, mineralisation, geometry and density, two regression models were employed to adjust for covariates previously identified as influencing childhood bone: In the first model child's sex, age at gestation and age at scan were included as covariates; in the second model maternal influencers, age at delivery, height and weight, and a further childhood covariate, pubertal stage at 13.5 years, were additionally included. It was hypothesised that child's pubertal stage, and height and weight at DXA or pQCT examination might be on the causal pathway, and so additional models included these variables separately. In a further model child's height and weight at DXA or pQCT examination were included. Child's height was not included in every analysis for the reason that as bones grow, there is an increase in not only length, but width as well. By analysing the data with and without child's height as a covariate, it is possible to assess whether any associations seen are mediated by length or are independent of length (and thus potentially associated with other dimensions of bone size, such as bone width).

Placental measurements and sex interactions were examined, however these provided little evidence of sex differences and we therefore analysed boys and girls together. In line with convention, DXA-derived whole body bone variables were analysed "less head". To enable comparison of effect sizes across relationships, all predictor and outcome variables were standardised to z-scores with a mean of 0 and an SD of 1. Regression coefficients are therefore representative of SD change in outcome per unit SD change in predictor, and may be interpreted as partial correlation coefficients.

5.4 Results

5.4.1 Baseline characteristics

5152 children underwent pQCT at the 15.5 year assessment. Of these, 518 (10%; 230 boys and 288 girls) had complete placental, DXA and pQCT measurements. Table 5.1 shows the offspring, placental and maternal characteristics. Offspring DXA indices at 9.9 and 15.5 years are shown in Table 5.2. Mean (SD) age for boys and girls was 15.3 (0.2) and 15.4 (0.2) years respectively. Mean (SD) maternal age at delivery was 29.3 (4.4) years; 50.8% of women were primiparous. At birth, boys were heavier and longer than girls, however by 9.9 years of age there was no difference in height, weight nor any of the DXA variables between the sexes. At age 15.5 years boys were taller and heavier, and had higher whole body (less head) BA, BMC and BMD (all $p<0.001$) than the girls. Similarly boys had higher cortical area, cortical thickness, cortical content, periosteal circumference and endosteal circumference at the tibial 50% site (p all <0.001); conversely boys had lower cortical density than girls ($p<0.001$). Placental measurements did not differ by offspring sex, but girls were on average at a greater stage of puberty than boys when assessed at 13.5 years.

Table 5.1: Baseline characteristics of mothers, placentas and children

	Women		
	n	Mean (%)	(SD)
Mothers			
Age (years)	518	29.3	(4.40)
Height (cm)	504	164.9	(6.6)
Weight (kg)	492	61.5	(9.9)
Body mass index (BMI; kg/m^2)	490	22.6	(3.4)
Parity			
Primiparous (Parity=0)	257	(50.8)	
Multiparous (Parity≥1)	249	(49.2)	

	Boys			Girls			
	n	Mean/ (%)	(SD)	n	Mean	(SD)	p-value
Child							
Birth weight (g)	228	3540.5	(553.3)	287	3414.4	(446.1)	0.004
Age (years)	230	15.3	(0.2)	288	15.4	(0.3)	0.2
Height (cm)	230	175.0	(8.2)	288	165.0	(5.9)	<0.001
Weight (kg)	230	64.1	(12.0)	288	59.1	(9.9)	<0.001
Gestation (weeks)	230	39.6	(1.6)	288	39.7	(1.4)	0.4
Tanner stage							
Stage 1	17	(11/8)		11	(5.2)		p<0.001
Stage 2	32	(22.4)		21	(9.9)		
Stage 3	42	(29.4)		50	(23.6)		
Stage 4	41	(28.7)		87	(41.0)		
Stage 5	11	(7.7)		43	(20.3)		
Placental measurements							
Area (cm^2)	230	286.1	(59.2)	288	284.8	(53.2)	0.8
Volume (cm^3)	230	793.8	(192.9)	288	797.1	(176.5)	0.8
No. of cotyledons/ cm^3	195	1.7	(0.6)	266	1.8	(0.7)	0.04
Tibial pQCT scan at 15 years (50% site)							
Cortical area (cm^2)	230	331.2	(47.7)	288	276.5	(35.8)	<0.001
Cortical BMD (mg/cm^2)	230	1076.2	(36.3)	288	1126.2	(24.5)	<0.001
Cortical thickness (mm)	230	5.7	(0.7)	288	5.3	(0.6)	<0.001
Cortical content (mg)	230	356.8	(54.6)	288	311.4	(40.4)	<0.001
Periosteal circumference (mm)	230	76.0	(5.2)	288	69.2	(4.4)	<0.001
Endosteal circumference (mm)	230	40.2	(5.0)	288	36.2	(4.8)	<0.001

Table 5.2: DXA indices at 9.9 and 15.5 years

	Boys			Girls			
	n	Mean	(SD)	n	Mean	(SD)	p-value
DXA at 9 years							
Total area (cm^2)	213	1141.5	(156.3)	268	1128.1	(163.8)	0.4
Total BMC (g)	213	900.5	(172.6)	268	880.6	(181.6)	0.2
Total BMD (g/cm^2)	213	0.8	(0.05)	268	0.8	(0.05)	0.06
DXA at 15 years							
Total area (cm^2)	230	2103.7	(276.2)	288	1918.1	(229.4)	<0.001
Total BMC (g)	230	2261.9	(478.5)	288	1945.8	(353.1)	<0.001
Total BMD (g/cm^2)	230	1.1	(0.1)	288	1.0	(0.1)	<0.001

All DXA variables presented "less head"

5.4.2 Placental size and offspring pQCT indices at age 15.5 years

The relationships observed between placental measurements and offspring bone mass are shown in table 5.3. Strong positive relationships between child's cortical area, periosteal circumference and endosteal circumference at age 15.5 years and each of placental area and volume were observed; these relationships remained robust after adjusting for gestational age, age at pQCT and sex (all $p<0.05$; Figure 5.1). Conversely there was a negative association between placental area and cortical BMD [β (95% CI) = -0.1 (-0.19, -0.02); p=0.01]; Figure 5.1). These relationships were attenuated but remained after additional adjustments for maternal age at delivery, maternal parity, height and weight, and also after inclusion of child's pubertal stage at 13.5 years, ($p\leq0.04$, except for placental area and cortical area, p=0.06). Adjustment for child's height and weight at 15.5 years did not materially alter the associations observed.

Table 5.3: Associations between placental characteristics and childhood pQCT measurements at 15.5 years

pQCT at 15.5 years (n=518)	Placental measurement															
	Area (SD)								Volume (SD)							
	B[1] (95% CI)	p	B[2] (95% CI)	p	B[3] (95% CI)	p	B[4]	p	B[1] (95% CI)	p	B[2] (95% CI)	p	B[3] (95% CI)	p	B[4]	p
Cortical area (SD)	0.10 (0.01,0.18)	0.03	0.08 (-0.01,0.17)	0.07	0.08 (-0.01,0.17)	0.06	0.01 (-0.06,0.08)	0.79	0.14 (0.05,0.23)	0.003	0.09 (-0.02,0.19)	0.06	0.10 (0.01,0.19)	0.04	0.06 (-0.02, 0.13)	0.12
Cortical BMD (SD)	-0.11 (-0.20,-0.03)	0.01	-0.14 (-0.22,-0.05)	0.003	-0.13 (-0.22,-0.05)	0.002	-0.16 (-0.24,-0.07)	<0.001	-0.09 (-0.18,0.004)	0.06	-0.10 (-0.20,-0.01)	0.04	-0.09 (-0.18,0.003)	0.06	-0.10 (-0.19,-0.01)	0.03
Cortical thickness (SD)	-0.04 (-0.13,0.04)	0.36	-0.07 (-0.16,0.02)	0.14	-0.07 (-0.15,0.02)	0.14	-0.11 (-0.19,-0.03)	0.01	-0.01 (-0.10,0.09)	0.90	-0.04 (-0.13,0.06)	0.46	-0.03 (-0.12,0.07)	0.55	-0.05 (-0.14,0.03)	0.22
Cortical content (SD)	0.07 (-0.02,0.15)	0.13	0.05 (-0.04,0.13)	0.30	0.05 (-0.04,0.13)	0.27	-0.03 (-0.09,0.04)	0.43	0.11 (0.02,0.20)	0.02	0.06 (-0.03,0.16)	0.18	0.07 (-0.02,0.16)	0.12	0.03 (-0.04,0.10)	0.39
Periost circum (SD)	0.19 (0.10,0.27)	<0.001	0.18 (0.10,0.27)	<0.001	0.18 (0.10,0.27)	<0.001	0.11 (0.04,0.18)	0.002	0.22 (0.13 0.31)	<0.001	0.17 (0.08,0.27)	<0.001	0.18 (0.08,0.27)	<0.001	0.13 (0.06,0.21)	<0.001
Endost circum (SD)	0.21 (0.13,0.30)	<0.001	0.24 (0.15, 0.32)	<0.001	0.24 (0.15,0.32)	<0.001	0.20 (0.12,0.29)	<0.001	0.21 (0.12,0.30)	<0.001	0.19 (0.10,0.29)	<0.001	0.19 (0.10,0.28)	<0.001	0.17 (0.08,0.26)	<0.001

[1] Model 1: Adjusted for child's age at gestation, age at pQCT and sex
[2] Model 2: As model 1 and maternal age at delivery, height, weight and parity
[3] Model 3: As model 2 and child's pubertal stage at 13.5 years
[4] Model 4: As model 2 and child's pubertal stage at 13.5 years, child's height and weight at 15.5 years

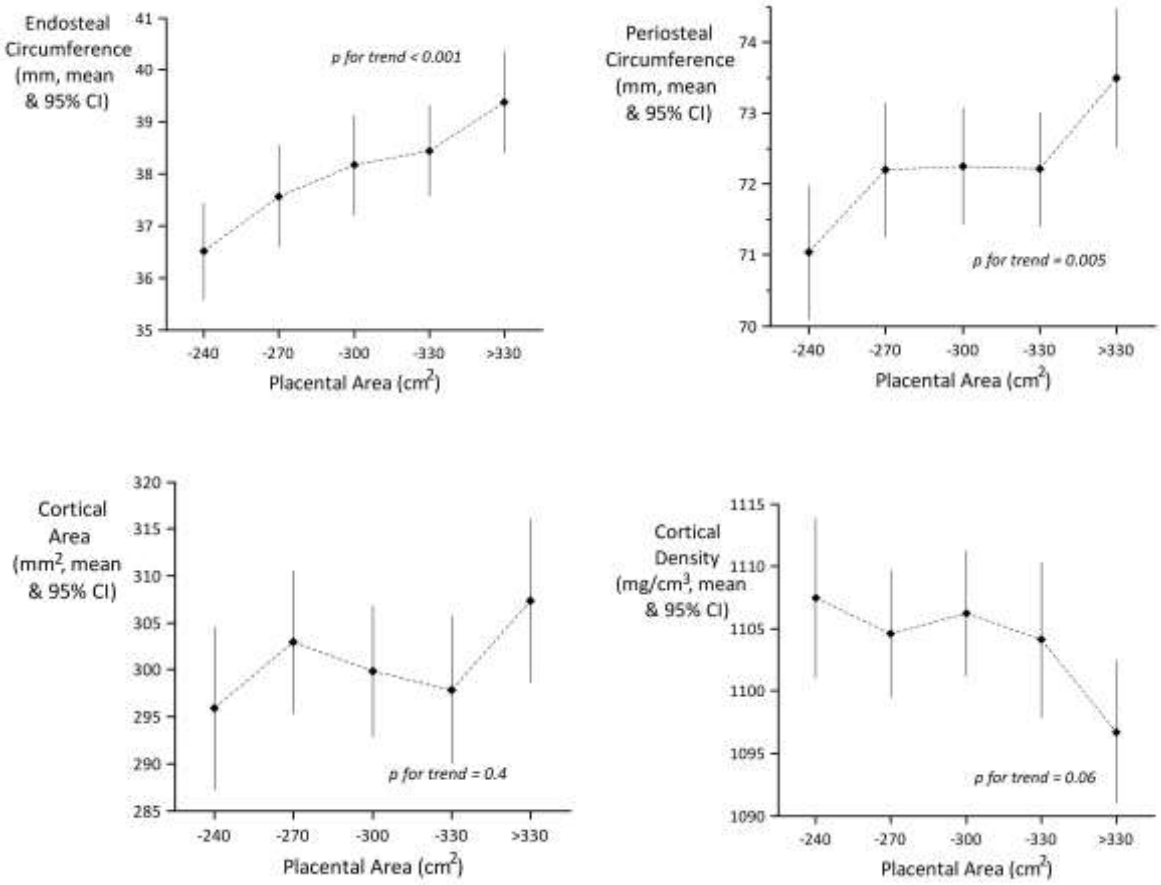


Figure 5.1: Associations between placental characteristics and childhood pQCT measurement at 15.5 years

Adjusted for child age at gestation, child age at pQCT, offspring sex; maternal age at delivery, maternal height, maternal weight, parity and child pubertal stage at 13.5 years

The strongest observed relationships were between placental area and measurements of endosteal and periosteal circumference [EC: β= 0.21 (95%CI: 0.13, 0.30); PC: β= 0.19 (95%CI: 0.10, 0.27)]. Similar relationships were observed between placental volume and child pQCT measurements. There was no association between placental size and cortical thickness; a weak association was observed between placental volume and cortical content, however this relationship was no longer present after maternal and pubertal covariates were incorporated into the regression model. When examined separately by offspring sex, relationships appeared similar in boys and girls, with the p-value for the interaction placental size*sex on pQCT outcomes >0.05.

In a sensitivity analysis using the complete case data, results were not materially different from those using imputed values where pubertal status was missing.

Table 5.4 demonstrates that mean placental area and volume did not differ by pubertal stage at 13.5 years.

Table 5.4: Associations between pubertal stage at 13.5 years and placental measurements (complete case analysis)

	Placental measurement			
Pubertal stage	Area (cm^2)		Volume (cm^3)	
Boys				
(n=143)	mean	SD	mean	SD
1 (n=17	272.6	43.9	764.7	163.6
2 (n=32)	296.0	59.8	855.5	218.2
3 (n=42)	281.4	59.0	757.1	176.8
4 (n=41)	279.4	55.0	767.7	172.2
5 (n=11)	283.1	36.6	758.4	127.9
Test for linear trend (p value)	0.8		0.2	

	Placental measurement			
Pubertal stage	Area (cm^2)		Volume (cm^3)	
Girls				
(n=212)	mean	SD	mean	SD
1 (n=11)	307.8	36.7	799.1	157.8
2 (n=21)	272.9	47.4	771.3	195.3
3 (n=50)	277.3	46.1	813.8	132.7
4 (n=87)	285.4	57.1	782.2	168.1
5 (n=43)	289.3	66.6	807.4	226.3
Test for linear trend (p value)	0.8		0.9	

Table 5.5 similarly summarises the mean pQCT indices (represented as SD scores) by pubertal status at 13.5 years. Here, there was a trend for greater cortical area, thickness, content and density with later pubertal stage, both in boys and girls ($p \leq 0.01$).

Table 5.5: Association between pubertal stage at 13.5 years and pQCT measurements at 15.5 years (complete case analysis)

	pQCT measurement (mean (SD))					
Pubertal stage Boys (n=143)	Cortical area (cm^2)	Cortical BMD (mg/cm^2)	Cortical thickness (mm)	Cortical content (mg)	Periosteal circumference (mm)	Endosteal circumference (mm)
1 (n=17)	305.3 (40.6)	1055.9 (42.3)	5.4 (0.4)	322.2 (44.1)	73.7 (5.5)	40.0 (4.7)
2 (n=32)	331.3 (44.8)	1048.4 (28.9)	5.6 (0.6)	347.4 (48.8)	76.7 (6.2)	41.2 (7.3)
3 (n=42)	328.4 (42.0)	1076.1 (35.3)	5.6 (0.6)	353.5 (47.5)	76.3 (4.9)	41.1 (5.0)
4 (n=41)	339.8 (48.2)	1098.2 (28.1)	5.8 (0.7)	373.3 (53.9)	76.6 (5.0)	40.0 (4.6)
5 (n=11)	345.9 (57.9)	1104.8 (24.6)	6.0 (0.8)	381.5 (59.3)	76.4 (5.8)	38.6 (5.8)
Linear test for trend (p value)	**0.01**	**<0.001**	**0.003**	**<0.001**	0.2	0.4

	pQCT measurement (mean (SD))					
Pubertal stage Girls (n=212)	Cortical area (cm^2)	Cortical BMD (mg/cm^2)	Cortical thickness (mm)	Cortical content (mg)	Periosteal circumference (mm)	Endosteal circumference (mm)
1 (n=11)	265.4 (34.1)	1093.4 (24.5)	5.0 (0.6)	290.2 (38.0)	69.1 (3.2)	37.8 (3.9)
2 (n=21)	261.9 (36.4)	1115.6 (25.7)	5.0 (0.6)	292.2 (40.4)	67.8 (4.4)	36.1 (4.6)
3 (n=50)	276.3 (34.8)	1128.2 (18.8)	5.2 (0.6)	311.8 (40.0)	69.2 (4.0)	36.2 (4.2)
4 (n=87)	283.3 (38.5)	1126.8 (23.4)	5.3 (0.6)	319.2 (43.4)	69.8 (5.0)	36.2 (5.6)
5 (n=43)	282.2 (30.9)	1135.5 (20.0)	5.4 (0.5)	320.0 (33.2)	69.5 (4.1)	35.8 (4.5)
Linear test for trend (p value)	**0.01**	**<0.001**	**0.005**	**0.001**	0.2	0.4

5.4.3 **Placental size and offspring pQCT indices at 17.7 years**

Table 5.6 summarises the relationships observed between placental measurements and offspring bone mass at age 17.7 years. Although the previously observed associations were attenuated, in the adjusted models, positive relationships remained between placental size (area and volume) and endosteal circumference and periosteal circumference (all $p<0.01$). The negative associations between placental size and cortical density remained, but only achieved statistical significance between placental volume and cortical density in the unadjusted model.

5.4.4 **Placental size and offspring DXA measurements of bone mass**

At age 9.9 years, positive relationships were observed between each of placental area and volume, and offspring WB (less head; LH) BA and WB(LH) BMC (Table 5.7). No associations between placental measures and child BMD(LH) were seen. At 15.5 years, similar trends were observed for positive associations between placental area or volume and DXA indices, but these were attenuated and the only significant relationship observed were between WB BA(LH) and placental size (Table 5.7).

Table 5.6: associations between placental characteristics and childhood pQCT measurements at 17.7 years

pQCT at 17.7 years (n=312)	Placental measurement															
	Area (SD)								Volume (SD)							
	B[1] (95% CI)	p	B[2] (95% CI)	p	B[3] (95% CI)	p	B[4]	p	B[1] (95% CI)	p	B[2] (95% CI)	p	B[3] (95% CI)	p	B[4]	p
Cortical area (SD)	0.05 (-0.04, 0.13)	0.27	0.05 (-0.04, 0.13)	0.30	0.05 (-0.04, 0.13)	0.30	-0.01 (-0.09, 0.07)	0.82	0.07 (-0.02, 0.16)	0.11	0.05 (-0.04, 0.14)	0.27	0.05 (-0.04, 0.14)	0.26	-0.003 (-0.08, 0.08)	0.94
Cortical BMD (SD)	-0.06 (-0.14, 0.03)	0.18	-0.06 (-0.15,0.03)	0.18	-0.06 (-0.14, 0.03)	0.17	-0.05 (-0.13, 0.04)	0.26	-0.10 (-0.19, -0.02)	0.02	-0.09 (-0.18, 0.003)	0.06	-0.08 (-0.17, 0.01)	0.08	-0.07 (-0.16, 0.02)	0.14
Cortical thickness (SD)	-0.04 (-0.12, 0.05)	0.39	-0.05 (-0.14, 0.04)	0.27	-0.05 (-0.14, 0.04)	0.27	-0.09 (-0.17,-0.01)	0.04	0.003 (-0.08, 0.09)	0.94	-0.02 (0.11, 0.07)	0.69	-0.02 (-0.11, 0.08)	0.75	-0.05 (-0.14, 0.03)	0.22
Cortical content (SD)	0.04 (-0.05, 0.12)	0.37	0.04 (-0.05, 0.12)	0.43	0.04 (-0.05, 0.12)	0.43	-0.02 (-0.10, 0.06)	0.66	0.05 (-0.03, 0.14)	0.23	0.04 (-0.06, 0.13)	0.44	0.04 (-0.05, 0.13)	0.41	-0.02 (-0.10, 0.07)	0.72
Periosteal circum (SD)	0.14 (0.05, 0.22)	0.002	0.15 (0.06, 0.23)	<0.001	0.14 (0.06, 0.23)	<0.001	0.08 (0.01, 0.15)	0.02	0.15 (0.07, 0.24)	<0.001	0.13 (0.04, 0.22)	0.004	0.13 (0.04, 0.22)	0.004	0.07 (-0.01, 0.14)	0.08
Endosteal circum (SD)	0.17 (0.08, 0.25)	<0.001	0.19 (0.10, 0.27)	<0.001	0.19 (0.10, 0.27)	<0.001	0.15 (0.07,0.24)	<0.001	0.15 (0.06, 0.23)	0.001	0.14 (0.05, 0.23)	0.003	0.13 (0.04, 0.23)	0.004	0.10 (0.01, 0.19)	0.03

[1] Model 1: Adjusted for child's age at gestation, age at pQCT and sex
[2] Model 2: As model 1 and maternal age at delivery, height, weight and parity
[3] Model 3: As model 2 and child's pubertal stage at 13.5 years
[4] Model 4: As model 2 and child's pubertal stage at 13.5 years, child's height and weight at 17.7 years

Table 5.7: Associations between placental characteristics and childhood bone DXA measurements

Placental measure	9 years			15.5 years			17.7 yrs		
	WB BA (SD)	WB BMC (SD)	WB BMD (SD)	WB BA (SD)	WB BMC (SD)	WB BMD (SD)	WB BA (SD)	WB BMC (SD)	WB BMD (SD)
	B (95%CI)	B (95%CI)	B (95%CI)	B (95%CI)	B (95%CI)	B (95%CI)	B (95%CI)	B (95%CI)	B (95%CI)
Area (SD)	0.12** (0.03, 0.2)	0.10* (0.01, 0.18)	0.05 (-0.04, 0.14)	0.09* (0.01, 0.18)	0.07 (-0.01, 0.16)	0.03 (-0.06, 0.11)	0.10 (0.02, 0.18)*	0.08 (-0.001, 0.16)	0.04 (-0.04, 0.12)
Volume (SD)	0.14** (0.05, 0.23)	0.12** (0.03, 0.22)	0.08 (-0.01, 0.17)	0.12** (0.04, 0.21)	0.09 (-0.001, 0.18)	0.02 (-0.07, 0.11)	0.04 (-0.01, 0.08)	0.02 (-0.03, 0.07)	-0.02 (-0.09, 0.05)

WB = Whole body less head; BA= Bone Area; BMC = Bone Mineral Content; BMD = Bone Mineral Density; All associations adjusted for age at gestation, age at DXA and sex

*p<0.05; **p<0.01; ***p<0.001

5.5 Summary of findings

In summary, the previously described observations between placental size and offspring bone size persisted into late childhood. Positive associations between placental size and DXA-derived whole body BA and BMC at 9 years were observed, with weaker associations for BMD. Although the direction of association was maintained, the magnitude of the placenta-bone relationships was much attenuated by the age of 15.5 years, suggesting that pubertal transition might modify these relationships.

Using pQCT to assess children at 15.5 and 17.7 years enabled detailed measurements of bone indices without the effect of overall size that confounds DXA measures. At 15.5 and 17.7 years, tibial periosteal and endosteal circumference were positively associated with placental size, but an inverse association between placental area or volume, and volumetric cortical BMD at the tibia was observed. These findings suggest a disparity between influences on bone size and volumetric density.

The mechanisms which might underlie the observed associations between placental size and offspring bone development are poorly characterised, but may comprise direct effects of the placenta on long term postnatal growth trajectories, shared determinants of placental size and bone indices, or mediation through factors such as age at pubertal onset. These are discussed further in Chapter 8.

6. Parental associations with childhood bone mass at 8 years: DXA findings from the SWS

6.1 Background and aims

Although there is evidence that measures of bone size, mineralisation and density may be partly inherited, there are scant data available from which to elucidate independent influences of mother and father.

The aim of this study was to document the relationships between DXA-derived indices of bone mass in childhood and the corresponding measures in the mother and father, using a prospective cohort, the SWS.

6.2 Methods

This study used observational data collected in the SWS. Children attending the SWS 8 year follow-up completed a lifestyle questionnaire and underwent DXA assessment of their whole body, hip and spine. The child's parents were also invited to attend for DXA assessment where measurements, including BA. BMC and aBMD were made of whole body, lumbar spine and hip bone mass. To reduce the influence of bone size on DXA measurements, the method developed by Prentice et al (201) was used to calculate scBMC . A lifestyle questionnaire was also completed by each parent, facilitated by a member of the research team. Full methodology is described in 3.1.

6.3 Statistical analysis

All data from the questionnaires were anonymised, coded and double-punched onto a computer. The DXA scans were analysed at the visit by a trained DXA technician using automated software. All scans were reviewed for movement and other artefacts; those with significant artefact were excluded from analysis. The data collected from the parent visit was amalgamated with the maternal

pre-, early and late pregnancy data and the childhood data at birth and 8 years of age.

All data were analysed using Stata V13.0 (StataCorp, Texas, USA). All data were checked for normality. Initial statistical analysis utilised tests for comparing means between groups. Maternal and paternal characteristics were compared using a combination of paired t-test (for continuous parametric variables), Wilcoxon signed-rank test (for continuous non-parametric variables) and McNemar's test (for categorical variables). Sex difference between offspring characteristics were compared using unpaired t-test (for continuous parametric variables) and Mann-Whitney rank sum test (for continuous non-parametric variables). Linear regression and Pearson's correlation coefficient were then then used to assess parent-parent relationships and parent-child relationships. Multivariate models (multiple linear regression) were used to assess independent parent-child relationships. Sex interactions were examined between parent and offspring using linear regression and a sex interaction term. The Hausman test was used to compare the magnitude of regression coefficients (218).

DXA values obtained consisted of BA, BMC and BMD for the participant's whole body, lumbar spine and non-dominant hip. In line with convention, all offspring whole body bone variables were expressed less head (LH), and adjusted for gestational age, age at DXA and sex. Parental whole body bone variables were also expressed LH. To adjust for skeletal size, scBMC was calculated by using linear regression to adjust BMC for BA, height and weight.

6.3.1 Power calculation

6.3.1.1 Total BMC

Based on data from a previous study (195), the correlation coefficients (r) between parental and child's total BMC was likely to be approximately r_{mc}=0.36, r_{fc}=0.38 and r_{fm}=0.20 (m - mother; f - father; c - child), where the standard deviations of mother, father and child BMC (g) were SDm=223.45, SDf=319.15 and SDc=104.56. To carry out a power estimation for our study, we generated

random data with the above correlation structure and a sample size n=500. A regression analysis with child BMC as the outcome variable, and maternal and paternal BMC as the predictors was carried out on these randomly generated data. The results suggested that in order to have 90% power to detect an effect size of 0.06g change in child BMC per one gram change in the mother's BMC, also allowing for the father's BMC, using a test at the 5% level of statistical significance we would require n=300 mother-child pairs. Similarly, our study would have 90% power to detect an effect size of 0.04g change in child BMC per one gram change in the father's BMC, also accounting for the mother's BMC.

6.3.1.2 **Total BMD**

Using the same method, we generated random data for the parent and child total BMD, with the following correlation structure: r_{mc}=0.39, r_{fc}=0.33 and r_{fm}=0.09, where the standard deviations of mother, father and child BMD were SDm=0.066, SDf=0.076 and SDc=0.039. We found that our study of n=300 would have 90% power to detect an effect size of 0.075g/cm^2 change in child BMD per one g/cm^2 change in the mother's BMD, also allowing for the father's BMD, using a test at the 5% level of statistical significance. In addition, the results suggested that an effect size of 0.065g/cm^2 change in child BMD per one g/cm^2 change in the father's BMD, also accounting for the mother's BMD, could be detected with 90% power using a test at the 5% level of significance.

6.4 Results

6.4.1 **Recruitment**

1214 children attended for the SWS 8 year follow-up study, 1013 of whom underwent DXA assessment. The mothers of all 1013 children were invited to

attend for DXA along with 982 fathers; the remaining fathers were not invited as it was not possible to obtain contact details. 43% of parents replied to the invitation of which 72% agreed to participate and underwent DXA assessment. After mother, father and child scans were matched, 279 mother-father-child trios were available for analysis. 6 maternal whole body DXA scan results were excluded due to significant artefact; 13 paternal whole body, 15 hip and 18 lumbar spine DXA results were also excluded for the same reason. Figure 6.1 illustrates a consort diagram for parent and child recruitment.

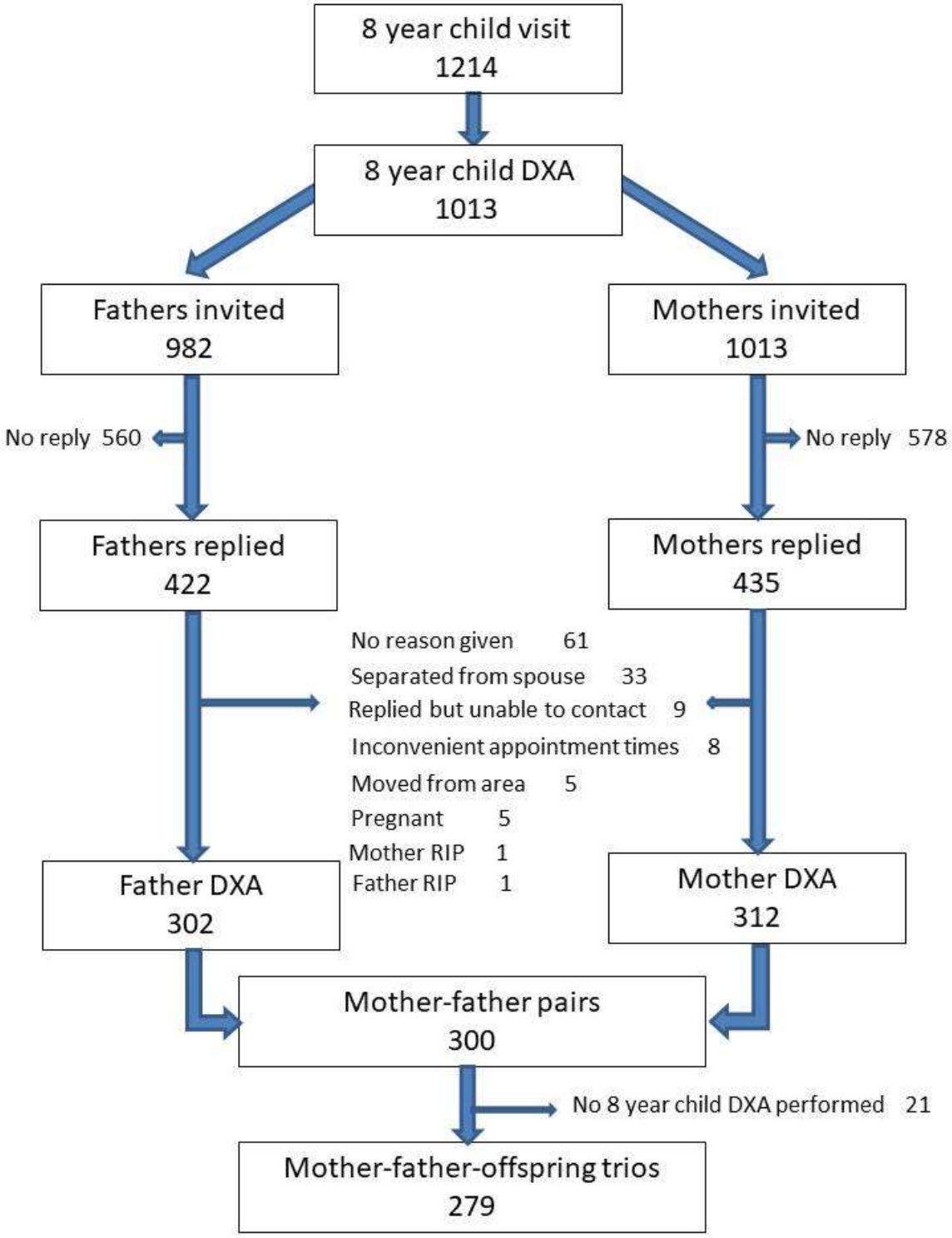


Figure 6.1: Consort diagram for child and parent recruitment

6.4.2 Baseline demographics

6.4.2.1 Offspring demographics

Baseline characteristics of the children are shown in Table 6.1. 51% of the children were male. Mean (SD) age was 9.2 (0.2) years. Girls were significantly taller and heavier than boys, although BMI was similar between the two groups. After adjusting the child's height for the height of parents, a significant difference in height between the sexes was no longer seen. Birthweight was similar between the two groups.

Whole body bone variables were similar between boys and girls, however lumbar spine BA and hip BMD were significantly higher in boys. Conversely, lumbar spine BMD and hip area were significantly higher in girls (all $p<0.05$).

6.4.2.2 Parental demographics

Baseline characteristics of the parents are shown in Tables 6.2 and 6.3. Mean (SD) age of the mothers [41.2 (3.4) years] was significantly lower than that for the fathers [43.8 (5.1) years); $p<0.0001$]. Fathers were also significantly taller, heavier and had higher BMI than the mothers (all $p<0.005$). Although there was no significant difference in current smoking between the two groups ($p=0.4$), fathers were significantly more likely to have smoked regularly in the past ($p=0.007$). Fathers consumed significantly more alcohol ($p<0.0001$) and milk ($p=0.004$) per week, had higher rates of previous fracture ($p<0.001$), and undertook more hours of strenuous per week ($p=0.03$). There was no significant difference in previous steroid exposure ($p=0.24$) or ethnicity ($p=1$). This cohort contained a very low proportion on non-Caucasian participants, with only 8 non-white mothers and 9 non-white fathers.

DXA derived bone outcomes (table 6.3) were all significantly higher in the fathers than the mothers ($p<0.0001$).

Table 6.1: Offspring baseline characteristics

	Overall			**Boys**			**Girls**			**P value**
	n	Mean/ median	SD /IQR	n	mean	SD/ IQR	n	mean	SD/ IQR	
Age (yrs)	279	9.2	0.2	146	9.2	0.2	133	9.2	0.2	0.7
Height (cm)	279	135.6	5.8	146	135.1	5.2	133	136.2	6.4	0.1
Height (z-score)	279	0.3	1.0	146	0.2	0.9	133	0.4	1.0	**0.03**
Height (adj for both parent's height)	262	135.4	4.8	140	135.2	4.6	122	135.6	5.0	0.5
Weight (kg)	279	29.8	(26.7-33.2)	146	29.2	(22-32.5)	133	30.6	(27.9-35.1)	**0.005**
Weight (z-score)	279	0.2	1.0	146	0.1	1.0	133	0.3	1.0	**0.03**
BMI (kg/m^2)	279	16.1	(12.7-23.3)	146	15.8	(14.8-17.4)	133	16.4	(15.2-18.2)	**0.006**
BMI (z-score)	279	-0.03	1.1	146	-0.1	1.1	133	0.1	1.1	0.09
Birthweight (g)	277	3437.1	550.9	144	3468.2	47.0	133	3403.5	535.9	0.3
Bone outcomes										
WB BA less head (cm^2)	272	1107.8	143.7	141	1094.6	131.4	131	1122.1	155.1	0.1
WB BMC less head g)	272	717.3	113.3	141	711.0	109.0	131	724.1	117.8	0.3
WB BMD less head (g/cm^2)	272	0.6	0.1	141	0.6	0.1	131	0.6	0.1	0.6
L spine BA (cm^2)	279	39.2	4.2	146	39.9	4.2	133	38.4	4.1	**0.004**
L spine BMC (g)	279	23.2	3.9	146	23.2	3.9	133	23.3	3.8	1.0
L spine BMD (g/cm^2)	279	0.6	0.1	146	0.5	0.1	133	0.6	0.1	**0.002**
Hip area (cm^2)	279	21.1	2.8	146	20.3	2.7	133	21.5	2.8	**0.004**
Hip BMC (g)	279	14.9	2.7	146	15.0	2.8	133	14.7	2.6	0.3
Hip BMD (g/cm^2)	279	0.7	0.1	146	0.8	0.1	133	0.7	0.1	**<0.0001**

Table 6.2: Baseline parental characteristics

	Mothers (n=279)			Fathers (n=279)			P value
	n	Mean/ median/ %	SD / IQR	n	Mean/ median/ %	SD / IQR	
Age (years)	279	40.9	3.5	279	43.7	5.3	**<0.0001**
Height (cm)	279	164.8	6.2	279	176.9	6.7	**<0.0001**
Weight (kg)	279	68.1	(61.1-79.4)	279	85.5	(76.4-96.2)	**<0.0001**
BMI (kg/m^2)	279	25.2	(22.7-29.3)	279	27.2	(25.1-30.5)	**<0.0001**
Parity	262						
Primiparous	23	8.8%					
Multiparous	239	91.2%					
Current smoking	22/248	8.9%		30/254	11.8%		0.4
Ever smoked regularly	86/262	32.8%		116/265	43.8%		**0.007**
Smoked in EP	19/276	6.9%					
Smoked in LP	17/276	6.3%					
Serum 25(OH)D EP (nmol/l)	219	65.3	26.6				
Serum 25(OH)D LP (nmol/l)	257	71.1	33.4				
Alcohol consumption (units per week)	263	3	(1-7.5)	126	7	(2.2-16)	**<0.0001**
Pre-menopausal	272/279	97.5%					
Previous fracture	101/263	38.4%		157/265	59.2%		**<0.0001**
≥ 0.5Pints of milk/day	153/262	58.4%		192/261	73.6%		**0.0002**
Hours strenuous activity/week	260	0.25	(0-1.5)	262	0.5	(0-2.1)	**0.03**
Previous oral steroid use	11/ 262	4.2%		3/ 265	1.1%		0.24
Ethnicity	263			265			
White	255	97.0%		256	96.6%		1.00
Non-white	8	3.0%		9	3.4%		

EP = Early pregnancy; LP = Late pregnancy

Table 6.3: Baseline parental bone indices

	Mothers			Fathers			P value
	n	Mean	SD	n	Mean	SD	
WB BA less head (cm^2)	273	1731.5	142.6	265	2032.9	168.3	**<0.0001**
WB BMC less head (g)	273	1802.9	247.2	265	2384.2	370.9	**<0.0001**
WB BMD less head (g/cm^2)	273	1.0	0.08	265	1.2	0.1	**<0.0001**
L spine BA (cm^2)	279	60.4	5.5	260	70.0	7.1	**<0.0001**
L spine BMC (g)	279	65.3	11.1	260	73.2	14.0	**<0.0001**
L spine BMD (g/cm^2)	279	1.1	0.1	260	1.0	0.1	**0.001**
Hip BA (cm^2)	279	34.2	2.9	263	45.0	4.4	**<0.0001**
Hip BMC (g)	279	33.9	5.1	263	47.3	8.6	**<0.0001**
Hip BMD (g/cm^2)	279	1.0	0.1	263	1.0	0.1	**<0.0001**

6.4.2.3 Characteristics of the participants compared to non-participating members of the SWS

Differences between the mothers who attended this phase of the SWS (n=279) compared to the rest of the SWS cohort (n=2845) are shown in Table 6.4. Mothers who participated were significantly older, taller and had lower BMI at the early pregnancy visit. Additionally, participating mothers were significantly less likely to have smoked in early or late pregnancy and were of higher social class. Late pregnancy serum 25(OH)D when measured was significantly higher in those mothers who participated in this phase of the SWS compared to the rest of the group. There were no significant differences in maternal ethnicity, triceps skinfold thickness in late pregnancy, walking speed in late pregnancy or offspring birthweight between the two groups.

Table 6.4: Differences between attending and non-attending SWS mothers

	Mothers who attended (n=279)			Mothers who did not attend (n=2845)			P value
	n	Mean/ median/ %	SD / IQR	n	Mean/ median/ %	SD / IQR	
Age EP (years)	253	30.5	3.4	1969	29.9	3.8	**0.02**
Height (cm)	279	164.8	6.2	2830	163.1	6.5	**0.001**
Weight EP (kg)	250	65.3	(59.4-75)	1909	67.2	(59.8-76.7)	0.14
BMI EP (kg/m^2)	250	24.3	(22.0-27.2)	1909	25.1	(22.6-28.6)	**0.003**
Smoked in EP	19/276	6.9%		420/2486	16.9%		**<0.0001**
Smoked in LP	17/276	6.3%		379/2343	16.2%		**<0.0001**
Serum 25(OH)D EP (nmol/l)	219	65.3	26.6	1777	62.0	25.5	0.08
Serum 25(OH)D LP (nmol/l)	257	71.1	33.4	2043	63.4	30.4	**0.0001**
Triceps skinfold thickness LP (mm)	279	21.0	6.8	2279	21.6	6.7	0.18
Walking speed LP	300			2343			0.1
Very slow	35	11.7%		410	17.5%		
Easy paced stroll	166	55.3%		1172	50.0%		
Normal speed	80	26.7%		617	26.3%		
Fairly brisk	19	6.3%		138	5.9%		
Fast	0	0%		6	0.3%		
Ethnicity	263			2844			
White	255	97.0%		2712	95.4%		0.9
Non-white	8	3.0%		132	4.6%		
Social class	308			2753			**0.001**
I	23	7.5		124	4.5		
II	131	42.5		935	34.0		
IIIN	108	35.1		1063	39.0		
IIIM	18	5.8		237	8.6		
IV	22	7.1		336	12.2		
V	6	2.0		58	2.1		
Offspring BW (g)	279	3424.7	31.0	2808	3432.1	10.7	0.83

EP = Early pregnancy; LP = Late pregnancy; BW = Birthweight

6.4.3 **Relationship between maternal and paternal anthropometry and bone mass**

In general, there was little significant correlation observed between maternal bone variables and the corresponding indices in the father, aside from lumbar spine bone area and lumbar spine BMC [r = 0.18 and 0.14 respectively (Table 6.5)]. There was a significant correlation between maternal and paternal height, suggesting that taller mothers had paired with taller fathers (r=0.21; p<0.001). This phenomenon, in which individuals of similar phenotypes pair with each other, is known as assortative mating.

Table 6.5: Relationship between maternal and paternal height and bone variables

	Father variables									
Mother variables	WB BA (cm^2)	WB BMC (g)	WB BMD (g/cm^2)	Spine area (cm^2)	Spine BMC (g)	Spine BMD (g/cm^2)	Hip area (cm^2)	Hip BMC (g)	Hip BMD (g/cm^2)	Height (cm)
WB BA (cm^2)	0.13 (0.03)									
WB BMC (g)		0.07 (0.26)								
WB BMD (g/cm^2)			0.08 (0.21)							
Spine area (cm^2)				**0.18 (0.01)**						
Spine BMC (g)					**0.14 (0.02)**					
Spine BMD (g/cm^2)						0.07 (0.28)				
Hip area (cm^2)							0.08 (0.19)			
Hip BMC (g)								0.09 (0.89)		
Hip BMD (g/cm^2)									-0.01 (0.83)	
Height (cm)										**0.21 (<0.001)**

Numbers are Pearson correlation coefficients (p value); Numbers in bold indicate statistical significance ($p<0.05$)

6.4.4 Relationships between baseline characteristics and bone indices

6.4.4.1 Offspring baseline characteristics - offspring bone indices

Child height, and to a lesser extent, age at DXA had significant positive associations with childhood bone mass (Table 6.6). A much weaker, but still significant positive association was observed for moderate-vigorous physical activity (measured when the child was 6 years of age, using an Actiheart machine) and whole body scBMC, and hip BMD and scBMC.

6.4.4.2 Maternal baseline characteristics: maternal bone indices

The most consistent positive association observed was between maternal height and maternal bone indices ($p<0.03$ across all bone variables; Table 6.7). Significant positive relationships were also seen between maternal triceps skinfold thickness in late pregnancy and maternal whole body and hip bone mass, however this was not robust across all the measured variables, and was not observed at the lumbar spine. No other consistent significant associations were observed between maternal bone parameters and other maternal characteristics, including age, social class, smoking, parity, walking speed in late pregnancy, physical activity and serum 25(OH)D in late pregnancy.

Significant positive correlations were observed between mid-placental volume (measured at 19 weeks) and several maternal bone variables, including maternal whole body BA ($r=0.12$), BMC ($r=0.14$) and BMD ($r=0.12$), and maternal hip BMC ($r=0.13$) (all $p<0.05$).

6.4.4.3 Paternal baseline characteristics: paternal bone indices

Similar to the described maternal relationships, paternal height was strongly positivity associated with paternal bone indices (all $p<0.004$; Table 6.8). Additionally, paternal vigorous activity was significantly positively associated with multiple paternal bone variables (whole body scBMD, all spine variables, and hip BMC, BMD and scBMD, In contrast to the mothers, a significant negative

association was observed between paternal age and several paternal bone variables (whole body bone area and BMC, hip BMD and scBMD).

Table 6.6: Relationships between offspring characteristics and offspring bone indices

	n	WB BA LH (cm^2)	WB BMC LH (g)	WB BMD LH (g/cm^2)	WB sc BMC LH (g)	Spine area (cm^2)	Spine BMC (g)	Spine BMD (g/cm^2)	Spine sc BMC (g)	Hip area (cm^2)	Hip BMC (g)	Hip BMD (g/cm^2)	Hip sc BMC (g)
		β (p value)	β (p value)	β (p value)	β (p value)	β (p value)	β (p value)	β (p value)	β (p value)	β (p value)	β (p value)	β (p value)	β (p value)
Child covariates													
Age (yr)	272	−56.43 (0.17)	40.41 (0.21)	**0.06 (<0.001)**	0.02 (0.08)	**2.54 (0.03)**	**3.06 (0.003)**	0.03 (0.05)	0.001 (0.14)	1.20 (0.11)	**1.46 (0.04)**	003 (0.17)	0.0003 (0.55)
Height (cm)	272	**13.59 (<0.001)**	**14.70 (<0.001)**	**0.01 (<0.001)**	−0.0001 (0.89)	**0.47 (<0.001)**	**0.46 (<0.001**	**0.20 (<0.001)**	0.00001 (0.78)	**0.33 (<0.001)**	**0.31 (<0.001)**	**0.003 (<0.001)**	0.00003 (0.81)
Milk intake (pints per day)	272	14.5 (0.57)	28.3 (0.16)	0.017 (0.08)	**16.72 (0.04)**	0.15 (0.84)	1.00 (0.15)	0.02 (0.05)	0.74 (0.10)	0.20 (0.69)	0.44 (0.36)	0.02 (0.20)	0.18 (0.47)
Mod/vig/v.vig physical activity (hours per day)	155	−0.64 (0.04)	(−0.29) 0.22	0.0001 (0.52)	**0.0003 (0.01)**	−0.01 (0.23)	−0.004 (0.62)	0.0001 (0.65)	0.01 (0.12)	−0.003 (0.58)	0.002 (0.75)	**0.000 (0.01)**	**0.00001 (0.001)**

Numbers are β co-efficients (p value); Numbers in bold indicate statistical significance (p<0.05)
LH= Less head

Table 6.7: Relationships between maternal characteristics and maternal bone indices

	n	WB BA LH (cm^2)	WB BMC LH (g)	WB BMD LH (g/cm^2)	WB scBMC LH (g)	Spine area (cm^2)	Spine BMC (g)	Spine BMD (g/cm^2)	Spine scBMC (g)	Hip area (cm^2)	Hip BMC (g)	Hip BMD (g/cm^2)	Hip scBMC (g)
Maternal covariates		B (p value)	B (p value)	B (p value)	B (p value)	B (p value)	B (p value)	B (p value)	B (p value)	B (p value)	B (p value)	B (p value)	B (p value)
Triceps skinfold thickness (LP; mm))	261	**6.99 (<0.001)**	**0.15 (<0.001)**	0.001 (0.11)	−0.002 (0.05)	0.02 (0.61)	0.11 (0.26)	0.002 (0.15)	−0.0001 (0.41)	0.05 (0.06)	**0.18 (<0.001)**	**0.004 (<0.001)**	−0.00003 (0.31)
Smoking (LP)	268	15.55 (0.68)	28.02 (0.67)	0.005 (0.82)	0.01 (0.79)	0.90 (0.51)	−0.03 (0.99)	−0.02 (0.48)	−0.001 (0.62)	0.76 (0.29)	0.91 (0.49)	0.002 (0.95)	0.001 (0.61)
Current smoking	242	65.85 (0.06)	111.40 (0.06)	0.02 (0.19)	0.01 (0.59	1.47 (0.23)	1.80 (0.47)	0.005 (0.86)	−0.0004 (0.79)	1.55 (0.20)	2.37 (0.20)	0.02 (0.38)	0.001 (0.54)
Walking speed (LP)	261	2.15 (0.85)	6.56 (0.74)	0.003 (0.62)	0.01 (0.52)	0.68 (0.12)	0.29 (0.75)	−0.01 (0.36)	−0.001 (0.29)	0.25 (0.29)	0.31 (0.46)	0.003 (0.76)	0.0004 (0.25)
Current vigorous activity (hours/week)	254	0.10 (0.99)	10.62 (0.27)	**0.01 (0.03)**	**0.01 (0.01)**	0.36 (0.09)	0.48 (0.26)	0.001 (0.79)	0.0002 (0.99)	0.12 (0.27)	0.18 (0.36)	0.002 (0.64)	0.0002 (0.10)
25(OH)D (LP) (nmol/l)	257	0.26 (0.34)	0.71 (0.13)	0.0002 (0.10)	0.0003 (0.14)	0.01 (0.42)	0.02 (0.37)	0.0001 (0.52)	0.00001 (0.51)	0.005 (0.33)	0.01 (0.20)	0.0002 (0.34)	0.00001 (0.10)
Parity	273	−11.69 (0.50)	−16.96 (0.57)	−0.002 (0.83)	−0.01 (0.72)	−0.36 (0.59)	−0.34 (0.80)	−0.0002 (0.99)	−0.00002 (0.98)	0.23 (0.50)	0.25 (0.69)	0.001 (0.97)	−0.0002 (0.69)
Social class	274	−2.95 (0.72)	−1.92 (0.89)	0.001 (0.75)	0.002 (0.77)	−0.16 (0.62)	−0.39 (0.54)	−0.002 (0.73)	−0.0004 (0.31)	0.02 (0.89)	0.18 (0.53)	0.005 (0.51)	−0.0001 (0.66)
Age at DXA (yr)	273	−0.29 (0.91)	−0.45 (0.99)	0.00003 (0.98)	0.00002 (0.99)	0.05 (0.63)	−0.01 (0.98)	−0.001 (0.52)	−0.0001 (0.65)	0.07 (0.19)	0.30 (0.74)	−0.001 (0.56)	−0.00001 (0.95)
Height (cm)	273	**17.55 (<0.001)**	**24.75 (<0.001)**	**0.004 (0.002)**	0.0002 (0.88)	**0.52 (<0.001)**	**0.78 (<0.001)**	**0.004 (0.003)**	0.00002 (0.82)	**0.29 (<0.001)**	**0.33 (<0.001**	**0.002 (0.002)**	0.00001 (0.73)

Numbers are β co-efficients (p value); Numbers in bold indicate statistic significance (p<0.05)
LP = Late pregnancy; LH = Less head
Parity: 2 groups- nulliparous (reference), multiparous; Smoking: 2 groups- No (reference value), yes
Walking speed: 5 groups- very slow, easy paced stroll, normal speed, fairly brisk, fast
Social class: 6 groups- unskilled (v), partly skilled (IV), skilled manual (IIIM), skilled non-manual (IIIN), management and technical (II), professional (I)

Table 6.8: Relationships between paternal characteristics and paternal bone mass

	n	WB BA LH (cm^2)	WB BMC LH (g)	WB BMD LH (g/cm^2)	WB scBMC LH (g)	Spine area (cm^2)	Spine BMC (g)	Spine BMD (g/cm^2)	Spine scBMC (g)	Hip area (cm^2)	Hip BMC (g)	Hip BMD (g/cm^2)	Hip scBMC (g)
Paternal covariates		B (p value)	B (p value)	B (p value)	B (p value)	B (p value)	B (p value)	B (p value)	B (p value)	B (p value)	B (p value)	B (p value)	B (p value)
Current vigorous activity (hrs/ week)	248	1.13 (0.84)	12.2 (0.31)	0.01 (0.08)	**0.01 (0.02)**	**0.45 (0.04)**	**1.16 (0.01)**	**0.01 (0.01)**	**0.001 (0.04)**	0.07 (0.62)	**0.45 (0.08)**	**0.01 (0.03)**	**0.001 (0.001)**
Current Smoking	240	−18.58 (0.58)	−53.78 (0.47)	−0.02 (0.48)	−0.02 (0.57)	−0.80 (0.59)	−3.50 (0.23)	−0.04 (0.20)	−0.001 (0.54)	−0.15 (0.87)	−3.14 (0.07)	−0.06 (0.02)	−0.001 (0.20)
Social class	217	2.86 (0.74)	6.59 (0.73)	0.002 (0.68)	−0.002 (0.84)	0.05 (0.87)	−0.27 (0.70)	−0.004 (0.60)	−0.0005 (0.33)	−0.38 (0.10)	−0.53 (0.24)	−0.002 (0.81)	−0.0002 (0.45)
Age at DXA (yr)	256	**−4.42 (0.03)**	**−9.98 (0.02)**	−0.002 (0.06)	−0.002 (0.29)	−0.14 (0.11)	−0.29 (0.09)	−0.002 (0.15)	−0.0001 (0.61)	0.09 (0.07)	−0.12 (0.24)	**−0.005 (0.004)**	**−0.004 (0.004)**
Height (cm)	265	**20.03 (<0.001)**	**34.40 (<0.001)**	**0.005 (<0.001)**	0.001 (0.52)	**0.61 (<0.001)**	**0.91 (<0.001)**	**0.004 (0.003)**	0.00002 (0.85)	**0.37 (<0.001)**	**0.61 (<0.001**	**0.004 (0.001)**	0.00001 (0.99)

Numbers are β co-efficients (p value); Numbers in bold indicate statistical significance ($p<0.05$)

LH = Less head
Social class: 6 groups- unskilled (v), partly skilled (IV), skilled manual (IIIM), skilled non-manual (IIIN), management and technical (II), professional (I)

6.4.5 Parental - offspring associations

Table 6.9 shows the relationships between parental non-bone baseline characteristics and offspring bone mass. The only measured parental demographic that had robust relationships with childhood bone mass was parental height, which had strong positive associations for both mother and father. Relationships with other measured parental variables, such as age, social class, smoking, physical activity and maternal 25(OH)D in late pregnancy did not achieve statistical significance.

Whilst positive relationships were observed between mid-pregnancy placental volume and offspring bone mass, these were not found to be significant. When the analysis, however, was expanded to include all participating SWS mothers and children with a placental volume measurement and child DXA measurement at 9 years (n=996), significant positive relationships remained between placental volume at 19 weeks and child spine and hip bone area [β (95% CI)=0.004 (0.001-0.01), p = 0.01; 0.002 (0.001-0.004), p = 0.04].

6.4.5.1 Maternal - offspring bone mass associations

Strong positive associations were observed between maternal whole body (LH), hip and lumbar spine bone variables (BA, BMC and BMD), and the corresponding indices in the offspring (all p<0.001). This is illustrated in Table 6.10 and Figures 6.2-6.4. In each case, as the predictor and outcome variables are in the same units, the regression coefficients can be considered dimensionless, enabling effect sizes to be compared across associations. The strongest relationships observed were between maternal-offspring BA, with the relationship between maternal-offspring hip BA being strongest of all (β=0.39; p<0.0001). Additional, strong positive relationships were observed between maternal and offspring whole body and lumbar spine bone area respectively (β=0.26-0.35). Maternal BMC and BMD were less strongly associated with the corresponding offspring variables (β=0.17-0.25), but still significant with p values all <0.01. In general, maternal-offspring BMD was more strongly associated than BMC. Maternal-offspring bone relationships were attenuated after adjustment for size, either by calculating scBMC (Figure 6.5) or adjusting

for both maternal and offspring height (Figures 6.6, 6.7), however significant bone associations remained.

Table 6.9: Relationship between offspring bone outcomes and parental non-bone characteristics

	n 6.4.5.2	WB BA LH (cm^2)	WB BMC LH (g)	WB BMD LH (g/cm^2)	WB sc BMC LH (g)	Spine area (cm^2)	Spine BMC (g)	Spine BMD (g/cm^2)	Spine sc BMC (g)	Hip area (cm^2)	Hip BMC (g)	Hip BMD (g/cm^2)	Hip sc BMC (g)
Maternal factors		B (p value)	B (p value)	B (p value)	B (p value)	B (p value)	B (p value)	B (p value)	B (p value)	B (p value)	B (p value)	B (p value)	B (p value)
Triceps skinfold thickness (LP; mm)	261	1.02 (0.43)	0.44 (0.66)	0.00002 (0.96)	−0.80 (0.06)	0.01 (0.79)	−0.01 (0.77)	−0.0003 (0.60)	−0.39 (0.08)	−0.001 (0.96)	−0.01 (0.67)	−0.0004 (0.54)	−0.01 (0.29)
Smoking (LP)	268	18.73 (0.62)	23.32 (0.33)	0.012 (0.39)	23.83 (0.05)	0.22 (0.83)	0.77 (0.43)	0.01 (0.43)	0.78 (0.23)	0.68 (0.32)	0.67 (0.32)	0.01 (0.74)	0.45 (0.23)
Walking speed (LP)	261	−13.92 (0.24)	−12.76 (0.16)	−0.004 (0.37)	−6.38 (0.09)	0.26 (0.44)	−0.14 (0.66)	−0.01 (0.16)	−0.20 (0.32)	−0.13 (0.56)	−0.14 (0.50)	−0.001 (0.80)	−0.07 (0.56)
25(OH)D (LP; nmol/l)	250	0.01 (0.42)	0.01 (0.97)	0.0001 (0.19)	0.09 (0.30)	0.01 (0.04)	0.01 (0.04)	0.0001 (0.27)	0.01 (0.12)	0.002 (0.65)	0.004 (0.45)	0.0001 (0.39)	0.002 (0.37)
Parity	272	(−21.4) 0.22	−15.97 (0.25)	−0.002 (0.73)	0.30 (0.96)	−0.59 (0.24)	−0.46 (0.32)	−0.003 (0.71)	−0.02 (0.95)	−0.25 (0.45)	−0.43 (0.18)	−0.43 (0.18)	−0.21 (0.22)
Social class	268	−2.64 (0.75)	−3.23 (0.62)	−0.001 (0.78)	1.82 (0.49)	−0.29 (0.22)	−0.31 (0.15)	−0.004 (0.36)	−0.10 (0.48)	−0.10 (0.50)	−0.07 (0.63)	0.0003 (0.93)	0.02 (0.85)
Age at DXA (yr)	268	−4.77 (0.06)	−3.53 (0.08)	−0.0003 (0.71)	−0.001 (0.53)	**−0.16** **(0.02)**	−0.13 (0.06)	−0.001 (0.48)	−0.00003 (0.99)	−0.05 (0.27)	−0.09 (0.06)	−0.002 (0.06)	−0.002 (0.06)
Placental volume (cm^3)	259	0.09 (0.38)	0.08 (0.28)	0.00003 (0.37)	−0.00001 (0.68)	0.003 (0.23)	0.002 (0.42)	0.0001 (0.90)	−0.00001 (0.46)	0.003 (0.15)	0.002 (0.20)	0.00002 (0.63)	0.00002 (0.63)
Height (cm)	272	**6.43** **(<0.001)**	**5.89** **(<0.001)**	**0.002** **(0.002)**	−2.90 (0.52)	**0.27** **(<0.001)**	**0.20** **(<0.001)**	0.001 (0.13)	−0.001 (0.96)	**0.15** **(<0.001)**	**0.12** **(<0.001**	0.001 (0.37)	−0.004 (0.77)
Paternal factors													
Height (cm)	272	3.55 (0.006)	**4.44** **(<0.001)**	**0.002** **(<0.001)**	0.03 (0.94)	**0.13** **(0.001)**	**0.15** **(<0.001)**	**0.002** **(0.003)**	0.01 (0.63)	**0.08** **(0.001)**	**0.09** **(<0.001)**	**0.001** **(0.02)**	0.02 (0.16)
Age at DXA (yr)	273	1.43 (0.39)	0.84 (0.53)	0.00002 (0.97)	−0.27 (0.65)	−0.23 (0.63)	−0.01 (0.80)	0.0001 (0.91)	−0.004 (0.89)	0.05 (0.12)	0.02 (0.58)	−0.001 (0.34)	−0.03 (0.11)

LP = Late pregnancy; LH = Less head
Parity: 2 groups- nulliparous (reference), multiparous; Smoking: 2 groups- No (reference value), yes
Walking speed: 5 groups- very slow, easy paced stroll, normal speed, fairly brisk, fast
Social class: 6 groups- unskilled (v), partly skilled (IV), skilled manual (IIIM), skilled non-manual (IIIN), management and technical (II), professional (I)
Numbers in bold indicate statistical significance (p<0.05)

6.4.5.3 Paternal-offspring bone associations

Strong positive associations were also observed between paternal and offspring bone mass (Table 6.11; Figures 6.2-6.5). In general, paternal bone mass variables were less strongly associated with the corresponding offspring variables compared to maternal-offspring associations. The only exception to this was observed with parental hip BMD which appeared to predict offspring hip BMD at a similar magnitude for both mother and father (β=0.17).

The strongest father-offspring association observed was between paternal and offspring lumbar spine BMD (β=0.21; p<0.001). Paternal BA and BMD predicted the corresponding indices in the offspring at a similar magnitude (BA β=014-0.20; BMD β=0.15-0.21). The relationship between paternal and offspring BMC was also consistently significant but appeared weaker than the other observed bone mass relationships (β=0.10-0.11). Although still significant, the paternal-offspring bone mass relationships were attenuated when adjusted for body size to estimate vBMD of both parent and child (p<0.01; Figure 6.5).

6.4.5.4 Independent associations of parent-child bone mass

To try and establish the independent relationships between parental and offspring bone mass, a regression model was fitted with the other parent's corresponding bone variable as a covariate. Although significant relationships were observed throughout, the strength of the observed maternal-offspring relationships was weakened when the corresponding paternal variable was incorporated into the maternal-offspring regression model (β=0.10-0.33; Table 6.11); this observation was consistent across all mother-offspring variables (all p<0.05). Similarly, when maternal bone mass was incorporated into the paternal-offspring regression model, the predictive value of paternal bone mass was reduced, although remained significant (β=0.09-0.18); all p<0.05).

Table 6.10: Relationships between DXA derived parental and offspring bone indices (model 1; unadjusted)

		Offspring DXA												
	n	WB BA LH (cm²)	WB BMC LH (g)	WB BMD LH (g/cm²)	WB scBMC LH (g)	n	Spine area (cm²)	Spine BMC (g)	Spine BMD (g/cm²)	Spine scBMC (g)	n	Hip area (cm²)	Hip BMC (g)	Hip BMD (g/cm²)	Hip scBMC (g)
Maternal DXA		B	B	B	B		B	B	B	B		B	B	B	B
BA (cm²)	268	**0.29*** **	**0.29*** **	**0.0001*** **	0.01	279	**0.29*** **	**0.25*** **	**0.002***	0.05	279	**0.33*** **	**0.25*** **	0.001	-0.02
BMC (g)	268	**0.14*** **	**0.16*** **	**0.0001*** **	**0.03***	279	**0.10*** **	**0.15*** **	**0.002*** **	**0.07*** **	279	**0.10** **	**0.12*** **	**0.003*** **	**0.04***
BMD (g/cm²)	268	**238.75***	**376.66*** **	**0.20*** **	**156.05*** **	279	2.62	**11.01*** **	**0.24*** **	**7.83*** **	279	-0.18	**2.66***	**0.14*** **	**2.76*** **
scBMC (g)	268	0.01	0.10	**0.0001** **	**0.10*** **	279	-0.04	**0.14*** **	**0.004*** **	**0.15*** **	279	-0.02	0.07	**0.005*** **	**0.10*** **
Adjusted for paternal															
BA (cm²)	255	**0.27*** **	**0.26*** **	**0.0001*** **	0.01	260	**0.26*** **	**0.21*** **	0.001	0..03	263	**0.28*** **	**0.21*** **	**0.0004**	-0.03
BMC (g)	255	**0.13*** **	**0.15*** **	**0.0001*** **	**0.03***	260	**0.09*** **	**0.13*** **	**0.002*** **	**0.06*** **	263	**0.08** **	**0.11*** **	**0.003*** **	**0.04***
BMD (g/cm²)	255	214.64	**342.27*** **	**0.19*** **	**145.39*** **	260	2.02	**10.17*** **	**0.23*** **	**7.29*** **	263	-0.46	2.35	**0.14*** **	**2.63*** **
scBMC (g)	255	0.004	0.07	**0.0001***	**0.07** **	260	-0.05	**0.12*** **	**0.004*** **	**0.13*** **	263	-0.02	0.07	**0.004*** **	**0.09*** **
Paternal DXA															
BA (cm²)	258	**0.13***	**0.17*** **	**00001*** **	0.02	260	**0.13*** **	**0.15*** **	**0.002** **	0.04	263	**0.15*** **	**0.15*** **	**0.002***	0.03
BMC (g)	258	**0.05***	**0.09*** **	**0.00005*** **	**0.02** **	260	**0.05** **	**0.09*** **	**0.002*** **	**0.05*** **	263	**0.07*** **	**0.10*** **	**0.002*** **	**0.04*** **
BMD (g/cm²)	258	123.30	**262.13*** **	**0.15*** **	**117.00*** **	260	1.71	**7.34*** **	**0.16*** **	**5.42*** **	263	2.22	**4.55*** **	**0.15*** **	**2.74*** **
scBMC (g)	258	0.03	**0.12** **	**0.0001*** **	**0.08*** **	260	0.01	**0.09*** **	**0.002*** **	**0.08*** **	263	0.03	**0.12*** **	**0.004*** **	**0.09*** **
Adjusted for maternal															
BA (cm²)	255	**0.11***	**0.15*** **	**0.0001*** **	0.02	260	**0.10** **	**0.12*** **	**0.002** **	0.03	263	**0.14*** **	**0.14*** **	**0.002***	0.03
BMC (g)	255	**0.05***	**0.08*** **	**0.00004*** **	**0.02** **	260	**0.04***	**0.08*** **	**0.001*** **	**0.04*** **	263	**0.07*** **	**0.10*** **	**0.002*** **	**0.04*** **
BMD (g/cm²)	255	107.98	**235.89*** **	**0.14*** **	**104.17*** **	260	1.59	**6.74*** **	**0.15*** **	**4.81*** **	263	2.21	**4.59*** **	**0.15*** **	**2.61*** **
scBMC (g)	255	0.03	**0.11** **	**0.0001*** **	**0.07*** **	260	0.02	**0.09*** **	**0.002*** **	**0.07*** **	263	0.04	**0.12*** **	**0.04*** **	**0.08*** **

Numbers are regression coefficients. *p<0.05, **p<0.01, ***p<0.001; Numbers in bold indicate statistical significance (p<0.05)

Offspring variables adjusted for sex and age. LH = Less head; Shaded box= corresponding bone indices

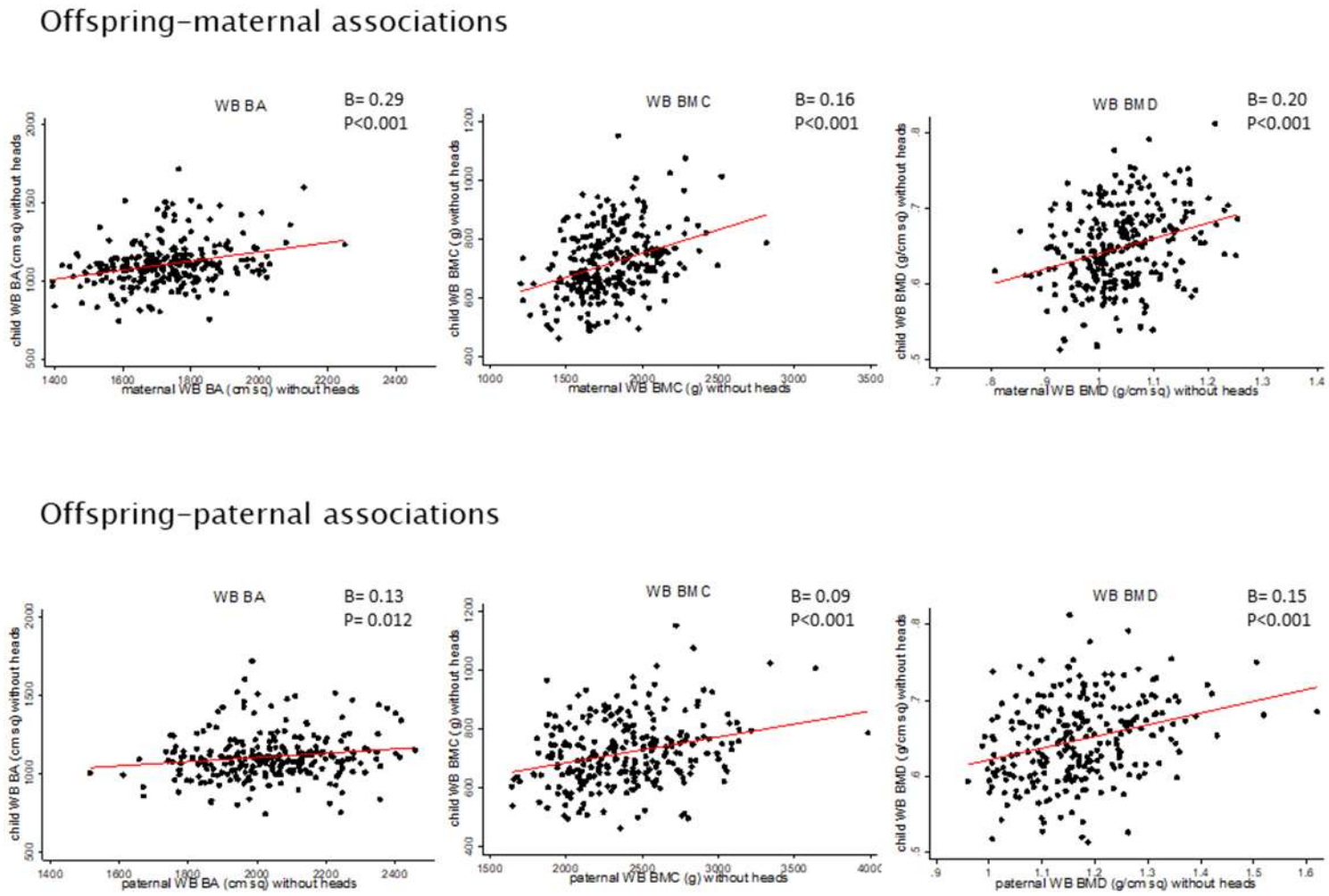


Figure 6.2: Scatterplots illustrating the relationship between parental and offspring whole body bone indices

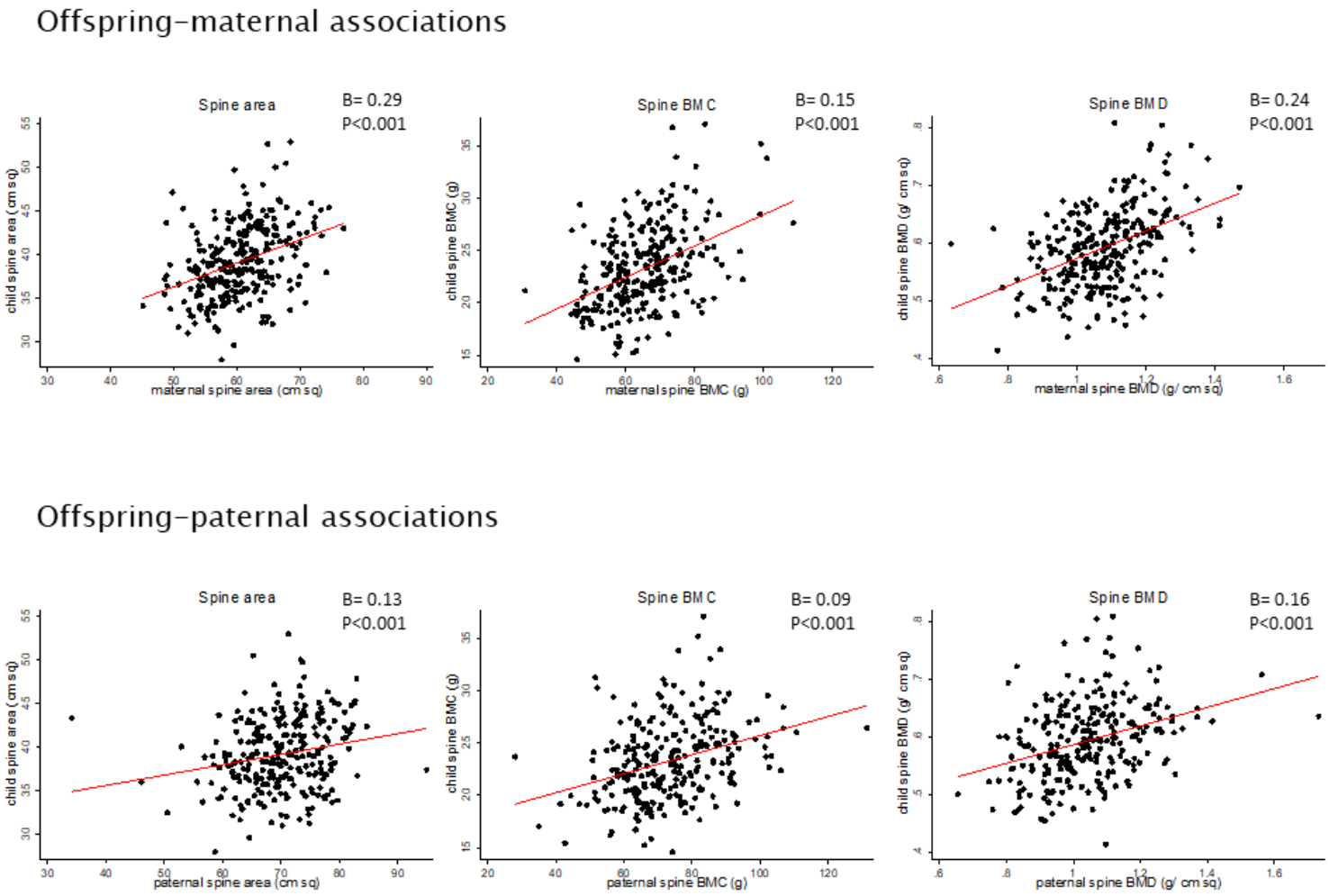


Figure 6.3: Scatterplots illustrating the relationship between parental and offspring spine bone indices

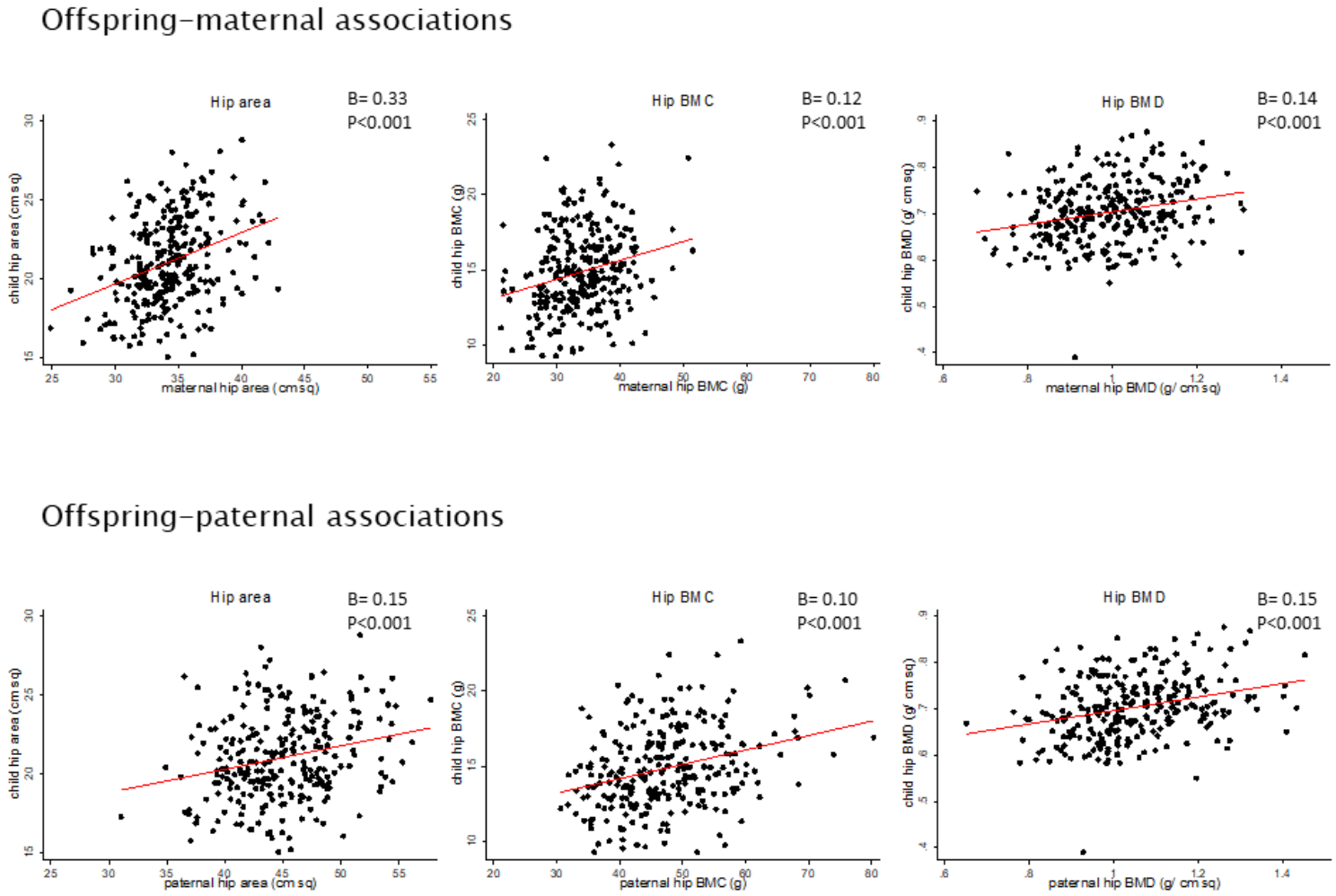


Figure 6.4: Scatterplots illustrating the relationship between parental and offspring hip bone indices

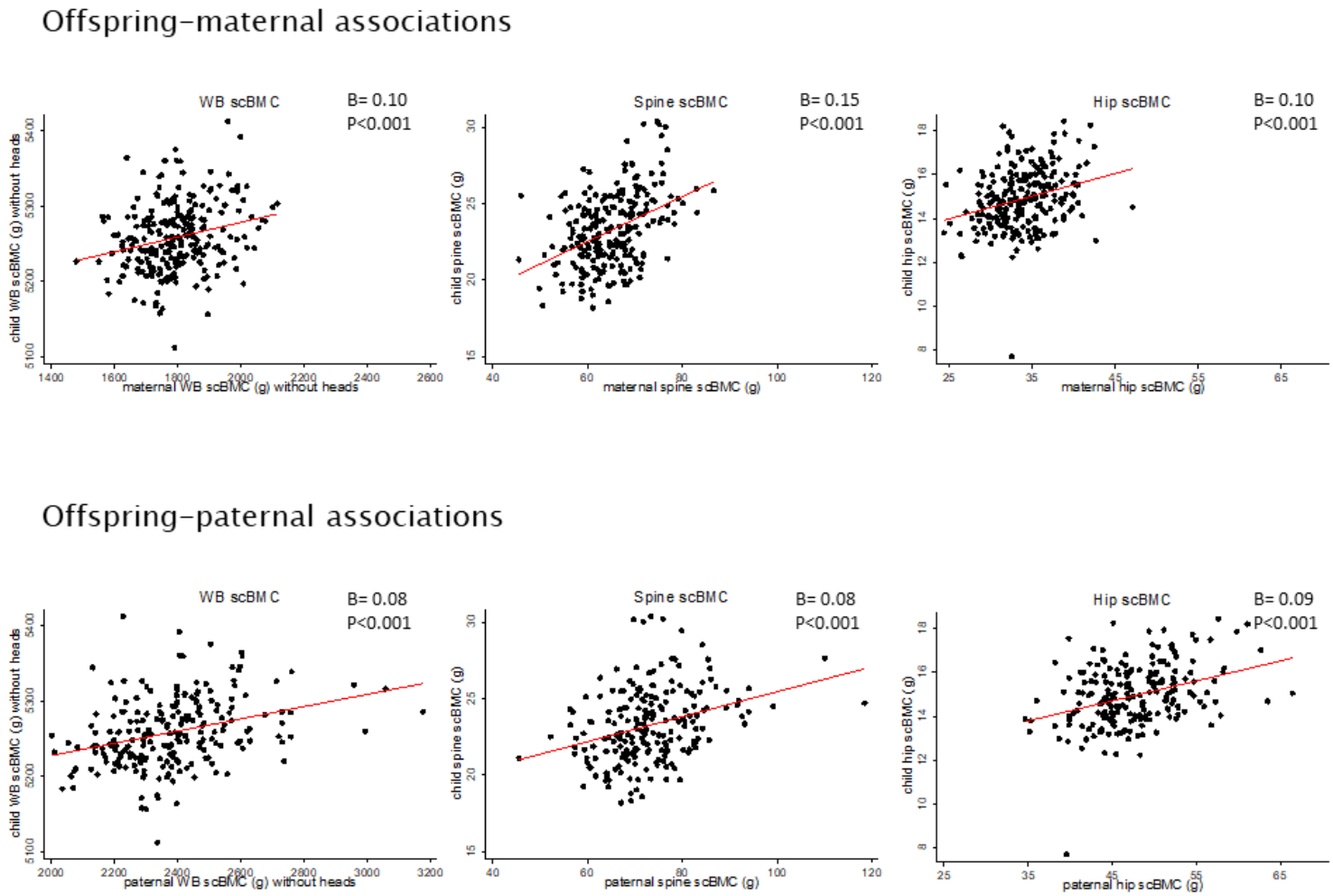
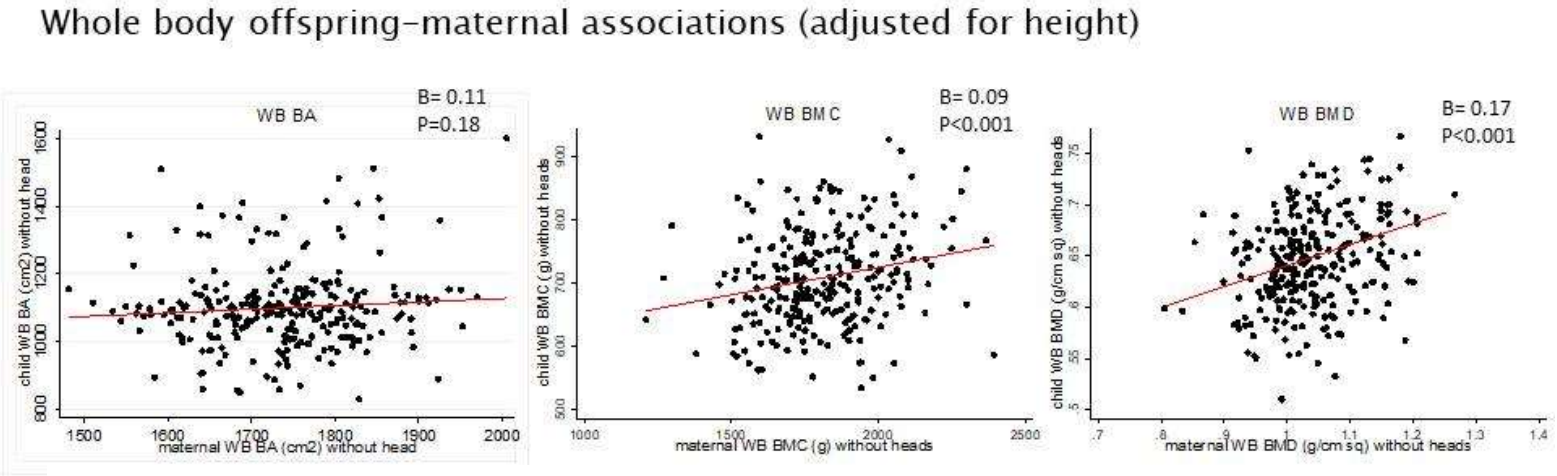
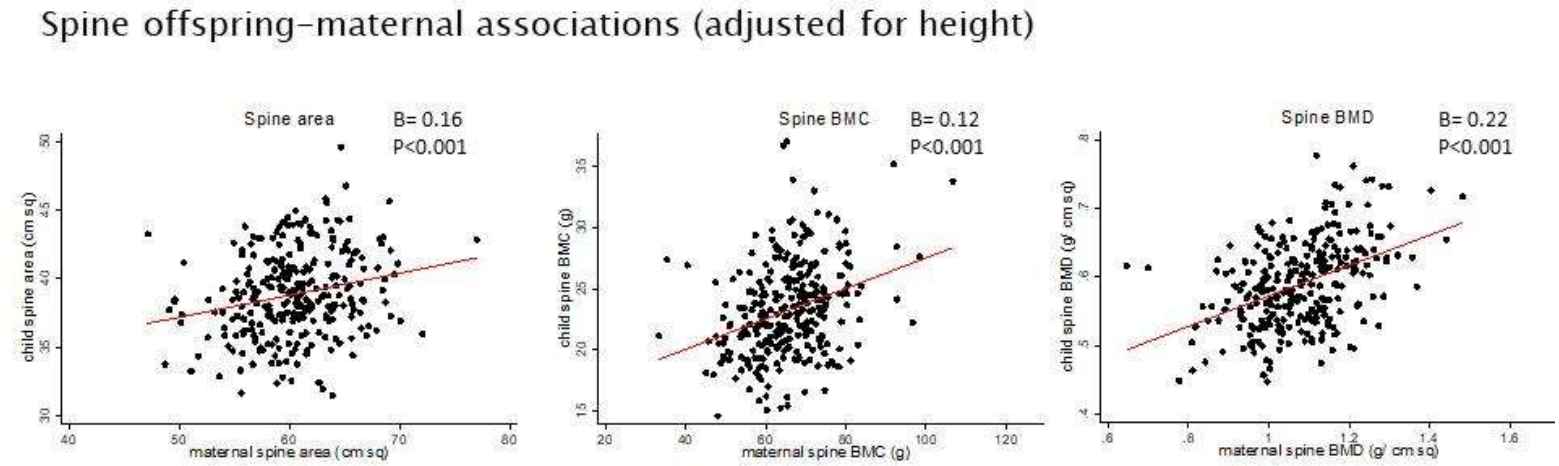


Figure 6.5: Scatterplots illustrating the relationship between parental and offspring scBMC

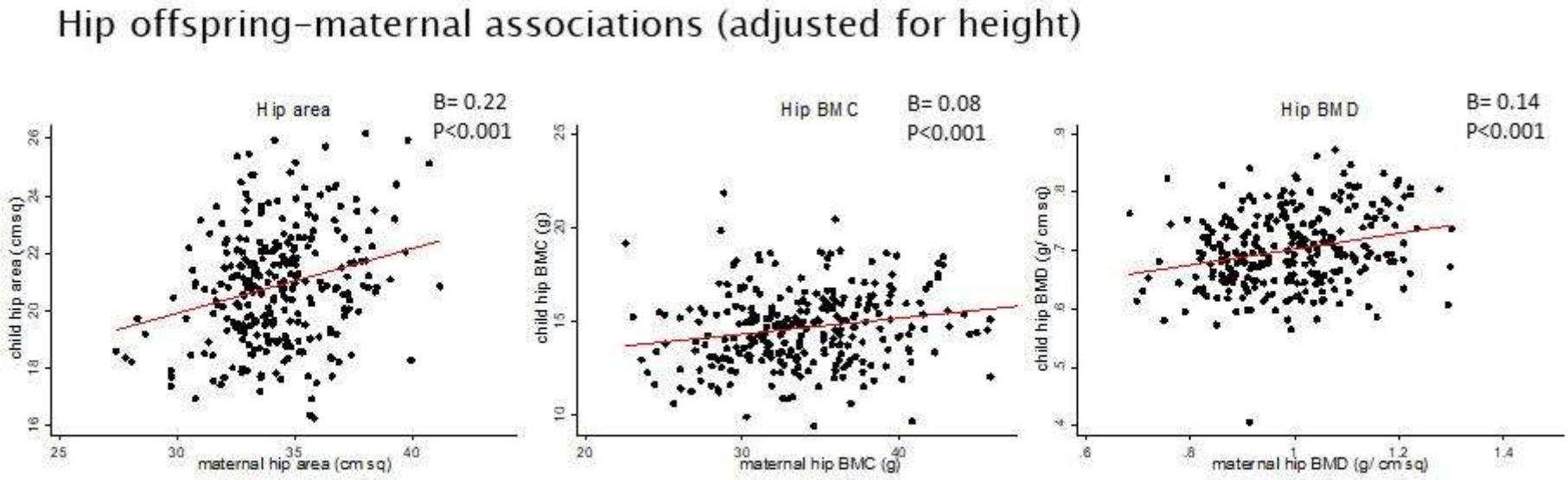


Maternal variables adjusted for maternal height, offspring variables adjusted for offspring height



Maternal variables adjusted for maternal height, offspring variables adjusted for offspring height

Figure 6.6: Scatterplots illustrating the relationship between mother and child whole body and spine bone variables after adjustment for height



Maternal variables adjusted for maternal height, offspring variables adjusted for offspring height

Figure 6.7: Scatterplots illustrating the relationships between mother and child hip bone variables after adjustment for height

6.4.5.5 Differences in parental bone associations with childhood bone indices

To determine whether the observed differences in the parental associations with offspring bone mass were significant, regression co-efficients were compared using a Hausman test. The relationships between mother and child whole body BA and BMC, spine BA, BMC, BMC and scBMD, and hip BA were found to be of significantly larger magnitude compared to those between father and child (Table 6.11a, Figure 6.8). The greatest observed difference in the magnitude of parent-child association was on offspring bone area, with regression co-efficients for mother-child more than twice that for father-child at all 3 sites (whole body: 2.15; spine 2.30; hip 2.16). These trends remained even after adjusting for the other parental variable (Table 6.11b)

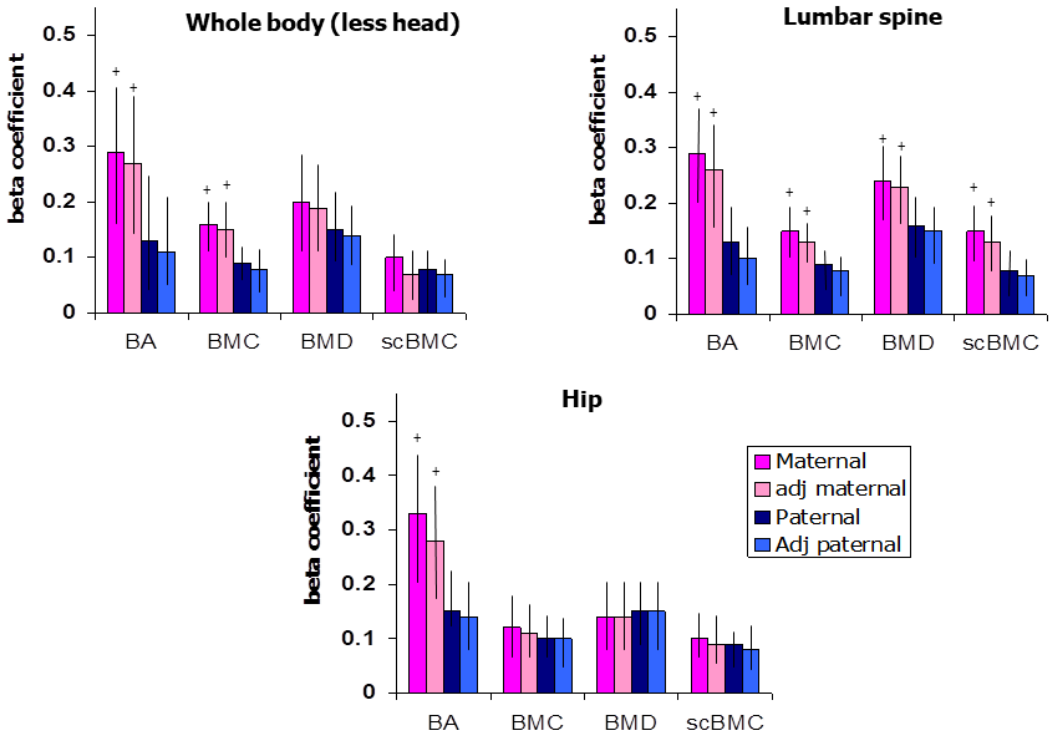


Figure 6.8: Differences in the relationships between offspring and parental bone mass

+ denotes significant difference in β coefficient between mother-child and father-child ($p<0.05$)
Maternal bone mass adjusted for corresponding paternal variable and vice versa

Table 6.11: Differences in β coefficients between mother-child versus father-child bone associations

(a) unadjusted; (b) after adjustment for the reciprocal parental variable

(a)

Parental DXA (whole body)	Offspring DXA – whole body				Parental DXA (spine)	Offspring DXA - spine				Parental DXA (hip)	Offspring DXA - hip			
	BA	**BMC**	**BMD**	**scBMC**		**BA**	**BMC**	**BMD**	**scBMC**		**BA**	**BMC**	**BMD**	**scBMC**
BA	**2.15 (0.03)**	**1.63 (0.02)**	1.16 (0.63)	1.98 (0.74)	**BA**	**2.30 (0.002)**	**1.68 (0.02)**	1.05 (0.91)	1.22 (0.77)	**BA**	**2.16 (0.003)**	1.67 (0.11)	5.26 (0.48)	1.39 (0.09)
BMC	**2.58 (0.02)**	**1.83 (0.01)**	1.43 (0.17)	1.18 (0.78)	**BMC**	**2.06 (0.04)**	**1.65 (0.01)**	1.41 (0.09)	1.39 (0.21)	**BMC**	1.45 (0.39)	1.31 (0.40)	1.22 (0.56)	1.02 (0.96)
BMD	1.94 (0.34)	1.44 (0.23)	1.32 (0.30)	1.17 (0.77)	**BMD**	1.53 (0.71)	1.50 (0.09)	**1.48 (0.04)**	1.51 (0.23)	**BMD**	120.1 (0.23)	1.71 (0.31)	1.06 (0.85)	1.01 (0.97)
scBMC	5.76 (0.72)	1.17 (0.77)	1.06 (0.86)	1.20 (0.55)	**scBMC**	3.68 (0.19)	1.51 (0.23)	**1.94 (0.002)**	**1.81 (0.01)**	**scBMC**	1.62 (0.36)	1.53 (0.49)	1.03 (0.89)	1.13 (0.67)

(b)

Parental DXA (whole body)	Offspring DXA – whole body				Parental DXA (spine)	Offspring DXA - spine				Parental DXA (hip)	Offspring DXA - hip			
	BA	**BMC**	**BMD**	**scBMC**		**BA**	**BMC**	**BMD**	**scBMC**		**BA**	**BMC**	**BMD**	**scBMC**
BA	**2.55 (0.05)**	**1.78 (0.04)**	1.20 (0.880)	1.51 (0.86)	**BA**	**2.73 (0.01)**	1.74 (0.09)	1.40 (0.47)	1.02 (0.99)	**BA**	**2.09 (0.02)**	1.51 (0.30)	5.27 (0.34)	1.19 (0.07)
BMC	**2.73 (0.04)**	**1.88 (0.02)**	1.46 (0.22)	1.24 (0.75)	**BMC**	2.29 (0.09)	**1.74 (0.03)**	1.43 (0.19)	1.42 (0.35)	**BMC**	1.20 (0.71)	1.16 (0.68)	1.16 (0.67)	1.07 (0.89)
BMD	1.98 (0.42)	1.45 (0.32)	1.35 (0.35)	1.15 (0.79)	**BMD**	1.27 (0.87)	1.51 (0.15)	**1.53 (0.05)**	1.52 (0.13)	**BMD**	4.80 (0.14)	1.95 (0.23)	1.05 (0.82)	1.01 (0.98)
scBMC	7.06 (0.78)	1.48 (0.63)	1.15 (0.79)	1.05 (0.91)	**scBMC**	3.03 (0.14)	1.34 (0.50)	**1.90 (0.01)**	**1.88 (0.02)**	**scBMC**	2.64 (0.35)	1.65 (0.46)	1.02 (0.94)	1.13 (0.74)

Numbers presented are the differences in multiples between mother-child versus father-child regression coefficients (p value)
Shaded box = $p<0.05$

6.4.5.6 Relationship between parental and offspring bone after adjustment for confounders

Three further regression models were generated to adjust for previously identified potential confounding factors. In the first of these regression models (Table 6.12) additional adjustments for maternal triceps skinfold thickness in late pregnancy and placental volume, and paternal age and physical activity, made little difference to the observed relationships, with consistently significant relationships between offspring bone and both maternal and paternal bone across all variables at all 3 sites, with the strongest associations observed between mother and child.

Adjusting for offspring physical activity (Table 6.13, model 3) and parental height (Table 6.14, model 4) reduced the number of participants included in the model (n=124), however significant independent parent-child associations still remained for BMD across all 3 sites and BMC at the spine in both parents. There was some disparity between parental influences at the hip site in these models; maternal hip BA remained a significant predictor of offspring hip BA, whereas paternal hip BMC remained a significant predictor of offspring hip BMC. Unlike in previous models, there was not a significant difference in the overall magnitude of the bone associations between mother-child versus father-child.

Table 6.12: Relationships between DXA derived parental and offspring bone mass (model 2) after adjustments

		Offspring DXA												
	n	WB BA LH (cm²)	WB BMC LH (g)	WB BMD LH (g/cm²)	WB scBMC LH (g)	n	Spine area (cm²)	Spine BMC (g)	Spine BMD (g/cm²)	Spine scBMC (g)	n	Hip area (cm²)	Hip BMC (g)	Hip BMD (g/cm²)	Hip scBMC (g)
Maternal DXA		B	B	B	B		B	B	B	B		B	B	B	B
BA (cm²)	**249**	**0.32*** **	**0.33*** **	**0.0001*** **	0.03	**259**	**0.32*** **	**0.29*** **	**0.002*** **	**0.07***	**259**	**0.37*** **	**0.30*** **	0.001	-0.02
BMC (g)	**249**	**0.14*** **	**0.18*** **	**0.0001*** **	**0.04*** **	**259**	**0.10*** **	**0.16*** **	**0.003*** **	**0.08*** **	**259**	**0.11****	**0.14*** **	**0.003*** **	**0.05****
BMD (g/cm²)	**249**	226.51	**387.92*** **	**0.22*** **	**171.25*** **	**259**	3.01	**11.70*** **	**0.25*** **	**8.20*** **	**259**	-0.43	**2.49**	**0.14*** **	**2.97*** **
scBMC (g)	**249**	0.01	0.12	**0.0001*** **	**0.10*** **	**259**	-0.04	**0.14*** **	**0.004*** **	**0.14*** **	**259**	-0.03	0.07	**0.004*** **	**0.09*** **
Adjusted for paternal															
BA (cm²)	**238**	**0.30*** **	**0.30*** **	**0.0001*** **	0.03	**241**	**0.29****	**0.26*** **	**0.002***	0.05	**244**	**0.33*** **	**0.26*** **	0.0001	-0.02
BMC (g)	**238**	**0.13*** **	**0.16*** **	**0.0001*** **	**0.04*** **	**241**	**0.09*** **	**0.15*** **	**0.002*** **	**0.07*** **	**244**	**0.09****	**0.12*** **	**0.003*** **	**0.04***
BMD (g/cm²)	**238**	198.51	**351.24*** **	**0.20****	**162.80*** **	**241**	2.39	**10.88****	**0.24****	**7.78*** **	**244**	-0.83	2.31	**0.15*** **	**2.93*** **
scBMC (g)	**238**	0.001	0.08	**0.0001***	**0.07****	**241**	-0.05	**0.12*** **	**0.004*** **	**0.13*** **	**244**	-0.03	0.06	**0.004*** **	**0.09*** **
Paternal DXA															
BA (cm²)	**238**	**0.14***	**0.19*** **	**00001*** **	0.02	**241**	**0.13*** **	**0.15*** **	**0.002*** **	**0.05***	**244**	**0.15*** **	**0.14*** **	0.002	0.02
BMC (g)	**238**	**0.06***	**0.09*** **	**0.00005*** **	**0.03****	**241**	**0.05***	**0.10*** **	**0.002*** **	**0.05*** **	**244**	**0.07*** **	**0.10*** **	**0.002*** **	**0.04*** **
BMD (g/cm²)	**238**	141.34	**277.48*** **	**0.16*** **	**117.93*** **	**241**	2.11	**7.71*** **	**0.17*** **	**5.41*** **	**244**	**2.84***	**4.91*** **	**0.14*** **	**2.56*** **
scBMC (g)	**238**	0.03	**0.12****	**0.0001*** **	**0.08*** **	**241**	0.02	**0.10*** **	**0.002*** **	**0.09*** **	**244**	0.05	**0.13*** **	**0.005*** **	**0.09*** **
Adjusted for maternal															
BA (cm²)	**238**	**0.11***	**0.16*** **	**0.0001*** **	0.02	**241**	**0.10****	**0.13*** **	**0.002****	0.04	**244**	**0.13*** **	**0.13*** **	0.002	0.02
BMC (g)	**238**	**0.05***	**0.08*** **	**0.00004*** **	**0.02****	**241**	**0.04***	**0.08*** **	**0.001*** **	**0.04*** **	**244**	**0.07*** **	**0.09*** **	**0.002*** **	**0.03*** **
BMD (g/cm²)	**238**	127.75	**250.45*** **	**0.14****	**103.28*** **	**241**	1.96	**7.02*** **	**0.15*** **	**4.77*** **	**244**	**2.81***	**4.98*** **	**0.15*** **	**2.46*** **
scBMC (g)	**238**	0.03	**0.11***	**0.0001*** **	**0.07*** **	**241**	0.02	**0.09*** **	**0.002*** **	**0.07*** **	**244**	0.06	**0.13*** **	**0.004*** **	**0.08*** **

Numbers are regression coefficients. *p<0.05, **p<0.01, ***p<0.001; Shaded box= corresponding bone indices; Numbers in bold indicate statistical significance (p<0.05)

Offspring variables adjusted for sex and age. LH = Less head

Adjusted for maternal variables: triceps skinfold thickness in late pregnancy and placental volume; paternal variables: age, physical activity

Table 6.13: Relationships between DXA derived parental and offspring bone mass (model 3)

		Offspring DXA												
	n	WB BA LH (cm²)	WB BMC LH (g)	WB BMD LH (g/cm²)	WB scBMC LH (g)	n	Spine area (cm²)	Spine BMC (g)	Spine BMD (g/cm²)	Spine scBMC (g)	n	Hip area (cm²)	Hip BMC (g)	Hip BMD (g/cm²)	Hip scBMC (g)
Maternal DXA		B	B	B	B		B	B	B	B		B	B	B	B
BA (cm²)	133	**0.21***	**0.21****	**0.0001***	−0.01	140	**0.19****	**0.25*****	**0.003*****	**0.08***	140	**0.35*****	**0.25*****	0.0003	−0.06
BMC (g)	133	0.10	**0.12****	**0.0001****	0.03	140	0.06	**0.13*****	**0.002*****	**0.07*****	140	0.10	**0.10***	**0.002***	0.03
BMD (g/cm²)	133	167.29	**300.64***	**0.18****	**152.94****	140	0.69	**8.31*****	**0.19*****	**6.60*****	140	−1.64	1.33	**0.13****	**2.87****
scBMC (g)	133	0.03	0.13	**0.0001***	**0.11****	140	−0.05	0.08	**0.003*****	**0.10*****	140	−0.03	0.06	**0.004****	**0.09****
Adjusted for paternal															
BA (cm²)	124	0.19	**0.19****	0.0001	0.004	132	**0.20****	**0.25*****	**0.003*****	0.07	131	**0.35*****	**0.24****	0.0001	−0.07
BMC (g)	124	0.09	**0.11****	**0.0001****	0.03	132	0.05	**0.12*****	**0.002*****	**0.05****	131	0.08	**0.10***	**0.002***	0.03
BMD (g/cm²)	124	147.65	**257.32***	**0.15****	**150.40****	132	0.04	**7.29****	**0.18****	**5.67*****	131	−1.28	1.74	**0.14****	**2.71***
scBMC (g)	124	0.05	0.09	0.0001	0.07	132	−0.07	0.05	**0.002****	**0.10****	131	−0.03	0.06	**0.004****	**0.08***
Paternal DXA															
BA (cm²)	124	0.04	0.10	**00001***	−0.01	132	**0.14****	**0.17*****	**0.002****	0.06	131	0.11	0.11	0.002	0.01
BMC (g)	124	0.02	**0.06***	**0.00005*****	0.02	132	**0.07****	**0.12*****	**0.002*****	**0.07*****	131	**0.08****	**0.10*****	**0.002****	0.03
BMD (g/cm²)	124	55.51	**245.61***	**0.18*****	**125.07****	132	4.91	**10.95*****	**0.20*****	**5.73*****	131	**4.61****	**5.86*****	**0.13****	**2.26***
scBMC (g)	124	0.002	**0.14***	**0.0001*****	**0.11*****	132	0.05	**0.13****	**0.002*****	**0.09*****	131	**0.11***	**0.18*****	**0.005*****	**0.11*****
Adjusted for maternal															
BA (cm²)	124	0.02	0.07	0.0001	−0.01	132	**0.14****	**0.16*****	**0.002****	0.06	131	0.11	0.10	0.002	0.01
BMC (g)	124	0.01	**0.06***	**0.00004****	0.02	132	**0.06***	**0.10*****	**0.001*****	**0.06*****	131	**0.09****	**0.10*****	**0.002****	0.03
BMD (g/cm²)	124	37.87	**206.40***	**0.15****	**101.53***	132	4.91	**10.07*****	**0.18*****	**6.45*****	131	**4.55****	**5.93*****	**0.14****	**2.10***
scBMC (g)	124	−0.02	0.09	**0.0001****	**0.05****	132	0.07	**0.13****	**0.002*****	**0.08*****	131	**0.13****	**0.19*****	**0.05*****	**0.09****

Numbers are regression coefficients. *p<0.05, **p<0.01, ***p<0.001; Shaded box= corresponding bone indices; Numbers in bold indicate statistical significance (p<0.05)
Offspring variables adjusted for sex, age and physical activity (aged 6). LH = Less head
Adjusted for maternal variables: triceps skinfold thickness in late pregnancy and placental volume; paternal variables: age, physical activity

Table 6.14: Relationships between DXA derived parental and offspring bone-mass after full adjustments (including parental height; model 4)

		Offspring DXA										
	n	WB BA LH (cm^2)	WB BMC LH (g)	WB BMD LH (g/cm^2)	n	Spine area (cm^2)	Spine BMC (g)	Spine BMD (g/cm^2)	n	Hip area (cm^2)	Hip BMC (g)	Hip BMD (g/cm^2)
Maternal DXA		B	B	B		B	B	B		B	B	B
BA (cm^2)	**133**	0.12	0.11	0.00003	**140**	**0.12****	**0.22*****	**0.003****	**140**	**0.31*****	**0.24***	0.001
BMC (g)	**133**	0.02	0.08	**0.0001***	**140**	0.02	**0.12*****	**0.002*****	**140**	0.02	0.08	**0.003***
BMD (g/cm^2)	**133**	53.83	226.55	**0.18****	**140**	0.32	**7.53****	**0.19*****	**140**	−1.85	1.24	**0.13****
Adjusted for paternal												
BA (cm^2)	**124**	0.07	0.08	0.00002	**131**	0.13	**0.24*****	**0.004*****	**132**	**0.29****	**0.23***	0.001
BMC (g)	**124**	0.09	0.06	**0.0005***	**131**	0.02	**0.10*****	**0.002*****	**132**	0.03	0.08	**0.003****
BMD (g/cm^2)	**124**	18.98	180.07	**0.15***	**131**	−0.81	**6.75****	**0.18*****	**132**	−1.64	1.57	**0.14****
Paternal DXA												
BA (cm^2)	**124**	0.13	0.11	00002	**131**	**0.12***	**0.17****	**0.002****	**132**	0.12	0.11	0.001
BMC (g)	**124**	0.03	0.06	**0.00004***	**131**	0.05	**0.1*****	**0.002*****	**132**	**0.08***	**0.10*****	**0.002****
BMD (g/cm^2)	**124**	23.91	191.87	**0.14****	**131**	3.53	**9.93*****	**0.20*****	**132**	**3.70***	**5.35****	**0.13****
Adjusted for maternal												
BA (cm^2)	**124**	0.14	0.11	0.00002	**131**	**0.13***	**0.17*****	**0.003****	**132**	0.12	0.11	0.001
BMC (g)	**124**	0.02	0.06	**0.00003***	**131**	0.05	**0.10*****	**0.001*****	**132**	**0.08****	**0.11*****	**0.002****
BMD (g/cm^2)	**124**	18.00	165.11	**0.12***	**131**	3.62	**9.19*****	**0.18*****	**132**	**3.63***	**5.42****	**0.14****

Numbers are regression coefficients. *p<0.05, **p<0.01, ***p<0.001; Shaded box= corresponding bone indices; Numbers in bold indicate statistical significance (p<0.05)
Offspring variables adjusted for sex, age and physical activity (measured at age 6). LH = Less head
Adjusted for maternal variables: triceps skinfold thickness in late pregnancy, placental volume and height; paternal variables: age, physical activity and height

6.4.5.7 Differences in parental association according to offspring sex

To determine whether the parental relationship with offspring bone parameters differed according to the sex of the child we performed two separate analyses. Firstly, separate univariate regression was performed according to offspring sex, and secondly univariate regression was performed incorporating a sex interaction term. Using the first method, non-significant trends were observed between, maternal-son lumbar spine scBMC (p=0.07), father-daughter whole body BA (p=0.10), father-daughter whole body scBMC (p=0.23), father-son hip BA (p=0.08), father-daughter hip BMD (p=0.09) and father-son lumbar spine BA (p=0.13). When this was further investigated using the second approach, no significant sex interaction terms were observed. This difference may be due to small numbers in both groups.

6.5 Summary of findings

In summary, DXA-derived parental bone indices was positively associated with offspring bone indices at age 9 years, with no significant difference according to offspring sex. Strong, independent relationships were observed between both maternal and paternal bone and the corresponding indices in their child, with the strongest observed relationship between maternal and offspring whole body BA.

Placental volume was positively related to offspring bone mass, however in this smaller cohort of 259 mother-child pairs, these relationships were not found to be statistically significant.

A differential parent-child association was seen, with relationships of significantly larger magnitude observed between mother-child compared to father –child for WB BA, hip BA, and all measured bone indices at the spine.

These findings suggest that whilst parent of origin genetic effects are potential explanations, the differential maternal and paternal associations seen may reflect in-utero mechanisms.

7. Parental associations with childhood bone mass at 6 years: pQCT findings from the SWS

7.1 Background

Previous studies have suggested that measures of bone size, mineralisation and density may be partly inherited (71;72), however the independent parental influences on offspring bone mass remain poorly understood. Using DXA-derived data from the SWS cohort, strong positive associations were observed between parental WB, total hip and lumbar spine measures (BA, BMC, BMD) and the corresponding indices in the offspring (Chapter 6). Differential parent-child associations for DXA bone indices were observed, with mother-child associations being of greater magnitude than those between father-child for several measures of bone size and mineral content.

One of the major limitations of DXA is the size dependence of the measurement (as discussed in Section 1.7.1.2). aBMD measurements derived from DXA can be influenced by bone size and may not be a reliably accurate representation of volumetric mineral density. One advantage of bone assessment using pQCT is that it is able to directly measure true size-independent vBMD.

The aim of this study was to document the relationships between pQCT-derived bone indices in the mother, father and child at 6 years of age, using a prospective cohort, the SWS.

7.2 Methods

The methodology for the SWS has been described in detail in Chapter 3. A subset of children attending the SWS 6-year visit underwent pQCT assessment of their non-dominant lower leg. A proportion of the parents of these children underwent the same pQCT assessment when attending the 8-year parental visit. Four sites of

the tibia were scanned (4%, 14%, 38% and 66% of the total tibial length) using a Stratec XCT-2000 machine (Stratec Inc., Pforzheim, Germany) during the 5 minute scan time. The total radiation dose associated with pQCT for both adults and children was 0.43 μSv per slice (219), which is less than 2 hours natural background radiation in the United Kingdom (220). A lifestyle questionnaire was also completed for the child and both parents.

7.3 Statistical analysis

All data from the questionnaires were anonymised, coded and double-punched onto a computer. All pQCT scans were analysed at the visit by a trained DXA technician using automated software. All scans were reviewed and graded on a 0-5 visual scale for movement artefact (Figure 7.1)(221); those with significant movement artefact (Grade 3 or above) were excluded from the analysis. The data collected from the parent visit was amalgamated with the maternal pre-, early and late pregnancy data and the childhood data at birth and 6 years of age.

All data were analysed using Stata V13.0 (StataCorp, Texas, USA). A similar statistical approach to that used for the DXA data (described in Chapter 6) was employed. All data were checked for normality. Maternal and paternal characteristics were compared using a combination of paired t-test (for continuous parametric variables), Wilcoxon signed-rank test (for continuous non-parametric variables) and McNemar's test (for categorical variables). Sex difference between offspring characteristics were compared using unpaired t-test (for continuous parametric variables) and Mann-Whitney rank sum test (for continuous non-parametric variables). Linear regression was then used to assess parent-parent relationships and parent-child relationships. Multivariate models (multiple linear regression) were used to assess independent parent-child relationships. Sex interactions were examined between parent and offspring using linear regression and a sex interaction term. The Hausman test was used to compare the magnitude of regression coefficients.

In this analysis only a subset of the pQCT variables generated were included. They are as follows:

4% tibial site: Total area, total vBMD and trabecular vBMD

38% tibial site: Total area, cortical area, medullary area, cortical thickness, cortical vBMD, periosteal circumference, endosteal circumference, stress-strain index (SSI; a surrogate measure of bone strength)

The 14% site was not included in this analysis as the cortex at this site is poorly detected in children. The 66% site was not included as this gives little information on bone mass; it is most helpful in assessing muscle mass and body composition.

All offspring variables were adjusted for age at pQCT and sex.

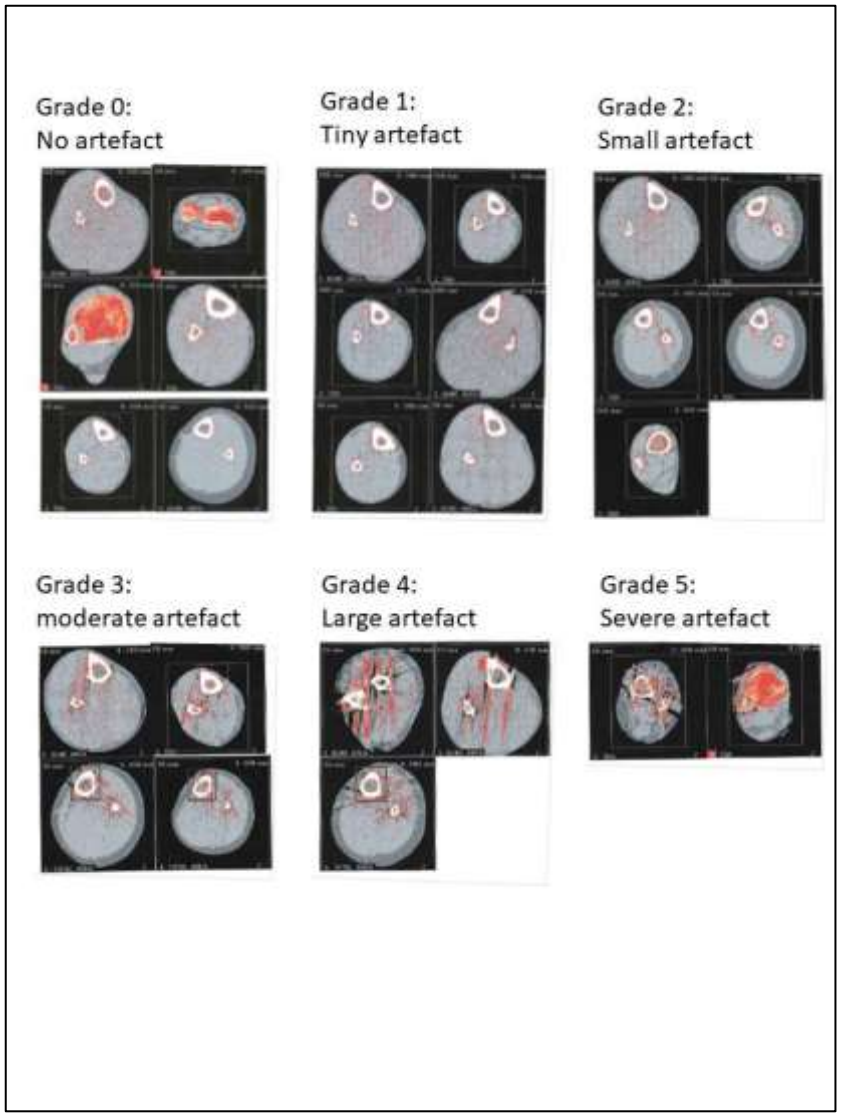


Figure 7.1: pQCT visual artefact grading score system

7.4 Results

513 children attended for the pQCT component of the 6 year follow-up study; 307 mothers and 297 fathers also underwent pQCT assessment. After mother, father and child scans were matched, 104 mother-father-child trios were available for analysis. 5 (1.6%) maternal and 8 (2.7%) paternal scans were excluded due to artefact.

7.4.1 Baseline demographics

7.4.1.1 Offspring demographics

Baseline characteristics of the children are shown in Table 7.1. 53% of the children were male, mean (SD) age was 7.0 (0.3) years. There was no statistical difference in height, weight, birthweight or BMI between the sexes. pQCT variables at the 4% site were similar between boys and girls. At the 38% site, cortical thickness was significantly lower, but endosteal circumference and medullary area significantly higher in girls compared to boys (all $p<0.05$).

7.4.1.2 Parental demographics

Baseline characteristics of the parents are shown in Tables 7.2 and 7.3. Mothers were significantly younger than the fathers (age (SD) 41.0 (3.5) vs 43.9 (5.5) years); as expected fathers were significantly taller and heavier, with higher BMI (all $p<0.05$). There were no significant differences in current or previous smoking, physical activity, ethnicity or social class between the two groups, however fathers consumed significantly more alcohol ($p<0.0001$) and milk ($p=0.0002$) per week, had higher rates of previous fracture ($p<0.02$), and undertook more hours of strenuous per week ($p=0.03$). All pQCT derived bone outcomes at both sites were significantly higher in the fathers than the mothers ($p<0.0001$; Table 7.3).

Table 7.1: Offspring baseline characteristics

	Overall			Boys			Girls			P value
	n	Mean/ median	SD /IQR	n	mean	SD/ IQR	n	mean	SD/ IQR	
Age (yrs)	104	7.0	0.3	55	6.9	0.3	49	7.0	0.3	0.5
Height (cm)	102	120.7	5.3	55	120.5	5.0	47	121.0	5.3	0.7
Height (z-score)	102	-0.1	0.9	55	-0.2	0.9	47	-0.03	1.0	0.4
Weight (kg)	102	22.9	(20.5-25.9)	55	21.9	(20.2-25.2)	47	23.9	(21.3-26.9)	0.2
Weight (z-score)	102	0.03	1.0	55	-0.1	1.0	47	0.2	1.0	0.2
BMI (kg/m²)	102	15.8	(14.9-16.9)	55	15.7	(14.7-16.6)	47	16.1	(15.0-17.5)	0.1
BMI (z-score)	102	0.1	1.0	55	0.05	1.0	47	0.3	0.1	0.3
Birthweight (g)	102	3408.3	569.8	53	3375.2	579.0	49	3444.1	563.5	0.5
pQCT variables										
4% Total bone area (mm²)	104	683.4	109.5	55	675.5	120.4	49	692.4	36.3	0.4
4% Total bone density (mg/cm³)	104	328.7	39.8	55	330.6	40.9	49	326.5	39.0	0.6
4% Trabecular density (mg/cm³)	104	313.1	55.7	55	306.4	61.0	49	320.6	48.7	0.2
38% Total bone area (mm²)	104	221.2	36.0	55	215.7	35.6	49	227.7	35.7	0.1
38% cortical area (mm²)	104	121.8	17.3	55	122.8	16.6	49	120.6	18.2	0.5
38% cortical thickness (mm)	104	2.8	0.3	55	2.9	0.3	49	2.7	0.4	**0.02**
38% cortical density (mg/cm³)	104	1041.3	36.2	55	1036.7	36.2	49	1046.6	36.0	0.2
38% periosteal circumference (mm)	104	52.6	4.2	55	51.9	4.2	49	53.3	4.2	0.1
38% endosteal circumference (mm)	104	35.0	4.7	55	33.9	4.5	49	36.4	4.7	**0.01**
38% medullary area (mm²)	104	99.4	27.7	55	92.9	26.5	49	107.1	98.4	**0.02**
38% SSI	104	459.8	89.2	55	463.6	84.9	49	455.6	94.8	0.7

Table 7.2: Parental baseline demographics

	Mothers (n=104)			Fathers (n=104)			P value
	n	Mean/ median/ %	SD / IQR	n	Mean/ median/ %	SD / IQR	
Age (years)	96	41.0	3.5	99	43.9	5.5	**<0.0001**
Height (cm)	103	163.8	6.1	104	176.8	6.7	**<0.0001**
Weight (kg)	103	68.1	(60.8-80.3)	104	83.9	(77.8-95.2)	**<0.0001**
BMI (kg/m^2)	103	25.9	(22.8-29.3)	104	26.9	(24.9-29.9)	**0.04**
Parity	94						
Primiparous	8	8.5%					
Multiparous	86	91.5%					
Current smoking	9/89	10.1%		12/96	12.5%		0.8
Ever smoked regularly	39/95	41.1%		52/98	53.1%		0.4
Smoked in early pregnancy	8/104	7.7%					
Smoked in late pregnancy	8/99	8.1%					
Serum 25(OH)D EP (nmol/l)	84	67.1	30.1				
Serum 25(OH)D LP (nmol/l)	94	77.1	34.9				
Alcohol consumption (units/ week)	95	3.5	(1-7)	126	7	(2.4-15)	**<0.0001**
Pre-menopausal	92/95	96.8%					
Previous fracture	40/95	42.1%		64/97	66.0%		**0.002**
≥ 0.5Pints of milk/day	61/104	58.7%		70/104	67.3%		**0.0002**
Strenuous activity/week (hours)	94	0.25	(0-1.5)	97	0.8	(0-5.3)	0.1
Previous used oral steroid	6/95	6.3%		1/98	1.0%		**0.03**
Ethnicity	95			98			
White	94	98.9%		97	99.0%		1.00
Non-white	1	1.1%		1	1.0%		
Social class	101			82			0.8
I	11	10.9%		13	15.9%		
II	41	40.6%		25	30.5%		
IIIN	32	31.7%		12	14.6%		
IIIM	6	5.9%		20	24.4%		
IV	9	8.9%		6	7.2%		
V	2	2.0%		6	7.3%		

EP = Early pregnancy
LP= Late pregnancy

Table 7.3: Baseline parental pQCT bone variables

	Mothers (n=104)			Fathers (n=104)			P value
	n	Mean/ median	SD / IQR	n	Mean/ median	SD / IQR	
pQCT variable							
4% Total bone area (mm^2)	104	1025.73	140.50	104	1282.37	192.89	**<0.0001**
4% Total bone density (mg/cm^3)	104	307.07	44.67	104	337.09	45.47	**<0.0001**
4% Trabecular density (mg/cm^3)	104	233.45	38.17	104	253.95	35.77	**0.0001**
38% Total bone area (mm^2)	104	382.48	45.61	104	494.38	5.89	**<0.0001**
38% cortical area (mm^2)	104	268.06	35.94	104	355.77	44.79	**<0.0001**
38% cortical thickness (mm)	104	4.63	0.58	104	5.41	0.56	**<0.0001**
38% cortical density (mg/cm^3)	104	1183.22	(1167.95-1197.09)	104	1162.52	(1147.61-1177.60)	**<0.0001**
38% periosteal circumference (mm)	104	72.61	5.48	104	82.83	5.96	**<0.0001**
38% endosteal circumference (mm)	104	43.50	6.52	104	48.82	6.52	**<0.0001**
38% medullary area (cm^2)	104	114.42	27.38	104	138.62	33.48	**<0.0001**
38% SSI	104	1460.96	266.61	104	2142.47	348.80	**<0.0001**

7.4.1.3 Characteristics of the participants compared to non-participating members of the SWS

In parallel with the DXA findings (Chapter 6), mothers who attended this phase of the SWS (n=104), compared to the rest of the SWS cohort (n=2845), were significantly older, taller and had lower BMI at the early pregnancy visit. Additionally, participating mothers were of higher social class and were less likely to have smoked in early or late pregnancy and were of higher social class. Late pregnancy serum 25(OH)D was significantly higher in those mothers who participated in this phase of the SWS compared to the rest of the group. There were no significant differences in ethnicity, triceps skinfold thickness in late

pregnancy and walking speed in late pregnancy; offspring birthweight was similar between the two groups.

7.4.2 Relationships between baseline characteristics and bone indices

7.4.2.1 Offspring baseline characteristics - offspring bone indices

Child height and age at pQCT demonstrated strong significant associations with pQCT derived bone indices at both tibial sites (Table 7.4). Child height demonstrated a significant positive association with bone area at the 4% site (β (95% CI) 77.12; 11.67-142.56); at the 38% site, significant positive associations were observed between child height and bone area, cortical area, medullary area, periosteal circumference, endosteal circumference and SSI; There were no significant associations between child height and any of the bone density measurements at either site.

With regards to child age at pQCT, a dichotomy was observed. Strong positive associations were seen between age and total bone area at the 4% and 38% sites, in addition to cortical area, cortical thickness, periosteal circumference and SSI measured at the 38% site, but significant negative associations were seen with age and total and trabecular vBMD at the 4% site [β (95% CI) -32.75 (-56.32,-9.18); -49.03 (-81.81, -16.25)]. As larger bones are inherently stronger and therefore do not need to be so densely mineralised, we investigated whether size attenuated the observed negative relationship between trabecular vBMD and age, however these associations were still observed after adjustment for either 38% periosteal circumference or endosteal circumference. The same associations were seen when the group was divided by sex.

Child birthweight was positively associated with bone area, medullary area, periosteal circumference and endosteal circumference, but the magnitude of the observed relationships was much less than that observed for age and height. Physical activity and milk intake were not consistently associated with any of the tibial pQCT variables.

Table 7.4: Relationships between child characteristics and child tibial pQCT measurements

		Child tibial bone mass – 4%			Child tibial bone mass – 38%							
Child characteristics	**n**	**Total bone area (mm^2)**	**Total vBMD (mg/cm^3)**	**Trabecular vBMD (mg/cm^3)**	**Total bone area (mm^2)**	**Cortical area (mg/cm^2)**	**Medullary area (mg/cm^2)**	**Cortical vBMD (mg/cm^3)**	**Cortical thickness (mm)**	**Periosteal circ. (mm)**	**Endosteal circ. (mm)**	**SSI**
		B (p value)	B (p value)	B (p value)	B (p value)	B (p value)	B (p value)	B (p value)	B (p value)	B (p value)	B (p value)	B (p value)
Age at pQCT (yr)	104	**77.12 (0.02)**	**–32.75 (0.01)**	**–49.03 (0.004)**	**39.07 (0.001)**	**22.97 (<0.001)**	16.09 (0.07)	–9.38 (0.43)	**0.30 (0.01)**	**4.66 (0.001)**	2.78 (0.07)	**90.14 (0.002)**
Birth weight (g)	102	0.23 (0.23)	–0.003 (0.63)	–0.002 (0.85)	**0.01 (0.03)**	0.003 (0.38)	**0.11 (0.03)**	–0.004 (0.55)	–0.0001 (0.42)	**0.002 (0.02)**	**0.002 (0.02)**	0.02 (0.22)
Height (cm)	99	**9.60 (<0.001)**	1.02 (0.14)	1.77 (0.07)	**3.49 (<0.001)**	**1.22 (<0.001)**	**2.27 (<0.001)**	–0.55 (0.44)	0.004 (0.54)	**0.42 (<0.001)**	**0.39 (<0.001)**	**8.93 (<0.001)**
Milk intake (pints per day)	103	–3.99 (0.89)	12.23 (0.23)	9.99 (0.48)	–9.49 (0.33)	0.42 (0.93)	–9.92 (0.21)	10.41 (0.32)	0.08 (0.40)	–1.09 (0.34)	–1.60 (0.23)	–29.74 (0.23)
Physical activity 9hr per day)	58	–0.50 (0.20)	–0.03 (0.78)	**–0.37 (0.03)**	–0.05 (0.73)	0.01 (0.88)	–0.06 (0.61)	0.10 (0.44)	0.001 (0.48)	–0.005 (0.73)	–0.01 (0.54)	0.29 (0.40)

Numbers are β coefficients from linear regression
Numbers in bold represent statistical significance ($p<0.05$)
Child variables adjusted for sex

7.4.2.2 **Maternal baseline characteristics - maternal bone indices**

Maternal height was positively associated with maternal tibial total bone area at both the 4% and 38% sites, and also with cortical area and SSI at the 38% site; there was no significant association between maternal height and any of the bone density measurements at either site (Table 7.5).

At the 4% tibial site, significant positive correlations were observed between the number of hours per day of moderate and vigorous exercise, and maternal total and trabecular vBMD, with vigorous exercise having a greater magnitude of association [moderate exercise β (95% CI) total vBMD exercise: 3.36 (0.79-5.94), trabecular vBMD: 3.34 (1.19-5.48); vigorous exercise β (95% CI) total vBMD; 6.60 (0.57-12.62), trabecular vBMD: 6.09 (1.03-11.14)]. Significant positive relationships were also observed between maternal triceps skinfold thickness in late pregnancy and maternal tibial periosteal and endosteal circumference at the 38% site [β (95% CI) PC: 0.27 (0.08-0.46), EC: 0.37 (0.14-0.59)].

There were significant correlations between maternal placental volume (measured at 19 weeks gestation) and maternal 38% tibial total bone area, cortical area, cortical thickness and SSI, which remained robust after adjusting for maternal height (p all <0.05). No significant relationships were observed between placental volume and maternal tibial vBMD.

7.4.2.3 **Paternal baseline characteristics - paternal bone indices**

In parallel with the findings observed with the SWS mothers, paternal height was strongly associated with paternal tibial bone area at both the 3% and 38% sites ($p<0.0001$, $p=0.004$; Table 7.6); strong positive associations with paternal height were also seen for cortical area, cortical thickness and SSI.

The only other measured paternal characteristic that demonstrated a significant association with paternal tibial bone mass was vigorous exercise; a greater number of hours per week of paternal vigorous exercise was positively associated with paternal total and trabecular BMD at the 4% site [β (95% CI) total vBMD: 4.09 (0.35-1.73), trabecular vBMD: 3.73 (0.74-6.72)] and cortical area (β (95% CI) 3.86 (0.03-7.69), periosteal circumference [β (95% CI) 0.59 (0.08-1.12)] and SSI [β (95% CI) 32.17 (1.31-63.04)] at the 38% site; the

relationship between moderate exercise and tibial bone mass did not achieve statistical significance. Paternal smoking, milk intake or social class also did not appear to be associated with tibial bone mass.

Table 7.5: Relationships between maternal characteristics and pQCT derived maternal tibial bone mass

		Maternal tibial bone mass - 4%			Maternal tibial bone mass - 38%							
Maternal characteristics	**n**	**Total bone area (mm^2)**	**Total vBMD (mg/cm^3)**	**Trab vBMD (mg/cm^3)**	**Total bone area (mm^2)**	**Cortical area (mg/cm^2)**	**Medull area (mg/cm^2)**	**Cort vBMD (mg/cm^3)**	**Cort thick (mm)**	**Periosteal circ. (mm)**	**Endosteal circ. (mm)**	**SSI**
		B (p value)	B (p value)	B (p value)	B (p value)	B (p value)	B (p value)	B (p value)	B (p value)	B (p value)	B (p value)	B (p value)
Triceps skinfold thickness (LP; mm)	99	2.55 (0.23)	0.71 (0.29)	0.79 (0.16)	1.36 (0.11)	0.47 (0.48)	0.88 (0.09)	-0.94 (0.05)	-0.02 (0.16)	**0.27 (0.01)**	**0.37 (0.02)**	-0.10 (0.98)
Smoking (LP)	99	21.62 (0.68)	-3.43 (0.84)	1.09 (0.94)	-16.91 (0.31)	-3.17 (0.81)	-13.74 (0.18)	21.89 (0.02)	0.17 (0.44)	-2.28 (0.25)	-3.33 (0.16)	-18.64 (0.85)
Current smoker	89	82.63 (0.10)	0.59 (0.97)	8.21 (0.54)	2.33 (0.90)	13.43 (0.32)	-11.11 (0.30)	12.52 (0.20)	0.23 (0.31)	0.71 (0.74)	-0.71 (0.78)	80.10 (0.43)
Walking speed (LP)	99	6.06 (0.76	2.31 (0.72)	-0.27 (0.96)	11.31 (0.08)	10.15 (0.05)	1.17 (0.77)	-4.91 (0.20)	0.13 (0.13)	0.95 (0.23)	0.16 (0.87)	59.37 (0.11)
Mod exercise (hours/ day)	95	-5.92 (0.18)	**3.36 (0.01)**	**3.34 (0.003)**	-1.40 (0.33)	-0.64 (0.57)	-0.76 (0.38)	0.51 (0.53)	-0.003 (0.89)	-0.14 (0.44)	-0.12 (0.56)	-3.31 (0.69)
Vigorous exercise (hours / day)	94	-5.05 (0.62)	**6.60 (0.03)**	**6.09 (0.02)**	3.23 (0.33)	4.47 (0.08)	-1.24 (0.54)	0.25 (0.90)	0.08 (0.06)	0.22 (0.06)	-0.28 (0.56)	32.83 (0.09)
Milk intake (pints/ day)	94	10.71 (0.80)	22.15 (0.08)	18.52 (0.08)	2.95 (0.84)	15.21 (0.16)	-12.26 (0.16)	4.70 (0.57)	0.28 (0.11)	0.78 (0.65)	-0.99 (0.63)	24.85 (0.77)
25(OH)D (LP; nmol/l)	94	0.07 (0.87)	0.07 (0.61)	0.07 (0.53)	-0.02 (0.89)	-0.02 (0.84)	0.003 (0.97)	-0.06 (0.49)	-0.001 (0.61)	0.004 (0.82)	0.01 (0.64)	-0.24 (0.76)
Parity	104	18.69 (0.50)	2.87 (0.75)	4.28 (0.57)	-8.73 (0.35)	-2.15 (0.77)	-6.59 (0.23)	1.47 (0.78)	0.05 (0.69)	-1.19 (0.29)	-1.48 (0.26)	-29.369 (0.58)
Social class	101	8.50 (0.49)	2.10 (0.59)	1.53 (0.65)	0.50 (0.91)	2.32 (0.48)	-1.82 (0.47)	2.36 (0.32)	0.05 (0.40)	0.12 (0.82)	-0.17 (0.78)	7.08 (0.77)
Age at pQCT (yr)	104	3.42 (0.40)	-1.46 (0.26)	-1.56 (0.16)	0.04 (0.98)	-0.13 (0.90)	0.17 (0.83)	0.09 (0.91)	0.003 (0.86)	-0.04 (0.79)	-0.06 (0.74)	2.93 (0.71)
Height (cm)	103	**7.74 (<0.0001)**	-0.92 (0.20)	-1.05 (0.09)	**2.12 (0.01)**	**1.51 (0.01)**	0.61 (0.19)	0.46 (0.29)	0.02 (0.08)	0.17 (0.07)	0.06 (0.57)	**15.66 (<0.0001)**
Placental volume (cm^3)	102	0.02 (0.74)	0.21 (0.36)	0.01 (0.97)	**0.54 (0.02)**	**0.74 (0.01)**	0.24 (0.53)	-0.35 (0.39)	**36.35 (0.04)**	2.81 (0.14)	0.16 (0.92)	**0.10 (0.01)**

Numbers are β coefficients from linear regression
Numbers in bold represent statistical significance ($p<0.05$)
Parity: 2 groups- nulliparous (reference), multiparous; Smoking: 2 groups- No (reference value), yes
Walking speed: 5 groups- very slow, easy paced stroll, normal speed, fairly brisk, fast
Social class: 6 groups- unskilled (v), partly skilled (IV), skilled manual (IIIM), skilled non-manual (IIIN), management and technical (II), professional (I)

Table 7.6: Relationships between paternal characteristics and pQCT derived paternal tibial bone mass

		Paternal tibial bone mass - 4%			Paternal tibial bone mass - 38%							
Paternal characteristics	**n**	**Total bone area (mm^2)**	**Total vBMD (mg/cm^3)**	**Trab vBMD (mg/cm^3)**	**Total bone area (mm^2)**	**Cortical area (mg/cm^2)**	**Medull area (mg/cm^2)**	**Cort vBMD (mg/cm^3)**	**Cort thick (mm)**	**Periosteal circ. (mm)**	**Endosteal circ. (mm)**	**SSI**
		B (p value)	B (p value)	B (p value)	B (p value)	B (p value)	B (p value)	B (p value)	B (p value)	B (p value)	B (p value)	B (p value)
Current smoker	92	66.03 (0.26)	-19.33 (0.16)	-13.31 (0.23)	-27.73 (0.12)	-24.45 (0.08)	-3.28 (0.75)	4.60 (0.52)	-0.12 (0.51)	-3.73 (0.05)	-2.99 (0.16)	-136.78 (0.23)
Mod exercise (hours / day)	96	-4.83 (0.22)	-0.73 (0.43)	-1.14 (0.12)	-0.99 (0.41)	-1.24 (0.18)	0.25 (0.72)	-0.17 (0.73)	-0.02 (0.10)	-0.05 (0.70)	0.07 (0.61)	-6.08 (0.42)
Vigorous exercise (hours / day)	97	-2.67 (0.75)	**4.09 (0.03)**	**3.73 (0.02)**	4.66 (0.06)	**3.86 (0.048)**	0.80 (0.59)	0.07 (0.94)	0.01 (0.59)	**0.60 (0.03)**	0.51 (0.09)	**32.17 (0.04)**
Milk intake (pints / day)	97	-22.77 (0.60)	3.75 (0.71)	9.32 (0.25)	-13.75 (0.30)	-7.32 (0.48)	-6.43 (0.40)	5.76 (0.28)	-0.001 (0.99)	-1.54 (0.28)	-1.53 (0.33)	-47.96 (0.57)
Social class	82	-0.86 (0.95)	-2.11 (0.57)	-2.60 (0.34)	-7.97 (0.07)	-3.67 (0.30)	-4.31 (0.09)	-1.41 (0.43)	-0.004 (0.92)	-0.71 (0.13)	-0.68 (0.19)	-38.03 (0.18)
Age at pQCT (yr)	104	3.13 (0.37)	-0.95 (0.24)	-0.67 (0.30)	-0.30 (0.77)	0.23 (0.77)	-0.53 (0.37)	-0.03 (0.95)	0.02 (0.14)	-0.11 (0.31)	-0.21 (0.09)	6.37 (0.32)
Height (cm)	104	**11.87 (<0.0001)**	-1.06 (0.11)	-0.49 (0.35)	**2.38 (0.004)**	**1.88 (0.004)**	0.50 (0.32)	0.26 (0.46)	**0.02 (0.03)**	0.18 (0.04)	0.07 (0.49)	**18.74 (<0.0001)**

Numbers are β coefficients from linear regression
Numbers in bold represent statistical significance ($p<0.05$)
Parity: 2 groups- nulliparous (reference), multiparous; Smoking: 2 groups- No (reference), yes
Social class: 6 groups- unskilled (v), partly skilled (IV), skilled manual (IIIM), skilled non-manual (IIIN), management and technical (II), professional (I)

7.4.3 **Parental non-bone characteristics and offspring bone indices**

The relationships between parental baseline characteristics and offspring bone mass are shown in Tables 7.7 and 7.8. None of the measured maternal non-bone factors characteristics consistent significant relationships across all of the offspring bone outcomes, however late pregnancy triceps skinfold thickness was positively associated with offspring total and medullary bone area the 38% site [β (95% CI), p value= 1.36 (0.30-2.42), 0.013 and 1.31 (0.44-2.18), 0.004 respectively].

Maternal 25(OH)D measured in late pregnancy was significantly negatively associated with 38% tibial total bone area, medullary area, periosteal circumference and endosteal circumference (n=78, p all ≤ 0.01); these negative relationships remained after adjustment for offspring height. When maternal BMI was added to the regression model significant negative associations remained between maternal late pregnancy 25(OH)D and offspring total bone area and periosteal circumference at the 38% site. No significant relationships were observed between maternal late pregnancy 25(OH)D and offspring bone variables at the 4% tibial site.

Placental volume in mid-pregnancy appeared to have a positive relationship with trabecular density at the 4% site (β (95% CI), p value= 0.11 (0.01-0.21), 0.04; significant relationships were not observed for any of the other measured offspring bone variables.

In contrast to the maternal association, where a null relationship was found between maternal height and offspring bone mass, paternal height was strongly associated with offspring bone size at the 38% site, demonstrating significant positive relationships with offspring total bone area, medullary area, periosteal and endosteal circumference and SSI; these associations were not observed at the 4% site.

Table 7.7: Relationships between maternal non-bone characteristics and child pQCT derived bone indices

		Child tibial bone mass – 4%			Child tibial bone mass – 38%							
Maternal characteristics	**n**	**Total bone area (mm^2)**	**Total vBMD (mg/cm^3)**	**Trabecular vBMD (mg/cm^3)**	**Total bone area (mm^2)**	**Cortical area (mg/cm^2)**	**Medullary area (mg/cm^2)**	**Cortical vBMD (mg/cm^3)**	**Cortical thickness (mm)**	**Periosteal circ. (mm)**	**Endosteal circ. (mm)**	**SSI**
		B (p value)	B (p value)	B (p value)	B (p value)	B (p value)	B (p value)	B (p value)	B (p value)	B (p value)	B (p value)	B (p value)
Triceps skinfold thickness (LP; mm)	84	2.64 (0.10)	−0.39 (0.48)	0.10 (0.90)	**1.36 (0.01)**	0.05 (0.84)	**1.31 (0.004)**	−0.95 (0.12)	−0.01 (0.10)	**1.16 (0.01)**	0.22 (0.07)	2.08 (0.13)
Smoking (LP)	99	55.83 (0.16)	−10.29 (0.46)	12.01 (0.54)	0.45 (0.98)	−2.29 (0.75)	2.74 (0.83)	−2.25 (0.90)	−0.08 (0.60)	0.11 (0.95	0.64 (0.77)	28.69 (0.45)
Walking speed (LP)	99	−4.99 (0.97)	−1.00 (0.85)	1.16 (0.88)	−6.59 (0.18)	−0.77 (0.74)	−5.82 (0.16)	2.10 (0.70)	0.04 (0.47)	−0.79 (0.18)	−1.02 (0.14)	−3.46 (0.78
25(OH)D (LP; nmol/l)	94	−0.31 (0.35)	−015 (0.20)	−0.18 (0.25)	**−0.32 (0.003)**	−0.08 (0.12)	**−0.24 (0.01)**	0.11 (0.38)	0.0003 (0.78)	**−0.04 (0.003)**	**−0.04 (0.01)**	−0.46 (0.09)
Parity	104	−5.48 (0.80)	−6.27 (0.41)	−5.23 (0.62)	3.17 (0.66)	−1.55 (0.64)	4.72 (0.42)	−0.48 (0.95)	−0.08 (0.28)	0.35 (0.68)	0.83 (0.40)	−9.50 (0.61)
Social class	101	1.42 (0.88)	−6.11 (0.06	−8.63 (0.06)	2.03 (0.53)	0.05 (0.97)	1.97 (0.45)	−1.44 (0.68)	−0.01 (0.71)	0.26 (0.49)	0.34 (0.45)	−5.75 (0.49
Age at pQCT (yr)	104	0.86 (0.78)	−3.11 (0.78)	0.98 (0.53)	−0.60 (0.57)	−0.78 (0.11)	0.18 (0.83)	1.51 (0.18)	−0.02 (0.10)	−0.78 (0.52)	0.03 (0.84)	−2.94 (0.29)
Height (cm)	103	1.14 (0.51)	−0.05 (0.94)	0.46 (0.60)	0.63 (0.28)	0.21 (0.43)	0.41 (0.39)	0.42 (0.51)	0.0003 (0.95)	0.08 (0.25)	0.08 (0.34)	2.17 (0.15)
Placental volume (cm^3)	102	0.05 (0.67)	0.06 (0.10)	**0.11 (0.04)**	0.01 (0.84)	0.001 (0.97)	0.01 (0.83)	−0.01 (0.78)	−0.0001 (0.77)	0.001 (0.81)	0.002 (0.74)	0.04 (0.66)

Numbers are β coefficients from linear regression; Numbers in bold represent statistical significance ($p<0.05$)
Offspring variables adjusted for sex and age at pQCT
LP = late pregnancy
Parity: 2 groups- nulliparous (reference), multiparous; Smoking: 2 groups- no (reference), yes
Walking speed: 5 groups- very slow, easy paced stroll, normal speed, fairly brisk, fast
Social class: 6 groups- unskilled (v), partly skilled (IV), skilled manual (IIIM), skilled non-manual (IIIN), management and technical (II), professional (I)

Table 7.8: Relationships between paternal non-bone characteristics and child pQCT derived bone mass variables

		Child tibial bone mass – 4%			Child tibial bone mass – 38%							
Paternal characteristics	**n**	**Total bone area (mm^2)**	**Total vBMD (mg/cm^3)**	**Trabecular vBMD (mg/cm^3)**	**Total bone area (mm^2)**	**Cortical area (mg/cm^2)**	**Medullary area (mg/cm^2)**	**Cortical vBMD (mg/cm^3)**	**Cortical thickness (mm)**	**Periosteal circ. (mm)**	**Endosteal circ. (mm)**	**SSI**
		B (p value)	B (p value)	B (p value)	B (p value)	B (p value)	B (p value)	B (p value)	B (p value)	B (p value)	B (p value)	B (p value)
Age at pQCT (yr)	104	0.84 (0.66)	−0.3 (0.66)	−0.33 (0.73)	−0.50 (0.43)	−0.11 (0.70)	−0.39 (0.45)	−0.05 (0.95)	0.0003 (0.96)	−0.06 (0.45)	−.06 (0.50)	−0.44 (0.79)
Height (cm)	104	2.52 (0.11)	0.45 (0.43)	0.74 (0.34)	**1.60 (0.002)**	0.35 (0.15)	**1.25 (0.003)**	−0.47 (0.41)	−0.003 (0.55)	**0.19 (0.002)**	**0.21 (0.003)**	**2.81 (0.04)**

Numbers are β coefficients from linear regression; Numbers in bold represent statistical significance ($p<0.05$)
Offspring variables adjusted for sex and age at pQCT

7.4.4 **Maternal-offspring bone mass associations**

At the 4% site, the strongest association seen was between maternal and offspring total tibial bone area [β (95% CI), p value = 0.27 (0.12-0.41)), <0.0001]; this remained robust after adjustment for several other possible associated factors including maternal height, triceps skinfold thickness in late pregnancy, late pregnancy 25(OH)D and offspring height [β (95% CI)=0.29 (0.15-0.43)]. No other significant maternal-offspring relationships were observed at this site (Table 7.9).

The strongest significant association seen at the 38% site, was between maternal and offspring cortical BMD [Tables 7.10a and 7.10b; β (95% CI) = 0.34 (0.03-0.65), p=0.03]. Positive trends were also observed across all the remaining maternal and offspring variables at this site, however significant associations were only seen for maternal-child endosteal circumference and SSI. After adjustment for offspring and maternal height, the maternal-offspring SSI relationship remained significant, but the relationship between maternal and offspring endosteal circumference failed to achieve statistical significance (β (95% CI) = 0.09 (-0.04-0.25), p=0.25).

Table 7.9: Relationships between pQCT derived measurements of maternal and child bone – 4% tibial site

	Child Total bone area (mm^2)		**Child Total vBMD (mg/cm^3)**		**Child Trabecular vBMD (mg/cm^3)**	
Maternal	β1 (95% CI) p value	β2 (95% CI) p value	β1(95% CI) p value	β2 (95% CI) p value	β1 (95% CI) p value	β2 (95% CI) p value
Total bone area (mm^2)	**0.41 (0.12-0.61) <0.0001**	**0.42 (0.13-0.62) <0.0001**	-0.01 (-0.06-0.05) 0.78	-0.01 (-0.06-0.05) 0.85	0.04 (-0.03-0.12) 0.24	0.05 (-0.03-0.12) 0.19
Total vBMD (mg/cm^3)	-0.22 (-0.69-0.25) 0.36	-0.22 (-0.69-0.26) 0.37	0.11 -0.06-0.28 0.19	0.10 (-0.07-0.27) 0.23	0.02 (-0.21-0.26) 0.85	0.01 (-0.22-0.25) 0.92
Trabecular vBMD (mg/cm^3)	-0.15 (-0.70-0.40) 0.60	-0.15 (-0.70-0.41) 0.60	0.13 -0.06-0.33 0.19	0.13 (-0.06-0.33) 0.19	0.03 (-0.24-0.31) 0.81	0.03 (-0.24-0.31) 0.81

Child variables adjusted for age at pQCT and sex

Number are β coefficients (95% CI), p value. Shaded boxes = corresponding bone indices

B1: unadjusted; β2: adjusted for the corresponding father's variable

Table 7.10a: Relationships between pQCT derived measurements of maternal and child bone - 38% tibial site

	Child									
	Total bone area (mm^2)		**Cortical area (mm^2)**		**Medullary area (mm^2)**		**Periosteal circum (mm)**		**Endosteal circum (mm)**	
Maternal	β1 (95% CI) p value	β2 (95% CI) p value	β1 (95% CI) p value	β2 (95% CI) p value	β1 (95% CI) p value	β2 (95% CI) p value	β1 (95% CI) p value	β2 (95% CI) p value	β1 (95% CI) p value	β2 (95% CI) p value
Total bone area (mm^2)	0.11 (-0.38-0.26) 0.14	0.10 (-0.04-0.25) 0.16	0.05 (-0.18-0.13) 0.14	0.05 (-0.02-0.13) 0.17	0.06 (-0.06-0.18) 0.35	0.05 (-0.06-0.17) 0.35	0.13 (-0.004-0.03) 0.12	0.01 (-0.004-0.03) 0.14	0.01 (-0.01-0.03) 0.27	0.01 (-0.01-0.03) 0.28
Cortical area (mm^2)	0.06 (-0.13-0.25) 0.51	0.04 (-0.15-0.23) 0.65	0.06 (-0.03-0.15) 0.18	0..05 (-0.04-0.15) 0.25	0.001 (-0.15-0.16) 0.98	-0.01 (-0.16-0.14) 0.88	0.01 (-0.01-0.03) 0.48	0.01 (-0.02-0.03) 0.62	0.001 (-0.03-0.03) 0.93	-0.001 (-0.03-0.02) 0.94
Medullary area (mm^2)	0.22 (-0.04-0.49) 0.09	0.20 (-0.06-0.47) 0.13	0.05 (-0.08-0.18) 0.46	0.05 (-0.08-0.18) 0.47	0.18 (-0.04-0.39) 0.10	0.15 (-0.05-0.35) 0.13	0.03 (-0.003-0.06) 0.08	0.03 (-0.01-0.06) 0.11	0.03 (-0.002-0.07) 0.07	0.03 (-0.005-0.06) 0.09
Cortical vBMD (mg/cm^3)	-0.05 (-0.32-0.21) 0.69	-0.10 (-0.37-0.16) 0.43	0.01 (-0.11-0.14) 0.85	0.02 (-0.11-0.15) 0.73	-0.07 (-0.28-0.15) 0.54	-0.13 (-0.33-0.07) 0.21	-0.01 (-0.04-0.03) 0.70	-0.01 (-0.04-0.02) 0.46	-0.01 (-0.05-0.02) 0.52	-0.02 (-0.06-0.01) 0.24
Cortical thickness (mm)	-4.10 (-16.50-8.29) 0.52	-5.17 9-17.61-7.270 0.41	1.65 (-4.33-7.64) 0.58	0.33 9-5.70-6.370 0.91	-5.76 (-15.69-4.17) 0.25	-5.50 9-14.97-3.960 0.25	-0.50 (-1.97-0.96) 0.50	-0.62 (-2.10-0.86) 0.40	-1.02 (-2.72-0.67) 0.23	-0.97 (-2.61-0.68) 0.25
Periosteal circum (mm)	1.14 (-0.14-2.42) 0.08	1.12 (-0.14-2.38) 0.08	0.46 (-0.16-1.08) 0.15	0.44 (-0.20-1.08) 0.18	0.68 (-0.35-1.72) 0.19	0.68 (-0.28-1.64) 0.16	0.14 (-0.01-0.29) 0.06	0.14 (-0.01-0.29) 0.07	0.13 (-0.04-0.31) 0.14	0.13 (-0.04-0.30) 0.12
Endosteal circum (mm)	1.13 (-0.003-2.26) 0.05	1.09 (-0.03-2.20) 0.06	0.27 (-0.28-0.83) 0.33	0.28 (-0.29-0.84) 0.33	0.86 (-0.06-1.77) 0.07	0.81 (-0.02-1.65) 0.06	**0.14 (0.01-0.27) 0.04**	**0.14 (0.003-0.27) 0.045**	**0.16 (0.005-0.32) 0.04**	**0.15 (0.01-0.30) 0.04**
SSI	0.01 (-0.02-0.03) 0.53	0.01 (-0.02-0.03) 0.59	0.01 (-0.004-0.02) 0.20	0.01 (-0.004-0.02) 0.21	0.0001 (-0.02-0.02) 0.99	-0.001 (-0.02-0.02) 0.91	0.001 (-0.002-0.004) 0.50	0.001 (-0.002-0.004) 0.56	0.0001 (-0.003-0.004) 0.92	-0.00003 (-0.003-0.003) 0.99

Child variables adjusted for age at pQCT and sex

Number are β coefficients (95% CI), p value. Shaded boxes = corresponding bone indices

B1: unadjusted; β2: adjusted for the corresponding father's variable

Table 7.10b: Relationships between pQCT derived measurements of maternal and child bone - 38% tibial site

	Child					
	Cortical vBMD (mg/cm³)		Cortical thickness (mm)		SSI	
Maternal	β1 (95% CI) p value	β2 (95% CI) p value	β1 (95% CI) p value	β2 (95% CI) p value	β1 (95% CI) p value	β2 (95% CI) p value
Total bone area (mm²)	-0.30 (-0.20-0.14) 0.72	-0.03 (-0.21-0.14) 0.69	0.0004 (-0.01-0.02) 0.64	0.0004 (-0.001-0.002) 0.64	**0.67** **(0.29-1.06)** **0.0001**	**0.62** **(0.23-1.00)** **0.002**
Cortical area (mm²)	0.03 (-0.18-0.24) 0.75	0.03 (-0.19-0.25) 0.79	0.001 (-0.001-0.003) 0.28	0.001 (-0.001-0.003) 0.89	**0.56** **(0.05-1.07)** **0.03**	0.46 (-0.05-0.98) 0.08
Medullary area (mm²)	-0.16 (-0.46-0.13) 0.28	-0.14 (-0.44-0.17) 0.37	-0.001 (-0.004-0.002) 0.49	-0.001 (-0.003-0.002) 0.60	**0.97** **(0.29-1.65)** **<0.01**	**0.85** **(0.16-1.55)** **0.02**
Cortical vBMD (mg/cm³)	**0.31** **(0.03-0.59)** **0.03**	**0.29** **(0.01- 0.58)** **0.046**	0.001 (-0.002-0.004) 0.51	0.001 (-0.001-0.004) 0.28	-0.08 (-0.78-0.62) 0.82	-0.15 (-0.85-0.56) 0.68
Cortical thickness (mm)	6.01 (-7.64-19.67) 0.38	4.79 9-9.41-18.990 0.50	0.08 (-0.04-0.21) 0.20	0.06 (-0.07-0.18) 0.37	3.96 (-29.39-37.32) 0.81	-3.35 (-36.65-29.94) 0.84
Periosteal circum (mm)	-0.08 (-1.52-1.35)	-0.12 (-1.61-1.36) 0.87	0.002 (-0.01-0.02) 0.81	0.001 (-0.01-0.01) 0.83	**5.60** **(2.21-8.97)** **0.001**	**5.15** **(1.71-8.59)** **0.004**
Endosteal circum (mm)	-0.40 (-1.67-0.88) 0.54	-0.38 (-1.69-0.93) 0.57	-0.003 (-0.02-0.01) 0.59	-0.003 (-0.01-0.01) 0.65	**4.04** **(1.03-7.06)** **<0.01**	**3.78** **(0.72-6.83)** **0.02**
SSI	0.003 (-0.02-0.03) 0.81	0.003 (-0.03-0.03) 0.85	0.0001 (-0.0001-0.0003) 0.31	0.0001 (-0.0001-0.0004) 0.27	**0.08** **(0.01-0.15)** **0.02**	**0.07** **(0.01-0.14)** **0.03**

Child variables adjusted for age at pQCT

Number are β coefficients (95% CI), p value. Shaded boxes = corresponding bone indices

β1: unadjusted; β2: adjusted for the corresponding father's variable

7.4.5 Paternal-offspring bone associations

In contrast to the described mother-child relationships, there were no significant associations observed between any of the paternal and offspring tibial bone variables at the 4% site (Table 7.11).

At the 38% site, a strong positive association was seen between paternal and offspring cortical density [β 995% CI); p value = 0.34 (0.03-0.65); 0.03] (Tables 7.12a and 7.12b). Weaker, positive relationships were also observed for paternal-offspring cortical thickness, endosteal circumference and SSI [β (95% CI); p value = 0.18 (0.05-0.3); 0.01; 0.17 (0.02-0.32); 0.03; 0.04 (0.01-0.10); 0.01]. After adjustment for potential confounders (paternal height, child height and paternal vigorous activity) significant relationships between father-offspring endosteal circumference and SSI were no longer seen, however the relationship between father-child cortical thickness remained [β (95% CI); p value= 0.2 (0.06-0.34); 0.005].

7.4.5.1 Independent relationships between parent and child bone indices

To help establish the independent relationships between parental and offspring bone mass, the same statistical technique used for the previously described parent-child DXA relationships (Chapter 6) was performed, i.e. a regression model was fitted with the other parent's corresponding bone variable as a covariate. This had very little effect on the observed mother-child relationships with significant positive relationships remaining for 4% tibial total area and 38% endosteal circumference and SSI (Tables.7.9, 7.10a, 7.10b). Similarly when maternal bone mass was incorporated into the paternal-offspring regression model, there was very little change in the strength of the observed father-child relationships (Tables 7.11, 7.12a, 7.12b; Figure 7.2).

Table 7.11 Relationships between pQCT derived measurements of paternal and child bone – 4% tibial site

	Child Total bone area (mm^2)		**Child Total vBMD (mg/cm^3)**		**Child Trabecular vBMD (mg/cm^3)**	
Paternal	β1 (95% CI) p value	β2 (95% CI) p value	β1(95% CI) p value	β2 (95% CI) p value	β1 (95% CI) p value	β2 (95% CI) p value
Total bone area (mm^2)	0.01 (-0.10-0.12) 0.80	-0.02 (-0.13-0.08) 0.69	-0.01 (-0.05-0.03) 0.59	-0.01 (-0.05-0.03) 0.63	-0.01 (-0.07-0.04) 0.58	-0.02 (-0.08-0.03) 0.44
Total vBMD (mg/cm^3)	-0.03 (-0.49-0.44) 0.90	-01 (-0.48-0.46) 0.96	0.14 (-0.03-0.30) 0.1	0.13 (-0.36-0.29) 0.12	0.10 (-0.11-0.31) 0.33	0.12 (-0.11-0.35) 0.29
Trabecular vBMD (mg/cm^3)	-0.19 (-0.78-0.40) 0.52	-0.19 (-0.78-0.40)	0.10 (-0.11-0.31) 0.33	0.10 (-0.11-0.31) 0.34	-0.03 (-0.32-0.26) 0.84	-0.03 (-0.32-0.26) 0.84

Child variables adjusted for age at pQCT

Number are β coefficients (95% CI), p value. Shaded boxes = corresponding bone indices

β1: unadjusted; β2: adjusted for the corresponding mother's variabl

Table 7.12a: Relationships between pQCT derived measurements of paternal and child bone - 38% tibial site

	Child									
	Total bone area (mm^2)		Cortical area (mm^2)		Medullary area (mm^2)		Periosteal circum (mm)		Endosteal circum (mm)	
Paternal	β1 (95% CI) p value	β2 (95% CI) p value	β1 (95% CI) p value	β2 (95% CI) p value	β1 (95% CI) p value	β2 (95% CI) p value	β1 (95% CI) p value	β2 (95% CI) p value	β1 (95% CI) p value	β2 (95% CI) p value
Total bone area (mm^2)	0.09 (-0.03-0.22) 0.15	0.10 (-0.02-0.23) 0.11	0.03 (-0.03-0.09) 0.33	0.02 (-0.04-0.09) 0.77	0.06 (-0.04-0.16) 0.21	0.08 (-0.02-0.17) 0.12	0.01 (-0.004-0.03) 0.14	0.01 (-0.003-0.03) 0.11	0.01 (-0.01-0.03) 0.19	0.01 (-0.003-0.03) 0.11
Cortical area (mm^2)	0.08 (-0.08-0.25) 0.32	0.10 (-0.07-0.27) 0.25	0.06 (-0.02-0.14) 0.16	0.05 (-0.03-0.13) 0.24	0.03 (-0.11-0.16) 0.70	0.05 (-0.08-0.18) 0.46	0.01 (-0.01-0.03) 0.33	0.01 (-0.01-0.03) 0.26	0.005 (-0.02-0.03) 0.69	0.01 (-0.01-0.03) 0.48
Medullary area (mm^2)	0.15 (-0.08-0.39) 0.20	0.12 (-0.11-0.36) 0.30	-0.01 (-0.13-0.11) 0.86	-0.03 (-0.15-0.09) 0.63	0.16 (-0.18-0.34) 0.08	0.15 (-0.03-0.33) 0.10	0.02 (-0.01-0.05) 0.18	0.02 (-0.01-0.04) 0.29	0.03 (-0.001-0.06) 0.06	0.03 (-0.004-0.06) 0.09
Cortical vBMD (mg/cm^3)	-0.04 (-0.32-0.25) 0.80	-0.08 (-0.37-0.21) 0.58	-0.01 (-0.15-0.13) 0.92	-0.02 (-0.16-0.13) 0.80	-0.03 (-0.25-0.19) 0.79	-0.06 (-0.29-0.16) 0.58	-0.005 (-0.04-0.03) 0.77	-0.01 (-0.04-0.02) 0.57	-0.01 (-0.05-0.03) 0.70	-0.01 (-0.05-0.03) 0.51
Cortical thickness (mm)	-0.43 (-13.98-13.12) 0.95	3.00 (-11.45-17.44) 0.68	6.37 (-0.16-12.90) 0.056	**7.37 (0.36-14.38) 0.04**	-6.80 (-17.27-3.66) 0.20	-4.37 (-15.36-6.62) 0.43	-0.65 (-1.67-1.54) 0.94	0.35 (-1.36-2.07) 0.68	-1.19 (-3.00-0.62) 0.20	-0.76 (-2.67-1.15) 0.43
Periosteal circum (mm)	0.91 (-0.32-2.15) 0.15	0.99 (-0.26-2.25) 0.12	0.11 (-0.51-0.72) 0.73	0.02 (-0.62-0.66) 0.95	0.81 (-0.15-1.77) 0.10	**0.97 (0.01- 1.93) 0.047**	0.11 (-0.04-0.25) 0.15	0.12 (-0.03-0.27) 0.13	0.14 (-0.03-0.31) 0.10	0.17 (-0.001-0.33) 0.05
Endosteal circum (mm)	0.78 (-0.35-1.91) 0.17	0.73 (-0.46-1.93) 0.23	-0.20 (-0.76-0.36) 0.49	-0.36 (-0.96-0.25) 0.25	**0.98 (0.11-1.85) 0.03**	**1.09 (0.19- 1.98) 0.02**	0.09 (-0.04-0.23) 0.17	0.08 (-0.06-0.23) 0.24	**0.17 (0.02-0.32) 0.03**	**0.19 (0.03-0.34) 0.02**
SSI	0.01 (-0.01-0.03) 0.25	0.02 (-0.01-0.04) 0.15	0.01 (-0.005-0.01) 0.30	0.01 (-0.004-0.02) 0.26	0.01 (-0.01-0.02) 0.41	0.01 (-0.01-0.03) 0.24	0.001 (-0.001-0.004) 0.25	0.002 (-0.001-0.004) 0.14	0.001 (-0.002-0.004) 0.40	0.002 (-0.001-0.004) 0.24

Child variables adjusted for age at pQCT

Number are β coefficients (95% CI), p value. Shaded boxes = corresponding bone indices

β1: unadjusted; β2: adjusted for the corresponding mother's variable

Table 7.12b: Relationships between pQCT derived measurements of paternal and child bone - 38% tibial site

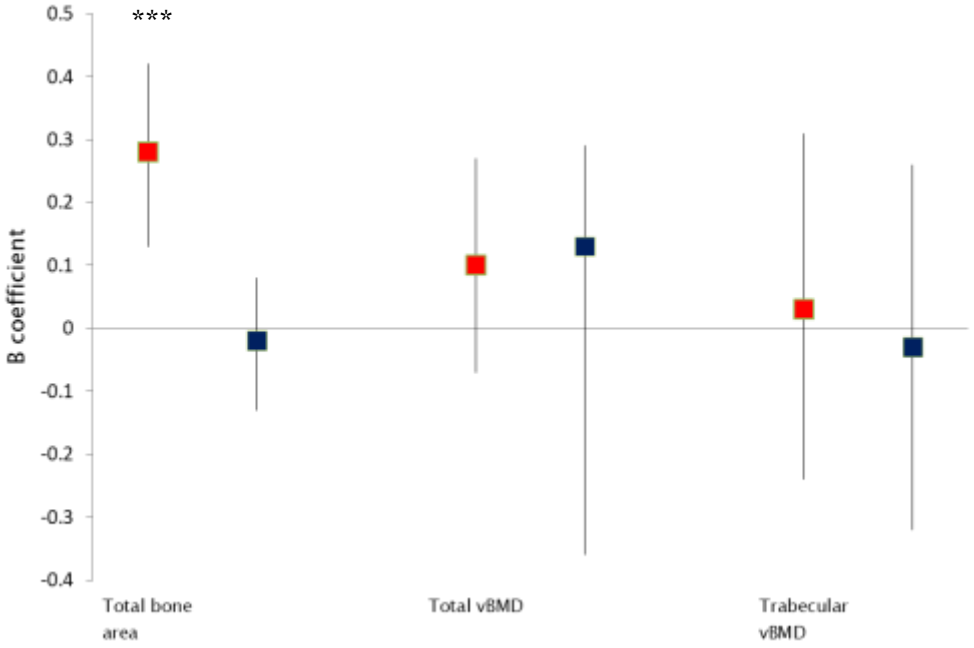
	Child					
	Cortical vBMD (mg/cm³)		Cortical thickness (mm)		SSI	
Paternal	β1 (95% CI) p value	β2 (95% CI) p value	β1 (95% CI) p value	β2 (95% CI) p value	β1 (95% CI) p value	β2 (95% CI) p value
Total bone area (mm²)	-0.04 (-0.18-0.10) 0.59	-0.05 (-0.20-0.10) 0.51	-0.00003 (-0.001-0.001) 0.97	0.0002 (-0.002-0.001) 0.72	0.31 (-0.02-0.65) 0.07	0.31 (-0.01-0.63) 0.06
Cortical area (mm²)	0.02 (-0.17-0.21) 0.82	0.01 (-0.19-0.20) 0.94	0.001 (-0.001-0.003) 0.33	0.001 (-0.001-0.002) 0.55	0.34 (-0.10-0.78) 0.13	0.30 (-0.15-0.74) 0.19
Medullary area (mm²)	-0.18 (-0.44-0.08) 0.18	-0.17 (-0.45-0.10) 0.22	-0.002 (-0.004-0.001) 0.15	-0.002 (-0.004-0.001) 0.12	0.39 (-0.24-1.02) 0.22	0.29 (-0.35-0.92) 0.37
Cortical vBMD (mg/cm³)	**0.34 (0.03-0.65) 0.03**	**0.34 (0.02- 0.66) 0.04**	0.0004 (-0.003-0.003)	0.0005 (-0.003-0.003) 0.77	-0.13 (-0.92-0.66) 0.74	-0.29 (-1.12-0.54) 0.49
Cortical thickness (mm)	6.99 (-8.06-22.04) 0.36	5.86 (-10.63-22.34) 0.48	**0.18 (0.05-0.3) 0.01**	**0.18 (0.03-0.32) 0.02**	27.38 (-8.27-63.04) 0.13	33.44 (-4.82-71.70) 0.09
Periosteal circum (mm)	-0.29 (-1.68-1.11) 0.69	-0.04 (-1.88-1.09) 0.60	-0.01 (-0.02-0.01) 0.42	-0.01 (-0.02-0.01) 0.23	1.41 (-1.90-4.73) 0.40	1.18 (-2.10-4.46) 0.48
Endosteal circum (mm)	-0.55 (-1.82-0.72) 0.39	-0.66 (-2.06-0.75) 0.36	**-0.01 (-0.02- 0.001) 0.03**	**-0.02 (-0.03- -0.004) 0.01**	-0.04 (-3.08-3.00) 0.98	-0.71 (-3.90-2.47) 0.66
SSI	0.002 (-0.02-0.02) 0.88	0.001 (-0.02-0.02) 0.97	0.00004 (-0.0002-0.0002) 0.70	0.00003 (-0.0002-0.0002) 0.80	0.04 (-0.01-0.10) 0.10	**0.06 (0.003-0.11) 0.04**

Child variables adjusted for age at pQCT

Number are β coefficients (95% CI), p value. Shaded boxes = corresponding bone indices

β1: unadjusted; β2: adjusted for the corresponding mother's variable

a: 4% tibial site



b: 38% tibial site

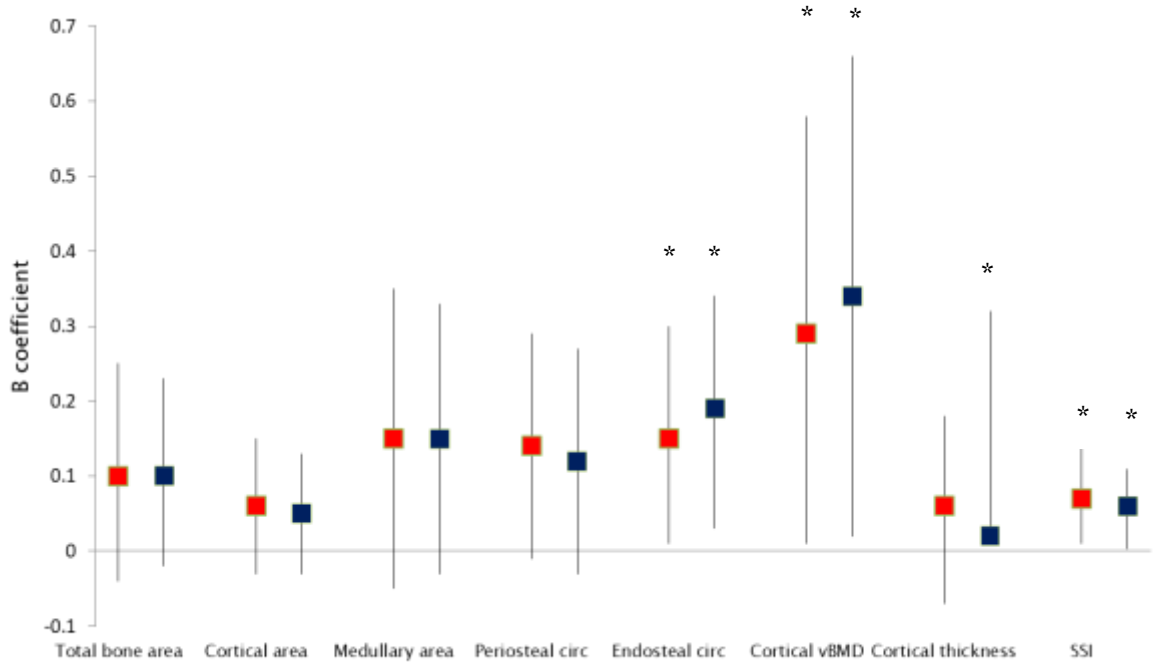


Figure 7.2: Relationships between parental and offspring tibial bone mass at the 4% site (a) and 38% site (b)

Shown as β coefficient (95% CI) after adjustment for corresponding parent variable
Red box denotes mother-child associations; blue box denotes father-child associations
* p<0.05; **p<0.01, ***p<0.001

7.4.5.2 Differences in parental bone relationships with childhood bone

The regression co-efficients from any analysis that demonstrated a significant relationship with both mother-child and father-child were compared, to determine whether there was any significant difference in the parental relationships with offspring bone; only 3 variables fulfilled these criteria: cortical density, endosteal circumference and SSI. No significant differences in the magnitude of relationships between maternal-child versus paternal-child were seen for any of the variables (p= 0.62-0.81). This approach was not used to compare regression co-efficients for parent-offspring 4% total bone area, as whilst this was highly significant for mother-child, did not achieve statistical significance for father-child. Likewise, cortical thickness at the 38% site was significantly associated between father and child, but not between mother and child, thus regression co-efficients were not compared.

7.4.5.3 Differences in parental according to offspring sex

To determine whether the parental effect on offspring bone mass differed according to the sex of the child, a univariate analysis incorporating a sex interaction term was performed. Using this method, no significant sex interaction was observed across any of the bone variables at either the 4% or the 38% tibial site.

7.5 Summary of findings

Both maternal and paternal tibial bone mass were shown to be independently associated with offspring tibial bone mass at age 7 years for some, but not all, size and density measurements; the largest significant association found was between maternal-child total tibial area at the 4% site; a similar significant association was not observed between father and child.

Different parental relationships were observed with offspring bone size and bone density at the two tibial sites measured. At the 4% site, whilst maternal total area was strongly independently related to offspring total bone area ($p<0.001$), parental vBMD was not found to be significantly associated with offspring vBMD. However, at the 38% site, significant parent-child trends were seen for both bone density and size. Independent positive relationships of similar magnitude were observed between mother-child and father-child for cortical vBMD, as well as tibial endosteal circumference and SSI.

Previously identified maternal predictors of offspring bone mass such as maternal height, walking speed and smoking in late pregnancy, were not significantly related to any of the child tibial bone outcomes. Maternal triceps skinfold thickness in late pregnancy was positively associated with some measurements of offspring bone size at the 38% tibial site (total bone area, medullary area and periosteal circumference).

Mid-pregnancy placental volume was significantly associated with offspring trabecular vBMD at the 4% site; whilst positive trends were observed for other measures of offspring bone size and density these did not achieve statistical significance.

These findings suggest that whilst the greatest parental association with offspring bone is primarily with bone size, there is also a positive relationship with volumetric bone density. The differential maternal and paternal association seen at the 4% site may reflect in-utero influences on child bone size, and possible underlying mechanisms are discussed win Chapter 8.

8. Discussion

8.1 Main findings

This work has explored several objectives in relation to the parental relationships with offspring bone using two large mother-offspring cohorts – ALSPAC and SWS. This has generated a number of novel and interesting findings.

1. Placental size measured in mid-pregnancy is positively associated with offspring bone mass at birth, with a larger association on bone size than bone density.

2. The positive association between placental size and offspring bone size persists through childhood into late adolescence.

3. There appears be a disparity in the observed placental relationships with later childhood/ adolescence bone mass, with a positive association with bone size, but a negative association with cortical volumetric density.

4. Previously identified maternal determinants of offspring bone mass (maternal serum 25(OH)D, late pregnancy triceps skinfold thickness, walking speed in late pregnancy and smoking in late pregnancy) are not related to placental size, raising the possibility that their effect is through modification of placental function rather than size.

5. Parental bone mass has a strong independent association with offspring bone mass when assessed by both DXA and pQCT; the strongest relationships are with parent-child bone size over parent-child bone density.

6. There appears to be a differential parent-child bone association, with significantly larger effect sizes observed for maternal- than paternal-offspring relationships for several DXA derived variables such as WB BA, hip BA, all spine indices, and total bone area of the 4% tibia site.

7. The observed parent-child bone associations are independent of placental size.

8.2 Relationships between placental size and offspring bone

8.2.1 Placental size and offspring bone size

Larger bones in early life are likely to lead to larger, stronger bones in older adulthood, which reduces the risk of osteoporosis and fracture in later life (2). Several parental factors have been previously demonstrated to influence childhood bone mass (6;164). Understanding the relationships between the placenta and offspring bone mass is important in trying to understand possible mechanisms whereby factors in pregnancy such as maternal diet, smoking, physical activity and vitamin D may influence offspring bone development.

Using data from two large observational UK cohorts, placental size was positively associated with offspring bone size. In the SWS cohort, mid-pregnancy placental area and volume (estimated from static ultrasound images obtained at 19 weeks gestation) were positively associated with neonatal DXA-derived BA and BMC. In the ALSPAC cohort, placental area and volume (measured post-delivery) were positively associated with cortical area, endosteal circumference and periosteal circumference at the tibia when measured by pQCT at a site 50% along its total length in children aged 15.5 years. These associations remained after adjusting for pubertal status, and although the magnitude was much attenuated, the direction of associations was maintained in the same cohort over 2 years later at age 17.7 years. Although one cannot determine from these studies that a larger placenta directly causes greater offspring bone mass, these findings may help to understand the possible

mechanisms underpinning the influence of prenatal factors on offspring bone mass.

Several studies have investigated the association between the placenta and offspring birthweight, but this analysis is the first to examine the relationship between placenta size and offspring bone size. Placental volume measured by 3D ultrasound scanning in the first trimester was positively associated with birth weight in one study of 199 women (222). Likewise, placental volume derived from ultrasound measurements in mid-pregnancy and by direct measurement at birth have been shown to correlate positively with birth weight (223-226). The rate of placental growth appears to be an important determinant of birth weight, with the rate between 17 to 20 weeks gestation being a predictor of fetal abdominal and head circumference, femoral length and biparietal diameter; weaker associations are observed for placental growth earlier in pregnancy (at 14-17 weeks) (227).

The mechanisms that might underlie the observed associations between placental size and offspring bone development are poorly characterized, however there are several theories that may explain this observation. The first is that the relationship between placental and offspring size is through shared determinants of placental size and bone indices. For example, mothers with higher BMC are likely to have children with higher BMC through direct genetic inheritance, and additionally will have larger placentas, as BMC and placental volume are positively correlated to maternal size (228). Taller mothers are also more likely to have a greater pelvic diameter, thus allowing for more space for placenta and baby to grow without constraint.

Certainly, in the SWS cohort it was observed that placental size is positively related to maternal height, however the relationships between both placental volume and cross-sectional area and neonatal BA and BMC remained after adjusting for maternal height, suggesting that maternal height is not the major driver of this relationship. Clearly, maternal height is only one aspect of maternal size, and it is possible that other measures of maternal size do attenuate the placenta: neonatal bone size relationships. Using subsequent parental DXA data, after maternal BMC was added to the model, although the direction of relationships were maintained, the magnitude of the placenta-offspring bone associations were significantly reduced and no longer achieved

the pre-defined cut-off for statistical significance (p values for placenta-offspring: BA = 0.06, BMC = 0.10; BMD 0.68).

A second hypothesis is that the mechanisms that underpin these relationships comprise of direct effects of the placenta on long-term postnatal growth trajectories. There is scant evidence to inform this hypothesis, although previous studies have found significant associations between patterns of intrauterine growth and postnatal skeletal development (229-231), early growth, adult hip morphology (148;232), and risk of hip fracture (4;233); and positive relationships between expression of placental calcium transporters and offspring BMC at birth (52).

Previous studies using data from mother-child cohorts including SWS, Birthright and The Princess Anne Cohort have found that certain maternal factors are positively associated with offspring bone size including maternal serum 25(OH)D, late pregnancy fat stores (assessed by triceps skinfold thickness) and physical activity (assessed by self-categorisation of walking speed (6;163;164). In this analysis, the relationship between placental size and childhood bone size did not appear to be influenced by these maternal factors, suggesting that they do not exert their effects on the offspring via an increase in placental size; a possible explanation may be that these factors modulate aspects of placental function, such as utero-placental blood flow or maternal nutrient concentrations. This concept is further strengthened by findings from the SWS 8 year parent study. Here, stronger bone relationships were observed between mother-child compared with father-child. These associations remained robust after adjustment for placenta size, and one could theorise that intra-uterine environmental effects acting on placental function is the potential mechanism.

In this study utero-placental blood flow or maternal nutrient concentrations in pregnancy have not been measured, however in an attempt to explore this theory further, placental “efficiency” was calculated in the SWS cohort, by dividing offspring birthweight by placental volume; a placenta of low volume associated with a child of high birthweight would be considered to be an efficient placenta, and vice-versa. Placental “efficiency” was positively associated with offspring BA and BMC in univariate regression modelling, but in the adjusted models, after the aforementioned maternal factors were incorporated, these relationships were no longer significant. This potentially

supports the idea that maternal influences are acting through aspects of placental function rather than size alone.

Nutrient transport is one of the many functions of the placenta. Up to 30g of calcium crosses to the fetus in a successful pregnancy; in the third trimester calcium transport quadruples to around 140mg/kg per day to sustain adequate mineralisation of the fetal skeleton (38). Placental calcium transport occurs in the synctiotrophoblast (50) where calcium crosses the placenta bound to calcium transport proteins before being actively extruded from the basal plasma membrane of the trophoblast layer to the fetal circulation via a number of pumps and exchangers, such as plasma membrane Ca^{2+}-ATPase (PMCA). It has previously been demonstrated in animal models that a 2-3 fold increase in PMCA gene expression is associated with a 72-fold increase in calcium transport across the placenta in late gestation (51).

Maternal serum 25(OH)D concentration appears to influence offspring bone mineral accrual though effects on the concentration of umbilical venous calcium (5), and it is possible that regulation of placental transport may be important in the relationships between placenta and offspring bone mass. It has previously been shown that expression of one of the isoforms of PMCA (PMCA 3) is positively related to neonatal whole body BMC (52), and several studies have demonstrated the importance of nutrient transport across the placenta, even after adjustment for overall size. Maternal vitamin D concentration may thus exert its effects on offspring bone mass through PMCA 3 expression (52), however mechanistic confirmation is required to determine whether the effects are due to altered presentation of nutrient to the placenta (substrate dependent) or a direct action on transport processes. It is difficult to distinguish between the two potential mechanisms, and the results from the SWS study would be consistent with either, rather than an effect purely on placental size per se.

Whilst the positive associations between placental size and offspring bone size appear modest, they are potentially of biological significance. In the SWS cohort it was observed that for every 1 SD increase in placental volume, neonatal BMC increased by 3.6 grams. Placental volume accounted for 6.25% of the variation in BMC and 1.2% in the variation of BMD at birth. The difference in mean BMC for those individuals who were in the top compared with bottom quartile of

placental volume at 19 weeks was 0.7 SD and 0.3 SD respectively. If these differences were to be sustained into adulthood, they may equate to a 15% difference in risk of fracture (234). This figure is similar to the 13% increased risk of vertebral fractures in women who smoke (a risk factor incorporated into the standard international method of risk stratification (FRAX®) (235)) compared to women who do not. Therefore these findings may well be relevant in terms of later bone health.

8.2.2 **Placental size and offspring bone mineral density**

The strongest associations detected in both the SWS neonatal study and ALSPAC study were between placental size and neonatal skeletal size. In the SWS placental volume predicted neonatal BA and BMC more strongly than aBMD, with a regression coefficient for placental volume-BA more than double that for placental volume-BMD. AS previously discussed, one of the major limitations of DXA, particularly in children, is the size dependence of the measurement. The aBMD calculation derived from DXA is based on a two-dimensional projection of a three-dimensional structure and is affected by bone size. In the SWS neonatal study, the method of Prentice was used whereby offspring BMC was adjusted for bone area, offspring length and weight, to give scBMC. When this was applied to the SWS data, the positive relationships between either placental area or volume and scBMC were no longer seen, suggesting the importance of size in the associations seen. Nevertheless, in adult studies, bone size and BMC perform well as predictors for fracture risk suggesting that the overall size of the skeletal envelope will have longer term implications (236).

One of the advantages of pQCT over DXA is that it allows detailed measurements of bone indices without the effect of overall size that confounds DXA measures; volumetric bone mineral density is directly measured, without having to rely on mathematically derived estimates or make assumptions about the shape of the bone being investigated. To my knowledge this is the only study to investigate the relationships between placenta size and offspring bone indices using pQCT.

In parallel with the SWS cohort findings, the relationship between placental size and offspring bone size was also of a higher magnitude than that between placental size and bone density in the ALSPAC cohort; the regression coefficients for the relationship between placental volume and pQCT derived measures of bone size such as periosteal circumference and endosteal circumference were more than five times higher than that observed with bone density (cortical density at the 50% site). This finding is consistent with previous studies showing that skeletal size, rather than volumetric density is influenced by early life factors (237); density tends to be more dependent on environmental influences later in the life course, such as loading and nutrition (3;238).

In contrast to the SWS findings, an inverse association between placental size and volumetric cortical BMD at the tibia was observed in the ALPAC cohort, suggesting a disparity between influences on bone size and volumetric density; this has been observed with other aspects of intrauterine growth (230). There may be a potential maturational explanation for the ALSPAC findings. These children were assessed toward the end of the pubertal period, during which substantial linear growth had occurred. The concept of “cortical consolidation” describes the way in which mineralization may lag behind growth in bone size during modelling, with mineralization and volumetric density catching up with skeletal size by the time of peak bone mass (237). Indeed, late puberty is a time of rapid bone remodelling, with increased cortical porosity and active periosteal apposition—both characteristics that would be consistent with our findings.

One hypothesis, therefore, is that greater placental size leads to earlier onset of puberty, resulting in larger bones at age 15.5 years, but with cortical density lagging behind proportionate to bone size (with larger bones having lower cortical density compared with smaller bones). Such a mechanism was proposed in a recent study from the ALSPAC cohort, based on all children who underwent pQCT at ages 15.5 and 17.7 years, linking birth weight to bone outcomes (239). Here, relationships between birth weight and pQCT measures were somewhat attenuated by adjustment for puberty, and those with cortical density were not apparent at age 17.7 years. We found that associations between placental size and pQCT measures at age 15.5 years were not appreciably changed by adjustment for puberty; however, relationships between placental size and pQCT measures at age 17.7 years, although robust for PC and EC, were much

weaker for cortical density, consistent with a maturational aetiology and further supported by the conditional models, showing that the strongest placental associations were with the earlier time points of follow-up. Conversely, whereas increasing pubertal stage at age 13.5 years was associated with larger bones by pQCT, there was no evidence of placental size having been greater in children who were at a later stage of puberty at age 13.5 years. Additionally, increasing pubertal stage at age 13.5 years was associated with increasing rather than decreasing cortical density. It must be noted however that the 2-year interval between pubertal staging and pQCT measures somewhat limits the inferences that can be made. Furthermore, the correlation between birth weight and placental area was 0.4, suggesting much scope for relationships between placental size and outcomes independent of birth weight, consistent with previous documentation of the role of placental size versus function (38;240). Inclusion of birth weight in the base model removed associations between placental size and DXA BA, most likely due to the strong association between birth weight and overall size, thus potentially on the causal pathway. In contrast, associations between placental size and pQCT measures of PC, EC, and cortical density, although attenuated, remained similar to those without the inclusion of birth weight, suggesting relationships over and above those mediated through size at birth. Consistent with these findings, although placental size was weakly correlated with height in childhood, and whereas the DXA associations were removed by addition of height in the models, those with the pQCT indices remained statistically significant, further supporting the notion that the placenta pQCT relationships were not purely mediated via linear growth.

Second, it is notable from pQCT studies that bone size, for example, PC, tends to be inversely related to cortical density (241). The bending strength of a bone is proportional to the fourth power of the radius (242) and thus greater diameter bones require lower cortical density to achieve the same strength as narrower bones (243). Because the skeleton adapts its structure to the prevailing loads imposed on it, and cortical density encompasses cortical porosity as well as tissue mineralization, this then provides a second possible mechanism. Certainly, when both PC and cortical density were regressed simultaneously on placental volume or area, the predominant association was

with PC, suggesting that the primary effect is on bone size—an observation that could support either of these two maturational hypotheses.

Both of these potential explanations would be compatible with the observed increased incidence of childhood fractures during the transition into puberty, where an increase in bone size appears to outstrip mineralization (244); reassuringly, however, the relative catch-up in mineralization by young adulthood (245) suggests that by the time peak bone mass has been achieved, a larger placenta is likely to be associated with greater adult bone strength.

There are several maternal factors that influence placental size, including the maternal skeleton, hence it is important to elucidate the relationships between parent and offspring bone indices, taking into account placental size. This is discussed in section 8.3

8.3 Relationships between parental and offspring bone mass

To investigate further the relationships between offspring bone mass, maternal size and placental size, family trios of mother, father and child underwent investigation of bone mass using both DXA and pQCT.

8.3.1 Parent and offspring bone size

In the SWS cohort, using data from 259 mother-father-offspring trios, parental bone size measured by DXA was strongly positively associated with offspring bone size at age 9 years. This finding was consistent across all 3 sites measured – whole body (LH), hip and lumbar spine and remained robust after adjusting for possible confounding factors including those maternal factors previously found to be associated with offspring bone mass. Similarly the relationships remained after adjusting for mid-pregnancy placental volume, suggesting that the relationships are independent of placental volume. Adding the other parent's corresponding bone mass indices into the model, with the intention of demonstrating independent parental associations, attenuated the

magnitude of the observed relationships, but did little to change the direction of association, with statistical significance observed still across all measures.

A similar pattern was observed using data from tibial pQCT analysis in a subset of the parent-offspring trios (n=104). Again, positive independent relationships were observed between measures of both maternal and paternal bone size and offspring bone size, achieving statistical significance for endosteal circumference and SSI at the 38% tibial site.

This study is unique, as there have not been any other studies investigating the familial relationships in bone between mother, father and pre-pubertal offspring using both DXA and pQCT in a western cohort. The majority of published familial bone mass studies have focused primarily on mother-child relationships (246;247); there have been far fewer studies where father-child bone relationships have been examined, and fewer still where both sets of parents and their offspring have participated. In this latter group the Pune Maternal Nutrition Study (195) related parental bone mass to childhood bone mass at age 6 years; most other familial trio studies have used an adult offspring cohort.

Whilst there are similarities between this study and the Pune study, there are notable differences. Firstly the cohort characteristics are different - all participants in the Pune study were from rural villages in a developing country; secondly, the Pune study only used DXA to investigate bone mass; and thirdly whilst prenatal data was obtained in the Pune study, there was very little information collected from the father and children postnatally.

Congruous to this study, strong relationships between parental bone mass and child bone mass have been identified in the other published studies. There are several possible mechanisms underlying this association: family inheritance, imprinted genes, epigenetic factors, independent parental influences in early life and shared environmental exposures postnatally (Figure 8.1).

8.3.1.1 **Familial inheritance**

The role of genetic factors in bone mass has been well defined. The most recent and largest GWAS study identified more than 300 conditionally independent SNPs linked with BMD (248) (30). Depending on skeletal site and age, between 41-85% of the variation in DXA-derived bone mass measurements have been

attributed to genetic factors (58;249;250). There is a paucity of studies using techniques other than DXA to assess the genetic influence on bone mass, and those published have focused on older families, or middle-aged female twins (251-253). A recent Australian study used HRpQCT at the distal radius and tibia to measure bone mass in 177 mother-offspring pairs from the T-Bone cohort (246); mean offspring age in this cohort was 25 years. Strong positive relationships were observed across all the parameters measured, and heritability estimates ranged from 42%-74% at the tibia and 24%-67% at the radius.

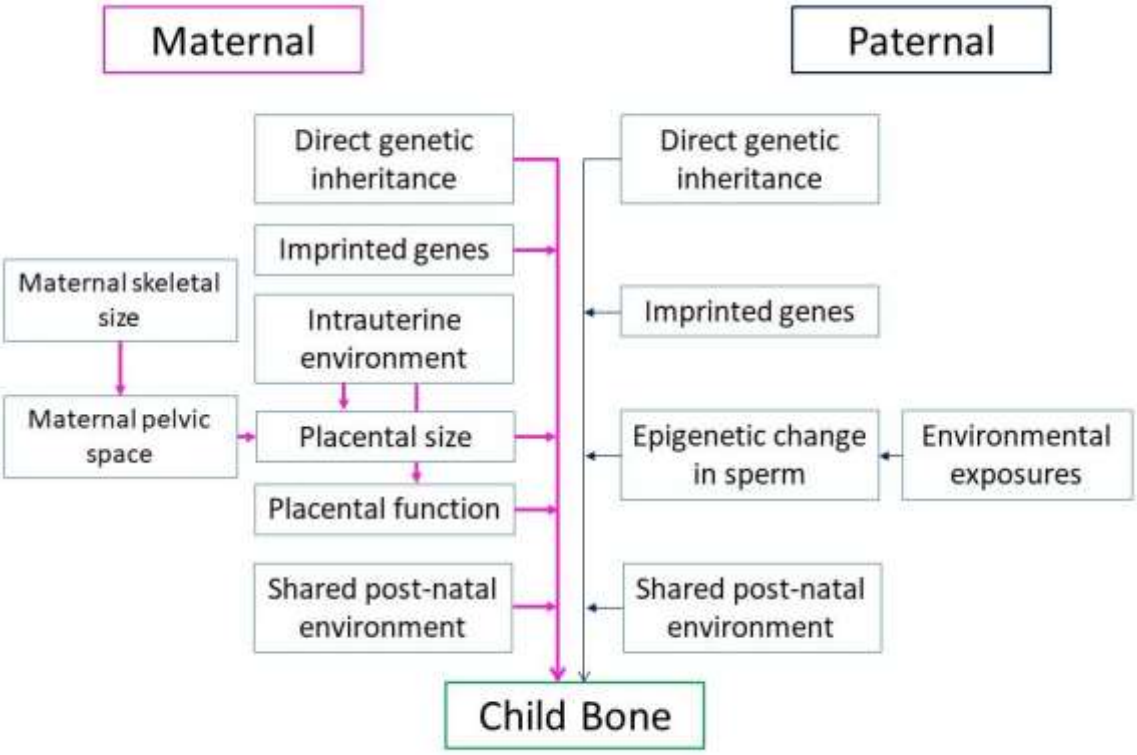


Figure 8.1: Parental influences on offspring bone

It might be expected that if the parent-child bone mass relationships were primarily genetic in origin, similar magnitudes of bone mass relationship between either mother or father and their offspring would be observed. In the Pune study (195) this pattern was seen, with associations of approximately equal magnitude of paternal and maternal bone outcomes with those of the

offspring. In the SWS cohort, the bone relationships between mother-child were of significantly greater magnitude than those of father-child across numerous variables and at different sites. This differential relationship was predominantly observed for the size-dependent variables BA and BMC (where a significant difference between mother-child and father-child associations were seen at all 3 sites) over the more size-independent variables, BMD and scBMC. For these latter variables, a significant difference between mother-child and father-child bone was only observed at the spine, with relatively equal strengths of relationship at the hip and whole body sites. This draws into question other possible mechanisms that may be a playing a role in the observed relationships.

8.3.1.2 **Genomic imprinting**

An additional mechanism that might explain these findings is genomic imprinting. This is an epigenetic phenomenon, independent of classical Mendelian inheritance, that causes genes to be expressed in a parent-of-origin specific manner and has been demonstrated in animals and humans; there are around 80 known imprinted genes in humans. There is some evidence that imprinting may have a role in childhood growth and subsequent low BMD and fracture (254). For example, in the SWS cohort, expression of the imprinted gene PHLDA2 has been found to be associated with lower fetal femur growth velocity between 19-34 weeks and lower offspring BMC at 4 years of age (255).

A widely accepted hypothesis for the evolution of genomic imprinting is the "parental conflict hypothesis" (256), which states that the inequality between parental genomes due to imprinting is a result of the differing interests of each parent in terms of the evolutionary fitness of their genes (257;258). The father's genes that encode for imprinting gain greater fitness through the success of the offspring, at the expense of the mother. The mother's evolutionary imperative is often to conserve resources for her own survival while providing sufficient nourishment to current and subsequent offspring. Accordingly, paternally expressed genes tend to be growth-promoting whereas maternally expressed genes tend to be growth-limiting (256). Whilst this is in contrast to the findings from this study, where the maternal-child associations were generally stronger than the paternal-child associations, most of the evidence underpinning the “parental conflict hypothesis comes from animal studies and further studies are

needed to determine the role of genomic imprinting in human offspring bone mass development.

8.3.1.3 Maternal environmental factors and placental function in early life

In this study, previously identified prenatal environmental factors did not alter the observed parent-child bone mass relationships; likewise the relationships remained robust after adjusting for mid-pregnancy placental volume. Similarly, whilst placental size was strongly associated with offspring bone mass at birth (SWS) and in later childhood (ALSPAC), the relationships remained robust after adjusting for the aforementioned prenatal environmental factors.

It can been theorised that environmental factors may exert their influence prenatally through epigenetic changes resulting in altered placental nutrient transport, rather than an effect on placental size. This is thought to be one of the mechanisms underlying the principle of maternal constraint, where maternal and uteroplacental processes act to limit the growth of the fetus.

The lack of an observed relationship between offspring bone mass in childhood and prenatal environmental factors in this study may be due to a number of reasons such as a low number of participants with prenatal environmental data, or postnatal environmental factors exerting significant effects on offspring bone mass at later ages, therefore modulating the relationships.

In contrast to the ALPSPAC study, a significant association between offspring bone size in mid-childhood and placental volume was not observed in the SWS cohort. Again, the reasons for this are not clear, but may be due to the lower number of participants in the SWS cohort or differences in the timing or technique of placental measurement (derived from 19 week ultrasound images in SWS versus direct measurement from placentas collected at birth in ALSPAC).

Nevertheless, it remains possible that one of the processes underlying the significant differences in bone mass relationships between mother-child and father-child are prenatal maternal environmental factors acting through effects on placental function, rather than on placental size.

Another concept of how the maternal environment might modulate offspring bone is indirectly through maternal size. Whilst a significant proportion of offspring bone mass may result from direct inheritance of parental genes coding for bone size, other maternal genes coding for maternal size, whilst not necessarily inherited by the offspring, may result in the mother being of a larger size. As a result of the larger body size, maternal BMC is likely to be higher and her pelvis bigger. This larger pelvis is likely to be able accommodate higher offspring growth, without constraint, and will result in a larger child at birth, with larger BA and BMC as a result. This may explain the differential parent–child associations observed, and the tendency for size-dependent measures of offspring bone to have a stronger maternal association. However strong relationships remained across multiple variable even when adjusted for maternal height (a surrogate for maternal size), suggesting the potential importance of other mechanisms.

8.3.1.4 **Shared post-natal environmental factors**

A fourth concept that may explain some of the parent-child bone mass relationships is that of shared post-natal environmental factors. It has been well documented that certain parental activities are highly correlated to those of their offspring, such as physical activity and diet (259-261), which may in turn confound the relationship with bone mass. Additionally, differences in the strength of parental effect have been observed (261). In this study, there was no significant correlation between parental moderate, vigorous or very vigorous activity and that of their child. Similarly, milk intake (as a surrogate for calcium intake) between parent and child was not significantly related. These observations are limited by a low number of participants (especially those with offspring physical activity data), differences in data collection techniques (Actiheart assessment in children at age 6 versus parental self-reported physical activity) and inadequate dietary records to make accurate conclusions.

8.3.2 **Parent and offspring bone density**

Significant positive relationships were observed in the SWS cohort between parental and offspring bone density measurements when assessed by both DXA and pQCT. One of the major drawbacks with DXA is that aBMD derived from DXA still has a size dependency, which needs to be considered when interpreting results; pQCT on the other hand is able to measure true size-independent vBMD.

Using DXA, positive parent-child associations were seen for aBMD at all 3 sites measured, and these remained robust after adjusting for the corresponding parents bone indices. Relationships were generally of lower magnitude than those for parent-child bone size, and unlike with BA and BMC, there was little difference between the relationships of mother-child versus father-child; the only exception to this being aBMD at the spine which did appear to be more strongly related to maternal than paternal lumbar spine aBMD (β= 0.23 vs 0.16).

These relationships remained robust after adjusting for possible environmental confounders and were changed little by incorporating mid-pregnancy placental volume into the multiple regression models. These observations are concordant with those observed in the Pune study (195) where parent-child BA and BMC relationships appeared stronger than those for aBMD, and associations were little altered when placental weight was included in the model. These findings are consistent with previous studies showing that size, rather than density is more strongly influenced by early life factors (3;238).

As previously discussed, due the size dependence of DXA, DXA-derived aBMD measurements are influenced by the overall size of the individual being assessed, and to overcome this, scBMC was calculated using the Method of Prentice (described in 1.7.1.2). It should be noted that whilst scBMC acts as an indicator of vBMD, it is not an actual measure of vBMD. One of its limitations is that the incorporation of the individual's height, weight and BA into the calculation may actually result in over-adjustment and weaken the strength of any associations seen. Using scBMC attenuated the relationships seen, however robust positive relationships remained for both mother and father-child, suggesting a possible size-

independent association between parental and offspring BMD. Using this approach, again there was little difference in relationships according to the parent of origin, with scBMC at the spine being the only exception (mother-child statistically stronger than father-child).

There was some disparity in findings from the SWS pQCT data with regards to parent-child bone density associations. A small but positive relationship was observed between parent-child cortical vBMD measured at the 38% tibial site, which remained robust after adjusting for the other parent's corresponding variable; relationships varied little between mother-child and father-child. At the 4% site however, there was no significant relationship seen between either parent and their offspring for total vBMD and trabecular vBMD.

This result is surprising as previous studies have shown high levels of correlation between parent and offspring bone mass when measured by pQCT (252). In a recent study using HR-pQCT to measure vBMD at the radius and tibia in 1047 adult relatives from the Framingham Heart Study, positive correlations were seen between familial vBMD measures, with strong correlation for total vBMD and trabecular vBMD at the radius (252).

Our findings may simply reflect the smaller numbers in the current study. Certainly, the numbers of mother-father-child trios that had complete pQCT data in this study (n=104) is less than half of those who had complete DXA data (n=255). Secondly, precision of measurement may have a role; in children the precision of pQCT at a cortical site (38% site) is likely to be better than at a predominantly trabecular site (4% site) (262)

The parental-child bone density associations are likely to be explained by the same mechanisms discussed in section 8.3.1. In contrast to the observed parent-child BA and BMC associations, parent of origin appeared to have less of an impact on parent-child aBMD relationships. Whilst similar maternal and paternal relationships with offspring bone density do not exclude an intrauterine mechanism, these findings are suggestive that genetic inheritance may play more of a role in familial associations of bone density than bone size. The exception to this trend was observed for spinal aBMD, where the magnitude of the mother-child relationship was significantly larger than father-child, even after adjusting for bone size. This finding may represent a possible differential parental influence on

different bone types, for example maternal bone density influences may be stronger for offspring trabecular bone density (the predominant bone type in lumbar vertebrae) than for cortical bone density (found at the hip). However, as a significant parental-offspring trabecular BMD relationship was not observed in the SWS pQCT dataset, larger familial studies are needed before firm conclusions can be made.

8.3.3 The effect of size correction (maternal and child) in DXA and pQCT outputs, and their effects on interpreting offspring data

To explore the influence of size on the observed relationships in this thesis, results have been presented both unadjusted, and then after adjustment for potential confounding factors, including parental and offspring size. In the SWS study, the strong positive associations between placental size and DXA-derived offspring bone size and density persisted after adjustment for maternal height, however these relationships were no longer seen when child's height was also added into the regression model. Paternal height was not associated with placental size.

When the relationships between offspring bone mass and placental size were explored in the ALSPAC cohort using pQCT of the mid-tibia in adolescence, again the strong positive relationships between placental size and offspring tibial PC and EC, and the strong negative association between placental size and cortical VBMD were unaffected after adjustment for maternal height. The relationships also remained robust after adding offspring height and weight into the regression models (as surrogate measures for offspring size). Whilst it would be expected that any relationship with vBMD would not be affected by adjustment for offspring size (as one of the advantages of pQCT over DXA is that it can measure "true" volumetric bone density and is not influenced by size), the fact that the placental relationships with EC and PC also remained robust suggest that the observed relationships may not just simply a case of big mothers having big placentas and consequently big children. One may argue that it is not necessary to present data for pQCT-derived vBMD adjusted for size, however this has been included merely to be consistent with the adjustments made for other size dependent variables within a given table (e.g. Table 5.3)

The latter two results chapter in this thesis have explored the relationships between parental and offspring bone mass assessed initially by DXA and subsequently by pQCT. Adjusting for maternal and offspring size did little to change the observed strong positive relationships between maternal bone size and density and offspring size and density; whilst the magnitude of the relationship was diminished, albeit only slightly, consistently significant findings were still observed across all 3 measured sites. The only exception to this was the relationship between maternal and child whole body bone area, which was no longer significant after adjusting for maternal and child's height (p=0.17). This is unsurprising as whole body bone area is well known to be influenced by body size.

In the final results chapter, the significant relationship between maternal and tibial bone area at the 4% site remained robust after adjusting for maternal and child height [β (95% CI); p value = 0.30 (0.16-0.44; p<0.001). Similarly the relationships between maternal and child cortical vBMD and SSI at the 38% site remained after maternal and child size adjustment [β (95% CI); p value = 0.33 (0.04-0.64); 0.03 and β (95% CI); p value = 0.08 (0.02-0.14; 0.001 respectively. However the relationship between maternal and child EC at the 38% site was no longer significant. As previously discussed, it is unsurprising that vBMD relationships were not affected by size, due to the very nature of the measurement.

8.4 Strengths and limitations of this work

8.4.1 **Study cohorts**

Both cohorts used in this analysis, ALSPAC and SWS, are large and rigorously conducted with detailed characterization of the participating mothers and their children; however, they are not without limitations. The strengths and limitations of the studies will be discussed here.

8.4.1.1 SWS

The SWS is a large birth cohort, unique in that mothers were recruited and assessed pre-pregnancy. 75% of women invited to take part in the SWS consented to participate in the study. These women are self-selected and thus more likely to be healthy, although they do still encompass a wide range of demographic characteristics. At the 9 year study, participating women were taller and had lower BMI, were less likely to have smoked in pregnancy, had higher late pregnancy 25(OH) D and were of higher social class than non-participating mothers. Additionally, there was very little ethnic diversity within the participating mothers; 97% of mothers were White. This reflects the local population from which the women were recruited and gives more homogeneity to the study population, but needs to be considered in the generalisability of the findings to the wider population. As the results are based on internal comparisons within the cohort, there is no reason to expect that this would have erroneously led to the observed associations.

8.4.1.1.1 ALSPAC

The main strengths of the ALSPAC cohort are: its sample size; the duration of follow-up and the availability of repeat measurements. Like the SWS, the majority of participants in ALSPAC are of White ethnicity, which again limits the generalisability to other ethnic groups. Attrition in ALSPAC has been greater for those who experienced adversity during the index pregnancy (such as early pregnancy complications, lack of social support and inadequate housing); women participating in later studies within the cohort are older, less socio-economically deprived and were healthier at recruitment than those invited but did not attend (263). In contrast to SWS, ALSPAC recruited women during pregnancy, rather than pre-pregnancy, so does not contain any phenotypic information on mothers before they fell pregnant.

8.4.2 Causality

Both the SWS and ALSPAC studies are observational, meaning that it is not possible to deduce the direction of association between any of the relationships

seen, and one cannot infer a causal relationship. The wealth of data collected in the SWS and ALSPAC studies has enabled a number of parental and offspring covariates to be include in statistical models, but cannot eliminate all potential sources of confounding. Despite this, there is biological plausibility behind the associations seen, but further studies are needed to examine the relationships further.

8.4.3 **Placental assessment**

Placental measurements in the SWS and ALSPAC were performed at different times using different approaches. The placental measurements in the SWS study are limited in that they were obtained from static ultrasound images obtained at 19 weeks gestation; whilst this enabled direct measurement of placental area, placental volume was estimated using an algorithm that assumed the placenta to be ellipsoid in shape. This method did however demonstrate good correlation with placental volume measured by 3D ultrasound ($r=0.64$, $p<0.0001$) in a subset of 28 pregnancies at 19 weeks gestation. A second limitation is that intrauterine ultrasound measurements are prone to reproducibility error; however scans were performed by two experienced ultrasonographers following standard guidelines.

In the ALSPAC study, although placentas were collected at delivery, they were not measured immediately, but stored for several years in formaldehyde. The effect of this on placental size and shape is uncertain; nevertheless, because all placentas were stored identically, this is unlikely to have affected the relationships observed between placental size and offspring bone mass.

8.4.4 **Pubertal assessment**

In the ALSPAC cohort, pubertal stage was assessed at 13.5 years, and not at the times of DXA or pQCT scanning (9 years or 15.5 years). Information was missing for 42.6% of individuals and in these cases data were imputed; those with missing pubertal assessment were given a value close to the measured mean. This may have led to an under- or over-estimate of the true pubertal spread within the group, however similar findings were observed from a

sensitivity analysis where the data was restricted to only include those with documented pubertal status.

In the SWS cohort, puberty was not assessed at the 8 year visit. As the mean age of participants assessed was 9.2 years (the oldest child being 9.9 years), it is probable that some children may have entered the early stages of puberty; the average of onset of puberty ranging from 8 to 13 years in girls, and from 9 to 14 years in boys (264;265). Certainly in the 8 year DXA analysis, girls had a significantly greater height and weight z-score than boys. One explanation for this could be that more girls had entered puberty, therefore leading to an acceleration of growth. However, when the children's heights were adjusted for their parent's height, a sex difference in size was no longer seen, suggesting that in this cohort, it is the parental influence on height that is causing this effect, rather than puberty. In a sensitivity analysis, removing the top 5% of children for height and weight from the analysis had no effect on the observed associations.

8.4.5 **Number of participants**

Using bone mass data from a previously published mother-father-offspring trio study (195), it was calculated that 300 family trios would be needed for 90% power to detect a similar effect size. This was not achieved in either of the SWS familial DXA (n=255) or pQCT (n=104) analyses, thus both analyses were under-powered. This is unlikely to have had a substantial effect on the DXA analyses, where strong relationships were observed across multiple variables, but may account for the lack of parent-child association observed for certain variables in the pQCT analysis. In retrospect, to have increased the numbers of families with complete pQCT data, parents of children who had already undergone pQCT at age 6 years could have been specifically targeted and prioritised.

8.4.6 **Parental data**

With respect to paternal data, objective evidence of paternity was not obtained. A previous study has estimated true paternity at 85-90% (262); one could speculate that there may be an even higher rate of true-paternity in this study,

as the vast majority of fathers recruited were still partnered and co-habiting with the respective mother. In addition, high rates of non-paternity would tend towards the null hypothesis, making significant associations less likely to be seen.

With respect to maternal data, the mean age of mothers who underwent DXA and pQCT assessment was 41 years. Only 3 mothers (<3%) identified themselves as post-menopausal, however by the age of 41 years, it is possible that some women may have entered the peri-menopause, which may have an implication on their bone mass results. Ideally, maternal bone mass should have been measured earlier to ensure peak bone mass was captured, however this was not possible as this was the first time since the early post-partum period that maternal assessments had been made. Secondly, the majority of women (92%) recruited in the SWS went on to have further pregnancies; it is obviously not possible to perform DXA or pQCT in pregnant women due to the risks of radiation exposure, and important that bone mass is not measured too closely to a recent pregnancy due to the known temporary effects pregnancy and breastfeeding can have on maternal bone mass (263). Excluding post-menopausal mothers from the bone mass analysis did not alter any of the relationships observed.

8.4.7 **Questionnaire data**

Using interview-led, self-reported questionnaires has advantages and disadvantages. It allows large amounts of data on demographics, diet and health characteristics to be collected reasonably quickly, but relies on participants being accurate and honest to provide the correct information. There may be a tendency for individuals to under-report certain behaviours known to be associated with poorer health outcomes, such as smoking and alcohol consumption, and over-report beneficial habits, such as physical activity. Whilst there may have been significant recall bias, a previous study using the Princes Anne Cohort found a good correlation between retrospective nutrient intake assessed by questionnaire with prospective 4 day food diaries (211).

Due to time constraints within the parental SWS 8 year visit, the questionnaire administered did not contain sufficient information from which to calculate daily calcium intake, a possible important confounding factor in the observed relationships. Instead milk intake has been calculated and whilst this may be a reasonable surrogate for calcium intake in childhood, is less reliable in an adult population, where consumption of other dairy items such as cheese may account for higher proportions of daily calcium intake (268).

8.4.8 **Anthropometry data**

Anthropometric measurements are prone to inter-observer error. All measurements performed in SWS and ALSPAC were performed by trained research nurses or doctors, following a detailed protocol and using specific landmarks to improve the accuracy of measurements and reduce measurement bias. Regular IOV sessions were undertaken, with further training if needed. As children have a tendency to move, children's measurements (except weight) were repeated three times and an average taken to obtain a precise a measurement as possible.

8.4.9 **Physical activity data**

Physical activity has been assessed differently in parents and child. In the SWS parent study it was collected by a self-reported interview led questionnaire. Childhood physical activity was not assessed at the childhood 8 year visit, therefore childhood 6 year physical activity data, measured with an Actiheart monitor, has been used as a surrogate. Relatively few children had recorded activity data, thus significantly reducing numbers when physical activity was incorporated into statistical models. To explore the impact of physical activity further, the same assessment in parent and child should be performed, ideally with an objective tool, such as Actiheart.

8.4.10 **DXA measurements**

DXA is considered the gold standard for the measurement of adult bone mass and body composition as it is highly reproducible, easy to perform and is associated with low radiation exposure. DXA in children, however, can be challenging for several reasons. For some children, especially at a very young age, remaining still for the duration of the scan may be difficult; any scans with significant movement artefact were thus excluded from the study.

Bone edge detection is more difficult in smaller children due to their lower absolute BMD, however specific paediatric software with increased sensitivity for edge detection was use to minimise this limitation. It was not possible to perform repeat DXA assessments on the neonates to determine values for the coefficient of variation of DXA in children. However, DXA measurements of bone mass have been shown to correlate well with whole body calcium content in studies of small animals such as piglets (269).

The size dependence of DXA has already been discussed at length in section 8.2.2. To correct for this, mathematic adjustments were made by using the Method of Prentice, or by incorporating height and/or BMC into regression models

Finally, whilst movement was not a significant cause of artefact among the SWS parent scans, a significant number of parents had metalwork within their skeleton, often as a result of previous limb fracture (and joint replacement in 1 individual). Rather than excluding these scans altogether, and reducing numbers further, limb cross-imputation was performed, whereby the bone values for the limb containing metalwork was replaced by the values for the native limb on the contralateral side. This strategy is backed up by a study by Micklesfield et al, which demonstrated a lack of significant side to side differences in adult BMC measured by DXA (270).

8.4.11 **pQCT measurements**

pQCT has the advantage of being able to directly measure volumetric bone mineral density without the influence of size, and has been validated in children

as young as 3 years of age (119). Whilst movement of children undergoing scanning occurred frequently, movement artefact was able to be reduced by good positioning, tibial restraint and child distraction using television. Scans with significant movement artefact were excluded, however this was only a small proportion of the total scans performed (<3% of SWS 6 year scans). The SWS 6 year visit initially included pQCT of the radius, however movement artefact was so high at this site that the protocol was amended to remove this procedure and focus on the tibia.

In children the growth plate is still visible; therefore the pQCT reference line should be placed to bisect the medial border of the distal metaphysis. For the SWS 6 year visit pQCT scans the reference line was positioned to bisect the medial border of the articular surface. In children of this age, it is inevitable that the 4% measurement may have gone through the growth plate in a small proportion of cases. As the growth plate is an area of provisional calcification, this may lead to falsely elevated readings and add inaccuracies to the measurement. Despite this, the direction and magnitude of parent-child bone mass associations were not significantly altered when data acquired from the 14% site were substituted for the 4% site.

There is a difference in the published literature regarding the tibial sites scanned. This difference was observed between ALSPAC, where the 50% site was scanned, compared to SWS where the 4%, 14%, 38% and 66% sites were scanned in accordance with the machine's pre-sets. This variety and inconsistency of sites scanned, particularly in children, make comparison of results between studies problematic. In addition, because only the 50% site was scanned in the ALSPAC cohort, it was not possible to explore relationships between placental size and trabecular parameters.

With regard to the SWS parent scans, due to excessive calf size compared to the scanning aperture it was not possible to undertake complete scans in all parents. This was only a problem for a handful of parents and in most cases scanning of the 4% and 38% sites could be completed, but the final scan at the 66% site (which has not been included in these analyses) had to be abandoned.

Finally, in these analyses parental-child DXA relationships have been evaluated in offspring of mean age 9 years, whereas the parental-child pQCT relationships have been evaluated in offspring at 7 years of age, due to offspring pQCT not being part of the "8 year SWS child visit". Whilst this 2 year difference in mean

age of the offspring between imaging modalities is unlikely to have altered the relationships observed, and all scans were all adjusted for age, it would have been ideal to have obtained data on family trios with the same baseline demographics for both scanning modalities.

8.4.12 **Statistical methods**

Both the parent-child DXA and pQCT analysis are likely to be under-powered. Whilst the number of recruits in the SWS parent-child DXA analysis was close to the anticipated target of 300 trios to achieve 90% power (based on data from a previous parent-child trio (195)), the number of SWS pQCT parent-child trios, after exclusions was less than half of this (n=104). This may account for the lack of some of the expected associations seen in the pQCT analysis.

The statistical methods used in this thesis did not account for multiple testing. As a result there is a higher risk of rejecting the null hypothesis and getting a high rate of false positive findings. Several methods have been developed to deal with this problem; one method commonly used is the Bonferroni correction, where the p value is multiplied by the number of tests performed. The reasons for not using this method are two-fold. Firstly, the Bonferroni correction is not valid when exposures and outcomes are correlated (271), and secondly, the method can be too conservative and can lead to inflation of false negatives. For these reasons the data in this thesis has not been corrected for multiple testing. Instead we adopted a strategy on interpreting multiple analyses by giving weight for a priori hypotheses and overall patterns of association for bone mass.

8.5 Future research

SWS and ALSPAC are ongoing cohorts which will enable repeat analysis on offspring at later time points, to assess whether the observed relationships change with the age of the child. In SWS, children are currently being invited for further assessment at age 11-13 years. The assessment of bone mass at this

age, together with the parental data already obtained, will allow further exploration of how the effect of puberty modifies the relationships seen.

In the SWS, DXA data are available for offspring in the neonatal period, and at ages 4, 6 and 8 years. In this thesis we have only looked at parent-child relationships at a single time-point. By investigating the parent-child relationships at several time points, longitudinal changes in parent-child bone mass relationships can be assessed.

Until recently, ALSPAC has not obtained any parental bone mass data. Parental DXA and pQCT data is now being collected and when complete, the relationships between parent and offspring can be explored in this different larger cohort. The size of this cohort would hopefully reduce the risk of findings being limited by the power of the study to detect association.

This thesis has predominantly focused on relationships with offspring bone mass. DXA and pQCT also provide information on other aspects of body composition, such as fat and lean mass, which has also been collected. Additionally, muscle strength (obtained from measurement of grip strength) has been collected in parents and child. The relationships between placental size, parental size and offspring body composition, and parent-child muscle strength can be further explored using this data.

Finally, pQCT data is presently being collected on children at age 4 years within MAVIDOS (Maternal Vitamin D Osteoporosis Study), a double-blind randomised placebo-controlled trial of Vitamin D supplementation in pregnancy. Further exploration of mother-father-child relationships within this cohort may be possible to examine the effect serum 25(OH)D in pregnancy and supplementation with cholecalciferol has on parent-child bone relationships

8.6 Conclusions

In summary, my work, presented in this thesis has shown that placental volume is positively associated with offspring bone mass at birth, with associations remaining during puberty into late childhood. Parent and offspring bone is positively associated, with a greater magnitude of relationship observed for measures of bone size, than bone density. Parent-child bone mass associations are significantly stronger for mother-child than father-child across several variables, again predominantly those associated with bone size. These

relationships were not influenced by placental size or other environmental factors previously shown to affect offspring bone mass. Whilst parent of origin genetic effects are potential explanations, these associations may reflect in-utero environmental effects through changes in placental function

Appendices

Appendix1: Parent invitation letter

Appendix 2: Parent information booklet

Appendix 3: Parent consent form

Appendix 4: Parent questionnaire

Appendix 5: Copy of parent DXA result

Appendix 6: LREC approval letter

Appendix 1: Parent invitation letter

SOUTHAMPTON WOMEN'S SURVEY
MRC Epidemiology Resource Centre
(University of Southampton)
Southampton General Hospital
Southampton SO16 6YD

Freephone: 0800 7834503

«Title» «Inits» «Surname» **«SWSID»**
«Address1»
«Address2»
«Address3»
«Address4» «Postcode»

Dear «Title» «Surname»

You and your child [child name] recently assisted us in the Southampton Women's Survey 8-year follow-up, in which we are studying the relationship between women's diets and health before and during pregnancy, and the growth and development of their children.

We would like to now invite you and [child name] father to participate in an additional part of the study, looking at how parent's body composition (the amount of fat, muscle and bone) affects their child's body composition. This will involve a visit to a research clinic at Southampton General Hospital. During the clinic visit, we will carry out some simple tests to measure your body composition and your bone density. Firstly we will measure your height, weight and assess how strong your grip is. Then two tests will be performed – a DXA scan and a pQCT scan. The clinic should take around 1 hour. All these measurements are safe and painless. We shall also ask you some questions about your diet, medical history, family history and physical activity.

Full details are given in the enclosed booklet. A separate invitation letter, information sheet and reply slip is also enclosed for you to kindly pass onto [child name] father.

If you are able to take part in this study, please complete the enclosed reply slip and post back to us using the enclosed pre-paid envelope. Upon receiving a reply, a member of the research team will contact you within the next month to arrange the visit. **You can also telephone us at any time on the free phone number above**.

With many thanks

Yours sincerely

Dr Christopher Holroyd BM MRCP
Clinical Research Fellow, Southampton Women's Survey

Appendix 2: Parent information booklet

Southampton Women's Survey

Body Composition and Bone Health:

CLINIC VISIT

For mums

What will happen to the results of the research study?
The results of the study will be published in medical journals and presented at medical meetings so that doctors and health professionals all over the world can understand more about the parental influence on body composition. We may also arrange for local papers (e.g. The Daily Echo) to write about the study results so that you know what we have found.

The SWS has a website that is kept updated with the findings from this study: http://www.swsurvey.soton.ac.uk

Who is organising and funding the research?
This research is funded by the Medical Research Council. The study is organised by the MRC Epidemiology Resource Centre and University of Southampton.

Who has reviewed the study?
This study was given ethical approval by the Southampton Local Research Ethics Committee.

Do you have to take part?
It is up to you to decide whether or not to take part. If you do decide to take part, you will be asked to sign a consent form, but you are still free to withdraw at any time and without giving a reason. This will not affect the standard of care you receive. You may want to do some parts of the study but not others. If you are happy to have all the tests, your visit will last around 45 minutes.

What measurements will we be taking?
The tests will be discussed with you in detail at the clinic. Firstly, your height and weight will be measured. Next, we will measure the skin-fold thickness at your arms and hips using callipers. Then we will measure the strength of your grip in both hands by asking you to grip and squeeze against a small handheld device. A doctor or nurse will also go through a questionnaire with you regarding your lifestyle, medical history, diet and physical activity.

Bone Density Scan
You will have a scan of your skeleton using DXA (dual x-ray absorptiometry machine). This will give us important information about the size and strength of your bones. It also tells us how much muscle and fat is present. This scan takes approximately 10 minutes to perform. Your will lie on a table and a small scanning arm will pass overhead, about 2 feet in the air. It does not touch you. The dose of x-rays is small; it is about the same amount of x-rays that we are exposed to over 3 days in normal every day life. The DXA is very safe and causes no discomfort. You will be given a picture of your skeleton if you wish.

pQCT Scan
You will then have a scan of your lower leg using pQCT (peripheral quantitative computed tomography). This also gives us important information about the amounts of bone and muscle and fat in the lower leg. This scan takes around 20 minutes and involves you sitting on a chair and putting your lower leg into an open metal tube. It does not touch your skin. The dose of X-rays

open metal tube. It does not touch your skin. The dose of X-rays is equivalent to a day of natural sunlight. The scans will not cause any pain or harm.

What do you need to do?
Please avoid wearing clothing with metal belts or zips, as this will interfere with the scan results**.**
Please could you bring along any medicines that you take, so that we can accurately document them

Expenses and payments
Before you leave the clinic, you will be provided with an exit ticket for the hospital car park, or public transport costs will be reimbursed.

What are the possible benefits of taking part?
The main benefit is the knowledge that you are taking part in a unique study that will help improve our knowledge of parental influence on childhood body composition.
Also, if there are any problems with your bones identified during the scans you will be referred for further assessment and possible treatment.

What if there is a problem?
Any complaint about the way you have been dealt with during the study or any possible harm you might suffer will be addressed. Detailed information on this is given in Part 2.

Will you taking part in the study be kept confidential?
As always, all information about your participation in this study will be kept confidential. Details are included in Part 2.

Contact Details:
If you have any questions, or if you need to contact the study team at any time, please contact the research team on the freephone number 0800 783 4503.

This completes Part 1 of the Information Sheet. If you wish to take part, please continue to read the extra information in Part 2 before making a decision.

Part 2

What if there is a problem?
If you are worried about any aspect of this study, please speak to the researchers who will do their best to answer your questions.

Under our formal research procedures we are required to give you the following information:

If you remain unhappy and wish to complain formally, you can do this through the NHS Complaints Procedure. Details can be obtained from the study coordinator. We are an experienced research team, and aim never to cause harm to any volunteer. As outlined in Part 1, the planned investigations are considered safe. In the very unlikely event that something does go wrong and you are harmed due to someone's negligence then you may have grounds for a legal action for compensation against the University of Southampton but you may have to pay your legal costs.

Will your taking part in this study be kept confidential?
All information collected about you during the course of the research will be kept strictly confidential. If we discover information that may be useful for your family doctor (e.g. low bone strength), with your permission we will contact your doctor.

Appendix 3: Parent consent form

MRC Lifecourse Epidemiology Unit
Southampton General Hospital
Tremona Road
Southampton SO16 6YD

Tel: 023 80777624
Fax: 023 80704021

Study Number:
Patient Identification Number for this trial:

CONSENT FORM

Title of Project: Using DXA and pQCT to assess parental influence on childhood bone mass

Name of Researcher: Dr Christopher Holroyd

Please initial box

1. I confirm that I have read and understand the information sheet dated August 2009 (version 1) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐

2. I understand that participation is voluntary and that I can withdraw at any time, without giving any reason, without their medical care or legal rights being affected. ☐

3. I understand that relevant sections of my medical notes and data collected during the study, may be looked at by responsible individuals from Southampton University Hospitals NHS Trust, where it is relevant to taking part in this research. I give permission for these individuals to have access to my records. ☐

4. I agree that information collected from the study may be passed on to my General Practitioner (GP) or the hospital consultant in charge of my care. ☐

5. I agree to take part in the above study. ☐

Name of Participant	Date	Signature
Name of Person taking consent (if different from researcher)	Date	Signature
Researcher	Date	Signature

SWS serial number:

8 year Parental

QUESTIONNAIRE

Forename, _____

Surname) _____

Date of Birth: d d m m y y

Name of child enrolled in SWS: Forename _____

Surname _____

Relationship to child

1. Mother
2. Father

Interviewer:

Date of interview: d d m m y y

1. Food frequency

I am going to ask you about **a few** of the **foods** you have eaten in the **past 3 months**. I will ask you how often you have eaten certain foods.

	food	never	less than once per month	1-3 times per month	number of times per week							more than once per day	no. of times per day
					1	2	3	4	5	6	7		
1.1	**white bread, rolls, toast**	0	0.3	0.5	1	2	3	4	5	6	7	8	
1.2	**brown bread, rolls, toast**	0	0.3	0.5	1	2	3	4	5	6	7	8	
1.3	**breakfast cereals**	0	0.3	0.5	1	2	3	4	5	6	7	8	

What are the main types of breakfast cereal used?	type	brand
	type	brand
	type	brand

	food	never	less than once per month	1-3 per month	number of times per week							more than once per day	no. of times per day
					1	2	3	4	5	6	7		
1.4	**eggs & omelettes**	0	0.3	0.5	1	2	3	4	5	6	7	8	
1.5	**cheese**	0	0.3	0.5	1	2	3	4	5	6	7	8	
1.6	**yoghurt & fromage frais**	0	0.3	0.5	1	2	3	4	5	6	7	8	
1.7	**meat & meat dishes**	0	0.3	0.5	1	2	3	4	5	6	7	8	
1.8	**oily fish**	0	0.3	0.5	1	2	3	4	5	6	7	8	
	What are the main types of oily fish eaten?	1											
		2											
		3											
1.9	**butter & margarine**	0	0.3	0.5	1	2	3	4	5	6	7	8	

	What are the main types of spread?	1/ …….. 2/ …….. 3/ ……..

Now I would like to ask in more detail about your milk intake.

1.10 Which types of liquid milk have you used regularly in drinks and added to breakfast cereals over the past 3 months? *(list up to 3 below)*

0. None
1. Whole pasteurised & UHT
2. Semi-skimmed pasteurised & UHT
3. Skimmed pasteurised & UHT
4. Other (record as much detail as possible)

Milk 1 ☐ If "Other", *specify* _____

Milk 2 ☐ If "Other", *specify*_____

Milk 3 ☐ If "Other", *specify* _____

1.11 On average over the last 3 months how much of each milk have you consumed per day? *(1 average glass = 0.5 pints (225mls) ; 1 pint = 20oz)*

Milk 1 ☐ ☐☐ pints

Milk 2 ☐ ☐☐ pints

Milk 3 ☐ ☐☐ pints

1.12 Which types of dried milk have you used regularly in drinks (or used as reconstituted liquid milk) over the past 3 months? *(list up to 3 below)*

0. None
1. Dried skimmed milk (eg Marvel, Tesco's, Sainsbury)
2. Dried whole milk
3. Coffeemate, coffee whitener
4. Vending machine milk powder
5. Other

Milk 1 ☐ If "Other", *specify* _____

Milk 2 ☐ If "Other", *specify*_____

☐

Milk 3 If "Other", *specify* _____

1.13 On average over the last 3 months how much of each dried milk have you consumed per day? *(1 vending machine cup = 1 teaspoon)*

Milk 1 ☐ ☐☐ teaspoon

Milk 2 ☐ ☐☐ teaspoon

Milk 3 ☐ ☐☐ teaspoon

1.14 Have you regularly consumed any of the following foods over the past 3 months? *(see prompt card)*

0. No *go to 1:15*
1. Yes ☐

Product	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**more than once per day**	**no of times per day**
…….. ☐☐☐☐☐	1	2	3	4	5	6	7	8	
…….. ☐☐☐☐☐	1	2	3	4	5	6	7	8	
…….. ☐☐☐☐☐	1	2	3	4	5	6	7	8	
…….. ☐☐☐☐☐	1	2	3	4	5	6	7	8	

1.15 During the past three months have you taken any pills, tonics or tablets to supplement your diet? (e.g. vitamins, minerals, iron tablets, folic acid, fish oils etc.)

0. No *go to 2:*
1. Yes ☐

If yes, please state which:
(for number per day record number of tablets/capsules/teaspoons per day, as appropriate)

Supplement	Number per day	How many days in the last 90?
☐☐☐☐		
☐☐☐☐		
☐☐☐☐		
☐☐☐☐		
☐☐☐☐		
☐☐☐☐		
☐☐☐☐		
☐☐☐☐		
☐☐☐☐		
☐☐☐☐		

2: HEALTH AND MEDICATION

2.1 How would you rate your health in general? Would you say it is?

1. Very good
2. Good
3. Fair
4. Bad
5. Very bad

☐

2.2 Do you take any regular medicines *(not including the supplements recorded)* either from the chemist, doctor, or alternative therapies? Please include inhalers for asthma.

0. No *go to 2.3*
1. Yes, please list them in the table below

☐

USE BLOCK CAPITALS & COPY NAMES DIRECTLY FROM BOTTLES IF POSSIBLE

1	
2	
3	
4	
5	
6	
7	
8	
9	
10	

2.3 Have you ever taken steroid tablets or a steroid inhaler?

0. No *Go to 2.6*
1. Yes – Tablets
2. Yes – Inhaler
3. Yes - Both
4. Not known

☐

2.4 How long ago did you take steroids? ☐☐ Years ☐☐ Months ☐☐ Weeks

2.5 For how long ?
a/ Inhaler ☐☐ Years ☐☐ Months ☐☐ Weeks ☐☐ Days

b/ Tablets

☐☐ Years ☐☐ Months ☐☐ Weeks ☐☐ Days

2.6 Do you have any of the following conditions?

0. No
1. Yes
9. Not known

Diabetes Mellitus	
Inflammatory bowel disease	
Liver disease (e.g. cirrhosis)	
Malabsorption	
Osteoarthritis	
Rheumatoid arthritis	
Thyroid disease	
Coeliac disease	
Asthma	
Kidney disease	
Osteoporosis	

2.7 Do you have any other long-standing medical conditions? By long-standing I mean anything that has troubled you over a period of time, or that is likely to affect you over a period of time. Please write name of condition in the relevant box.

0. No *go to 3*
1. Yes ☐

Other disease	
Other disease	
Other disease	

3 FRACTURE HISTORY

3.1 Have you ever broken or fractured a bone?

0. No *go to 3:2*
1. Yes

☐

Which bone did you break / fracture	At what age did you break their bone?	How did it happen e.g. fell of bicycle

3.2 Has either of your parents ever broken or fractured a bone?

0. No *go to 3.3*
1. Yes
9. Not known

☐

Which parent?	Which bone did they break / fracture?	At what age did they break their bone?	How did it happen? e.g.: fell whilst walking

3.3 Have any of your grandparents ever broken or fractured a bone?

0. No *go to 3.4*
1. Yes
9. Not known

☐

Which grand parent?	Which bone did they break / fracture?	At what age did they break their bone?	How did it happen? e.g.: fell whilst walking

3.4 Have any of your siblings ever broken or fractured a bone?

0. No *go to 4*
1. Yes
2. No siblings *go to 4*
9. Not known

Which sibling?	Which bone did they break / fracture?	At what age did they break their bone?	How did it happen? e.g.: fell whilst walking

4: Reproductive History

<u>WOMEN ONLY (*men go to 5*)</u>

4.1 How many times have you been pregnant?

4.2 I am now going to ask bout the details of these pregnancies and whether they resulted in a live born child, stillborn or miscarriage.

Pregnancy Number	Year	Liveborn (L) Stillborn (S) Miscarriage (M) Termination (TOP)	If liveborn:	
			Male (M) Female (F)	Birthweight
1				
2				
3				
4				
5				
6				
7				
8				

4.3 How old were you when your periods started?

4.4 If your periods have stopped, how old were you when this happened?

4.5 Have you ever taken the oral contraceptive pill? ☐

0. Yes
1. No go to *4.12*

4.6 How old were you when you started taking the contraceptive pill? ☐☐

4.7 How long did you take it for (months)? ☐☐

4.8 Have you ever used any form of contraceptive implant? ☐
(e.g. depo-provera) 0. Yes
1. No

4.9 How old were you when you started using this? ☐☐

4.10 How long did you use it for (months)? ☐☐

5: ACTIVITY AND EXERCISE

5.13 Which of the following best describes your usual walking speed? ☐

1. Very slow
2. Stroll at an easy pace
3. Normal speed
4. Fairly brisk
5. Fast

5.14 During the past three months, how often have you done the following kinds of exercise or activities?

a) **strenuous exercise w**hich normally makes your heart beat rapidly **AND** leaves you breathless and exhausted e.g. jogging, vigorous swimming or cycling, aerobics.

FFQ categories . ☐☐ x1 ☐☐

and **on average** about how long does each period of activity last? ☐☐ hrs ☐☐ mins

b) **moderate exercise** which normally leaves you tired but not breathless, and makes your heart beat rapidly, e.g. brisk walking, dancing, easy swimming or cycling, badminton, sailing.

FFQ categories . ☐☐ >x1 ☐☐

and **on average** about how long does each period of activity last? ☐☐ hrs ☐☐ mins

c) **gentle exercise** which normally leaves you tired, e.g. walking, heavy housework (including washing windows and polishing, child and family care), gardening, DIY, golf.

FFQ categories . ☐☐ >x1 ☐☐

and **on average** about how long does each period of activity last? ☐☐ Hrs ☐☐ mins

5.15 Do you generally use sunblock if outside in sunny weather?

0. No *Go to 6* ☐
1. Yes

5.16 What factor do you use most frequently? ☐☐

6: LIFESTYLE

6.1 Have you ever smoked regularly?
(i.e. at least once a day for a year or more) ☐
0. No *go to 6.8*
1. Yes

6.2 At what age did you start smoking? ☐☐

6.3 If you added up all the years that you smoked, how many would that make in total? ☐☐

6.4 What was the average amount you smoked over this time?

Cigarettes/day ☐☐

Roll-ups (ozs/week) ☐☐

Cigars/week ☐☐

Pipe tobacco (ozs)/week) ☐☐

6.5 Do you still smoke regularly? ☐
0. No *Go to 6.6*
1. Yes *Go to 6.7*

6.6 How old were you when you last smoked regularly? ☐☐

6.7 Have much do you smoke now?

Cigarettes/day ☐☐

Roll-ups (ozs/week) ☐☐

Cigars/week ☐☐

☐☐

Pipe tobacco (ozs)/week)

6.8 Are you regularly exposed to tobacco smoke at home by other members of your household? ☐

0. No *Go to 7*
1. Yes

6.9 How many people (excluding yourself) in your household smoke regularly? ☐

7: ALCOHOL CONSUMPTION

7.1 Do you ever drink alcohol? ☐

0. No *Go to 8*
1. Yes

During the past three months:

7.2 a) How often have you drunk **Shandy or Low Alcohol Beer/Lager/Cider**? (don't include alcohol **free** lager etc)
FFQ categories ☐☐.☐☐ >x1 ☐☐

b) When you drank these how many pints did you normally have? ☐☐ ☐☐
(if range given code mid-point)

7.3 a) How often have you drunk **Beer/Stout/Lager/Cider/Alcopops?**
FFQ categories ☐☐.☐☐ >x1 ☐☐

b) When you drank these how many pints did you normally have? ☐☐ ☐☐
(if range given code mid-point)

7.4 a) How often have you drunk **Low alcohol wine?** FFQ categories ☐☐.☐☐ >x1 ☐☐

b) When you drank this how many glasses did you normally have? ☐☐ ☐☐
. *(if range given code mid-point)*

7.5 a) How often have you drunk **Wine/Sherry/Martini/Cinzano?**
FFQ categories ☐☐.☐☐ >x1 ☐☐

b) When you drank these how many glasses did you normally have?
(if range given code mid-point)

7.6 a) How often have you drunk **Spirits/Liqueurs?**

FFQ categories . >x1

b) When you drank these how many measures did you normally have*?*
(if range given code mid-point)

8: ETHNIC GROUP

8.1 To which of the ethnic groups listed on this card do you consider you belong?

1. White
2. Black Caribbean
3. Black African
4. Black Other
5. Indian
6. Pakistani
7. Bangladeshi
8. Chinese
9. Other Asian group
10. Other (specify) _____

9: BODY MEASUREMENTS

9.1 Measurer [ ][ ]

9.2 Which hand do you write with?

1. Right
2. Left
3. Completely ambidextrous

[ ]

9.3 Weight [ ][ ][ ] . [ ] kgs

9.4 Height [ ][ ][ ] . [ ] cms

9.5 GRIP STRENGTH **(to nearest 0.5kg)**

	RIGHT	**LEFT**
	[ ][ ] . [ ]	[ ][ ] . [ ] kgs
	[ ][ ] . [ ]	[ ][ ] . [ ] kgs
	[ ][ ] . [ ]	[ ][ ] . [ ] k

Appendix 5: Copy of parent DXA result

Osteoporosis Centre
Southampton General Hospital
Level C West Wing SO16 6YD

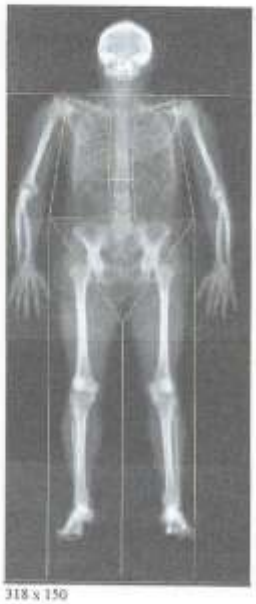
Telephone: 02380794696 E-Mail: pat.taylor@suht.swest.nhs.uk Fax: 02380798995

Name: [redacted]
Patien
DOB:

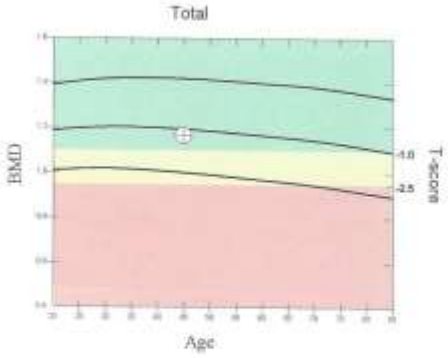
Sex: Male
Ethnicity: White

Height: 168.0 cm
Weight: 69.4 kg
Age: 44

Referring Physician: Holroyd



318 x 150



Scan Information:

Scan Date: 04 October 2012 ID: A10041208
Scan Type: a Whole Body
Analysis: 04 October 2012 13:23 Version 13.0
Auto Whole Body
Operator: akb
Model: Discovery W (S/N 80019)
Comment:

DXA Results Summary:

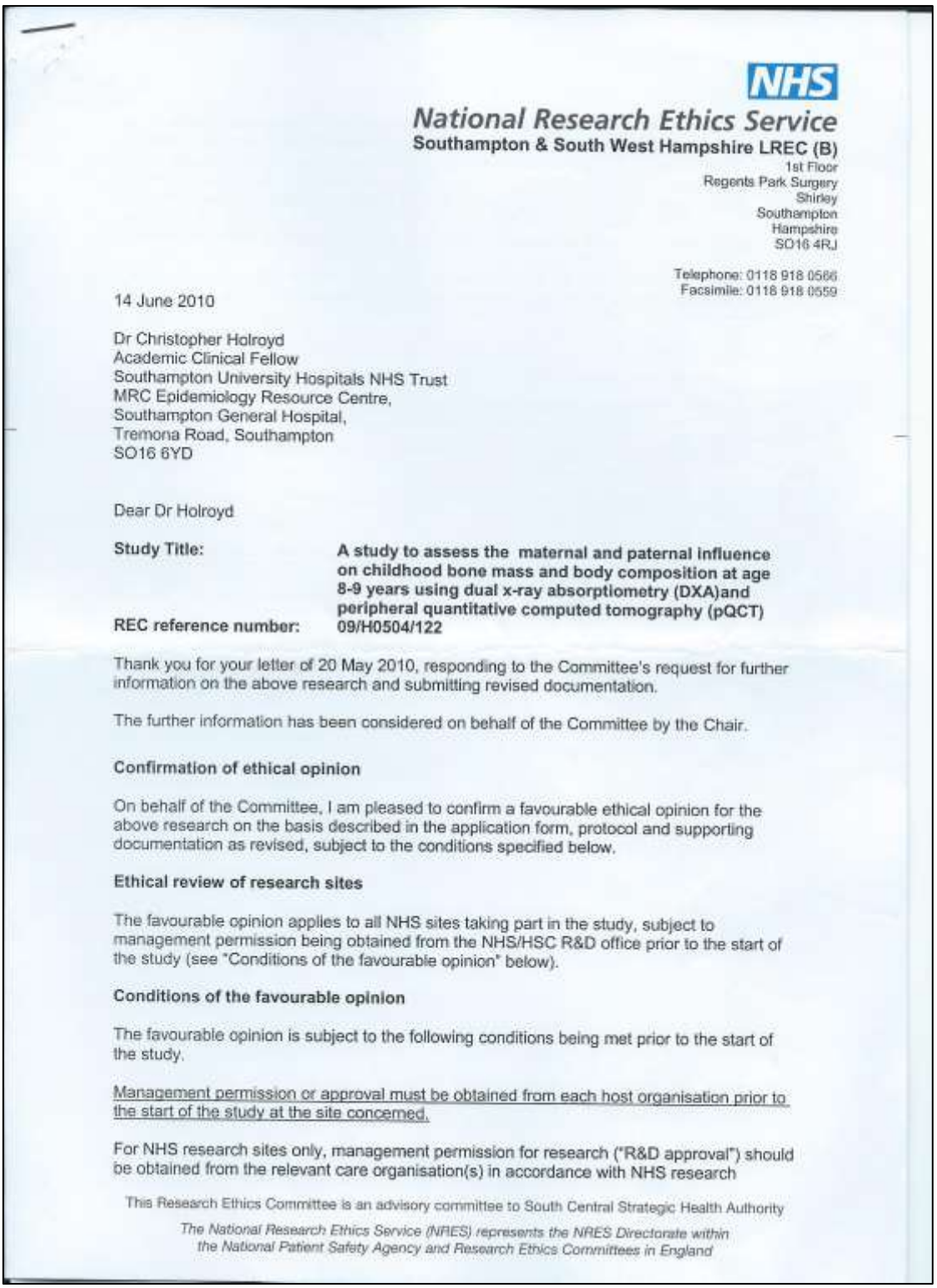
Region	Area (cm²)	BMC (g)	BMD (g/cm²)	T-score	PR (%)	Z-score	AM (%)
L Arm	222.89	193.25	0.867				
R Arm	226.77	207.59	0.915				
L Ribs	114.55	75.91	0.663				
R Ribs	140.96	93.70	0.665				
T Spine	127.37	108.91	0.855				
L Spine	51.65	48.03	0.930				
Pelvis	192.21	203.00	1.056				
L Leg	332.78	455.06	1.367				
R Leg	340.16	490.62	1.442				
Subtotall	749.33	1876.06	1.072				
Head	245.41	445.40	1.815				
Total	**1994.75**	**2321.47**	**1.164**	**-0.3**	**97**	**-0.3**	**98**

TBAR2695 - NHANES BCA calibration

T-score vs. White Male; Z-score vs. White Male. Source:2008 NHANES White Male

HOLOGIC

Appendix 6: LREC approval letter



NHS
National Research Ethics Service
Southampton & South West Hampshire LREC (B)
1st Floor
Regents Park Surgery
Shirley
Southampton
Hampshire
SO16 4RJ

Telephone: 0118 918 0586
Facsimile: 0118 918 0559

14 June 2010

Dr Christopher Holroyd
Academic Clinical Fellow
Southampton University Hospitals NHS Trust
MRC Epidemiology Resource Centre,
Southampton General Hospital,
Tremona Road, Southampton
SO16 6YD

Dear Dr Holroyd

Study Title: **A study to assess the maternal and paternal influence on childhood bone mass and body composition at age 8-9 years using dual x-ray absorptiometry (DXA)and peripheral quantitative computed tomography (pQCT)**

REC reference number: **09/H0504/122**

Thank you for your letter of 20 May 2010, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Ethical review of research 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.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

For NHS research sites only, management permission for research ("R&D approval") should be obtained from the relevant care organisation(s) in accordance with NHS research

This Research Ethics Committee is an advisory committee to South Central Strategic Health Authority

The National Research Ethics Service (NRES) represents the NRES Directorate within the National Patient Safety Agency and Research Ethics Committees in England

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