UNIVERSITY OF SOUTHAMPTON

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How do early environment, diet and physical activity interact to determine bone development in young children?

by

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ABSTRACT

FACULTY OF MEDICINE

Doctor of Philosophy

HOW DO EARLY ENVIRONMENT, DIET AND PHYSICAL ACTIVITY INTERACT TO DETERMINE BONE GROWTH IN YOUNG CHILDREN?

By Zoë Annalise Cole

Aims: To examine the interaction of maternal factors (body composition, physical activity, diet and cigarette consumption) with childhood factors (body composition, diet & physical activity) in the determination of bone mineral accrual by aged 6 years, assessed by a) bone densitometry b) hip structural analysis c) pQCT measurement of the tibia in children born to mothers from the Southampton Women's Survey.

Methods: Children were recruited at 6 years old from the Southampton Women's Survey. Their mothers' diet, lifestyle and anthropometry had previously been characterised before and during pregnancy. The children underwent measurement of bone mass by DXA, including hip structure analysis (HSA), and by pQCT at the tibia. Physical activity was assessed by accelerometry (Actiheart) for 7 continuous days. Diet was assessed using a validated food frequency questionnaire and detailed anthropometric data was also collected.

Results: There were 530 children who attended for a DXA scan. Of these, 148 also underwent pQCT assessment. Increased childhood height, weight and milk intake were associated with increased measures of bone size; increased physical activity levels and greater lean mass were positively associated with increased volumetric BMD. Fat mass was negatively associated with volumetric BMD. Whilst maternal height, weight, exercise in late pregnancy and pre pregnancy calcium intake were associated with increased bone size in the offspring, this association was removed after adjusting for childhood factors suggesting that maternal body composition and lifestyle may predict the child's body composition and lifestyle.
On assessment of growth patterns in this cohort, children were who born small tended to remain small at aged 6 years. Increased catch up growth was associated with increased maternal height and total milk intake at aged 3 years. Rapid weight gain during childhood was associated with maternal smoking during pregnancy.

Conclusions: We have demonstrated that maternal and childhood factors influence bone mineral accrual and bone strength, in the developing child. Whilst many important maternal determinants measured (such as physical activity levels) were shown to influence the corresponding determinants in the offspring, other factors such as maternal cigarette smoking were shown to have persistent independent effects on post-natal growth and body composition.

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Academic Thesis: Declaration of Authorship

I, Zoë Annalise Cole, declare that the thesis entitled

"HOW DO EARLY ENVIRONMENT, DIET AND PHYSICAL ACTIVITY INTERACT TO DETERMINE BONE GROWTH IN YOUNG CHILDREN?"

and the work presented in it are my own and has been generated by me as the result of my own original research. I can confirm that:

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7. None of this work has been published before submission.

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Date……

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Glossary

DXA (Dual X ray absorptiometry) indices

BA:	Bone area
BMC:	Bone mineral content
aBMD:	Areal bone mineral density
vBMD:	Volumetric bone mineral density
BMAD:	Bone mineral apparent density
WB:	Whole body
LS:	Lumbar spine

HSA (Hip axis software) indices

CSA:	Cross sectional area
CSMI:	Cross sectional moment of inertia
Z:	Sectional modulus (bending strength)
NN:	Narrow neck
IT:	Intertrochanteric
FS:	Femoral shaft

PQCT (peripheral quantitative computed tomography) indices

SSI:	Stress strain index

Biochemical measurements

IGF 1:	Insulin like growth factor 1
PTH:	Parathyroid hormone
PTHrP:	Parathyroid hormone related protein
GH:	Growth hormone
HPA:	Hypothalamic pituitary adrenal axis

Other

TSF:	Triceps skinfold thickness
PBM:	Peak bone mass
FFQ:	Food frequency questionnaire

1 BACKGROUND

1.1 Introduction

Osteoporosis is a major cause of morbidity and mortality through their association with age related fractures. Bone strength in later life depends upon the peak bone mass accrued during childhood and adolescence, and the subsequent rate of bone loss. Whilst most treatment strategies for osteoporosis have been targeted at retarding bone loss, optimising peak bone mass remains an equally effective preventative strategy.

Evidence is accruing to suggest that environmental factors in early life have a critical influence on the magnitude of peak bone mass achieved, and on the subsequent risk of fractures. The underlying hypothesis (often termed programming) is that persisting changes in structure and function are caused by environmental stimuli at critical periods during early development.

Skeletal bone consists of both trabecular and cortical bone. Bone structures that withstand vertical loading, for example vertebrae, derive a substantial proportion of their strength from a system of horizontal, cross bracing trabeculae. Severance of such connections in postmenopausal women results in an increased risk of fracture. An increased risk of fracture is also associated with altered bone geometry, in particular shorter hip axis length and increased cross sectional area are associated with an architecturally stronger structure for any given BMD.

This thesis explores the influence of environmental factors, both *in utero* and during early childhood, important for skeletal growth and body composition using DXA. It also explores the relationships between both cortical and trabecular bone densities, area and geometry to further understand the mechanisms behind how bones develop their strength, using pQCT and hip structure analysis, which may influence an individual's future risk of fracture.

1.2 Osteoporosis

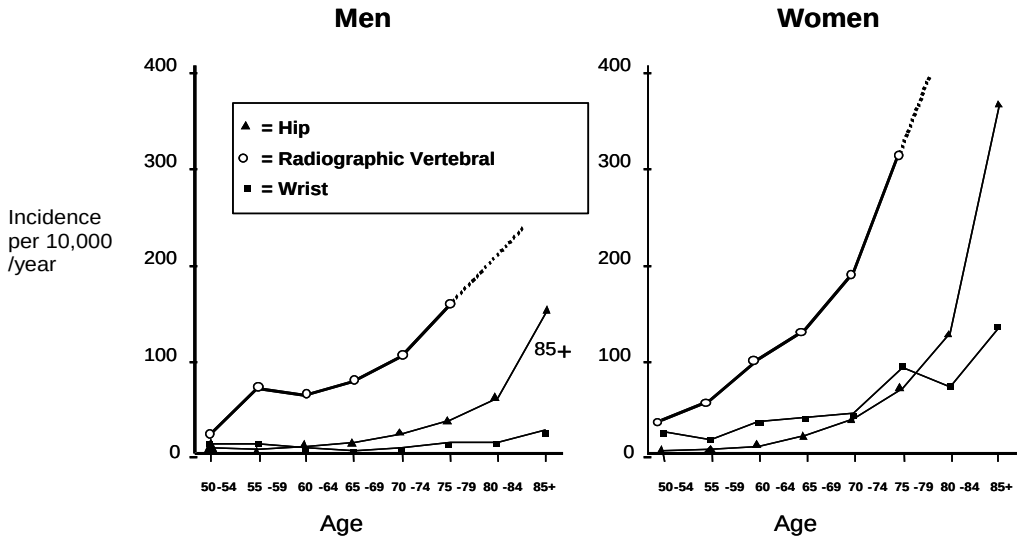
1.2.1 Definition

Osteoporosis is a systemic disorder characterised by low bone mass and micro-architectural deterioration of bone tissue with a consequent increase in bone fragility and susceptibility to fracture[1]. The World Health Organisation have defined osteoporosis clinically as a DXA derived T score of less than –2.5[2]. It is important to realize that this definition does not reflect decline in bone micro-architecture.

1.2.2 Epidemiology

Fracture incidence in the community is bimodal, showing peaks in youth and the very elderly (figure 1). In young people, fractures of the long bones predominate, usually after substantial trauma, and they are more frequent in males than females. Over the age of 35 years fracture incidence in women rises steeply so that rates become twice those of men. At age 50 years a UK study has shown that one in two women will have an osteoporotic fracture in their remaining lifetime; the figure for men is one in five[3]. The combined annual costs of all osteoporotic fractures have been estimated to be $20 billion in the USA and $30 billion in the European Union[4]

Figure 1: Incidence of osteoporotic fractures[3]



The most common sites for osteoporotic fracture are the vertebrae, hip and distal forearm although prospective studies have shown a heightened risk of almost all types of fracture in individuals with low bone density. The most frequent site of fracture is the thoraco-lumbar spine, with the age standardised prevalence in Europe 12.2% for men and 12.0% for women aged 50-79 years[5]. Only a third of all radiographically identified vertebral deformities come to clinical attention acutely. Hip fractures are the most devastating result of osteoporosis; resulting in inevitable hospital admission and significant morbidity and mortality. The remaining lifetime risk of hip fracture for a 50 year old in the UK is 11.4% and 3.1% for women and men respectively. Most of this risk is accrued in old age, such that a 50 year old woman's 10 year risk of hip fracture is 0.3% rising to 8.7% at aged 80 years[3].

Wrist fractures show a different pattern of occurrence to hip and vertebral fractures. There is an increased incidence between the ages of 45-60 years followed by a plateau. This may relate to altered neuromuscular reflexes with aging, and as a result, a tendency to fall sideways or backwards, thus not breaking the fall by an outstretched arm.

Whilst all fractures are associated with significant morbidity, both hip and vertebral fractures are also associated with excess mortality. Although this may represent complications of the fracture and subsequent surgery for hip fractures, it is likely to reflect coexisting co morbidity in persons experiencing vertebral fracture. By two years after hip fracture morality rates decline back to baseline, however mortality after vertebral fracture seems to increase progressively after diagnosis of the fracture probably as a result of their co-morbid conditions[3].

1.2.3 Pathophysiology

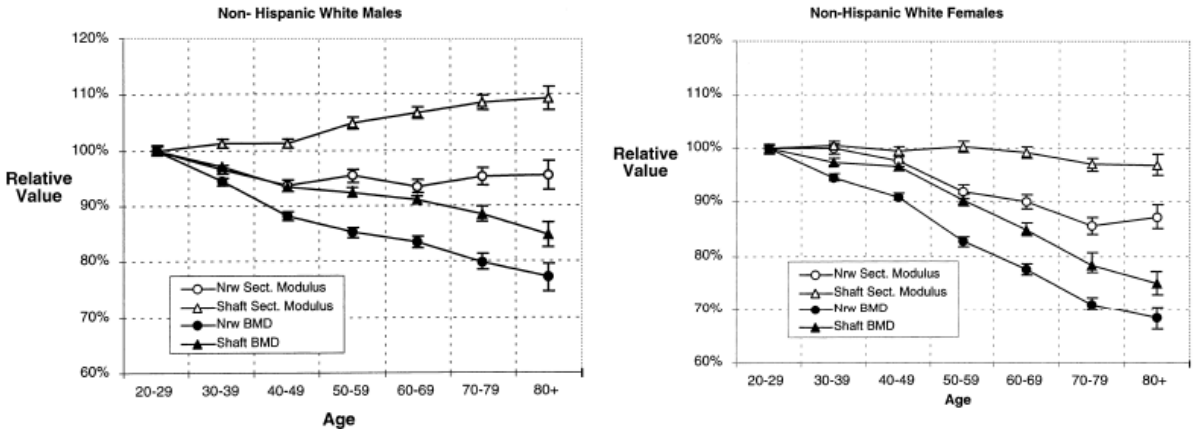
Fracture risk ultimately depends upon two factors: the mechanical strength of the bone and the forces applied to it. During the first three decades of life, fractures are considered to arise from a higher energy trauma compared to fractures occurring in later life. Most hip and forearm fractures occur when falling from standing height whereas vertebral fractures occur from routine activities such as bending and lifting light objects.

Bone mineral density is the major determinant of bone strength and risk of fracture. There is evidence for a genetic contribution for variation in bone density, with hereditability estimates between 0.6-0.8[6]. It is also influenced by environmental and medical factors. Other independent risk factors that are strongly associated with osteoporotic fractures include age, chronic glucocorticoid use, prevalent vertebral fracture and recent prior clinical fracture[7]. Maternal and paternal history of hip fracture[8], physical activity[9], impaired neuromuscular function and menopause before age 45[10] retain a moderate relationship with incident fracture whilst cigarette smoking has only a weak relationship[11].

Bone density in later life is a function of both the peak bone mass attained during childhood and adolescence and the subsequent rate of bone loss. Even in the 7th decade, half of the variance in bone mineral density is accounted for by peak bone mass[12]. Other aspects of bone structure that determine bone strength include geometry, micro-architecture and turnover.

There is growing realisation that adult bones do not merely lose mass as they age, but that they alter the distribution of the remaining material in order to preserve strength. Long bones generally expand their outer dimensions with age due to periosteal apposition . In both genders BMD trends downward more quickly than bone mass. There is a significant upward trend in the femoral neck bone area (explained by an expansion in the outer diameter of the femoral neck). Figure 2 shows that whilst the BMD declines with age the resulting change in structure leads to a relative preservation of section modulus (strength)[13].

Figure 2 Weight corrected age trends in BMD and section moduli in non-Hispanic white males and females[13].

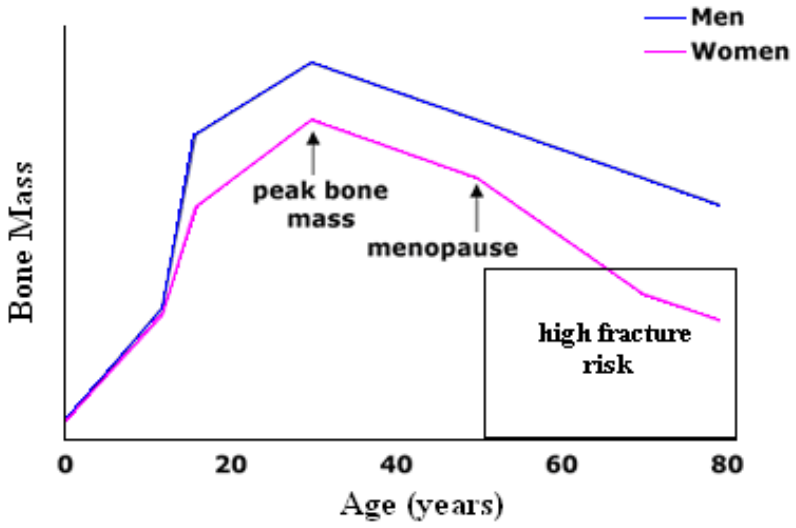


In adults both intertrochanteric and femoral shaft sub-periosteal width (hazard ratio 1.61 and 1.43 for each SD increase) and buckling ratio (hazard ratio 1.36 and 1.24 for each SD increase) were predictors of hip fracture independent of body size, age, clinical risk factors and conventional aBMD in 10,290 postmenopausal women followed up over 11 years as part of the Women's Health Initiative[14].

1.3 Peak bone mass

The human foetal skeleton accretes four fifths of the total calcium during the third trimester of pregnancy. Bone mass then increases during childhood largely as a result of longitudinal growth. A rapid gain occurs during adolescence and up to 25% of peak bone mass (PBM) is accreted during the 2 year period across peak height velocity[15]. At least 90% of PBM is acquired by the age of 18 years, the rest being achieved in the twenties. However the exact timing appears to vary with skeletal site and gender. Following achievement of peak bone mass there is a steady decline, accelerated in women at the menopause due to loss of the protective effect of oestrogen (figure 3).

Figure 3: Bone mass with age in men and women



1.3.1 Bone growth in utero

In utero, the skeletal system develops in a carefully coordinated series of events from the aggregation of mesenchymal cells to the laying down of osteoid and subsequent mineralisation to form mature bone.

The skeleton develops in two distinct components, intramembraneous (the skull and facial bones) and endochondral (the remainder of the skeleton) ossification.

1.3.2 Intramembraneous ossification

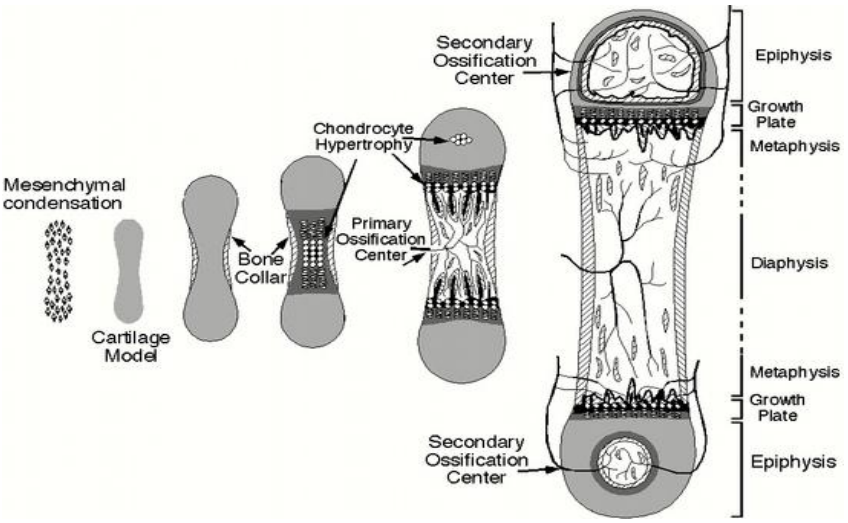
Intramembraneous ossification begins with a layer of mesenchymal cells which become highly vascular, the mesenchymal cells then differentiate into isolated osteoblasts, which begin to secrete osteoid. The osteoid matrix is mineralised at the end of the embryonic period to form bony spicules, which are precursors of the lamellae of the Haversian systems. There is no cartilage model preceding ossification in this type of bone development.

1.3.3 Endochondral ossification

Endochondral ossification is responsible for the formation of bones that are the main sites of fragility fracture in later life. This begins with condensation of the mesenchyme to form a cartilaginous model of the bone to be formed. Mesenchymal

cells undergo division and differentiate into prechondroblasts and then into chondroblasts. Beginning in the centre of the cartilage model, at what is to become the primary ossification centre, chondrocytes differentiate and become hypertrophic. During this process, hypertrophic cells deposit an extracellular matrix rich in cytokines, which facilitate vascular invasion and mineralization. Mesencymal progenitor cells in the perichondrium differentiate into osteoblasts and form a bone collar around the diaphysis of the cartilage analogue. Following calcification of this bone, blood vessels, preceded by the osteoclasts entering the primary ossification center, will penetrate this bone and the calcified cartilage, forming the blood supply which will allow seeding of the hematopoietic bone marrow and invasion of osteoclasts to resorb the calcified cartilage. Secondary ossification centers begin to form at the epiphyseal ends of the cartilaginous model, and by a similar process, trabecular bone and a marrow space are formed at these ends. Between the primary and secondary ossification centres, epiphyseal cartilage remains until adulthood. The continued differentiation of chondrocytes, cartilage mineralisation and subsequent remodelling cycles allows longitudinal bone growth to occur.

Figure 4 Endochondral Ossification, adapted from endotext.org



Chondrocyte differentiation is regulated by a number of factors, the first being parathyroid related peptide (PTHrP) which is secreted by the perichondral cells[16].

This factor prolongs chondrocyte proliferation. Other proliferative stimuli include cytokines of the GH/IGF axis[17], 1,25(OH)^2 vitaminD3[18], tri-iodothyronine[19], FGF[20] and bone morphometric proteins[21;22].Cbfa1 mediates mesenchymal differentiation into osteoblast progenitors as well as permitting terminal differentiation of chondrocytes[23].

1.3.4 Mineralization of the foetal skeleton

Whilst a miniature version of the skeleton is laid down in the embryonic period and primary ossification centres form in the vertebrae and long bones between the 8th and 12th weeks it is not until the third trimester that the bulk of mineralization occurs[24]. The main determinant of skeletal mineralization *in utero* appears to be the foetal plasma calcium concentration. To supply this demand, there is a requirement for an adequate maternal supply of calcium to the placenta and increased placental calcium transfer to maintain a higher foetal calcium concentration than the mother. This materno-foetal gradient emerges as early as 20 weeks gestation[25].

Low levels of foetal PTH activity influence foetal calcium levels[26]. Maternal PTH does not cross the placenta however both hypo and hyperparathyoidism appear to affect the foetus via decreasing or increasing the calcium load presented to the foetal circulation and suppression of foetal PTH.

PTHrP is a polyhormone coded on chromosome 12. It is produced by the foetal parathyroid gland, with some production by the placental syncytial and trophoblasts, it plays multiple roles during embryonic and foetal development. It is a major determinant of placental calcium transport, possibility through its interactions with the calcium sensing receptor (CaSR), which appropriately suppresses PTH in response to elevated calcium.

It seems likely that other factors also affect maternal calcium levels and foetal mineralization, such as 1,25(OH)2 vitamin D3. There is evidence to suggest vitamin D deficiency during late pregnancy results in impaired BMC of the offspring at age 9 years[27]. In addition, expression of an active placental calcium transporter (PMCA3) is positively correlated with whole body BMC in the offspring at birth[28]. This

observation may suggest a possible mechanism for the influence of maternal vitamin D status on placental calcium transport and accrual of bone mineral.

Finally there is evidence that PTH and PTHrP differentially affect mineralization of cortical and trabecular bone and thus are also attractive candidates for the physiological investigation of programming[29].

1.3.5 Bone mineral accrual in infancy and childhood

Childhood and adolescence are characterised by longitudinal growth as well as changes in skeletal size and shape. Skeletal mass increases from ~70-95g at birth to 2400-3300g in young women and men respectively[30].

Bone length increases by either intramembranous ossification of the distal end of the craniofacial bones, or endochondrial ossification of the remainder of the axial skeleton, through the growth plate. Here chondrocyte division on the metaphyseal surface of the growth plate leads to longitudinal growth. A sleeve of cartilage around the epiphysis forms the perichondral ring, which influences both the diameter and shape of the growth plate[31]. During puberty, the rate of chondrocyte division slows more than endochondral ossification leading to complete replacement of the growth plate by bone and the achievement of skeletal maturation.

1.4 Determinants of postnatal bone growth

After birth, growth can be divided into three phases: infancy, childhood, and puberty reflecting changes in the height velocity during these ages.

The speed of physical growth is rapid during the first two years of life. Birth weight is doubled in the first four months, tripled by age 12 months, but not quadrupled until 24 months. Growth then proceeds at a slower rate during childhood until shortly before puberty (between about 9 and 15 years of age), when a period of rapid growth occurs. Growth is not uniform in rate and timing across all body parts. For example, at birth the head size is already relatively near to that of an adult, but the lower parts of the body are much smaller. In the course of development the head grows relatively little, whereas the torso and limbs undergo a great deal of growth[32].

Genetic factors play a major role in determining the growth rate. However, genetic factors can only produce maximum growth if the environmental conditions are adequate; for example, poor nutrition and chronic disease may both reduce an individual's adult stature.

1.4.1 Catch up growth

Catch-up growth is defined as height velocity above statistical limits of normality for age and/or accelerated maturity during a defined period, following a transient period of growth inhibition.

Tanner suggested that catch up growth occurs in two different temporal patterns. In the first, the individual shows an early, marked growth acceleration that reduces the deficit rapidly within a few years. The child then grows along this improved percentile until adult height is achieved. In the second pattern, the child stays at a low percentile for many years, growing at normal velocity for chronological age. However bone maturation remains delayed so that growth continues beyond the usual age, leading to improved adult height[32].

Babies who are short or light at birth may have experienced poor intrauterine growth and have a period of accelerated growth postnatally, this maybe described as catch up growth. However, it is becoming clear that this pattern of growth is distinct from the above examples of catch up growth. Firstly, the onset of accelerated height velocity is some time after the end of the insult and, more importantly, there may not be a period of regulated growth deceleration.

The tempo of postnatal weight gain is emerging as particularly important in the relationship between birth weight and adult disease. Barker showed that excessive weight gain during childhood and adolescence in individuals who were born small at birth predicted a heightened risk of coronary heart disease in later life[33]. It has also been shown that small for gestational age adults continue to gain greater fat mass in early adulthood suggesting that the consequences of foetal growth restriction on body composition are evolving beyond the period of early postnatal catch-up[34].

Recent systematic reviews have identified consistent associations between postnatal rapid weight gain during the first 1-2 years of life and later obesity in children and adults[35;36]. Overall there was a 2-3 fold increase in overweight or obesity risk in those individuals whose weight crossed upward by at least one major band between birth and ages 1 or 2 years[35]. There is still debate as to the exact timing of rapid postnatal weight gain, Ong et al found that faster weight gain between ages 0-2 and 2-9 months was associated with increased body fat mass relative to lean mass at aged 10 years, but not between 9-19 months in 2715 girls[37]. Whereas Yliharsilia et al showed that rapid gain in BMI before the age of 2 years increased adult lean body mass without excess fat accumulation. He also reported that rapid gain in BMI in later childhood, despite the concurrent rise in lean mass, resulted in relatively larger increases in fat mass in 885 men and 1032 women born during 1934-1944[38].

The endocrine mechanisms governing catch up growth are still poorly understood. However there is evidence to suggest that insulin like growth factor (IGF-1) plays a major role in the regulation of growth during infancy and childhood. Higher concentrations at 3 months predicted greater subsequent gains in body length and slower gains in BMI and adiposity[39]. Whilst there is evidence to suggest abnormally low IGF levels in infants born SGA, these levels increase rapidly after birth. However the levels remain lower than children born appropriate for gestational age[40], which may account for some of the increased risk of cardiovascular disease[41].

1.4.2 Genetic determinants

Heredity factors are important determinants of bone mass. Convergent data from mother daughter pairs, sibling pairs and twin studies have estimated the heredity of bone mass to account for 60 – 80% of its variance[42;43]. The magnitude of the effect varies with age and between skeletal sites; it is higher in the young than in the elderly and in the spine than in the extremities. Further support for this genetic influence comes from studies showing reduced bone mass in daughters of osteoporotic women compared with controls[43], and in men and women with first degree relatives that have osteoporosis[44]. However the magnitude of genetic effects on bone mass may be overestimated due to similarities in environmental influences between parents and offspring.

Currently, genetic polymorphisms have been found to make only a modest contribution to bone mass in populations[45]. Differences in Vitamin D receptor (VDR) polymorphisms at the BsmI restriction site accounted for differences in BMD in pre-pubertal and adolescent girls. Girls with BB genotype had significantly lower spinal BMD than girls with the Bb or bb genotypes[46]. In contrast polymorphisms at the start codon of the VDR gene show no association with BMD at any skeletal site[47].

Recent technological and scientific advances have provided the tools needed to rapidly scan the genome for genetic variants affecting osteoporosis. Genomewide association studies (GWASs) have identified several associations contributing to risk of fracture and related traits. These discoveries promise to illuminate important new pathways in bone metabolism, contribute to the development of novel therapeutics and possibly harbour prognostic value.

Since May 2008 10 GWASs have identified nearly 30 independent loci affecting BMD and/or fracture[48]. Strong associations for BMD have been confirmed in or near many previously suspected candidate genes, such as the estrogen receptor (*ESR1*)[45;49] TNF receptor superfamily, member 11a (*TNFRSF11A*; RANK)[45;50], TNF (ligand) superfamily, member 11 (*TNFSF11*; RANKL),[45;49]SP7 transcription factor (*SP7*),[45;50;51] and low-density lipoprotein receptor-related protein 5 (*LRP5*).[45;52]However, most of the associations exceeding stringent genome-wide significance thresholds have been with novel genes, such as family with sequence similarity 3, member C (*FAM3C*)[53] and MAP/microtubule affinity-regulating kinase 3 (*MARK3*),[50]among many others. These novel genes have no known connection to bone and, once validated, their discovery should highlight important new biological mechanisms impacting bone metabolism.

The results to date explain only a small fraction of the genetic component for traits such as BMD. For example, a large-scale meta-analysis of 19,195 individuals identified a total of 15 SNPs associated with lumbar spine BMD, however these only explained 2.9% of the variance[45]. The undiscovered genetic component is likely to consist of a combination of many more common variants with increasingly smaller effects and the contributions of rare variants. It is also likely that inherited epigenetic

modifications and gene by gene and gene by environmental interactions are significant sources of variation. The key limitation of GWASs is they are not capable of providing information on the context in which those genes function, their relationships with other genes, or how these relationships change over time, in different environments or during disease.

1.4.3 Nutrition

The earliest data suggesting an influence of dietary calcium on peak bone mass (PBM) came from a study of two Croatian populations with substantially different calcium intakes. The differences seen in bone mass were present at aged 30 years, suggesting that the effects of dietary calcium probably occurred during growth rather than adulthood[54]. Moreover some epidemiological studies have shown an increased prevalence of osteoporosis in regions where dietary calcium intake is low[55].

The nature of infant feeding has been shown to influence bone mineral accrual, with a positive correlation between formula fed babies and infant bone mass[56]. Much of this work has been carried out in premature infants, who tend to be small and have reduced BMD. However, one study in term infants found that, although at 6 months infants fed a high calcium formula had greater BMD that those fed breast milk, when they were all put onto normal formula for the next 6 months, the differences disappeared[57], consistent with post-natal tracking along the growth trajectory. Further studies have since confirmed no difference in bone mass at age 4 years and duration of breastfeeding[58].

The most convincing evidence that calcium consumption influences rates of bone mineral accrual rates comes from controlled supplementation trials in young healthy subjects. These studies have shown that subjects given additional calcium, whether as calcium salts, milk minerals or dairy products for 1-7 years had greater gains than controls[59-63]. Although bone size increased as a result of added dietary calcium, the response to calcium varied with skeletal site, pre-treatment calcium consumption and pubertal stage. Greater bone mineral gains were reported at cortical skeletal sites, in pre-pubertal subjects and in girls whose habitual dietary intake was <850mg/day[59;62]. Whether these short-term increases will translate into clinically relevant reduction in

osteoporosis risk is yet unknown. Most studies have suggested that the beneficial effect of calcium supplementation does not last and report that the benefits of intervention stopped once the treatment had stopped[64;65]. Studies that showed benefits which persisted 12 months after discontinuation, supported the use of milk products[66]. A key aspect of dairy food supplementation studies is the failure to influence dairy intakes in children after the study; participants typically return to their pre-supplementation dietary intake within one year[67]**.** In studies which have looked at habitual milk intake among women aged 20-49 y, bone mineral content was 5.6% lower in those with low intakes of milk during the ages 5-12 years. In addition low intakes were associated with a 2 fold greater risk of fracture indicating that childhood milk intake has persisting effects on the skeleton in adult life[68]. Studies looking at habitual milk intake have found effects of increased intake on skeletal size in children as young as 5 years. Interestingly they found the effect was only seen for milk and not other dairy products[69].

Scientifically there are credible explanations for why milk is a good supplement. Insulin like growth factor 1 (IGF-1) is part of the protein fraction of milk. It is a potent growth factor of bone, contributing to osteoclast proliferation, differentiation and matrix formation and in addition mediates the effect of growth hormone. In studies of children increased milk consumption, circulating IGF-1 and height are all positively correlated[70]. In addition milk supplementation has been shown to increase IGF-1 levels[61]. Another possible mechanism is that milk supplies calcium hydroxyapatite, which contains calcium and phosphate, a key constituent of bone mineral. The calcium: phosphate ratio may be important as dietary phosphate, found in carbonated drinks, is known to bind to calcium in the gut to produce a non-absorbable salt.

Although most studies have focused on the effect of calcium on bone accrual there is increasing evidence to suggest a role of dietary fruit and vegetables. Jones et al first reported cross sectional data that showed a positive link between the consumption of fruit and vegetables and BMD in 10 year old girls[71]. A further study in girls aged 8-13 years found a positive association between fruit and vegetable consumption and bone area and BMD[72]. A positive association has also been seen in a study of boys aged 8-20 years and whole body BMC[73]. In addition a recent study of 198 mother

child pairs showed that dietary patterns consistent with a healthy diet during pregnancy was associated with increased BMC, BA and aBMD in her offspring at aged 9 years[74]. One explanation to account for some of this effect is that fruit and vegetables provide organic salts of potassium and magnesium that have a buffering effect against the acid load from the ingestion of western type diets which is believed to lead to bone loss[75;76]. Natural antioxidants and phytoestrogen compounds in some vegetables may also have some bone protective effects[77].

1.4.4 Exercise

The beneficial effects of exercise on bone mass have been well documented through multiple observational and retrospective studies indicating that weight bearing activities increase bone mass. Bone adapts to increased loading in order to maintain efficiency in providing structural, functional support to the skeleton without injury or fracture. The adaptation of the bone to loading will be to increase its size, change geometry and increase the amount of mass within the periosteal envelope.

Studies of pre-pubertal gymnasts showed a larger cross sectional area of the forearm despite a shorter stature[78]. They have also shown a greater cortical area and thickness in both the forearm and tibia as well as increased lumbar BMC and BMAD[79]. In addition there was no diminution across the twenty years since retirement with aBMD higher than the controls at all sites except the skull despite the lower frequency and intensity of exercise[78]. A study of Australian children confirmed that childhood fitness levels at aged 9 years were associated with increased bone mass as measured by calcaneal ultrasound densitometry 20 years later, independent of adult performance[80]. This suggests that increased skeletal loading in early childhood leads to an increase in peak bone mass. The Iowa bone development study provides some limited data on hip structural analysis (HSA) use in children aged 4-12 years, The data adjusted for height, age and weight showed that children who participated in 40 minutes of moderate and vigorous activity had 3% greater cross sectional area (CSA) and 5% greater bending strength (Z modulus) compared to those who did only 10 minutes daily[81]

Studies comparing the effects of different physical exercises on bone indicate that it is the high impact exercise that results in the greatest increases in bone mass; one

example is the finding of higher whole body aBMD among amateur athletes involved in weight bearing sports (rugby, football, endurance training, bodybuilding) than amateur sportsmen involved in active loading activities (swimming, rowing)[82].

Several randomised trials involving weight bearing interventions on bone mass have been conducted in children and adolesence[83-88]. Overall weight bearing exercise appeared to enhance bone mineral accrual. Adjusting studies to 6 months enabled a comparison of effects between studies. Increases in bone mass (BMC and aBMD) were 0.9-3.9% for studies conducted in pre-pubertal children, 0.9-6.2% in early pubertal children and 0.4 to 1.4% in pubertal children[89]. While it is not yet possible to see if these beneficial effects are maintained into and throughout adulthood, Fuchs and colleagues undertook a randomised control trial of jumping in children, and reported that 14 months after the finish of this trial after a period of detraining, the actively treated group maintained a 4% increased BMC and bone area at the hip[90]. The combination of exercise with calcium supplementation appeared to increase BMC more than exercise alone[83;85;86;88]. The reason for the combination is unclear. However the most likely explanation is that exercise induced osteogenesis requires calcium and thus the osteogenic adaptation may be comprised in the presence of inadequate calcium.

What these studies have failed to show is the specific type of exercise, intensity and duration that will provide the optimum stimulus for peak bone mineral accretion. This requires further investigation as well as the measurement of bone quality parameters and volumetric BMD to provide a greater insight into the mechanisms implicated in the adaptation of bone to exercise.

1.4.5 **Childhood obesity**

Obesity is now a major cause of preventable health problems in the UK and worldwide. The rise in the prevalence of overweight and obesity has extremely serious implications, not only for individual health, but also for the nations health and economy. There has been a rapid increase in the prevalence of obesity in all age groups across the UK over the last 20 years. For example, according to the latest Health survey for England between 1993 and 2002 the proportion of overweight and obese adults rose from 62 to 70% among men and from 56 to 63% among women.

Obesity in children is also increasing at an alarming rate. In 2-4 year old children rates almost doubled (5-9%) between 1989-1998 and obesity in 6-15 year old trebled (5-16%) between 1990-2001. Overweight children have a 50% chance of being an overweight adult. If current trends continue at least one fifth of boys and one third of girls will be obese by 2020.

Weight and body composition, particularly lean mass, are among the strongest determinants of bone mass throughout life, largely reflecting adaptation of skeletal modelling to loading. However whether fat mass affects skeletal development independently of lean mass remains controversial.

When regarding the crude values, obese children seemed to have denser bones than controls suggesting that body weight might improve bone mineralization by increasing the mechanical loading on weight bearing bones[91;92]. However more recent studies suggest that when adjusted for body size the skeleton may be under mineralised. Weiler et al showed in girls aged 10-19 years increasing percentage body fat was positively associated with bone area but had a negative impact on BMC, mineral content corrected to bone area ($r=0.33$, $p<0.01$) and bone density[93]. This was supported by another study of children aged 3-5 years[94], in which the authors report an association between percent body fat and bone area, but not BMC indicating that children with higher body fat will have larger bones which are undermineralized, Periosteal and endosteal circumferences were inversely correlated with body fat resulting in reduction of cortical bone area[94].

Whether fat mass has differing effects on trabecular and cortical bone is unclear. A study by Rocher et al, who looked at obese pre-pubertal children, showed that whilst BMC adjusted for weight and BMAD were significantly reduced compared to the controls, lumbar spine BMAD was in fact increased[92]. However a study comparing obese children with a history of fracture found that lumbar spine BMAD was reduced by 2-3SD compared to the non obese but history of fracture control group. Worryingly 18% of the obese children in this study fulfilled the criteria for osteoporosis[95]. A recent study by Wetzsteon demonstrated significantly higher bone strength at the distal and midshaft sites of the tibia in overweight children aged 10 years[96]. These differences were accounted for by higher total and cortical area, but

not cortical density. Over a 16 month follow up, the bone strength increased more in the overweight children due to greater changes in total and cortical area and not cortical density, thus the overweight children had a greater increase in both periosteal appostion and cortical thickness over this period[97]. Once adjusted for overall weight or fat mass, the bone strength index was reduced in the overweight children (19% and 50% respectively)

Cohort studies have likewise yielded conflicting results. In 1068 men aged 19 years, fat mass was positively correlated with tibial cross sectional area, whereas a negative association was seen at the radius, suggesting that adipose tissue acts to stimulate growth of weight bearing bones only. In contrast a study of 3032 children aged 9.9 years showed that fat mass was positively related to BMC at the total body, upper and lower limbs.

A possible explanation for these conflicting results is that the relationship between fat and bone mass is subject to confounding, which distinct studies may adjust for to differing degrees, for example; diet, physical activity, socio economic factors, puberty, lean mass and illness. In terms of the true nature of any functional relationship, overweight children probably stimulate bone growth through a direct mechanical action of increased load as a result of their increased lean mass[98]. Furthermore adipose tissue is known to express aromatase enzymes that convert steroid precursors to oestrogen, which has been reported to both stimulate[99] and suppress[100] periosteal bone growth in children. Increased leptin levels secondary to higher fat mass have been suggested to mediate the negative association between fat mass and periosteal growth observed at non weight bearing sites[101]. Defects in the leptin-proopiomelanocortin pathway cause severe obesity; the commonest defect is the melanocortin 4 receptor (MC4R)[102]. In a study of 5300 children aged 9.9 years MC4R polymorphisms mirrored the effect between bone mass and fat mass[103]. Another gene polymorphism (Fat mass and obesity associated gene**,** also known as FTO) is associated with increased weight gain in both childhood and adults[104]. This was also seen to mirror the effects between bone mass and fat mass in the 5300 children, suggesting that fat mass is on the causal pathway for bone mass[103].

There are no studies looking at the long-term consequences of childhood obesity on subsequent adult bone mass and geometry. It is also unclear whether there is a persistent increase risk of future fracture in individuals that have been overweight since early childhood.

1.5 Childhood fracture

Fractures during childhood cause pain and loss of mobility and independence. They also result in time off school, activity restricted days and long term consequences arising from complications such as secondary osteoarthritis[105]. A large study within the UK between 1988-1998 of 84,129 boys and girls suggest that fractures are a common problem[106]. By the age of 16, 42% of boys and 27% of girls had suffered at least one fracture. The male incidence rates peak later than those among females (14 years vs. 11 years respectively). Indeed, at this age, the incidence of childhood fractures (3% among boys and 1.5% among girls) is only surpassed at 85 years of age among women and never among men. The most common site affected in both sexes is the radius/ulna (almost 30%), closely followed by the small bones of the hand and wrist. Within the UK there was pronounced geographic variation in childhood fracture incidence with almost 50% higher rates observed in Northern Ireland, Scotland, Wales, and north England compared with London and southeast England. This might reflect a contribution of socio-economic status to fracture risk (accidents as a whole are known to be highly correlated with social class)[107].

Whilst historically fractures are associated with trauma, studies have also shown other risk factors including lower BMD[108;109], lower milk intake[110], lower levels of physical activity[109], a higher BMI [111]and a higher consumption of carbonated beverages[109].

In one large prospective cohort study of 6213 children age 9 years there was a small inverse relationship between BMD and subsequent fracture risk (OR per SD decrease 1.12, CI 1.02-1.25). Fracture risk was also inversely related to BMC adjusted for bone area, height and weight (OR = 1.89, CI 1.18-3.04)[108]. In a follow up of 2692 children from this study, children that fractured in the next two years participated in

higher amounts of time spent in vigorous activity. This was despite higher bone mass associated with increased physical activity; however this increased mass did not adequately compensate for the risk caused by increased exposure to injuries[112]. In a smaller case controlled study of 90 children aged 5-19 years, who had sustained two or more fractures, four risk factors were identified: early age of first fracture (27.7% vs. 11.3%) adverse symptoms to cow milk (22.2% vs 6.7%) low dietary calcium (20% vs. 4.5%) and overweight (33.3% vs. 15.5%)[111]. In a case controlled study by Manias et al, 100 children aged 4-16 years who had sustained a fracture had lower BMC and aBMD, lower milk intake, higher BMI and lower levels of physical activity compared to controls. There was, however, no difference in adjusted bone mass between children with one and those with recurrent fractures. Similarly not having been breastfed, maternal smoking and carbonated drink intake were associated with recurrent fractures[109].

Whether peak bone mass is low among children with fractures remains uncertain. In a cohort of 125 girls followed over 8.5 years, 42 subjects reported 58 fractures. Among those, BMC gain at multiple sites and vertebral bone size at pubertal maturity were significantly decreased[113]. Hence, childhood fractures may be markers of low peak bone mass acquisition and persistent skeletal fragility.

1.6 Developmental plasticity and intrauterine programming

1.6.1 Overview

The term developmental plasticity describes the ability of a single genotype to produce more than one alternative form of structure, physical state or behaviour in response to environmental conditions[114]. This enables the production of phenotypes that are better suited to their environment than would be possible if the same phenotype was produced regardless of their genotype, hence improving the survival of the species. In the natural world there are numerous examples of developmental plasticity allowing organisms to adapt to the prevailing environmental conditions. One example of this is the water flea Daphnia; if the mother is exposed to traces of a predator; the young are born with a protective helmet[115]. The problem arises when the developing organism is then exposed to a mismatch between the expected and

actual environment: the protective helmet of the water flea actually reduces reproductive competitiveness in the absence of a predator.

Programming is defined as persisting changes in structure and function caused by environmental stimuli acting at critical periods during early development[116]. Programming of adult disease is a consequence of growth strategies made by the developing foetus and infant in response to the early environment, causing permanent changes to structure or physiology. Whilst such adaptations may be appropriate during early life, they may be inappropriate in later life and lead to increased disease in adulthood; low birth weight, a surrogate marker for an adverse early intra uterine environment, has been shown to be associated with coronary heart disease, hypertension, type II diabetes and hypercholesterolaemia[116].

Evidence is accruing that for diseases like osteoporosis, where genetic variance makes a relatively small contribution, that environmental factors in early life have a critical influence on the magnitude of peak bone mass achieved, and on the subsequent risk of fractures. During early life there are tissue specific periods of rapid cell division called critical periods. Tissues differ in the timing of their critical window; for example the long bones accelerate their rate of growth during the second trimester, while bone mineralization occurs during the third trimester. The main adaptive response to a lack of nutrients and oxygen during this period of growth is to slow the rate of cell division. This reduction in cell division is either direct or mediated through altered concentrations of growth factors or hormones. The programming of bone growth during these critical periods is likely to explain some of the differences in bone mineral accrual during subsequent childhood and adolescence.

The data to support the programming of bone mass and the subsequent risk of osteoporotic fractures will now be reviewed. These include epidemiological studies of BMD and fracture in cohorts whose early life records have been preserved, physiological studies exploring relationships between candidate endocrine systems that might be programmed and age related bone loss, exploration of maternal determinants of childhood growth and studies of potential underlying mechanisms using animal models.

1.6.2 Prenatal growth, infant growth and bone mass

The first epidemiological evidence that osteoporosis risk might be programmed came from a study of 153 women born in Bath during 1968-69 who were traced and studied at age 21 years[117]. Data on childhood growth were obtained from linked birth and school records. There were statistically significant associations between weight at one year and adult BMC but not density, at the lumbar spine and femoral neck independent of adult weight and BMI. The association between weight in infancy and adult bone mass was replicated in a second cohort study of 238 men and 201 women aged 60-75 years, who were born and still lived in Hertfordshire[118]. In this study, there were highly significant relationships between weight at one year and adult bone area at the spine and hip, the relationships with BMC at these two sites were weaker but remained statistically significant. They also remained after adjustment for lifestyle characteristics in adulthood which might have influenced bone mass (physical activity, dietary calcium intake, cigarette smoking and alcohol consumption) and genetic markers including polymorphisms in the gene for the vitamin D receptor[119] and for collagen 1A1.

Further work has looked at critical periods, which may be involved in programming and their relative contribution to bone mass in later life. In a further study from the Hertfordshire cohort, birth weight was associated with lumbar spine and hip BMC in both men and women. A weaker relationship was seen for hip BMD in men only. Relationships between weight at one year and adult BMC were even stronger In men, 18% of the variance in proximal femoral bone area was explained by a model that included birth weight, weight at one year and adult weight, with the relative contributions attributed to each being 2.8%, 6.8% and 8.2% respectively. In women, similar modelling produced figures of 6.7%, 4.2% and 3.9% (overall variance of 15% in proximal femoral bone area). Hence weight at each of these three points in the life course was important in the determination of adult bone mass, with greater contributions of earlier growth to skeletal size than to volumetric bone mineral density[120]. Data using pQCT in 313 men and 318 women from this cohort showed that birth weight and weight at one year were strongly related to radial and tibial length in both sexes and to measures of bone strength (fracture load X, fracture load Y and polar strain index) at both of these sites, but not volumetric density[121].

These finding have been replicated in other countries; Yarborough et al found, in 305 postmenopausal Caucasian women (mean age 70 years)[122], that birth weight was positively correlated with BMC at the forearm, hip and lumbar spine, and that the age-adjusted mean BMC increased significantly from the lowest to the highest tertile of birth weight. Adjusting for adult weight diminished this association at the forearm and hip, but not at the spine. Birth weight was not independently correlated with BMD. A similar dichotomy between BMC and BMD, related to birthweight, was found in a cohort of adolescent boys and girls in Sweden[123]. Table 1 shows the results from a meta analysis of 10 observational studies from different populations around the world confirming the significant associations of body build in early life and skeletal status in individuals in childhood, young adulthood and the elderly [124].

TABLE 1: Growth in Infancy and adult bone mass

Site		Birth weight	Weight at one year
Adult BMC	Lumbar spine	0.15 (0.10 - 0.20)	0.25 (0.19 - 0.32)
	Femoral neck	0.12 (0.07 - 0.18)	0.2 (0.14 - 0.27)
	Whole body	0.19 (0.10 - 0.28)	0.44 (0.35 - 0.52)
Adult BMD	Lumbar spine	0.12 (0.07 - 0.16)	0.11 (0.04 - 0.18)
	Femoral neck	0.12 (0.07 - 0.16)	0.05 (-0.02 - 0.12)
	Whole body	0.24 (0.17 - 0.30)	0.25 (0.15 - 0.35)

Legend: Correlation coefficients with 95% C.I. are shown

Data are derived from published studies (n=10) relating weight in infancy and adult bone mass

Both the genome and the intrauterine environment influence birth weight. In twins only 10% of the variance in birth weight is thought to be heritable. A study using 4008 white female twins confirms that differences in birth weight do lead to differences in adult bone mass and density after adjustment for height and weight even among monozygotic twin pairs[125]. These observations support the important environmental influences on both foetal growth and persisting alterations in postnatal growth.

A further study from Hertfordshire assessed proximal femoral geometry. Weight at one year in the 333 men and women was associated with increased femoral width as well as intertrochanteric cross sectional moment of inertia (CSMI) at ages 60-70 years, independent of current body weight and BMC, supporting the hypothesis that early growth leads to persisting differences in proximal femoral geometry[126].

1.6.3 Childhood growth and hip fracture

Most evidence relating the intrauterine environment to later osteoporosis, stems from studies utilising non-invasive assessment of bone accrual. The clinically important consequence of reduced bone mass is fracture; data is available which directly link growth rates in childhood with the subsequent risk of later hip fracture. Studies of a unique Finnish cohort in whom birth and childhood growth data were linked to a later hospital discharge records for hip fracture[127] have permitted follow up of around 7000 men and women who were born in Helsinski University Central hospital during 1924-33. Body size at birth was recorded and an average of 10 measurements were obtained of height and weight throughout childhood. After adjustment for age and sex, there were two major independent determinants of hip fracture risk: tall maternal height and low rate of childhood growth. In addition hip fracture risk was also elevated among babies born short but of average height by age 7 years. There was no relationship observed between birth weight and risk of fracture however there was a suggestion that babies who measured less than 49 centimetres at birth had an increased risk of fracture (HR: 1.5 CI: 0.9-2.5). Levels of crowding in the house during childhood or social class also made no difference to fracture risk.

Further work in this Finnish cohort showed a relationship between poor growth in infancy and increased risk of hip fracture in later life[128], with a 6.4 fold increase in risk for those subjects in the lowest quartile of weight gain between 1 and 12 years. These findings are interesting as they suggest several paths to increased fracture risk. Thus a low rate of childhood growth, both in early and late childhood, could lead to poorer mineralization of bone tissue, and/ or decreased bone width and thus lower bending strength. Greater maternal height may act via a longer femoral neck, or faster catch-up growth, particularly in those children who were smaller at birth and of average size by age 7 years, whose skeletal growth may have been pushed beyond its capacity to mineralise. This concept is supported by the observation that fractures

in children most frequently happen in early puberty, where linear growth velocity is high and ahead of volumetric mineralisation[129].

1.6.4 Maternal influences during pregnancy

The third piece of epidemiological evidence that osteoporosis might arise in part through developmental maladaption stems from the investigation of a series of mothers through pregnancy. In 145 infants born at term in Southampton UK, the birthweights of both parents and the height of the father positively correlated with neonatal whole body BMC, independent of the infant's duration of gestation. In addition mothers who smoked during pregnancy had on average, babies with a 7.1g (11%) lower whole body BMC than mothers who did not smoke. Mothers who indulged in vigorous activity in late pregnancy, had a faster walking pace, or had lower triceps skin fold thickness (reflecting lower fat stores) had babies with a lower BMC and BMD[130]. Similar results were found in a more recent mother offspring cohort, the Southampton's Women's Survey[131]. In this study of 448 mother baby pairs the independent predictors of greater neonatal whole body BA and BMC, after adjustment for gestational age and age at DXA scan, included greater maternal birthweight, height, parity, triceps skinfold thickness, and lower walking speed. There was also a weaker trend toward lower percentage fat and greater percentage lean in the offspring of mothers who smoked during pregnancy. The authors postulate that the relationship with maternal height is likely to be largely genetic, although taller mothers might have greater capacity to nourish the foetus and thereby directly influence foetal growth. Maternal smoking has previously been shown to impair calcium transport by trophoblast cells[132], which make up the transporting epithelium of the placenta, it also influences placental vascular function through the effect of carbon monoxide. It is postulated that this results in the reductions in both birth weight and size and the increased risk of intrauterine growth retardation[133]. Triceps skinfold thickness is a reflection of current maternal nutritional status, together with the effect of exercise, the association between maternal fat stores and neonatal BMC may result from competition between the maternal and foetal skeleton for finite mineral resource.

Recent evidence from a study of 380 mother offspring from the Southampton Women's Survey suggests that differing patterns of growth *in utero* predict bone

mass; hence the velocity of foetal femur length growth from 19-34 weeks gestation predicted childhood skeletal size, whereas velocity of abdominal growth (a measure of liver volume and adiposity) predicted volumetric density at age 4 years[134].

These data provide evidence that environmental modulation *in utero*, in combination with genetic factors, has an effect on neonatal bone indices.

1.6.5 Physiological and mechanistic studies

The exact mechanisms that underlie the programming of bone mass are unknown at present. One hypothesis suggests local control of bone growth. As bone growth *in utero* is determined by the expansion of the growth plate by proliferating chondrocytes, such a mechanism could involve alteration in the number of cells in the proliferating chondrocyte zone by altering chondrocyte apoptosis, changing the growth trajectory of an individual throughout life. Alternatively the mechanism may involve resetting endocrine responses that alter the balance between proliferation and differentiation of chondrocytes. For an endocrine axis to be involved, it must firstly be able to influence bone growth and secondly be able to be set by early environmental factors. Hormones that satisfy these criteria are the glucocorticoids, growth hormone, leptin and vitamin D. Two possible explanations, which explain differences in hormone levels are firstly genetic polymorphisms and secondly epigenetic modification of DNA resulting in altered phenotypes.

1.6.5.1 *Hypothalalamic-pituitary-adrenal axis*

The relationship between birthweight as an indicator of the adult hypothalalamic-pituitary axis (HPA) is unclear, partly as a result of the use of different cortisol measures. A recent meta analysis of eleven studies and 2301 subjects has showed a significant inverse relationship between low birth weight and circulating cortisol level. A 1 kg decrease in birth weight was associated with a 23.5nmol/l higher cortisol level[135]. A further study of 6470 subjects from the 1958 British birth cohort showed that reduced head circumference at birth as well as short stature at aged 7 years was associated with greater cortisol levels at aged 45 years[136], suggesting that delayed growth resulting from early life deprivation has long lasting effects on cortisol metabolism.

A study has suggested that glucocorticoid receptor (GR) gene polymorphisms from 163 men and 274 women born in Helinski, Finland during 1924-1933, may modify this link[137].

A relationship between adult skeletal status and cortisol secretion has been demonstrated in a series of 151 men and 96 women aged 61 to 73 years[138]. In this prospective study over four years there was a significant association between elevated peak plasma cortisol levels and accelerated loss of lumbar BMD in men ($r=0.22$, $p=0.01$) after adjustment for testosterone, ostradiol, 25(OH) vitamin D3, and PTH levels. In contrast, in women elevated peak plasma cortisol was associated with lower baseline femoral BMD ($r = -0.23$, $p=0.03$) and greater femoral neck loss ($r=0.24$, $p=0.02$).

1.6.5.2 *Growth hormone/Insulin like growth factor-1*

Growth hormone, both directly and through the promotion of IGF-1 secretion is a major regulator of growth in late infancy and abnormalities of GH metabolism are known to give rise to osteoporosis. Data looking at growth hormone concentrations and bone mass appear contradictory. In the UK 37 men aged 63-73 years, whose weight gain in infancy had been recorded, had venous blood samples taken every 20 minutes over 24 hours. A statistically significant association was shown between both peak GH concentration and fasting IGF-1 concentration with femoral neck BMD. After allowing for peak GH concentration, median GH was negatively associated with BMD. Weight at 1 year was related to median, but not peak GH concentration[139]. These observations are consistent with a dual effect of GH secretion on bone density. High peak GH values drive IGF-1 production and maintain bone mineralization in adult life; while integrated GH secretion (after adjusting for the effect of pulse amplitude), is negatively associated with bone density in later life. A later study in 38 women from the same cohort found that lumbar spine BMD and BMC were positively associated with all measures of GH concentration, although relationships were strongest for BMC with trough GH. Total daily GH concentration tended to increase with rising birth weight, while IGF-1 concentration fell with rising birth weight, lending further support to a role for the GH/ IGF-1 axis in the programming of adult bone mass[140].

In order to further understand the possible role of GH/IGF-1 in the programming of bone mass a study of 119 newborn infants in Southampton UK, whose mothers pregnancies had been characterised were enrolled in a population based study to look at the relationship between cord serum IGF-1 and neonatal body composition as measured by DXA[141]. There were strong positive associations between cord serum IGF-1 concentration and whole body BMC ($r = 0.38$, $p<0.001$), whole body lean mass ($r = 0.40$, $p<0.001$) and whole body fat mass ($r = 0.5$, $p<0.001$) after adjusting for gestational age and sex. There was no association between IGF-1 and BMC adjusted for bone size for which the authors concluded that cord serum IGF-1 is more closely related to the size of the neonatal skeleton than to its degree of mineralization. Documented maternal determinants of neonatal bone mass seemed to mediate their effects independently of variations in cord serum IGF-1.

Further substudies of the Hertfordshire cohort have subsequently looked for associations between common single nucleotide polymorphisms in the growth hormone 1 gene (GH1), growth hormone releasing hormone gene (GHRH), growth hormone releasing hormone receptor gene (GHRHR), the growth hormone secretagogue receptor gene (GHSR) and the growth hormone receptor gene (GHR) and weight in infancy, adult bone mass and bone loss rates[142;143]. Homozygotes at loci GH1 A5157G and T6331A displayed low baseline bone density and accelerated bone loss: there was also a significant interaction among weight at 1 year, GH1 genotype and bone loss rate. Furthermore there was a graded association between alleles and circulating GH concentration among men[142]. Allelic variation in the gene encoding GHRH was associated with BMC and BMD at the proximal femur and lumbar spine. In women, the mean BMC lumbar spine within the GHRH 11 genotype was 56.9 g, while that of the GHRH 12 genotype was 68.4 g. Corresponding figures for BMD lumbar spine (GHRH 11 genotype) were 0.96 g/cm^2 versus 1.10 g/cm^2 [143] .

1.6.5.3 *Vitamin D*

Vitamin D is a key hormone for the regulation of bone growth and mineralization during life leading to rickets or osteomalacia in cases of deficiency. It can be synthesised in the skin to form vitamin D3, or absorbed from the diet and is metabolised in the hepatic and renal parenchyma to form 1,25 (OH) vitamin D3, the

most active moiety. Vitamin D deficiency and insufficiency are common; in one study of young women in Southampton 31% of women were classed as insufficient and 17% as deficient[27].

The first evidence to suggest that vitamin D levels in early life might be associated with change in bone mass in healthy children came from a study by Zamora et al. In a retrospective cohort study of 106 Caucasian girls aged 7-9 years they showed that vitamin D supplementation in the first year of life was associated with an 8.5% increase in areal BMD ($p = 0.03$) at the femoral neck and a 9% increase in lumbar spine BMC ($p<0.05$) after adjusting for potential confounders[144].

Further support for the role of vitamin D came from a longitudinal study of 198 children aged 9 years whose mothers' pregnancies had been characterised for body build, nutrition and vitamin D status. Reduced maternal concentration of 25(OH)-vitamin D during late pregnancy was associated with reduced whole-body and lumbar-spine BMC in children at age 9 years[27]. This association seemed to be partly mediated by venous umbilical cord calcium. Mothers who delivered in the winter months had lower estimated exposure to ultraviolet B radiation during late pregnancy. Both the estimated exposure to 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. Further work looking at 424 normal pregnancies within Southampton UK found that insufficient/deficient maternal 25-hydroxyvitamin vitamin D concentrations found in over one third of these women and was associated with greater femoral metaphyseal cross-sectional area and a higher femoral splaying index as early as 19 weeks gestation[145]. A recent study from Finland looked at pQCT measures of the tibia in 125 newborns whose mothers had been characterised for 25(OH) vitamin D in the first trimester, postpartum and from umbilical blood. The median levels of these three timepoints were taken and the infants compared to above and below the median value. Tibia bone mineral content was 0.047g/cm higher and cross-sectional area was 12.3 mm^2 larger in above median compared with below median group, however no difference in bone mineral density was observed[146].

Serum 1,25 (OH) vitamin D concentration have been shown to be higher in those with lower birthweight and weight at 1 year in data from the Hertfordshire UK cohort, suggesting an increased sensitivity of renal 1 alpha hydrolylase in those who were small in early life[147]. Further work in this cohort looked at the association between polymorphisms of the gene for the vitamin D receptor (VDR) and adult bone mass and VDR genotype[148]. Among individuals in the lowest third of birthweight, spine BMD was higher ($p = 0.01$) in individuals of genotype 'BB' after adjustment for age, sex and weight at baseline. In contrast, spine BMD was reduced ($p = 0.04$) in individuals of the same genotype who were in the highest third of the birthweight distribution. A significant ($p = 0.02$) statistical interaction was also found between VDR genotype and birthweight as determinants of BMD. These results suggest that genetic influences on adult bone size and mineral density may be modified by undernutrition *in utero*. Vitamin D supplementation of pregnant women, especially during winter months, could lead to long-lasting reductions in the risk of osteoporotic fracture in their offspring.

1.6.5.4 *Leptin*

Leptin is a peptide hormone encoded by the obese gene (ob) and is a candidate for involvement in foetal programming. Leptin is one of the most important adipose derived hormones, it plays a key role in regulating energy intake and energy expenditure, including appetite and metabolism. Leptin acts on receptors in the hypothalamus where it inhibits appetite. Absence of leptin or its receptor leads to uncontrolled food intake and resulting obesity. Although the role of leptin in bone metabolism is not fully elucidated, results from animal studies showed that mice deficient in leptin signalling have higher trabecular bone mass. There is also recent evidence that adults who had low birth weight have higher levels of leptin than would be expected from their adult level of obesity[149]. However data looking at serum leptin concentrations and bone mass appear contradictory. Data, again from Hertfordshire, showed a strong positive correlation between adult plasma leptin concentration and BMC ($r= 0.24$, $p<0.001$). However the negative association with rate of bone loss was significant only at the femoral neck in women ($p<0.01$) and all associations were explained by the association of leptin with obesity[150].

In Sweden a study of 1068 men aged 18-20 years showed that leptin was found to be a negative independent predictor of whole body, lumbar and femoral neck aBMD ($p<0.01$) as well as of the cortical bone size of both the radius and tibia ($p<0.01$) but not cortical or trabecular vBMD once adjusted for lean and fat mass, height, physical activity and smoking as covariates[101].

Data from a Southampton UK cohort of 117 neonates showed a positive association between umbilical venous leptin and whole body BMC ($r =0.42$, $p<0.001$) and estimated vBMD ($r =0.21$, $p=0.02$). Among the maternal determinants of neonatal bone mass, cord leptin explained the relationship with maternal fat stores implying that maternal fat stores may mediate their effect on foetal bone accrual through variation in foetal leptin[151].

1.6.5.5 *Epigenetic mechanisms*

The concept of environmental plasticity provides useful insights into potential mechanisms by which the environment may interact with the genome. Epigenetics is the study of inherited changes in phenotype or gene expression caused by mechanisms other than changes in the underlying DNA sequence. These changes may remain through cell divisions and for the remainder of the cells life and may also last for multiple generations. However there is no change in the underlying DNA sequence of the organism.

The molecular basis of epigenetics is complex. Three mechanisms are thought to contribute to epigenetic control; these are genomic imprinting, X chromosome inactivation, and metabolic differentiation. Metabolic differentiation is thought to mediate epigenetic imprinting by several interrelated processes[152]. The autoregulation of transcription factor levels produces a complex feedback mechanism for regulating gene expression within a cell. The DNA chromatin structure determines its configuration; and therefore the accessibility of protein binding sites to transcription factors. These mechanisms further interact with DNA methylation (which also blocks transcription factor binding) to produce an integrated control of gene transcription[152].

The gene silencing effect of DNA methylation is dependant on the formation of the DNA chromatin structure. This methylated chromatin structure binds transcriptional repressors such as MeCP2 and methylated CpG binding proteins (MBDs)[153] . During germ cell and preimplantation embryo development there are waves of genome wide demethylation and de novo methylation. This provides a vulnerable period during embryonic life in which the methylation status of the genetic code (and therefore its resulting phenotype) may be influenced by environmental factors.
The potential for nutrition and dietary supplementation during pregnancy to influence adult phenotype via DNA methylation has been demonstrated. For example Lillycrop et al showed that protein restriction of rat dams during pregnancy altered the methylation status of specific hepatic genes[154]. This same data also indicated that addition of folic acid to the diet of the protein restricted dams reversed the effect. Waterland, in an experiment using yellow agouti (A^{vy}/a) mice, indicted that the mutation could be silenced and a brown phenotype induced by adding methyl donors (including folic acid, vitamin B_{12}, choline and betaine) to the diet of the dams before and during pregnancy and lactation. Further more they showed that this change was due to an increase in methylation at the A^{vy} locus[155].

Further work is needed to explore possible epigenetic mechanisms in which both maternal and childhood diet and lifestyle affect the skeletal growth trajectory.

1.6.6 Animal models

Over the last 40 years, animal studies have proved informative over the role of nutrition in skeleton development. Seminal studies from Widdowson and McCance demonstrated that programming of growth may arise through nutritional modulation during critical windows of early life[156]. They demonstrated that rats undernourished early on in bone development (3-6 weeks after birth) lost weight compared to control groups, permanently remaining smaller, even after resumption of a normal diet. In contrast, rats undernourished later in bone development (9-12 weeks post birth) initially lost weight but regained their normal growth trajectory on resumption of a normal diet with catch up growth.

Amman et al investigated the effects of four isocaloric diets with varying levels of protein content (15, 7.5, 5, and 2.5% casein) on areal bone mineral density (BMD),

bone ultimate strength, histomorphometry, biochemical markers of bone remodeling, plasma IGF-1, and sex hormone status in adult female rats[157]. After 16 weeks on the lowest protein diet, BMD was significantly decreased at all skeletal sites assessed. Plasma IGF-1 was decreased by 29-34%. Using the same protocol the authors investigated the effect of protein restriction on ovariectomized and sham operated rats, pair-fed with isocaloric diets containing either 15 or 2.5% casein. Trabecular BMD was decreased by either manipulation, with effects appearing to be additive. Cortical BMD was decreased only in rats on a low-protein diet. This was accompanied by an increased urinary deoxypyridinoline excretion without any change in osteocalcin levels, suggesting an uncoupling of resorption and formation. Isocaloric protein undernutrition decreased bone mineral mass and strength. Thus there is good evidence of the importance of adequate dietary protein in an otherwise energy-replete diet.

Mehta et al looked at the effect of maternal protein restriction to evaluate bone density of the offspring in rats. The pregnant rats were fed a isocaloric diet containing 180g casein (normal protein) or 90g casein (low protein). After delivery all mothers were fed the control diet, as were the pups on weaning. The mean bone area of the offspring born to dams fed a low protein diet was around 10% lower than of the offspring born to those on a normal protein diet. A similar magnitude of difference was observed for whole body BMC, whereas there was no difference observed in BMD[158]. Furthermore, offspring of the protein-restricted mothers had abnormally widened growth plates compared with the offspring of controls ($p<0.001$). It was suggested that this reflected programming of chondrocyte function.

Oreffo et al examined the cellular mechanisms involved in the programming of bone development using pregnant rats that were either fed normal or low protein diets as described above. Offspring that were born to those who had a maternal restriction of protein had delayed skeletal maturity with a 40 % reduction in colony formation (colony-forming unit fibroblastic, CFU-F) indicative of the efficiency and proliferation potential of the mesenchymal stem cells at 8 weeks, compared to control, there was no difference at 12 weeks but a 111% increase at 16 weeks (catch up growth). Similar results were observed following examination of alkaline phosphatase positive CFU-F number, indicative of osteogenic potential and

differentiation. The addition of osteogenic growth factors (growth hormone, IGF-1 and 1,25 $(OH)_2$ vitamin D_3) were insufficient to overcome or reverse the effects of maternal dietary manipulation[159].

More recently the effect of maternal dietary fat excess was assessed using offspring from mouse dams fed either standard chow (C) or lifetime high-fat diet (HF). The offspring were maintained on a HF diet to adulthood. Femur samples were taken at 30 weeks of age and bone structure, adiposity and strength analysed. Offspring from HF-fed dams showed increased adiposity in the femur (bone marrow adiposity) in comparison to offspring from C-fed dams. Female offspring from HF dams exhibited altered trabecular structure indicative of *in utero* programming[160].

There is limited data on either maternal protein malnutrition or dietary fat excess in humans although healthier eating patterns with higher intake of protein and low saturated fats has been shown to increase bone mass in the offspring at aged 9 years[74]. This thesis aims to look further at maternal diet and bone mass of the offspring.

1.7 Measurement of childhood body mass

1.7.1 Dual energy X-ray absorptiometry

DXA is considered by most clinicians to be the gold standard in bone densitometry and is the most widely used technique for measuring bone mass and density. There are however several drawbacks to the use of DXA in children. In DXA the body is assumed to consist of two compartments, bone and non-bone. Using this model and measuring at two different energy levels we can calculate the amount of bone within the field of view. From this we can obtain BMC and bone area. BMD is determined by dividing the BMC by the projected area. Although this is suitable for an adult population, in which we assume that the volume of a bone remains stable over time, in children this approach is not suitable because as the child grows so does the volume of the bone. This means that for a constant bone density, a larger vertebra (seen in tall stature) would typically yield higher areal BMD results than a smaller one (short stature). There are many different reported methods for adjusting the

derived DXA measurements to estimate vBMD. These include using BMC adjusted for apparent volume to give bone mineral apparent density (BMAD) using the method of Carter[161], BMC adjusted for bone area using the method of Horlick and BMC adjusted for bone area /height and weight using method of Prentice[162]. The method of Prentice was used as the primary estimate of volumetric BMD as it was felt to most effectively control for body size. However all these adjustments are still only estimates of volumetric density as they assume the bone to be cylindrical in shape.

1.7.2 Peripheral quantitative computed tomography

There has been interest in using CT images to determine trabecular bone structure; however, ionising radiation dose constrains such applications in central skeletal sites. Dedicated peripheral CT (pQCT) scanners to measure BMD and bone morphology in the radius and tibia are now available. They are smaller, more mobile and less expensive that whole body CT scanners. They also use only very small doses of radiation.

Peripheral quantitative computed tomography (pQCT) differs in that it measures true volumetric density and is not affected by bone size since the images are 3-dimensional. It also has the ability to differentiate cortical from trabecular bone structure. This is important as a reduced BMD can be due to either to insufficient mineralization (mineral per volume) or a reduced structural density (decreased trabecular thickness) resulting from insufficient physical activity or muscular disorders. Other parameters such as cortical density, total cross sectional area, cortical bone area and cortical thickness can also be assessed using pQCT helping to determine the geometry of the bone as well as fracture load.

pQCT has been successfully validated in children as young as 3 years. A study of 101 children aged 3-5 years using the XCT 2000 accurately measured total cross-sectional area, cortical area, and cortical thickness in children. A coefficient of variation of 3.1% for total area, 4.5% for cortical area and 6.8% for cortical thickness was demonstrated[163] Total cross-sectional area, cortical area, and cortical thickness

correlated with weight and height. Furthermore, as the children got older, precision improved still further due to less movement artefact.

1.7.3 Hip structure analysis

The measurement of bone density is a surrogate for the measurement of bone strength. Bone strength is comprised of many components including bone architecture, geometry, cortical porosity and tissue mineralization density. A new application for DXA called hip structural analysis (HSA) allows the measurement of geometric contributions to bone strength in the proximal femur. With HSA, measurements or estimates of the mineralised bone surface cross sectional area (CSA) the cross sectional moment of inertia (CSMI), the section modulus (Z), the buckling ration (BR) and cortical thickness can be quantified.

The techniques were first described by Martin and Burr in 1984[164]. The bone profile generated during DXA can be used to derive information on the distribution of mass and diameter of the cross section. After making assumptions regarding the shape and symmetry of the bone in cross section, estimations of cortical thickness can also be obtained.

The technique uses three regions of interest at which the various geometric parameters are calculated. The first region of interest (ROI), the narrow neck (NN) is placed at the narrowest portion of the femoral neck. The intertrochanteric ROI is essentially a bisector of the intersection of the femoral neck and shaft axis. The last ROI, the shaft region, is placed 1.5 times the femoral neck width distal to the intersectional of the neck and shaft axes. The NN and IT regions contain cortical and trabecular bone whereas the shaft region is considered to contain cortical bone only.

Whilst this technique has been used extensively in adults, only one study to date has looked at children of similar age to our cohort. This group were able to successfully show a relationship between physical activity and cross sectional area and section modulus[81].

1.8 Outstanding areas of research

Whilst maternal lifestyle and anthropometric characteristics during pregnancy have been identified that predict neonatal bone mass, it is not known how they interact with early childhood lifestyle characteristics to influence childhood bone accrual. Furthermore there has been no research to date that determines whether maternal characteristics may be related to bone strength and geometry in the offspring. Finally it is not known how childhood and maternal characteristics interact to determine bone strength.

2 OBJECTIVES

The following hypotheses will be explored using the Southampton Women's Survey; a unique mother offspring study:

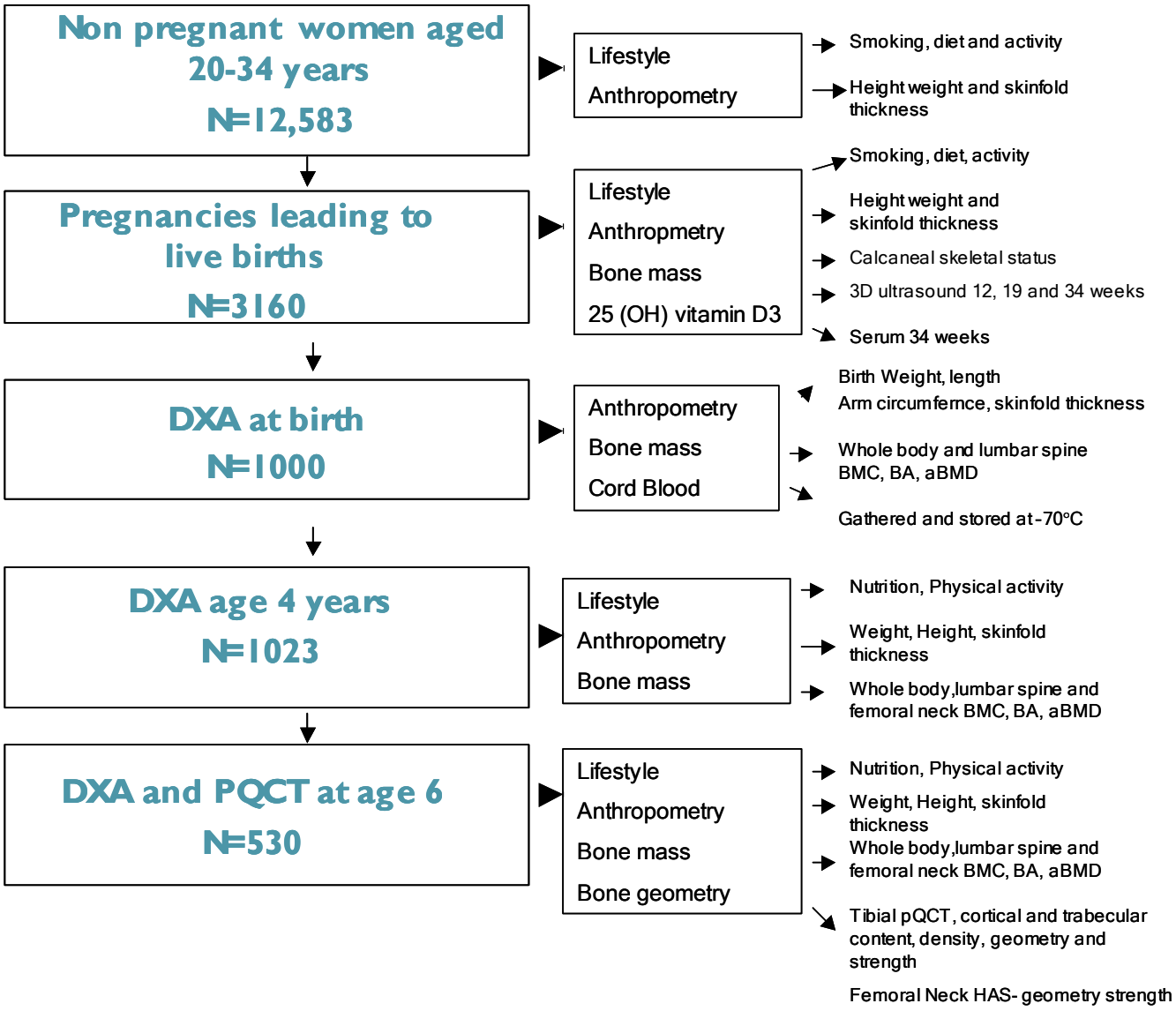
1) Do maternal lifestyle factors (diet, smoking, exercise) and body composition influence both childhood bone mineral accrual and the mechanical strength of bone?

2) Do childhood lifestyle (diet, activity) and body composition influence childhood bone mineral accrual and mechanical strength of bone?

3) Do both maternal factors (body build, physical activity, diet and cigarette consumption) and childhood factors (dietary calcium intake & physical activity) determine rates of childhood growth and bone mass?

3 OVERVIEW OF THE SOUTHAMPTON WOMENS SURVEY

Figure 5 Outline of SWS bone study, from preconception to 6 years



3.1 Pre conception phase

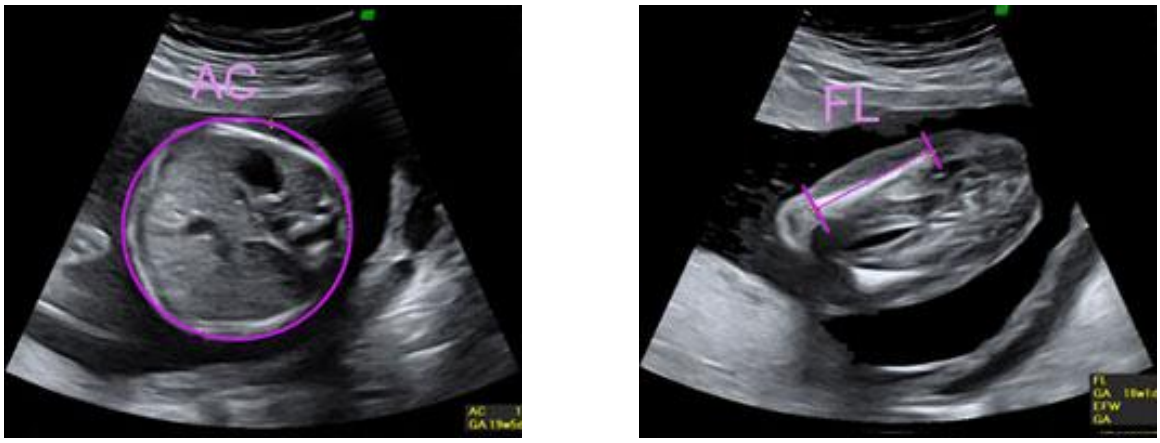
The Southampton Women's Survey (SWS) is a well-established prospective cohort of around 12,500 women aged 20- 34 years[165]. Run by the Medical Research Council (MRC) and the University of Southampton, the study was set up to assess diet, body composition, physical activity and hormone levels in a large group of non-pregnant women. For those women who became pregnant, the aim was to investigate the influence of these maternal factors on the development of the child throughout its early life.

Women were recruited via their general practitioners (GPs): a letter was sent to each woman from her GP's surgery and this was followed up with a telephone call. Self-referrals were encouraged via a local advertising campaign, with the aim of catching those women not registered with a GP, or whose contact details were out of date. Approximately 75% of women approached agreed to participate in the study. Because of out-of-date address information with GP practices, it was difficult to exactly quantify total number of possible participants.

The women underwent an initial interview by a trained research nurse. At this visit, an interviewer administered questionnaire was used to assess diet (validated 100 item food frequency questionnaire (FFQ)[166]), and other factors such as the woman's physical activity, smoking, family background, education, ethnicity, housing, household composition, childcare arrangements, benefits, general health, menstrual and obstetric history, and her own and her partner's occupation. Detailed body composition measurements were taken, including weight, height, waist and hip circumference, and skinfold thickness at four sites (triceps, biceps, subscapular and supra-iliac regions). The nurses were carefully trained and regular inter-observer variability studies performed to ensure measurements were as accurate as possible. Venous blood has been collected and stored at -70˚C.

3.2 Pregnancy follow up

The recruited women were asked to inform the study coordinators immediately if they became pregnant, and also to give written consent for their GP or hospital doctor to communicate this information. Pregnant women were then invited to attend interviews in early (11 weeks) and late (34 weeks) pregnancy. At these visits, diet and lifestyle factors were assessed in a similar way to the initial visit. The detailed anthropometry was repeated, and venous blood was collected and stored at -70˚C. The women additionally had high resolution ultrasound scans at 11, 19 and 34 weeks using a Kretz Voluson 730 or Acuson sequola 512 which were cross calibrated. After establishing correct positioning according to the standard anatomical landmarks, measurement of femur length (measure of skeletal size) and abdominal circumference (a composite of adiposity and liver size) were made on the frozen images using electronic callipers according to internationally and validated methodology. Each measurement was made in triplicate and the mean used for analysis. The coefficient of variation for triplicate measures of femur length was 0.6% at 19 weeks and 0.4% at 34 weeks. In women with a reliable date of last menstrual period, Royston models were fitted to foetal measurements of femoral length and abdominal circumference at 19 and 34 weeks to create Z scores for size and conditional growth.



Pictures 1: Ultrasound images of abdominal circumference (AC) and femoral length (FL)

AC is determined at the skin line on a true transverse view at the level of the junction of the umbilical vein, portal sinus, and fetal stomach. FS is most accurately measured with the beam of insonation being perpendicular to the shaft, excluding the distal femoral epiphysis

3.3 Childhood follow up

3.3.1 Birth

At birth, the babies were measured (length, head and abdominal circumference) weighed, and skinfold thickness measured (triceps, sub-scapular and thigh). Samples of cord blood were also collected and again stored at -70˚C. After birth, the mother was asked to agree for her baby to undergo assessment of bone mass, within 2 weeks of birth, using a Lunar DPX DXA instrument with specific paediatric software (GE Corporation, Madison, Wisconsin, USA). The instrument was located in the Princess Ann Maternity Hospital, Southampton, and underwent daily quality assessment, and was calibrated against a water phantom weekly. The mothers could attend either as an inpatient, or return from home within the two-week time period. At the visit to the scan room, the baby was pacified and fed if necessary, undressed completely, and then swaddled in a standard towel. It was placed on a waterproof sheet in a standard position on the scanner. Whole body measurement was performed first, followed by lumbar spine, using specific software protocols. The baby was kept in position using rice bags placed over the bottom end of the towel for whole body, and either side for the lumbar spine scan. A print out of the whole body scan was given to the mother as a momento of the occasion. The baby was weighed at the end of the visit on calibrated digital scales, and this weight and the previously recorded length were entered into the DXA record on the computer. The manufacturer's short-term and long-term coefficients of variation (CV) of the DXA instrument were 0.8% and 1.4% respectively. When a spine phantom was repeatedly scanned in the same position 24 times the CV was 0.15%.

3.3.2 6 and 12 month follow up

The mothers of the children were contacted when the child reached 6 months, 12 months, 2 and 3 years. Permission to contact the women by telephone was obtained when the baby was born. After an appointment was made, a trained research nurse visited the mothers and children in their own homes. A questionnaire was administered at each of these visits, which included questions about diet, feeding patterns, overall health of the child, activity and sleep patterns. In addition various infant measurements were taken which included weight, crown heel length or height,

head circumference, abdominal circumference, triceps skinfold and subscapular skinfold thickness. Each of these measurements were repeated in triplicate. The nurses who did these visits underwent regular training in anthropometric measurements in order to optimise accuracy and repeatability, with periodic assessment of inter-observer differences.

3.3.3 Determinants of 4 year bone mass

The mothers were once again contacted on their children becoming 4 years old by letter and information sheet telling them about this part of the study and inviting them to take part. Soon after this the mother was telephoned at home to see whether she was willing for her child to participate. If the response was positive, a time for the visit to the Osteoporosis Centre at Southampton General Hospital was organised and a letter confirming this appointment in writing was sent out.

At the visit to the Osteoporosis Centre, informed written consent for the DXA scan was obtained from the mother or father. The child's height (using a Leicester height measurer) and weight were measured and recorded. The child was then invited to lie down on the DXA couch. Whole body, lumbar spine and left hip scans were taken, using a Hologic Discovery instrument (Hologic Inc., Bedford, MA, USA). To make this more appealing, a suitably bright sheet with appropriate pictures was laid on the couch first. To help reduce movement artefact, the children were shown a suitable DVD cartoon. The whole body DXA scan took around 5 minutes. After this, the children underwent lumbar spine and left hip scans, each of which took around 20 seconds. The total radiation doses for the scans were as follows; whole body (paediatric scan mode) 4.7 microsieverts, spine (L1-L4) 1.5 microsieverts, hip 7.3 microsieverts (total dose 26.7 microsieverts). This is equivalent to three days background radiation and is significantly less than that for a chest radiograph. The manufactures coefficient of variation (CV) for the instrument was 0.75% for whole body, and the experimental CV when a spine phantom was repeatedly scanned in the same position 16 times was 0.68%.

After the scan the child's mid-upper arm circumference was measured in triplicate on the left side, and further measurements were taken until three readings within 5% of

each other were obtained. Grip strength was measured three times on either side, alternating between sides, with the child's arm in a standard position.

The child's diet (focusing on calcium and vitamin D intake), exercise and illnesses (including fractures) were assessed by an interviewer led questionnaire for the mother, father (if mother was absent) or carer .

In a subset of children and mothers, an Actiheart combined accelerometer and heart rate monitor (Cambridge Neurotechnology Ltd, Cambridge, UK) was fitted to both mother and child. They were asked to wear these continuously for 7 days and then post back in the envelopes supplied.

Pictures 2: Actiheart monitor

The unit comprised a small disc, 1.5 cm across and 3mm thick, and a short lead. Both of these parts were secured to the skin via clipping onto standard electrocardiograph electrode pads. The disc was positioned in the midline just below the xiphisternum and the lead going out horizontally to the left chest wall.

3.4 Determinants of 6 year bone mass

The parents of the children were then contacted again once the child had reached the age of 6 years giving them an information sheet about the next part of the study (appendix A and B). They were then contacted by telephone to see whether they were willing to take part and to organise the first part of this next study, the home visit. This study was part of a much larger study, which also looked at allergy and asthma in these children (appendix C: study protocol). A letter confirming this appointment in writing was sent out to the mother, containing a direct contact telephone number in case of problems attending.

At the home visit informed written consent was obtained from the parent, specifically related to skin prick testing and lung function that was also carried out at this visit. The child's diet using a food frequency questionnaire, exercise and illnesses were assessed by an interviewer led questionnaire from the parent or carer (appendix D).

The child's height (using a Leicester height measurer) and weight using calibrated digital scales (Seca Ltd)) were measured. After the scan the child's mid-upper arm circumference was measured three times on the left side, and further measurements were taken until three readings within 5% of each other were obtained.

The Actiheart combined accelerometer and heart rate monitor (Cambridge Neurotechnology Ltd, Cambridge, UK) was then fitted to the child and their mothers. This was positioned in the midline just below the xiphisternum and the lead going out horizontally to the left chest wall. They were asked to wear these continuously for 7 days and then post back in the envelopes supplied. A information sheet was provided giving further information of how to change the electrodes, they were also asked to record times were the monitor had been removed (appendix E).

At the end of this visit they were given further information about the next part of the study, the clinic visit (appendix F and G). If they agreed to this the research nurse arranged an appointment with them whilst they were still in the child's home. A letter confirming this visit was then sent out giving them directions to come to the osteoporosis centre (appendix H).

At the visit to the Osteoporosis Centre, informed written consent for the DXA scan was obtained from the mother or father (Appendix I). The child's height (using a Leicester height measurer) and weight (using calibrated digital scales (Seca Ltd)) were measured. The child was then invited to lie down on the DXA couch having taken off any metal items, which would appear on the scan. Whole body, lumbar spine and left hip scans were taken, using a Hologic Discovery instrument (Hologic Inc., Bedford, MA, USA). To make this more appealing, a suitably bright sheet with appropriate pictures was laid on the couch first. To help reduce movement artefact, the children were shown a suitable DVD, which they had been asked to bring with

them. The whole body DXA scan took around 5 minutes. After this, the children underwent lumbar spine and left hip scans, each of which took around 20 seconds. The total radiation dose for the scans were as follows; whole body (paediatric scan mode) 4.7 microsieverts, spine (L1-L4) 1.5 microsieverts, hip 7.3 microsieverts (total dose 26.7 microsieverts). This is equivalent to three days background radiation and is significantly less than that for a chest radiograph.

During the scan the parents were asked a very short questionnaire about their child's fracture history and whether there was a history of osteoporosis in the family (appendix J). They were also told about a substudy, which involved pQCT to look into the strength of the child bone in more detail. An information leaflet was given to the parent and child about this (Appendix K and L), the parent was then contacted by phone a short time after to see whether they wanted to take part in this substudy and to make an appointment.

At the end of this visit grip strength was measured three times on either side, alternating between sides, with the child's arm in a standard position. The child was then given a copy of their scan as a momento and a certificate of achievement (Appendix M and N).

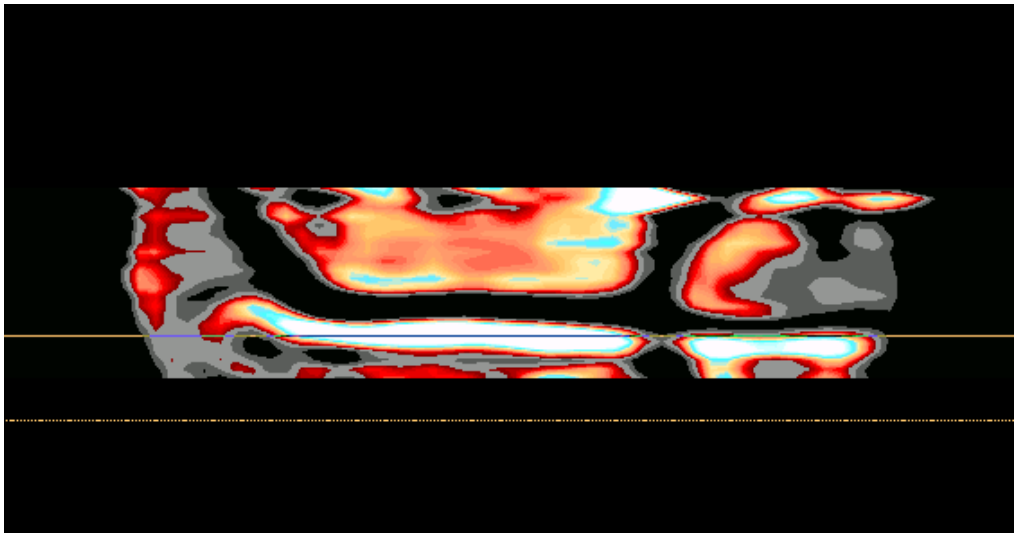
3.4.1 Determinants of volumetric bone mass and bone strength

If the parent had agreed to take part in the substudy at aged 6 years, an appointment reminder with directions to the osteoporosis centre was sent out to them by post. At this visit informed written consent for the pQCT scan was obtained from the mother or father (Appendix O). The child's height (using a Leicester height measurer) and weight, using calibrated digital scales (Seca Ltd) were measured as part of this visit. The child was then asked to put their right lower leg into the pQCT machine. The leg was secured into place in order to reduce movement artefact.

Pictures 3 PQCT in child

A suitable DVD was used in order to occupy the child and again reduce movement artefact. The scan took approximately 5 minutes and during this time four sites of the tibia were scanned (4%, 14%, 38% and 66% of the total length). The 4% and 14% sites were used to assess trabecular content and density, the 38% site cortical content, density and bending strength whereas the 66% site was used to look at the muscle, fat and bone ratios.

In order to obtain the exact measurements of the tibia the lower leg was first measured from the medial malleolus to the tibial tuberosity. When the lower leg was placed into the machine, it was positioned using a laser beam at the distal end of the medial malleolus. A scout view was then obtained to find the distal end of the tibia (picture 4). A reference line was then positioned to bisect the medial border of the articular surface; the 4 sites to be scanned were calculated from this line and the length measurement of the lower leg.



Pictures 4: Scout view of distal tibia with reference line placement

The total radiation dose for the scans were 1.5 microsieverts, which is equivalent to less than half a day background radiation.

The parent and child were then thanked and the child given a further certificate of achievement and copies of the scan results if requested (appendix P and Q).

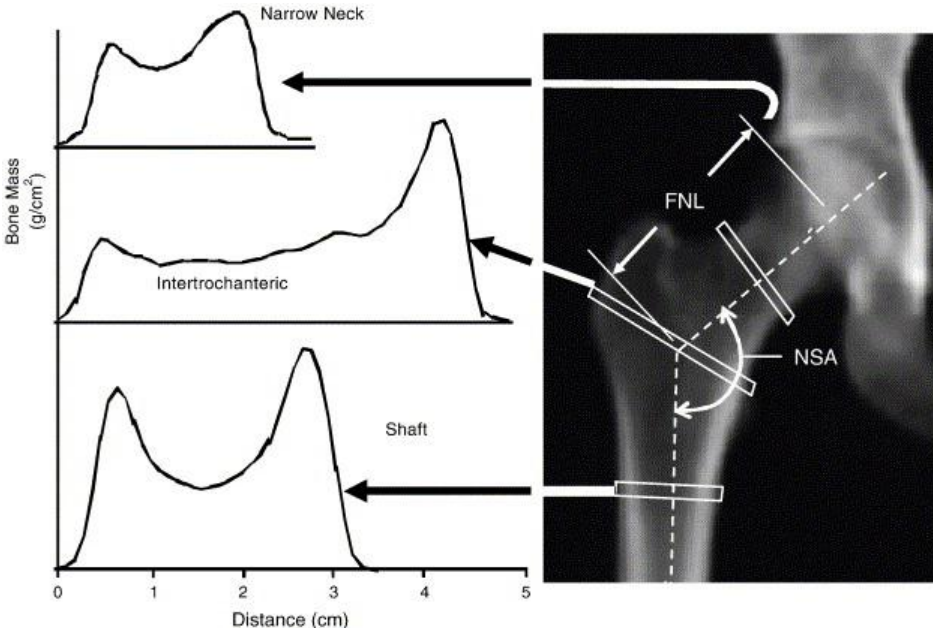
Full ethics and NHS R and D approval had been gained for this study (appendix R).

3.5 Analysis

The DXA and pQCT data were transferred regularly to secure servers at the MRC unit via an encrypted memory stick.

The DXA scans were analysed at the visit by a trained DXA technician using the automated paediatric software (Vertec Scientific Ltd, Reading, UK) In addition an interactive computer program (hip structural analysis, HSA) was used to derive a number of structural variables from the femoral DXA scans. The program analyses the proximal femur at three locations as shown in figure 6.

Figure 6: HSA position on scan



The regions that were analysed included the narrow neck (NN), measured across the narrowest diameter of the femoral neck, intertrochanteric (IT) along the bisector of the neck shaft angle and the femoral shaft (FS) 2cm distal to the midpoint of the lesser trochanter. For each region the HSA program generates a projection of the bone cross section from a line of pixel values traversing the bone width. The blur corrected sub periosteal bone width, bone cross sectional area (CSA) and cross sectional moment of inertia (CSMI) for bending in the image plane were measured. The section modulus (Z), an index of bending strength can be calculated from CSMI/dmax, where dmax is the maximum distance from the centre of mass to the medial or lateral cortical margin. An estimate of cortical thickness was calculated by modelling cortices of femoral shaft cross sections as concentric circles. In addition the method measures neck shaft angle and the femoral neck length. It should be noted that since the HSA algorithm assumes a tissue mineralization of adult cortical

bone, the CSA and Z will be systemically underestimated in the less mineralised bones of growing children; however experience in elderly adult populations indicates that the precision is 2.8% and 3.4% for CSA and Z respectively[167].

The pQCT data was analysed using an XCT-2000 scanner (Stratec, Pforzheim, Germany software version 5.50d) that showed any sign of movement were omitted from all analyses.

3.6 Statistical methods

All data was anonymised, coded and double-punched onto computer. The data were amalgamated with parental pre-, early and late pregnancy data, and neonatal, 4 and 6 year old bone mass, lifestyle and dietary data. Statistical analysis utilised tests for comparing mean in different populations and linear regression for univariate exploration. Multivariate models (multiple linear regression, logistic regression and conditional analyses) were used to study relationships between maternal factors, foetal and infant growth and bone mass/body composition at age 6 years.

Prior to starting the study a power calculation was performed. This suggested that by studying 228 children there was 90% power to detect a difference in whole body BMC of 10% between the highest and lowest quartiles of the distribution of maternal triceps skinfold thickness in early pregnancy, at the 5% significance level. We therefore aimed to study over 250 children as part of this thesis.

All data were analysed in Stata V11.0 (Statacorp, Texas, USA). All data were checked for normality and transformed if necessary. It was apparent that there was some unreliability in the DXA software analysis of measurements of bone mineral at the femoral neck, trochanter and Ward's triangle sites. Measurement at the total hip site appeared more uniformly reliable, and so this site was used exclusively. Movement artefact was most apparent at the head; this bone mineral accounts for a high percentage of whole body BMC, but not much to linear growth. Thus at 6 years, the whole body measurements are for whole body minus head.

3.7 Role of candidate

This thesis involved the 6 year follow up of children from the Southampton Women's study. The data from the mothers and children at younger ages had already been collected as part of the previous study.

My role in the 6 year study included the design and writing of protocols (Appendix C), the writing of parent and child information leaflets for both the DXA and pQCT sub studies (Appendix F, G, K and L) and ethics submission. In addition I attended over 300 clinic visits for DXA and all pQCT visits, where consent was obtained, the short questionnaire administered and measurements of anthropometry and grip strength obtained. While DXA was carried out by a trained technician, all femoral neck analyses were carried out my myself. I checked the DXA and pQCT data for outliers, movement and foreign objects. In addition I was responsible for contacting mothers for the pQCT study.

The dataset was checked for outliers and all statistical analysis was carried out myself using STATA V11.0. A trained statistician checked the results. The results interpretation is entirely my own work.

4 RESULTS: SIX YEAR FOLLOW UP OF THE CHILDREN IN THE SOUTHAMPTON WOMEN'S SURVEY

4.1 Aims

- To describe the women and children who took part in this follow up of the Southampton Women's Survey.

- To test the hypothesis that childhood lifestyle factors and body composition predict childhood bone mineral accrual.

- To test the hypothesis that maternal lifestyle factors (diet, smoking, exercise) and body composition influence childhood bone mineral accrual.

4.2 Methods

The parents of the children from the SWS cohort were contacted once their child had reached the age of 6 years. The children were visited by research nurses in their own home, in order to assess diet, lifestyle, anthropometry and fit an activity monitor. They were invited for a further clinic visit at the osteoporosis centre where measurements of bone mass and grip strength were obtained. The methods are described fully in chapter 3.

4.3 Results: Descriptive statistics

4.3.1 Study group: responders vs. non responders

1268 eligible families were contacted during the study period. Of these 780 (61.5%) agreed to a home visit. Of these 780 families, 530 (67.9%) attended a clinic visit. We were unable to contact a large number of the families despite numerous messages and postal reminders. Other reasons for non-attendance included parents working, parents reluctance to take children out of school and children not wanting to participate. A significant number gave no reason. Table 2 shows the maternal characteristics of responders/non responders for the clinic visit follow up.

TABLE 2: Maternal demographics between responders and non responders

		Responders		Non Responders		
			number		number	p value
Age, years (mean, sd)		28.4 (3.7)	530	27.8 (3.8)	738	0.99
Birthweight,g (mean,sd)		3243 (510)	486	3237 (562)	644	0.58
Percentage nulliparous		46.4	246	41.9	309	0.054
Qualifications %	None	1.89	10	4.88	36	
	CSE	9.06	48	10.99	81	
	O levels	29.62	157	29.85	220	
	A levels	28.49	151	28.63	211	
	HND	7.74	41	6.24	46	
	Degree	23.21	123	19.4	143	
			530		737	0.005
Social Class %	I	4.82	25	5.71	40	
	II	38.34	199	31.1	218	
	IIIN	37.19	193	37.38	262	
	IIIM	6.94	36	9.27	65	
	IV	11.37	59	13.55	95	
	V	1.35	7	3	21	
			519		701	0.01
Height, cm (mean, sd)		163.6 (0.28)	528	162.8 (0.24)	734	0.049
PP weight, kg (Median, IQR)		65.7 (59.2-73.7)	527	63.7 (57.2-72.4)	732	0.0072
PP BMI kg/m^2 (Median, IQR)		24.3 (22.4-27.5)	526	23.9 (21.5-27.2)	732	0.0315
PP triceps skinfold, mm (Median, IQR)		19.5 (15-25.3)	527	18.4 (14.3-23.5)	726	0.043
EP triceps skinfold, mm (Median, IQR)		19.3 (15.4-24.7)	422	18.3 (14.9-23.7)	568	0.059
LP triceps skinfold, mm (Median, IQR)		20.8 (16.5-25.8)	512	20.3 (16.3-25.1)	697	0.32
PP smoking,%		25.7	136	31.3	231	0.014
EP smoking, %		14.2	74	19.9	144	0.004
LP smoking, %		13.2	68	19.2	135	0.003
Units of alcohol per week (Median, IQR)		4.4 (1.5-10.3)	530	4.3 (1-10.5)	737	0.62
living with partner %		84.7	449	80.1	591	0.034

Mothers that agreed to participate in the 6 year follow up tended to be of higher social class (p=0.01), had a higher educational attainment (p=0.005), were less likely to smoke (p=0.014) and tended to be taller and heavier (p=0.049, p=0.007 respectively) (table 2).

Despite the differences in the mothers there was no difference in the anthropometry of the children when they were seen at birth or at aged 1 year (table 3).

TABLE 3: Childhood characteristics between responders and non responders

	Responders		Non Responders		
		number		number	p value
Gestational age (weeks) mean, sd	39.7 (1.8)	530	39.6 (2.1)	738	0.71
Birthweight (g) mean, sd	3442(536)	524	3404 (602)	728	0.88
Crown heel length birth(cm) mean, sd	49.8(2.1)	513	49.7 (2.2)	692	0.84
Weight at one year (kg) Median, IQR	10 (9.2-10.7)	526	10 (9.3-10.8)	652	0.31
Crown heel length age one (cm) mean, sd	75.5 (2.7)	520	75.7 (2.9)	639	0.08

4.3.2 Maternal characteristics

Data on 530 mother child pairs were available. The mean age of the mothers at the time of the child's birth was 30.6 year and 46% were in their first pregnancy. 42% of the mothers were in social class I and II, whereas only 12% were in social class IV and V. The majority of mothers (59%) had attained an educational qualification at A level or higher.

Table 4 shows maternal anthropometry prior to pregnancy. The average height and weight of the mothers was 163.6cm and 65.7kg respectively. The average weight gained during pregnancy up to 34 weeks was 11.9kg. Maternal triceps skinfold thickness increased from a median of 18.5mm prior to pregnancy to 20.8mm in late pregnancy.

TABLE 4: Maternal anthropometry before (PP) early (EP) and late pregnancy (LP)

Characteristic		n
Height, cm (mean, sd)	163.6 (6.5)	528
PP weight (Median, IQR)	65.7 (59.2-73.7)	526
PP BMI, kg/m^2 (Median, IQR)	24.3 (22.4-27.5)	526
Weight gain during pregnancy, kg (mean, sd)	11.9 (5.9)	516
PP triceps skinfold, mm (Median, IQR)	19.5 (15-25.3)	527
EP triceps skinfold, mm (Median, IQR)	19.3 (15.4-24.7)	422
LP triceps skinfold, mm (Median, IQR)	20.8 (16.5-25.8)	512

The majority of women consumed less than one unit of alcohol per week during early and late pregnancy; 29% consumed up to 7 units in early pregnancy and 27% in late pregnancy. Mothers tended to drink less alcohol in pregnancy compared to before. No association was seen between alcohol consumption and social class. Overall 25.7% of mothers smoked before pregnancy however this reduced to 14.1% in early pregnancy and 13.3% in late pregnancy. Mothers that smoked tended to be of lower social class. Women of lower social class were also less likely to give up smoking during pregnancy (table 5).

Mothers that had a high intake of milk prior to pregnancy were more likely to have a higher consumption during early and late pregnancy ($r=0.51$, $p<0.001$; $r=0.41$, $p<0.001$).

Both maternal walking speed and strenuous activity levels reduced during pregnancy. However women that exercised prior to falling pregnant were more likely to continue exercise particularly during early pregnancy.

TABLE 5: Maternal lifestyle characteristics

Characteristic		PP	EP	LP
Units of alcohol per week %	0-1.0	105(19.8)	283 (67)	366(71.9)
	to 7	270 (50.9)	124 (29.4)	138(27)
	to 14	83 (15.7)	5(1.2)	6 (1.2)
	>14	72 (13.6)	10 (2.4)	0
Pints milk per day %	<0.25	120 (22.6)	127 (30.1)	76 (14.8)
	to 0.5	201 (37.9)	125 (29.6)	161 (31.5)
	to 1.0	176 (33.2)	117 (27.7)	204 (39.8)
	>1	33 (6.23)	53 (12.6)	71 (13.9)
Walking Speed %	V slow	2 (0.4)	3 (0.7)	85 (16.6)
	Easy pace	32 (6.0)	49 (11.6)	260 (50.8)
	Normal	201 (37.9)	227 (53.8)	135 (26.4)
	Brisk	269 (50.8)	127 (30.1)	30 (5.9)
	Fast	26 (4.9)	16 (3.8)	2 (0.4)
Strenous activity, hrs/week (%)	0	203 (38.6)	233 (55.2)	369 (72.1)
	to 0.25	67 (12.7)	69 (16.35)	56 (10.9)
	to 1.5	178 (33.8)	85 (18.7)	64 (11.3)
	>1.5	78 (14.3)	35 (8.3)	23 (4.51)
Smoking % by social class	I and II	39 (17.4)	18 (8.1)	18 (8.2)
	III	66 (28.8)	35 (15.4)	29 (13.2)
	IV and V	27 (40.9)	19 (30.6)	19 (25.7)

table shows number and percentage

4.3.3 Childhood characteristics

There were 530 children with 6 year DXA data; of these 399 (75%) had undergone DXA assessment at age 4 years; 214 (40%) had undergone DXA assessment at birth and 170 (32%) had DXA at all three time points.

Not all the 6 year children had useable scans of whole body, lumbar and hip scans at the visit; one child was in a plaster cast, and several scans were excluded due to either movement artefact or metal found on clothing. In total 511 scans were available for whole body bone analysis, However due to clothing artefact only 499 were suitable for analysis of body composition. There were 526 good quality scans available for lumbar spine analysis and 526 available for hip analysis.

Despite similar height and weight at aged 6 years boys had higher grip strength scores compared to the girls ($p=0.01$), in addition to higher whole body and hip BMC ($p=0.008$ and 0.005 respectively), higher whole body ($p<0.001$), lumbar spine

(p=0.003) and hip aBMD (p<0.001) and higher bone area in the lumbar spine (p<0.001). Girls had higher fat mass (p=0.0001) compared to the boys and also had higher triceps skinfold thickness (p=0.0001) (table 6).

TABLE 6: Childhood characteristics of participants at age 6 years

Characteristic	Boys		Girls		P difference
		n		n	
Gestational age weeks, (mean, sd)	39.7 (1.6)	273	39.7 (2.0)	257	0.7
Birthweight g (mean, sd)	3482 (521)	268	3401 (549)	256	0.08
Age at DXA years, (mean, sd)	6.6 (0.2)	273	6.6 (0.2)	257	0.7
Height cm, (mean, sd)	120.3 (4.6)	257	119.7 (5.5)	235	0.2
Weight kg, (median, IQR)	22.7 (21.2-24.9)	256	23.2 (21.2-25.4)	236	0.33
BMI, kg/m^2, (median, IQR)					
Triceps skinfold thickness mm, (median, IQR)	8.7 (7.4-10.5)	214	11.0 (8.9-13.8)	196	0.0001
Grip strength R kg, (mean,sd)	9.0 (2.4)	224	8.5 (2.3)	228	0.01
Grip strength L kg, (mean,sd)	8.3 (2.3)	224	7.9 (2.3)	228	0.03
WB BMC (g) (mean, sd)	832.6 (95.1)	259	804.2 (95.8)	252	0.0008
WB Bone area cm^2 (mean, sd)	1139.0 (69.1)	259	1133.0 (73.4)	252	0.34
WB BMD g/cm^2, (mean, sd)	0.73 (0.05)	259	0.71 (0.05)	252	<0.00001
Total fat mass g, (median, IQR)	4605 (3795-5524)	253	5937 (4857-6504)	246	0.0001
Total lean mass g, (median, IQR)	17605 (16271-18940)	253	16660 (15106-18055)	246	0.0001
LS BMC g, (mean, sd)	18.1 (2.8)	272	17.6 (2.7)	254	0.07
LS Bone area cm^2, (mean, sd)	34.0 (3.0)	272	32.3(3.2)	254	<0.00001
LS BMD g/cm^2, (mean, sd)	0.53 (0.06)	272	0.55 (0.06)	254	0.003
Hip BMC g, (mean, sd)	11.4 (2.2)	272	10.9 (2.0)	254	0.005
Hip BA cm^2, (mean, sd)	16.6 (2.2)	272	16.9 (2.3)	254	0.1
Hip BMD g/cm^2, (mean, sd)	0.69 (0.06)	272	0.64 (0.06)	254	<0.00001

4.3.3.1 *Diet and lifestyle at age 6 years*

Daily milk was not normally distributed and was therefore grouped into quartiles. Total daily milk intake (a sum of all different types of milk drunk) was higher in boys compared to the girls (p=0.007) (table 7).

TABLE 7 Childhood milk intake at age 6 years (p value for trend 0.007)

Daily Milk Intake, pints	Boys	Girls	Total
<0.25	40 (18)	52 (25)	92 (22)
-0.5	87 (39)	89 (44)	176 (41)
-1	76 (34)	56 (27)	132 (31)
>1.0	18 (8)	7 (3)	25 (6)

table show number (percent)

Measurements of physical activity were available on 238 children using an actiheart monitor. Of these, 4 were excluded, as there was less than an average of 120

minutes/day; 19 were excluded as the subjects wore the monitor for less than 3 days. The main reason for taking the monitor off early was a skin reaction to the electrodes used to place the monitor. Minutes spent in vigorous and very vigorous activity were higher in boys compared to the girls (p= 0.008 and <0.0001 respectively) while girls spent more time in light activity. There was no difference between time spent in sedentary or moderate activity between the two sexes (table 8).

TABLE 8: Minutes spent in different types of activity per day (n=215)

Childhood activity Minutes per day	Boys	Girls	P value
sedentary	878 (78.1)	870 (83)	0.53
light	475 (66.7)	497 (69.1)	0.02
moderate	37.8 (12.4)	35.1 (12.4)	0.1
vigorous	20.3 (7.5)	17.6 (7.1)	0.008
very vigorous	28.7 (13.2)	21 (12.5)	<0.0001

table show number (percent)

4.3.3.2 *Fracture history*

In total 52 children reported a previous fracture. There was no significant difference seen between the boys and girls. A small number of these children had sustained more than one fracture (table 9). One child sustained two fractures at the time of injury. There were no statistically significant associations between childhood fracture and maternal social class, birthweight, diet, current activity levels and anthropometry. The small number of fractures limits the power to look at the determinants of fracture in this cohort.

TABLE 9: Total number of fractures

Number of fractures	Boys	Girls	Total
0	230	227	457
1	25	23	48
2	2	0	2
3	1	1	2

table show number (percent)

Table 10 displays fracture site by sex in this cohort. Wrist fractures was the most commonly reported, other upper limb fractures were also relatively common. Lower limb fractures were substantially less frequently reported.

TABLE 10 Subtypes of fractures seen in the girls and boys

Fracture Type	Boys	Girls	Total
Wrist	10	10	20
Elbow	6	1	7
Humerus	5	2	7
Clavicle	3	6	9
Metatarsal	2	0	2
Finger	2	2	4
Foot / Ankle	1	1	2
Femur	2	1	3
Tibia	0	1	1
Pelvis	0	1	1
Nose	1	2	3

4.4 Results: Determinants of 6 year bone mineral

4.4.1 Statistical analysis

Since both age at DXA and gender of the child were associated with bone mass (table 11), all DXA indices were adjusted for age at DXA and gender. For scans of the whole body the head was excluded in accordance with usual practice. All predictors and outcomes were checked for normality. Maternal and childhood triceps skinfold thickness, weight, BMI and fat mass were log transformed.

TABLE 11: Relationship of age and gender on whole body, lumbar spine and hip

	BA, cm2 β (CI)	BMC,g β (CI)	BMD, g/cm2 β (CI)
Whole body			
age (years)	59.9 (35.4,84.4)***	75 (48.2,101.9)***	0.04 (0.03,0.06)***
sex	7.5 (-3.9,18.9)	-0.09 (-12.7,12.5)	-0.005 (-0.01,0.003)
Lumbar spine			
age (years)	1.3 (0.09-2.5)*	1.6 (0.5,2.6)**	0.03 (1.1,2.7)*
sex	-1.7 (-2.2, -1.2)***	-0.4 (-0.9,0.04)	0.02 (0.005,0.03)**
Hip			
age (years)	2 (1.2,2.8)***	1.9 (1.1,2.7)***	0.03 (-0.05,-0.03)*
sex	0.3 (-0.06,0.7)*	-0.5 (-0.9,-0.2)**	-0.04 (-0.05,-0.03)***

table shows β; *p<0.05, **p<0.01, ***p<0.001

Childhood determinants of bone mass were explored first using univariate analysis. The determinants that showed statistically significant associations were then explored further using multivariate models. The univariate and multivariate maternal determinants were then explored before finally combining a multivariate model of childhood and maternal predictors.

4.4.2 Childhood determinants of 6 year bone mineral

The following childhood determinants of 6 year bone mass were considered; the child's lifestyle at age 6 years including diet and activity, and childhood anthropometry including height, weight, skinfold thickness and grip strength. (Early childhood anthropometry including birth weight and early growth will be considered separately in Chapter 7).

4.4.2.1 *Childhood dietary influences*

Milk intake was associated with increased whole body BMC, and bone area but neither aBMD nor vBMD. Milk intake was also associated with lumbar spine BMC and aBMD ($p<0.01$) and vBMD ($p<0.05$) and hip BMC and aBMD ($p=0.01$) Similarly, daily milk intake was associated with increased vBMD at both sites in this study ($p<0.05$) (table 12).

TABLE 12: Total milk intake at 6 years and whole body, lumbar spine and hip bone mass

	BA, cm2 β (CI) cm2/pt	**BMC,g** β (CI) g/pt	**BMD, g/cm2** β (CI) g/cm2/pt	**vBMD** β (CI) g/pt
Whole body				
Daily milk (pints)	9.9 (2.8-16.9)**	10 (2.3-17.7)*	0.005 (-0.0004-0.01)	0.2 (-2.7 3.1)
Lumbar spine				
Daily milk (pints)	0.3 (-0.06-0.62)	0.4 (0.14-0.72)**	0.008 (0.002-0.01)**	0.2 (0.03 0.4)*
Hip				
Daily milk (pints)	0.2 (-0.3-0.4)	0.3 (0.06-0.5)*	0.009 (0.002-0.02)*	0.1 (0.01 0.2)*

table shows β and 95% CI; *p<0.05, **p<0.01, ***p<0.001

There was a small association between fruit intake and whole body BMC ($r=0.1$, $p=0.04$), spinal BMC ($r=0.12$, $p=0.01$) and vBMD ($r=0.12$, $p=0.01$) and hip BMC ($r=0.11$, $p=0.03$) and vBMD, ($r=0.13$, $p=0.01$). No associations were seen between vegetable intake or carbonated drink intake and any measure of bone mass.

Duration of breastfeeding was not associated with the child's bone mass. However these children had reduced total fat mass (r=-0.1, p=0.02) and increased percentage lean mass (r=0.13, p=0.003) at aged 6 years. Whilst individual nutrients were not available for analysis, principal component analysis was used to obtain a measure of dietary patterns. A high score was associated with increased intake of fruit, vegetables and cereals whereas low score was associated with increased intake of fatty foods and low consumptions of fruit and vegetables. An increased score at aged 3 years was associated with increased whole body, lumbar spine and hip BMC and BA after adjustment for the child's weight at 6 years (table 13). These children also had a lower fat mass and higher percentage lean mass.

TABLE 13: Whole body, lumbar spine and hip BA, BMC, aBMD and vBMD at age 6 years per standard deviation increase in 3 year prudent diet score adjusted for weight

	BA, cm2 β (CI) cm2/pt	**BMC,g** β (CI) g/pt	**BMD, g/cm2** β (CI) g/cm2/pt	**vBMD** β (CI) g/pt
Whole body				
3 year prudent diet score (sd)	6.0 (0.9,11.0)*	6.7 (2.2,11.3)**	0.004 (0.0008,0.006)*	0.8 (-1.7,3.3)
Lumbar spine				
3 year prudent diet score (sd)	0.5 (0.2,0.8)***	0.4 (0.2,0.6)***	0.003 (-0.002,0.008)	0.05 (-0.1,0.2)
Hip				
3 year prudent diet score (sd)	0.3 (0.1,0.5)***	0.3 (0.1,0.5)***	0.005 (-0.0004,0.01)	0.02 (-0.08,0.1)

table shows p<0.05, **p<0.01, ***p<0.001

4.4.3 Influence of childhood activity

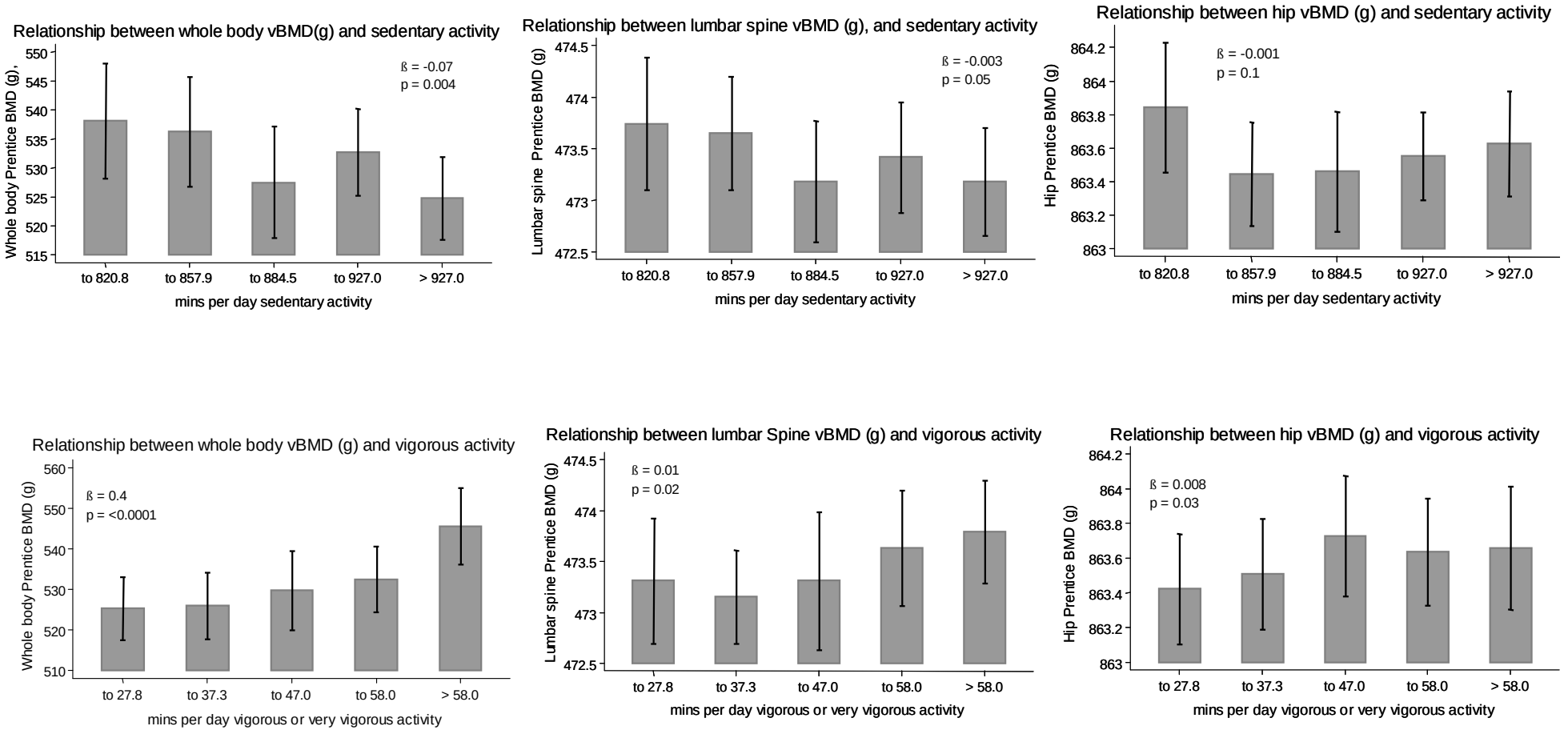
Valid activity data was available for 215 subjects. The results of different intensities of physical activity and measures of bone mass are summarised in table 14. High intensity exercise was associated with increased measures of vBMD for whole body ($r=0.32$, $p<0.001$) lumbar spine ($r=0.16$, $p=0.02$) and hip ($r=0.15$, $p=0.03$). Conversely high amounts of time spent in sedentary activity were associated with lower volumetric BMD for whole body and spine ($r=-0.21$, $p=0.004$; $r=-0.14$, $p=0.05$). Children engaged in higher intensities of activity appeared to have a lower whole body and lumbar spine bone area ($r=-0.16$, $p=0.02$; $r=-0.13$, $p=0.04$ respectively) compared to their contemporaries. Figure 7 uses barcharts to show the relationship between either vigorous or sedentary activity and whole body, lumbar and hip vBMD.

TABLE 14: Daily physical activity at age 6 years and whole body, lumbar spine and hip bone

Activity mins per day	BA, cm2 β (CI)	BMC,g β (CI)	BMD, g/cm2 β (CI)	vBMD, g β (CI)
Whole body				
sedentary	0.1 (-0.01,0.2)	0.04 (-0.09,0.2)	-0.00002 (-0.0001-0.00005)	-0.7 (-0.1,-0.02)
light	-0.06 (-0.2,0.07)	-0.03 (-0.2,0.1)	0.000007 (-0.00008,0.00010)	0.04 (-0.02,0.10)
moderate	-0.9 (-1.6,-0.2)*	-0.3 (-1.1,0.5)	0.0003 (-0.0002,0.0008)	0.7 (0.4,1.0)***
vigorous and very vigorous	-0.6 (-1,-0.09)*	-0.2 (-0.7,0.4)	0.0002 (-0.0001,0.0005)	0.4 (0.3,0.6)***
lumbar spine				
sedentary	0.004 (-0.001,0.009)	0.001 (-0.003,0.006)	-0.0004 (-0.0001,0.00007)	-0.003 (-0.006,-0.00005)
light	-0.002 (-0.008,0.003)	-0.001 (-0.007,0.004)	-0.00000003 (-0.0001,0.0001)	0.002 (-0.001,0.006)
moderate	-0.04 (-0.07,-0.004)*	-0.009 (-0.04,0.02)	0.0003 (-0.0003,0.0010)	0.02 (0.0007,0.04)*
vigorous and very vigorous	-0.02 (-0.04,-0.0006)*	-0.003 (-0.02,0.02)	0.0003 (-0.0001,0.0007)	0.01 (0.002,0.03)*
Hip				
sedentary	-0.0002 (-0.004,0.004)	-0.0008 (-0.004,0.003)	-0.0004 (-0.0001,0.00007)	-0.001 (-0.003,0.0005)
light	-0.0005 (-0.005,0.004)	-0.00008 (-0.004,0.004)	0.00001 (-0.0001,0.0001)	0.0008 (-0.001,0.003)
moderate	0.005 (-0.02,0.03)	0.009(-0.02,0.03)	0.0003 (-0.0004,0.001)	0.01 (-0.001,0.02)
vigorous and very vigorous	0.007 (-0.008,0.02)	0.01(-0.004,0.03)	0.0004 (-0.0001,0.0008)	0.008 (0.0006,0.02)*

table shows β and CI; *p<0.05, **p<0.01, ***p<0.001

Figure 7: Barcharts to show the relationships of time spent in sedentary and vigorous activity on volumetric BMD at age 6 years



4.4.4 Childhood anthrometric influences

At 6 years height, weight, BMI, total fat mass, total lean mass, triceps thickness and grip strength were all strongly associated with 6 year bone mass. (See table 15 and figure 8)

Height, weight and BMI were all positive predictors of whole body, lumbar spine and hip BA, BMC and aBMD (all $p<0.0001$) but not volumetric density.

Figure 8 shows scatterplots between fat and lean mass and whole body BMC and vBMD. Fat and lean mass were also positively associated with whole body, lumbar spine and hip BA, BMC and aBMD; however, whilst total lean mass was positively associated with whole body, spine and hip vBMD ($p<0.001$), fat mass was a negative predictor of both whole body and lumbar spine vBMD ($p<0.001$).

Triceps skinfold thickness was positively associated with whole body BMC, BA, aBMD and hip BMC, however there was a negative association between vBMD at all sites.

Figure 8: Scatterplots to show the relationship between total fat and lean mass and whole body BMC and vBMD

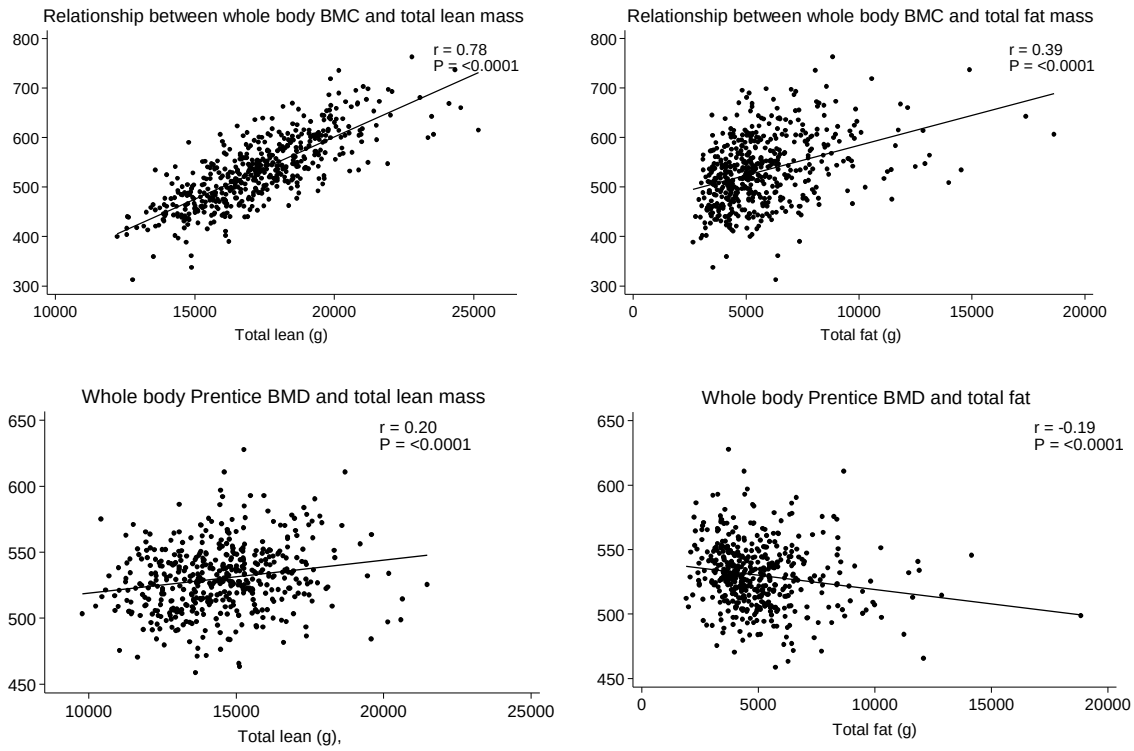
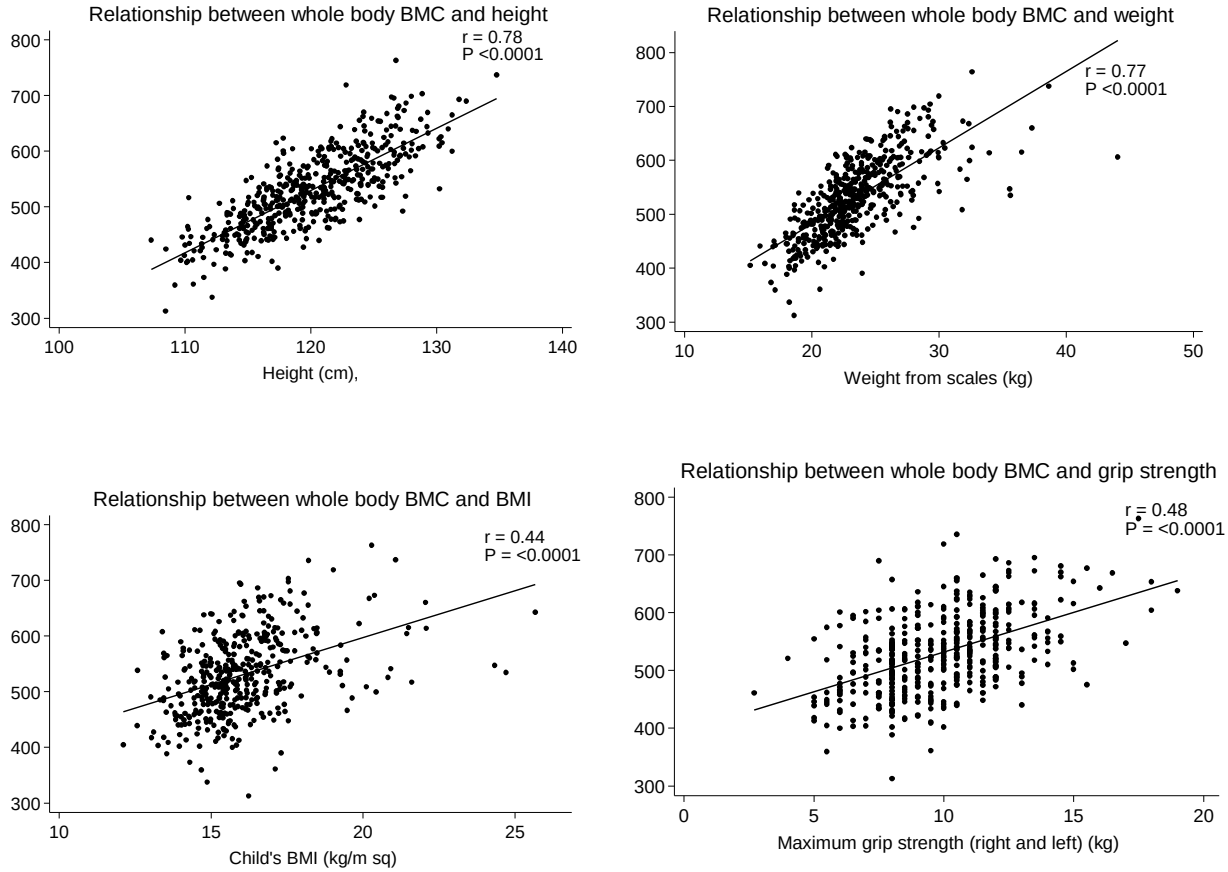


TABLE 15: Childhood anthropometry and 6 year whole body BMC, BA, aBMD and vBMD

	BA (cm^2)	BMC (g)	BMD (g/cm^2)	vBMD (g)
	Whole body			
Height (cm)	9.1 (8.4,9.9)***	10.4 (9.6,11.2)***	0.006 (0.005,0.006)***	-0.1 (-0.6,0.3)
Log: Weight (kg)	245.5 (212.7,278.3)***	350.5 (321.1,379.9)***	0.2 (0.2,0.2)***	-0.2 (-16.0,15.6)
BMI (kg/m^2)	7.8 (4.3,11.3)***	16.9 (13.3,20.5)***	0.01 (0.01,0.02)***	0.3 (-1.2,1.9)
Total fat (g)	0.007 (0.005,0.009)***	0.01 (0.010,0.01)***	0.000009 (0.000007,0.00001)***	-0.002 (-0.003,-0.0009)**
Total Lean (g)	0.02 (0.02,0.02)***	0.03 (0.02,0.03)***	0.00002 (0.00002,0.00002)***	0.002 (0.0010,0.003)***
Triceps thickness (mm)	2.2 (0.3,4.1)*	4.8 (2.7,6.8)***	0.004 (0.003,0.005)***	-1.2 (-2.0,-0.4)**
Grip strength (kg)	9.8 (7.5,12.0)***	13.7 (11.4,16.0)***	0.009 (0.007,0.01)***	2.2 (1.2,3.2)***
	Lumbar spine			
Height (cm)	0.4 (0.3,0.4)***	0.3 (0.3,0.4)***	0.004 (0.003,0.005)***	0.004 (-0.03,0.03)
Log: Weight (kg)	10 (8.3,11.7)***	10.6 (9.2,11.9)***	0.2 (0.1,0.2)***	-0.01 (-1.1,1.0)
BMI (kg/m^2)	0.3 (0.1,0.5)***	0.4 (0.3,0.5)***	0.007 (0.004,0.01)***	-0.01 (-0.1,0.09)
Total fat (g)	0.0002 (0.00007,0.0003)**	0.0002 (0.0001,0.0003)***	0.000004 (0.000001,0.000006)**	-0.0001 (-0.0002,-0.00006)***
Total Lean (g)	0.0008 (0.0007,0.0010)***	0.0009 (0.0008,0.0010)***	0.00001 (0.00001,0.00002)***	0.0002 (0.00008,0.0002)***
Triceps thickness (mm)	0.03 (-0.06,0.1)	0.04 (-0.04,0.1)	0.0007 (-0.001,0.002)	-0.1 (-0.1,-0.04)***
Grip strength (kg)	0.4 (0.3,0.5)***	0.5 (0.4,0.6)***	0.008 (0.006,0.01)***	0.1 (0.07,0.2)***
	Hip			
Height (cm)	0.3 (0.2,0.3)***	0.2 (0.2,0.3)***	0.003 (0.002,0.004)***	-0.005 (-0.02,0.01)
Log: Weight (kg)	7.8 (6.7,8.9)***	7.3 (6.3,8.4)***	0.1 (0.09,0.2)***	0.09 (-0.5,0.7)
BMI (kg/m^2)	0.3 (0.1,0.4)***	0.3 (0.2,0.4)***	0.006 (0.003,0.009)***	0.004 (-0.05,0.06)
Total fat (g)	0.0002 (0.0001,0.0003)***	0.0002 (0.00010,0.0003)***	0.000003 (0.0000002,0.000005)*	-0.00004 (-0.00008,0.000002)
Total Lean (g)	0.0006 (0.0005,0.0007)***	0.0006 (0.0006,0.0007)***	0.00001 (0.000010,0.00001)***	0.00006 (0.00002,0.0001)**
Triceps thickness (mm)	0.06 (-0.004,0.1)	0.05 (-0.01,0.1)	0.0005 (-0.001,0.002)	-0.03 (-0.06,-0.000009)*
Grip strength (kg)	0.3 (0.2,0.4)***	0.3 (0.2,0.4)***	0.006 (0.003,0.008)***	0.03 (-0.01,0.07)

table shows β and CI; *p<0.05, **p<0.01, ***p<0.001

Figure 9: Scattergraphs to show the relationship of childhood height, weight, BMI, fat mass, lean mass and grip strength aged 6years on whole body BMC(g)



4.5 Mutually independent determinants of childhood bone mass

The factors above which showed statistically significant associations with 6 year old bone mineral were explored in multivariate models.

Since childhood height and weight appeared to be the strongest predictors of bone mass in univariate analysis, all other significant predictors were explored separately and then in models including these. Triceps skinfold thickness and total fat mass were strongly correlated (r=0.79), as were grip strength and lean mass (r=0.55). Since these would show co-linearity in further analysis, grip strength and triceps thickness were chosen to be included in the multivariate analysis models.

Table 16 shows the multivariate analysis results from 157 subjects when grip strength, milk intake, triceps skinfold thickness and vigorous activity were all included in the model.

Grip strength was positively associated with whole body, lumbar spine and hip BA, BMC, a BMD and vBMD (all $p<0.05$). When height and weight were included in the model, grip strength remained positively associated with whole body and lumbar spine BA, BMC, aBMD and vBMD (all $p<0.0001$) and aBMD and vBMD of the hip (both $p=0.03$). Higher milk intake was positively associated with both whole body BA and BMC ($p<0.05$) once height and weight were included in the model, it remained associated with BA ($\beta=9.1$, $p=0.04$).

Triceps skinfold thickness was positively associated with whole body BA, BMC and aBMD (all $p<0.05$). Once height and weight were included, triceps skinfold thickness was negatively associated with lumbar spine aBMD ($p=0.05$) and hip BMC and BA (both $p=0.04$). There was a trend towards higher triceps skinfold thickness being associated with reduced whole body BMC and vBMD (both $p<0.1$).

Vigorous exercise was positively associated with whole body aBMD and vBMD ($p<0.001$). This association remained when height and weight were included in the model ($p<0.05$). In addition vigorous activity was also associated with lumbar spine vBMD ($\beta=0.02$, $p=0.02$) and hip BA, BMC, aBMD and vBMD (all $p<0.05$) when height and weight were included in the model.

TABLE 16: Mutually independent childhood determinants of bone mass at age 6 years

	BA, cm2	BMC,g	BMD, g/cm2	vBMD (prentice) (g)
	Whole body			
R^2 for model, %	**27**	**39.7**	**43.6**	**22.1**
Grip strength	13.8 (10,17.6)***	19.3 (15.4,23.3)***	0.01 (0.01-0.01)***	3.8 (2.2,5.3) ***
Milk intake	13.4 (2,24.7)*	13 (1.3,24.6)*	0.006 (-0.007,0.01)	1.7 (-9.8,13.1)
Triceps thickness	41.8 (7.1,76.5)*	67.3 (31.6,102.9)***	0.0004 (0.00004,0.0007)*	-4.6 (-19.5,10.3)
Vigorous activity	-0.3 (-0.8,0.2)	0.2 (-0.3,0.7)	0.04 (0.02, 0.07)***	.0.4 (0.2-0.6) ***
	Lumbar Spine			
R^2 for model, %	**13.7**	**26**	**21.2**	**13.7**
Grip strength	0.4 (0.3,0.6)***	0.6 (0.4,0.7)***	0.01 (0.007,0.01)***	0.2 (0.06,0.3)**
Milk intake	0.4 (-0.1,1)	0.2 (-0.2,0.7)	0.001 (-0.009,0.01)	-0.02 (-0.3,0.3)
Triceps thickness	1.6 (-0.9,3.3)	0.7 (-0.7,2.1)	-0.006 (-0.03,0.02)	-1.2 (-2.2,-0.3)*
Vigorous activity	-0.1 (0.04, 0.01	-0.002 (-0.02, 0.02)	0.0001 (-0.0003,0.0006)	0.009 (-0.007,0.04)
	Hip			
R^2 for model, %	**10.9**	**16**	**13**	**5.7**
Grip strength	0.2 (0.2,0.5)***	0.4 (0.2,0.5)***	0.01 (0.005,0.01)***	0.08 (0.01,0.1)*
Milk intake	0.3 (-0.1,0.7)	0.3 (-0.09,0.7	0.006 (-0.006,0.02)	0.05 (-0.15,0.2)
Triceps thickness	0.3 (0,1.6)	0.4 (-0.8,1.6)	0.1 (-0.02,0.05)	-0.2 (-0.77,0.4)
Vigorous activity	0.0008 (-0.02,0.02)	0.006 (-0.1,0.02)	0.0003 (-0.0002,0.0009)	0.006 (-0.004,0.02)

table shows β and 95% CI; *p<0.05, **p<0.01, ***p<0.001

Table 17 shows the effect when both height and weight are added to the four predictors in the multivariate model described in table 16. The child's height was positively associated with whole body BA, BMC (both p<0.001) lumbar spine BMC, BA (p<0.05) and hip BA and BMC (p<0.05). However a negative relationship was seen between the child's height and whole body vBMD (β=-2.1, p=0.002). Higher weight was positively associated with whole body BA, BMC and aBMD (all p<0.05), lumbar spine BMC and aBMD (p<0.05) and hip BA, BMC and aBMD (p<0.05).

TABLE 17: Mutually independent childhood determinants of bone mass at aged 6 years including height and weight

	BA, cm2	BMC,g	BMD, g/cm2	vBMD (prentice) (g)
	Whole body			
R^2 for model, %	**60**	**71**	**68**	**28**
Log child's weight	112.2 (2.3,222.1)*	291.6 (185.6,397.6)***	0.2 (0.2,0.3)***	60.6 (-2.5,123.7)
Child's height	6.1 (3.8,8.4)***	3.2 (1.0,5.4)**	-0.0003 (-0.002,0.001)	-2.1 (-3.4,-0.8)**
Grip strength	4.4 (0.8,7.9)*	7.2(3.8,10.6)***	0.005 (0.003,0.007)***	3.9 (1.9,5.9)***
Milk intake	9.1 (0.5,17.6)*	6 (-2.3,14.2)	0.001 (-0.004,0.007)	-0.8 (-5.6,4.0)
Triceps thickness	0.8 (-35.3,36.9)	-15 (-49.8,19.8)	-0.02 (-0.04,0.004)	-17.6 (-37.9,2.8)
Vigorous activity	-0.07 (-0.5,0.3)	0.3 (-0.1,0.6)	0.0003 (0.00007,0.0006)*	0.3* (0.05,0.5)
	Lumbar Spine			
R^2 for model, %	**43**	**47**	**26**	**15**
Log child's weight	5.1 (-0.8,11.0)	7.3 (2.4,12.3)**	0.1 (0.02,0.3)*	0.6 (-3.7,4.8)
Child's height	0.3 (0.1,0.4)***	0.1 (0.02,0.2)*	-0.0004 (-0.003,0.002)	-0.06 (-0.2,0.03)
Grip strength	0.05 (-0.1,0.2)	0.3 (0.09,0.4)**	0.007 (0.003,0.01)**	0.2 (0.07,0.3)**
Milk intake	0.3 (-0.2,0.8)	0.1 (-0.3,0.5)	-0.001 (-0.01,0.009)	-0.02 (-0.3,0.3)
Triceps thickness	-0.3 (-2.2,1.7)	-1.5 (-3.1,0.2)	-0.04 (-0.08,-0.0003)*	-1.3 (-2.7,0.05)
Vigorous activity	-0.004 (-0.03,0.02)	0.002 (-0.02,0.02)	0.0001 (-0.0003,0.0006)	0.006 (-0.01,0.02)
	Hip			
R^2 for model, %	**44**	**40**	**17**	**8**
Log child's weight	5.7 (1.3,10.0)*	6.6 (2.3,11.0)**	0.2 (0.010,0.3)*	1 (-1.7,3.6)
Child's height	0.2 (0.09,0.3)***	0.1 (0.01,0.2)*	-0.0008 (-0.004,0.002)	-0.05 (-0.1,0.009)
Grip strength	-0.01 (-0.2,0.1)	0.08 (-0.06,0.2)	0.006 (0.0006,0.01)*	0.09 (0.010,0.2)*
Milk intake	0.2 (-0.2,0.5)	0.2 (-0.2,0.5)	0.003 (-0.009,0.02)	0.04 (-0.2,0.2)
Triceps thickness	-1.5 (-2.9,-0.09)*	-1.5 (-2.9,-0.07)*	-0.03 (-0.08,0.02)	-0.3 (-1.2,0.5)
Vigorous activity	0.007 (-0.009,0.02)	0.009 (-0.007,0.02)	0.0003 (-0.0003,0.0008)	0.004 (-0.006,0.01)

table shows β and 95% CI; *p<0.05, **p<0.01, ***p<0.001

4.6 Maternal determinants of 6 year bone mass

4.6.1 Introduction

Maternal information was categorised as dietary, anthropometric and lifestyle data. The information was collected prior to conception, early pregnancy (14 weeks) and late pregnancy (34 weeks). Since much of the dietary data was not normally distributed, it has been log transformed where appropriate. As before 6 year DXA was adjusted for age and sex.

4.6.2 Maternal diet as a predictor of 6 year bone mass

Diet can be split into macro and micronutrients as well as individual food types and groups.

The only macronutrient that was associated with bone mass of the child was protein intake prior to pregnancy (table 18). On univariate analysis, pre pregnancy protein intake was associated with whole body BMC (r=0.1, p=0.02), BA (r=0.13, p=0.002), lumbar spine BMC (r=0.1, p=0.02) and BA (r=0.1, p=0.03) as well as hip BA (r=0.08, p=0.05). This relationship was not observed during early pregnancy and was only seen in late pregnancy for whole body BA (r=0.1, p=0.03).

For micronutrients all predictors were logged, as nutrient intake was not normally distributed. The overall results are displayed in table 19. Calcium intake prior pregnancy was positively associated with whole body BMC (r=0.13, p=0.002), BA (r=0.12, p=0.006) and aBMD (r=0.11, p=0.01). It was also associated with increased lumbar spine BMC (r=0.17, p<0.0001), BA (r=0.18, p<0.0001) aBMD (r=0.17, p=0.03) and hip BMC (r=0.11, p=0.01) and BA (r=0.11, p=0.009). There was no relationship seen in early pregnancy and only a weak relationship was seen for whole body BA in late pregnancy (r=0.09, p=0.04). Although calcium intake prior to pregnancy was strongly correlated with intake in early (r=0.52, p<0.0001) and late pregnancy (r=0.54, p<0.0001) there was some difference seen in the amount of calcium consumed.

A relationship was also observed for pre pregnancy intake of vitamin B12 for whole body BMC (r=0.11, p=0.01), BA (r=0.12, p=0.005) and lumbar spine BMC (r=0.11, p=0.009) and BA (r=0.11, p=0.01). No relationship was seen in early or late pregnancy.

Although dietary patterns were explored there was no significant relationship between a healthy diet (calculated using principal component analysis) and measures of bone mass. However mothers that had a higher score, consistent with a healthier diet had children with a higher percentage lean mass (r=0.11, p=0.01).

TABLE 18: Maternal macronutrient intake in pre, early and late pregnancy and bone mineral at age 6 years

	Whole body				Lumbar spine				Hip			
	BA, cm2	**BMC,g**	**BMD, g/cm2**	**vBMD, g**	**BA, cm2**	**BMC,g**	**BMD, g/cm2**	**vBMD, g**	**BA, cm2**	**BMC,g**	**BMD, g/cm2**	**vBMD, g**
Pre Pregnancy n=526												
Total protein (g/day)	31.5 (11.3,51.7)**	26.9 (4.6,49.1)*	0.008 (-0.006,0.02)	-1.8 (-10.5,6.9)	1.1 (0.1,2.1)*	1 (0.2,1.9)*	0.01 (-0.005,0.03)	0.2 (-0.4,0.8)	0.7 (-0.008,1.3)*	0.6 (-0.07,1.2)	0.008 (-0.01,0.03)	-0.06 (-0.4,0.3)
Total fat (g/day)	15.6 (-2.2,33.3)	11.8 (-7.7,31.3)	0.002 (-0.01,0.01)	1.9 (-5.7,9.4)	0.2 (-0.7,1.0)	0.6 (-0.1,1.4)	0.02 (-0.0007,0.03)	0.4 (-0.1,0.9)	0.08 (-0.5,0.7)	0.05 (-0.5,0.6)	0.000006 (-0.02,0.02)	-0.07 (-0.4,0.2)
Total carbohydrate (g/day)	17.3 (-1.4,36.1)	18.2 (-2.4,38.7)	0.008 (-0.005,0.02)	4.3 (-3.5,12.2)	0.6 (-0.3,1.5)	0.9 (0.06,1.6)*	0.02 (-0.0006,0.03)	0.5 (-0.05,1.0)	0.4 (-0.3,1.0)	0.2 (-0.4,0.8)	0.0004 (-0.02,0.02)	-0.1 (-0.4,0.2)
Total energy (kcal/day)	23.6 (3.0,44.3)*	21.7 (-1.0,44.4)	0.008 (-0.007,0.02)	3.1 (-5.7,11.8)	0.6 (-0.4,1.6)	1 (0.08,1.8)*	0.02 (0.0004,0.04)*	0.5 (-0.1,1.1)	0.4 (-0.3,1.1)	0.3 (-0.3,1.0)	0.002 (-0.02,0.02)	-0.1 (-0.5,0.2)
Early Pregnancy n=420												
Total protein (g/day)	12.0 (-8.6,32.7)	13.8 (-9.3,36.9)	0.007 (-0.008,0.02)	1.3 (-7.9,10.4)	0.6 (-0.3,1.6)	0.4 (-0.5,1.3)	0.0008 (-0.02,0.02)	-0.03 (-0.6,0.6)	0.4 (-0.3,1.2)	0.4 (-0.3,1.0)	0.005 (-0.02,0.03)	-0.1 (-0.5,0.2)
Total fat (g/day)	6.6 (-12.8,26.0)	5.4 (-16.3,27.2)	0.001 (-0.01,0.02)	-0.8 (-9.3,7.6)	0.4 (-0.6,1.3)	0.2 (-0.7,1.0)	-0.002 (-0.02,0.02)	-0.1 (-0.7,0.4)	0.2 (-0.4,0.9)	0.03 (-0.6,0.7)	-0.007 (-0.03,0.01)	-0.2 (-0.6,0.08)
Total carbohydrate (g/day)	6.3 (-15.3,27.9)	12.6 (-11.6,36.7)	0.009 (-0.006,0.02)	5.7 (-3.6,14.9)	0.06 (-0.9,1.1)	0.3 (-0.6,1.2)	0.007 (-0.01,0.03)	0.3 (-0.3,0.9)	0.2 (-0.6,0.9)	0.1 (-0.6,0.8)	-0.0006 (-0.02,0.02)	-0.05 (-0.4,0.3)
Total energy (kcal/day)	8.2 (-14.5,30.8)	11.6 (-13.7,36.9)	0.007 (-0.010,0.02)	2.8 (-7.1,12.6)	0.3 (-0.7,1.4)	0.3 (-0.6,1.3)	0.003 (-0.02,0.02)	0.1 (-0.5,0.8)	0.3 (-0.5,1.1)	0.2 (-0.6,0.9)	-0.003 (-0.03,0.02)	-0.2 (-0.5,0.2)
Late Pregnancy n=508												
Total protein (g/day)	23 (2.8,43.2)*	16.6 (-5.7,38.9)	0.002 (-0.01,0.02)	-4.1 (-12.8,4.6)	0.4 (-0.6,1.4)	0.3 (-0.6,1.2)	0.004 (-0.01,0.02)	0.1 (-0.5,0.7)	0.2 (-0.5,0.9)	-0.02 (-0.7,0.6)	-0.008 (-0.03,0.01)	-0.2 (-0.6,0.1)
Total fat (g/day)	16.1 (-2.3,34.6)	12.3 (-8.1,32.7)	0.002 (-0.01,0.02)	-0.03 (-7.9,7.9)	-0.08 (-1.0,0.8)	0.2 (-0.5,1.0)	0.009 (-0.008,0.03)	0.3 (-0.2,0.8)	-0.08 (-0.7,0.6)	-0.1 (-0.7,0.5)	-0.005 (-0.02,0.01)	-0.1 (-0.4,0.2)
Total carbohydrate (g/day)	17.7 (-1.4,36.9)	13.8 (-7.3,35.0)	0.003 (-0.01,0.02)	-0.5 (-8.7,7.6)	0.5 (-0.4,1.4)	0.5 (-0.3,1.3)	0.008 (-0.010,0.03)	0.4 (-0.2,0.9)	0.01 (-0.6,0.7)	-0.2 (-0.8,0.4)	-0.01 (-0.03,0.007)	-0.2 (-0.5,0.09)
Total energy (kcal/day)	21.6 (0.7,42.6)*	16.7 (-6.4,39.9)	0.003 (-0.01,0.02)	-0.9 (-9.8,8.1)	0.3 (-0.7,1.4)	0.5 (-0.4,1.4)	0.01 (-0.009,0.03)	0.4 (-0.2,1.0)	0.03 (-0.7,0.7)	-0.2 (-0.9,0.5)	-0.01 (-0.03,0.01)	-0.2 (-0.6,0.1)

table shows β; *p<0.05, **p<0.01, ***p<0.001

TABLE 19: Maternal micronutrient intake in pre, early and late pregnancy and bone mineral aged 6 years

	Whole body				Lumbar spine				Hip			
	BA, cm2	BMC,g	BMD, g/cm2	vBMD, g	BA, cm2	BMC,g	BMD, g/cm2	vBMD, g	BA, cm2	BMC,g	BMD, g/cm2	vBMD, g
Pre Pregnancy n=526												
Calcium mg/day	22.6 (6.7,38.5)**	27.2 (9.8,44.7)**	0.01 (0.004,0.03)*	2.9 (-3.9,9.6)	1.6 (0.8,2.3)***	1.4 (0.7,2.0)***	0.02 (0.002,0.03)*	0.3 (-0.1,0.8)	0.7 (0.2,1.2)**	0.7 (0.1,1.2)*	0.01 (-0.006,0.03)	-0.01 (-0.3,0.2)
Vitamin D mcg/day	8.8 (-0.03,17.6)*	7.9 (-1.8,17.6)	0.003 (-0.004,0.009)	-1.6 (-5.4,2.1)	0.3 (-0.1,0.7)	0.4 (0.02,0.8)*	0.007 (-0.0007,0.02)	0.08 (-0.2,0.3)	0.1 (-0.2,0.4)	0.1 (-0.2,0.4)	0.002 (-0.007,0.01)	-0.02 (-0.2,0.1)
B12 mcg/day	15.6 (4.8,26.4)**	15 (3.1,26.8)*	0.006 (-0.002,0.01)	-1 (-5.7,3.6)	0.7 (0.2,1.2)*	0.6 (0.2,1.1)***	0.008 (-0.002,0.02)	0.09 (-0.2,0.4)	0.3 (-0.03,0.7)	0.3 (-0.03,0.7)	0.006 (-0.005,0.02)	-0.003 (-0.2,0.2)
Folate mcg/day	5.5 (-7.2,18.2)	4.7 (-9.3,18.6)	0.001 (-0.008,0.01)	-2.1 (-7.4,3.2)	0.7 (0.1,1.3)*	0.5 (-0.02,1.0)	0.003 (-0.008,0.01)	-0.03 (-0.4,0.3)	0.1 (-0.3,0.5)	0.02 (-0.4,0.4)	-0.004 (-0.02,0.009)	-0.1 (-0.3,0.07)
Vitamin C mg/day	9.2 (0.7,17.7)*	6.8 (-2.5,16.1)	0.001 (-0.005,0.007)	-0.3 (-3.8,3.3)	0.3 (-0.06,0.7)	0.3 (-0.01,0.7)	0.005 (-0.002,0.01)	0.1 (-0.10,0.4)	0.2 (-0.05,0.5)	0.2 (-0.08,0.5)	0.001 (-0.007,0.010)	-0.03 (-0.2,0.1)
Total milk pts/day	6.6 (0.7,12.5)*	9.8 (3.4,16.3)**	0.006 (0.002,0.01)**	1.7 (-0.7,4.2)	0.4 (0.09,0.6)*	0.4 (0.1,0.6)***	0.005 (0.00009,0.01)*	0.1 (-0.05,0.3)	0.2 (-0.02,0.4)	0.2 (0.04,0.4)*	0.006 (0.0006,0.01)*	0.06 (-0.03,0.2)
Early Pregnancy n=420												
Calcium mg/day	9.6 (-6.1,25.3)	10.6 (-6.9,28.2)	0.005 (-0.006,0.02)	-0.5 (-7.4,6.4)	0.6 (-0.2,1.3)	0.2 (-0.4,0.9)	-0.003 (-0.02,0.01)	-0.1 (-0.6,0.3)	0.4 (-0.1,1.0)	0.2 (-0.3,0.7)	-0.004 (-0.02,0.01)	-0.2 (-0.5,0.05)
Vitamin D mcg/day	-6.4 (-15.4,2.5)	-4.6 (-14.6,5.5)	-0.001 (-0.008,0.005)	0.3 (-3.6,4.2)	0.08 (-0.3,0.5)	-0.01 (-0.4,0.4)	-0.002 (-0.010,0.007)	-0.06 (-0.3,0.2)	-0.05 (-0.4,0.3)	-0.02 (-0.3,0.3)	0.0001 (-0.009,0.009)	-0.03 (-0.2,0.1)
B12 mcg/day	2.4 (-8.7,13.5)	3.7 (-8.7,16.1)	0.003 (-0.005,0.01)	-1.2 (-6.2,3.8)	0.5 (-0.05,1.0)	0.3 (-0.2,0.8)	0.002 (-0.009,0.01)	-0.05 (-0.4,0.3)	0.2 (-0.2,0.6)	0.3 (-0.06,0.7)	0.01 (-0.0008,0.02)	0.05 (-0.1,0.2)
Folate mcg/day	-0.4 (-16.2,15.3)	-4.9 (-22.5,12.8)	-0.005 (-0.02,0.006)	-4 (-10.9,2.8)	0.2 (-0.5,0.9)	-0.2 (-0.8,0.5)	-0.007 (-0.02,0.007)	-0.3 (-0.7,0.2)	0.1 (-0.4,0.6)	-0.01 (-0.5,0.5)	-0.006 (-0.02,0.009)	-0.1 (-0.4,0.1)
Vitamin C mg/day	1 (-9.2,11.1)	-0.1 (-11.5,11.3)	-0.0008 (-0.008,0.007)	0.9 (-3.5,5.3)	0.1 (-0.4,0.6)	0.2 (-0.2,0.6)	0.004 (-0.006,0.01)	0.2 (-0.06,0.5)	-0.2 (-0.5,0.2)	0.03 (-0.3,0.4)	0.008 (-0.003,0.02)	0.1 (-0.03,0.3)
Mg mg/day	-5.5 (-26.1,15.2)	1.1 (-22.1,24.2)	0.004 (-0.01,0.02)	2.8 (-6.1,11.8)	0.2 (-0.8,1.1)	-0.06 (-0.9,0.8)	-0.005 (-0.02,0.01)	-0.2 (-0.8,0.3)	0.1 (-0.6,0.8)	0.09 (-0.6,0.8)	-0.0001 (-0.02,0.02)	-0.1 (-0.5,0.2)
Total milk pts/day	5.4 (-0.07,10.9)	6.2 (0.07,12.4)*	0.003 (-0.0008,0.007)	-0.2 (-2.6,2.2)	0.2 (-0.03,0.5)	0.2 (-0.08,0.4)	0.0006 (-0.004,0.006)	-0.01 (-0.2,0.1)	0.1 (-0.05,0.3)	0.1 (-0.08,0.3)	0.0002 (-0.005,0.006)	-0.05 (-0.1,0.04)
Late Pregnancy n=508												
Calcium mg/day	16.3 (0.6,32.0)	13.5 (-3.8,30.8)	0.004 (-0.008,0.01)	-1.1 (-7.8,5.5)	0.6 (-0.1,1.4)	0.4 (-0.3,1.0)	0.002 (-0.01,0.02)	0.04 (-0.4,0.5)	0.4 (-0.2,0.9)	0.05 (-0.5,0.6)	-0.01 (-0.03,0.005)	-0.2 (-0.5,0.01)
Vitamin D mcg/day	2.5 (-6.2,11.1)	-2.9 (-12.4,6.6)	-0.005 (-0.01,0.0010)	-3.1 (-6.7,0.6)	0.3 (-0.1,0.7)	-0.01 (-0.4,0.4)	-0.005 (-0.01,0.003)	-0.07 (-0.3,0.2)	-0.07 (-0.4,0.2)	-0.07 (-0.3,0.2)	-0.002 (-0.01,0.007)	-0.02 (-0.2,0.1)
B12 mcg/day	9.3 (-2.9,21.5)	5.7 (-7.8,19.2)	-0.00006 (-0.009,0.009)	-1.9 (-7.1,3.4)	0.4 (-0.2,1.0)	0.3 (-0.3,0.8)	0.001 (-0.010,0.01)	0.03 (-0.3,0.4)	0.09 (-0.3,0.5)	0.1 (-0.3,0.5)	0.003 (-0.009,0.02)	0.03 (-0.2,0.2)
Folate mcg/day	2 (-9.1,13.0)	-3.6 (-15.8,8.5)	-0.006 (-0.01,0.002)	-2.8 (-7.5,1.9)	0.4 (-0.1,0.9)	-0.07 (-0.5,0.4)	-0.009 (-0.02,0.001)	-0.2 (-0.5,0.1)	-0.1 (-0.5,0.3)	-0.2 (-0.5,0.2)	-0.007 (-0.02,0.004)	-0.09 (-0.3,0.09)
Vitamin C mg/day	5.4 (-4.5,15.3)	-0.5 (-11.4,10.4)	-0.004 (-0.01,0.003)	-2.5 (-6.7,1.8)	0.2 (-0.2,0.7)	0.04 (-0.4,0.5)	-0.002 (-0.01,0.007)	0.04 (-0.2,0.3)	-0.01 (-0.3,0.3)	-0.02 (-0.3,0.3)	-0.002 (-0.01,0.008)	-0.03 (-0.2,0.1)
Mg mg/day	14.1 (-6.1,34.2)	8.5 (-13.7,30.7)	-0.0006 (-0.02,0.01)	-2.9 (-11.5,5.7)	0.6 (-0.4,1.6)	0.3 (-0.5,1.2)	0.001 (-0.02,0.02)	0.1 (-0.5,0.7)	0.02 (-0.7,0.7)	-0.2 (-0.8,0.5)	-0.01 (-0.03,0.01)	-0.2 (-0.6,0.1)
Total milk pts/day	4.2 (-1.1,9.6)	5.7 (-0.1,11.6)	0.003 (-0.0003,0.007)	1 (-1.3,3.3)	0.1 (-0.1,0.4)	0.1 (-0.10,0.4)	0.002 (-0.003,0.007)	0.02 (-0.1,0.2)	0.2 (-0.03,0.3)	0.08 (-0.09,0.2)	-0.0007 (-0.006,0.005)	-0.04 (-0.1,0.05)

table shows b; *p<0.05, **p<0.01, ***p<0.001

4.6.3 Maternal lifestyle determinants of 6 year bone mineral

There was no relationship seen between maternal social class, maternal qualifications obtained or parity and childhood bone mass. Smoking was positively associated with whole body aBMD and BMAD but not vBMD using method of prentice (table 20).

TABLE 20: Maternal exercise and smoking status pre, early and late pregnancy and whole body bone mineral

	Whole Body			
	BA (cm^2)	**BMC (g)**	**aBMD (g/cm^2)**	**vBMD Prentice (g)**
Pre Pregnancy n=526				
freq strenous activity week	1.6 (-1.4,4.7)	1.2 (-2.2,4.5)	0.0003 (-0.002,0.002)	0.3 (-0.9,1.6)
walking speed	2.6 (-5.4,10.6)	2.1 (-6.7,10.8)	0.00002 (-0.006,0.006)	-0.7 (-4.0,2.6)
Current smoking	2.9 (-9.7,15.6)	8.9 (-5.0,22.8)	0.008 (-0.0009,0.02)	1.8 (-3.5,7.1)
Early pregnancy n-420				
freq strenous activity week	1.2 (-1.2,3.5)	0.3 (-2.4,2.9)	-0.0004 (-0.002,0.001)	-0.6 (-1.5,0.4)
walking speed	4.6 (-3.7,12.9)	5.6 (-3.7,14.9)	0.002 (-0.004,0.008)	0.9 (-2.6,4.5)
Current smoking	2.6 (-13.4,18.6)	14.5 (-3.0,32.0)	0.01 (0.003,0.03)*	2 (-4.6,8.7)
Late pregnancy n= 508				
freq strenous activity week	0.7 (-0.7,2.1)	0.9 (-0.6,2.4)	0.0005 (-0.0004,0.002)	0.4 (-0.2,1.0)
walking speed	0.5 (-6.3,7.4)	-0.8 (-8.4,6.8)	-0.002 (-0.007,0.003)	-0.7 (-3.6,2.2)
Current smoking	6.3 (-10.0,22.6)	17.4 (-0.5,35.3)	0.01 (0.003,0.03)*	1.9 (-5.1,8.8)

table shows β and 95% CI; *p<0.05, **p<0.01, ***p<0.001

Smoking was positively associated with both total and percentage fat at all time points during pregnancy (Table 21). It was conversely negatively associated with percentage lean mass (all p<0.0001) of the child at aged 6 years.

TABLE 21: Maternal exercise and smoking status pre, early and late pregnancy and body composition

	Total lean (g)	Total fat Z trans of log (g)	% Fat	% Lean
Pre Pregnancy n=526				
freq strenous activity week	33.8 (-59.1,126.7)	-0.04 (-0.09,0.009)	-0.05 (-0.10,-0.004)*	0.06 (0.008,0.1)*
walking speed	168.6 (-74.5,411.6)	-0.09 (-0.2,0.04)	-0.1 (-0.3,-0.002)*	0.1 (0.009,0.3)*
Current smoking	116.6 (-270.6,503.7)	0.3 (0.08,0.5)**	0.3 (0.1,0.5)**	-0.4 (-0.5,-0.2)***
Early pregnancy n-420				
freq strenous activity week	26.9 (-43.8,97.5)	-0.03 (-0.06,0.01)	-0.03 (-0.07,0.004)	0.03 (-0.006,0.07)
walking speed	202.1 (-51.2,455.3)	-0.1 (-0.3,-0.0009)*	-0.2 (-0.3,-0.07)**	0.2 (0.07,0.3)**
Current smoking	402.6 (-88.8,894.0)	0.5 (0.3,0.8)***	0.5 (0.3,0.8)***	-0.6 (-0.9,-0.4)***
Late pregnancy n= 508				
freq strenous activity week	17.6 (-23.9,59.2)	-0.02 (-0.04,0.005)	-0.02 (-0.05,-0.002)*	0.02 (-0.001,0.04)
walking speed	88.8 (-117.6,295.2)	-0.1 (-0.2,-0.01)*	-0.2 (-0.3,-0.05)**	0.2 (0.05,0.3)**
Current smoking	503.2 (12.2,994.2)*	0.5 (0.2,0.7)***	0.5 (0.2,0.7)***	-0.5 (-0.8,-0.3)***

table shows β and 95% CI; *p<0.05, **p<0.01, ***p<0.001

Whilst maternal exercise and walking speed were not associated with childhood bone mass, they were negatively associated with percentage fat and positively associated with percentage lean mass (table 21).

Increased frequency of strenuous activity in late pregnancy was positively associated with hip BMC (r=0.14, p=0.002), BA (r=0.09, p=0.04), aBMD (r=0.11, p=0.02) and vBMD (r=0.13, p=0.006). However no association with exercise levels was seen in either prior or during early pregnancy (table 20). Exercise in late pregnancy was positively correlated with vigorous activity in the child at aged 6 years (r=0.15, p=0.03).

4.6.4 Maternal anthropometric influences

The relationship between maternal anthropometry and bone mineral is summarised in table 22. Pre pregnancy maternal height and weight were both positively associated with 6 year whole body BMC ($r=0.28$, $p<0.0001$; $r=0.23$, $p<0.0001$), BA ($r=0.3$, $p<0.0001$; $r=0.17$, $p<0.0001$) and aBMD ($r=0.2$, $p<0.0001$; $r=0.24$, $p<0.0001$) but not vBMD. A weaker association was seen between maternal BMI and whole body BMC ($r=0.11$, $p=0.01$) and aBMD ($r=0.16$, $p<0.0001$). A similar association was seen for weight in late pregnancy (all $p<0.0001$); however there was no relationship between weight gained during pregnancy and any measure of bone mass.

A similar relationship was observed for pre pregnancy height and weight and 6 year lumbar spine BMC ($r=0.23$, $p<0.0001$; $r=0.16$, $p<0.0001$) , BA ($r=0.3$, $p<0.0001$; $r=0.16$, $p<0.0001$). However there was no relationship between BMI or triceps skinfold thickness and lumbar spine bone mass.

Whilst maternal height and weight remained significantly positively associated with 6 year hip BMC ($r=0.18$, $p<0.0001$; $r=0.18$, $p<0.0001$) and BA ($r=0.24$, $p<0.0001$; $r=0.16$, $p<0.0001$), maternal height appeared to be negatively associated with vBMD ($r=0.1$, $p=0.03$) whilst BMI was positively associated with vBMD ($r=0.1$, $p=0.03$).

TABLE 22: Maternal anthropometry pre and during pregnancy and whole body, lumbar spine and hip bone mass in the child at age 6 years

	Whole body BA (cm^2)	BMC (g)	aBMD (g/cm^2)	vBMD (prentice g)
Pre Pregnancy				
Weight (kg)	60.7 (30.7,90.7)***	87.6 (55.1,120.2)***	0.06 (0.04,0.08)***	0.8 (-12.1,13.6)
Height (cm)	2.9 (2.1,3.7)***	3 (2.1,3.9)***	0.001 (0.0008,0.002)***	-0.3 (-0.6,0.09)
BMI (kg/m^2)	16.3 (-16.6,49.2)	46.3 (10.6,82.1)*	0.04 (0.02,0.07)***	5.1 (-8.6,18.8)
Triceps skinfold (mm)	4.3 (-11.2,19.7)	13.1 (-3.9,30.1)	0.01 (0.002,0.02)*	0.3 (-6.2,6.8)
Early pregnancy				
Grip strength (kg)	1.3 (-0.3,3.0)	0.9 (-0.9,2.7)	0.0001 (-0.001,0.001)	-0.5 (-1.1,0.2)
Triceps skinfold (mm)	14 (-5.4,33.4)	16.3 (-5.4,38.0)	0.009 (-0.005,0.02)	-5.1 (-13.5,3.2)
Late pregnancy				
Weight (kg)	81.5 (48.7,114.4)***	111.9 (76.3,147.6)***	0.07 (0.05,0.10)***	0.2 (-14.1,14.5)
Triceps skinfold (mm)	14.1 (-3.1,31.4)	18.4 (-0.5,37.4)	0.01 (-0.0003,0.02)	-2.8 (-10.1,4.5)
Pregnancy weight gain (kg)	0.9 (-0.04,1.9)	0.8 (-0.3,1.8)	0.0003 (-0.0004,0.0010)	-0.2 (-0.6,0.2)
	Lumbar spine BA (cm^2)	**BMC (g)**	**aBMD (g/cm^2)**	**vBMD (prentice g)**
Pre Pregnancy				
Weight (kg)	2.7 (1.2,4.1)***	2.4 (1.1,3.7)***	0.03 (0.002,0.06)*	-0.1 (-1.0,0.7)
Height (cm)	0.1 (0.1,0.2)***	0.1 (0.06,0.1)***	0.0006 (-0.0001,0.001)	-0.1 (-1.0,0.7)
BMI (kg/m^2)	0.6 (-1.0,2.1)	1 (-0.4,2.4)	0.02 (-0.009,0.05)	0.1 (-0.8,1.0)
Triceps skinfold (mm)	0.4 (-0.3,1.1)	0.2 (-0.4,0.9)	0.002 (-0.01,0.02)	-0.1 (-0.5,0.3)
Early pregnancy				
Grip strength (kg)	0.05 (-0.03,0.1)	0.02 (-0.05,0.09)	-0.0002 (-0.002,0.001)	-0.03 (-0.07,0.01)
Triceps skinfold (mm)	0.4 (-0.5,1.3)	0.2 (-0.6,1.1)	0.0005 (-0.02,0.02)	-0.1 (-0.7,0.4)
Late pregnancy				
Weight (kg)	3.5 (1.9,5.1)***	3 (1.6,4.4)***	0.03 (0.004,0.06)*	-0.2 (-1.1,0.8)
Triceps skinfold (mm)	0.5 (-0.3,1.3)	0.09 (-0.6,0.8)	-0.005 (-0.02,0.01)	-0.3 (-0.8,0.2)
Pregnancy weight gain (kg)	0.04 (-0.008,0.08)	0.02 (-0.02,0.06)	-0.00009 (-0.0010,0.0008)	-0.008 (-0.04,0.02)
	Hip BA (cm^2)	**BMC (g)**	**aBMD (g/cm^2)**	**vBMD (prentice g)**
Pre Pregnancy				
Weight (kg)	1.9 (0.9,2.9)***	2.1 (1.1,3.0)***	0.05 (0.02,0.08)***	0.3 (-0.2,0.8)
Height (cm)	0.08 (0.05,0.1)***	0.06 (0.03,0.08)***	0.0003 (-0.0006,0.001)	-0.01 (-0.03,-0.001)*
BMI (kg/m^2)	0.7 (-0.4,1.8)	1.3 (0.3,2.4)*	0.05 (0.02,0.08)**	0.6 (0.06,1.1)*
Triceps skinfold (mm)	0.3 (-0.3,0.8)	0.4 (-0.04,0.9)	0.02 (0.002,0.03)*	0.2 (-0.07,0.4)
Early pregnancy				
Grip strength (kg)	0.02 (-0.04,0.07)	0.001 (-0.05,0.05)	-0.0005 (-0.002,0.001)	-0.02 (-0.05,0.006)
Triceps skinfold (mm)	0.4 (-0.3,1.0)	0.5 (-0.1,1.1)	0.01 (-0.005,0.03)	0.2 (-0.2,0.5)
Late pregnancy				
Weight (kg)	2.4 (1.3,3.5)***	2.4 (1.4,3.5)***	0.05 (0.02,0.09)**	0.2 (-0.2,0.5)
Triceps skinfold (mm)	0.3 (-0.3,0.9)	0.3 (-0.2,0.9)	0.008 (-0.009,0.02)	-0.0007 (-0.3,0.3)
Pregnancy weight gain (kg)	0.01 (-0.02,0.04)	0.004 (-0.03,0.03)	-0.0001 (-0.001,0.0008)	-0.005 (-0.02,0.01)

table shows β and 95% CI *p<0.05, **p<0.01, ***p<0.001

4.7 Mutually independent maternal determinants of childhood bone mass

The maternal factors that showed statistically significant associations with 6-year old bone mineral were explored in multivariate models.

Since once again height and weight seemed to be most strongly linked to childhood bone mass, other significant predictors were considered first. Table 23 describes the mutually independent maternal influences on childhood bone mass, with table 24 additionally showing height and weight as independent determinants.

When the two dietary components, protein and calcium consumption were looked at in isolation, maternal pre pregnancy calcium intake remained positively associated with whole body BMC and aBMD (both $p<0.05$) and lumbar spine BMC and BA (both $p<0.001$) Protein intake was no longer significant for any measure of bone mass. When calcium, protein, late pregnancy strenuous exercise and smoking status were put into a combined model, calcium intake remained significant for spinal BMC and BA only (table 23). When height and weight were additionally added to the model (table 24) maternal calcium intake prior to pregnancy remained positively associated with whole body aBMD ($\beta=0.02$, $p=0.05$) and lumbar spine BMC ($\beta=1.5$, $p=0.005$) and BA ($\beta=1.9$, $p=0.001$).

TABLE 23: Mutually independent maternal influences on childhood bone mass at age 6 years

	BA (cm^2)	BMC (g)	aBMD (g/cm^2)	vBMD (prentice g)
	Whole body			
R^2 for model, %	**1.9**	**2.3**	**2.3**	**1.2**
Calcium (mg/day)	4.6 (-19.8,28.9)	18.3 (-8.4,45.0)	0.02 (-0.0002,0.03)*	8.1(-2.3,18.4)
Protein (g/day)	24.0 (-6.8,54.9)	4.6 (-29.3,38.5)	-0.01(-0.03,0.01	-10.4(-23.7,2.8)
LP Freq strenous activity	0.6 (-0.7,2.0)	0.9 (-0.6,2.4)	0.0006 (-0.1,1.0)	0.5 (-0.1,1.0)
LP smoking	4.7 (-11.6,21.0)	15.3 (-2.6,33.2)	0.01(0.002,0.03)*	1.5 (-5.4,8.5)
	Lumbar spine			
R^2 for model, %	**3.2**	**2.6**	**1**	**0.5**
Calcium (mg/day)	1.9 (0.7,3.0)**	1.5 (0.5,2.5)**	0.01 (-0.010,0.03)	0.3 (-0.4,1.0)
Protein (g/day)	-0.6 (-2.0,0.9)	-0.5(-1.8,0.8)	-0.002 (-0.03,0.03)	-0.2 (-1.0,0.7)
LP Freq strenous activity	0.005 (-0.06,0.07)	0.02 (-0.04,0.08)	0.0006 (-0.0007,0.002)	0.02 (-0.02,0.06)
LP smoking	-0.2 (-0.06,0.07)	0.2 (-0.5,0.9)	0.01 (-0.005,0.02)	0.1 (-0.3,0.6)
	Hip			
R^2 for model, %	**1.9**	**2.6**	**1.4**	**1.7**
Calcium (mg/day)	0.6 (-0.2,1.4)	0.6 (-0.2,1.3)	0.008 (-0.02,0.03)	0.01 (-0.4,0.4)
Protein (g/day)	0.07 (-1.0,1.1)	-0.06 (-1.0,0.9)	-0.003 (-0.03,0.03)	-0.1 (-0.6,0.4)
LP Freq strenous activity	0.05 (0.003,0.09)*	0.07 (0.03,0.1)**	0.002 (0.0003,0.003)*	0.03 (0.009,0.05)**
LP smoking	0.04 (-0.5,0.6)	0.1 (-0.4,0.7)	0.006 (-0.01,0.02)	0.09 (-0.2,0.4)

table shows β and 95% CI; *p<0.05, **p<0.01, ***p<0.001; LP=late pregnancy

Late pregnancy strenuous activity was positively associated with hip BMC (β=0.07, p=0.002), BA (β=0.05, p=0.04), aBMD (β=0.002, p=0.01) and vBMD (β=0.03, p=0.006) once adjusted for calcium, protein and smoking status. This association remained once additionally adjusted for height and weight (p<0.05).

Smoking in late pregnancy was positively associated with whole body aBMD (β=0.01, p=0.02) and remained statistically significant after adjusted for height and weight.

Both maternal height and weight were positively associated with whole body BMC, BA and a BMD (all p<0.001) lumbar spine BMC and BA (all p<0.001) and hip BMC and BA (all p<0.001). In addition maternal weight was also associated with lumbar spine and hip aBMD (both p<0.01) whereas maternal height remained negatively associated with hip vBMD (β-0.01, p=0.04).

TABLE 24: Mutually independent maternal influences on childhood bone mass at age 6 years additionally including maternal height and weight

	BA (cm^2)	BMC (g)	aBMD (g/cm^2)	vBMD (prentice g)
	Whole body			
R^2 for model, %	**10.9**	**12.3**	**10.2**	**1.4**
log Calcium (mg/day)	4.6 (-19.8,28.9)	18.3 (-8.4,45.0)	0.02 (-0.0002,0.03)*	8.1 (-2.3,18.4)
log Protein (g/day)	24 (-6.8,54.9)	4.6 (-29.3,38.5)	-0.01 (-0.03,0.01)	-10.4 (-23.7,2.8)
LP Freq strenous activity	0.6 (-0.7,2.0)	0.9 (-0.6,2.4)	0.0006 (-0.0004,0.002)	0.5 (-0.1,1.0)
LP smoking	4.7 (-11.6,21.0)	15.3 (-2.6,33.2)	0.01 (0.002,0.03)*	1.5 (-5.4,8.5)
log maternal weight kg	65.1 (35.0,95.1)***	96.7 (64.2,129.2)***	0.07 (0.04,0.09)***	3.1 (-10.1,16.4)
Height	2.8 (2.0,3.6)***	3 (2.0,3.9)***	0.001 (0.0008,0.002)***	-0.3 (-0.6,0.1)
	Lumbar spine			
R^2 for model, %	**11.6**	**8.4**	**10.2**	**1.2**
log Calcium (mg/day)	1.9 (0.7,3.0)**	1.5 (0.5,2.5)**	0.01 (-0.010,0.03)	0.3 (-0.4,1.0)
log Protein (g/day)	-0.6 (-2.0,0.9)	-0.5 (-1.8,0.8)	-0.002 (-0.03,0.03)	-0.2 (-1.0,0.7)
LP Freq strenous activity	0.005 (-0.06,0.07)	0.02 (-0.04,0.08)	0.0006 (-0.0007,0.002)	0.02 (-0.02,0.06)
LP smoking	-0.2 (-1.0,0.6)	0.2 (-0.5,0.9)	0.01 (-0.005,0.02)	0.1 (-0.3,0.6)
log maternal weight kg	2.9 (1.4,4.3)***	2.7 (1.4,4.0)***	0.03 (0.007,0.06)*	-0.07 (-0.9,0.8)
Height	0.1 (0.10,0.2)***	0.09 (0.06,0.1)***	0.0006 (-0.0002,0.001)	-0.02 (-0.04,0.004)
	Hip			
R^2 for model, %	**9**	**8.4**	**3.6**	**3.6**
log Calcium (mg/day)	0.6 (-0.2,1.4)	0.6 (-0.2,1.3)	0.008 (-0.02,0.03)	0.01 (-0.4,0.4)
log Protein (g/day)	0.07 (-1.0,1.1)	-0.06 (-1.0,0.9)	-0.003 (-0.03,0.03)	-0.1 (-0.6,0.4)
LP Freq strenous activity	0.05 (0.003,0.09)*	0.07 (0.03,0.1)**	0.002 (0.0003,0.003)*	0.03 (0.009,0.05)**
LP smoking	0.04 (-0.5,0.6)	0.1 (-0.4,0.7)	0.006 (-0.01,0.02)	0.09 (-0.2,0.4)
log maternal weight kg	2.2 (1.2,3.2)***	2.3 (1.4,3.3)***	0.05 (0.02,0.08)***	0.3 (-0.2,0.8)
Height	0.08 (0.05,0.1)***	0.06 (0.03,0.08)***	0.0003 (-0.0006,0.001)	-0.01 (-0.03,-0.0008)*

table shows β and 95% CI; *p<0.05, **p<0.01, ***p<0.001; LP=late pregnancy

4.8 Mutually independent childhood and maternal predictors of bone mass.

Since childhood height and weight appeared to have the largest effect on the bone mass of the child at aged 6 the other childhood and maternal factors were considered first.

Table 25 summarizes the associations between various maternal and childhood determinants of bone mass excluding the child's height and weight. Table 26 additionally adjusts for the child's height and weight

Maternal height was positively associated with whole body BA, BMC and lumbar spine BA ($p<0.001$) however it was also negatively associated with vBMD at all three sites measured.

Maternal calcium intake was only associated with whole body aBMD ($\beta=0.02$, $p=0.04$), lumbar spine bone area ($\beta=1.9$, $p=0.007$) and BMC ($\beta=1.6$, $p=0.006$) when childhood vigorous activity was excluded from the model, allowing the number of observations to increase from 150 to 313. Maternal protein intake had a small negative association with vBMD at the hip ($\beta=-0.9$, $p=0.05$) when maternal determinants were additionally adjusted for all four childhood factors.

Strenuous activity in late pregnancy remained significantly associated with hip BMC ($\beta=0.06$, $p=0.03$) when adjusted for grip strength, triceps thickness and milk intake; when vigorous activity was added to the model the association was lost. Maternal lifestyle appeared to predict childhood lifestyle; for example, maternal strenuous activity in pregnancy was correlated with vigorous activity at aged 6 years ($r=0.15$, $p=0.03$). Maternal smoking remained positively associated with whole body aBMD ($\beta=0.02$, $p=0.05$).

TABLE 25: Mutually independent childhood and maternal predictors of 6 year bone mass

	BA (cm^2)	BMC (g)	aBMD (g/cm^2)	vBMD (prentice g)
	Whole body			
R^2 for model, %	**38.2**	**49.1**	**49.6**	**29.3**
Maternal determinants				
Calcium (mg/day)	-18.5 (-58.1,21.1)	-0.09 (-40.5,40.3)	0.01 (-0.01,0.04)	16.1 (-1.9,34.1)
Protein (g/day)	24.6 (-25.7,74.8)	-4 (-55.2,47.2)	-0.02 (-0.05,0.01)	-20.9 (-43.8,2.0)
LP Freq strenous activity	0.6 (-1.0,2.1)	0.7 (-0.9,2.3)	0.0004 (-0.0006,0.001)	0.07 (-0.6,0.8)
LP smoking	1.6 (-23.0,26.1)	15.8 (-9.2,40.8)	0.02 (0.0001,0.03)*	10.5 (-0.4,21.4)
Log. Maternal weight (kg)	45.3 (-7.7,98.3)	59.5 (5.5,113.5)*	0.03 (-0.0004,0.07)*	6.5 (-17.9,31.0)
Maternal Height (cm)	2.3 (0.9,3.7)**	2 (0.5,3.4)**	0.0007 (-0.0002,0.002)	-0.6 (-1.3,0.008)*
Childs determinants				
Vigorous activity mins/day	-0.3 (-0.8,0.2)	0.1 (-0.4,0.6)	0.0003 (-0.00002,0.0006)	0.3 (0.1,0.6)**
Milk intake pints/day	14.9 (4.1,25.7)**	14.6 (3.6,25.6)*	0.007 (0.0004,0.01)*	-0.2 (-5.1,4.6)
Grip strength (kg)	11.8 (8.0,15.6)***	17.4 (13.6,21.3)***	0.01 (0.009,0.01)***	4 (2.3,5.7)***
Triceps thickness (mm)	3.9 (0.6,7.3)*	5.7 (2.3,9.2)*	0.004 (0.001,0.006)**	-1.1 (-2.6,0.4)
	Lumbar spine			
R^2 for model, %	**26**	**32.3**	**25.9**	**22.5**
Maternal determinants				
Calcium (mg/day)	-0.04 (-2.0,1.9)	0.9 (-0.7,2.5)	0.03 (-0.008,0.06)	0.9 (-0.3,2.0)
Protein (g/day)	0.4 (-2.1,2.8)	-1.2 (-3.3,0.9)	-0.04 (-0.09,0.003)	-1.3 (-2.8,0.2)
LP Freq strenous activity	0.001 (-0.08,0.08)	0.02 (-0.05,0.09)	0.0006 (-0.0008,0.002)	0.007 (-0.04,0.05)
LP smoking	0.1 (-1.1,1.4)	0.6 (-0.5,1.6)	0.01 (-0.009,0.04)	0.5 (-0.2,1.2)
Log. Maternal weight (kg)	1.6 (-1.0,4.2)	1.5 (-0.7,3.8)	0.01 (-0.03,0.06)	0.3 (-1.4,1.9)
Maternal Height (cm)	0.1 (0.04,0.2)**	0.04 (-0.02,0.10)	-0.0005 (-0.002,0.0008)	-0.06 (-0.10,-0.01)*
Childs determinants				
Vigorous activity mins/day	-0.02 (-0.04,0.009)	-0.007 (-0.03,0.01)	0.00007 (-0.0004,0.0005)	0.006 (-0.009,0.02)
Milk intake pints/day	0.5 (-0.06,1.0)	0.3 (-0.2,0.8)	0.002 (-0.008,0.01)	-0.01 (-0.3,0.3)
Grip strength (kg)	0.4 (0.2,0.6)***	0.6 (0.4,0.7)***	0.01 (0.007,0.01)***	0.2 (0.10,0.3)***
Triceps thickness (mm)	0.2 (0.005,0.3)*	0.04 (-0.09,0.2)	-0.001 (-0.004,0.002)	-0.1 (-0.2,-0.05)**
	Hip			
R^2 for model, %	**22**	**23.4**	**17.6**	**14.8**
Maternal determinants				
Calcium (mg/day)	-0.9 (-2.4,0.6)	-0.2 (-1.6,1.1)	0.02 (-0.02,0.06)	0.4 (-0.3,1.1)
Protein (g/day)	1.1 (-0.8,3.0)	-0.2 (-2.0,1.6)	-0.05 (-0.1,0.007)	-0.9 (-1.9,-0.01)*
LP Freq strenous activity	0.03 (-0.03,0.10)	0.05 (-0.01,0.1)	0.001 (-0.0005,0.003)	0.02 (-0.01,0.05)
LP smoking	0.008 (-0.9,1.0)	0.3 (-0.6,1.2)	0.01 (-0.01,0.04)	0.2 (-0.2,0.7)
Log. Maternal weight (kg)	2.7 (0.6,4.7)*	2.7 (0.8,4.6)**	0.05 (-0.01,0.1)	0.4 (-0.6,1.4)
Maternal Height (cm)	0.04 (-0.01,0.10)	0.003 (-0.05,0.05)	-0.001 (-0.003,0.0002)	-0.04 (-0.06,-0.01)**
Childs determinants				
Vigorous activity mins/day	-0.002 (-0.02,0.02)	0.003 (-0.02,0.02)	0.0003 (-0.0003,0.0008)	0.006 (-0.003,0.02)
Milk intake pints/day	0.4 (-0.05,0.8)	0.4 (-0.03,0.8)	0.006 (-0.006,0.02)	0.05 (-0.2,0.3)
Grip strength (kg)	0.3 (0.1,0.4)***	0.4 (0.2,0.5)***	0.01 (0.005,0.01)***	0.09 (0.02,0.2)*
Triceps thickness (mm)	-0.02 (-0.1,0.1)	-0.002 (-0.1,0.1)	0.0004 (-0.003,0.004)	-0.02 (-0.08,0.04)

table shows β and 95% CI; *p<0.05, **p<0.01, ***p<0.001

The child's vigorous activity levels were positively associated with whole body vBMD when adjusted for all maternal and childhood factors in table 25 and 26. Milk intake remained positively associated with whole body BA, BMC, aBMD when adjusted for all factors in the current model. Grip strength remained a strong positive predictor of whole body, lumbar spine and hip BA, BMC, a BMD and vBMD (all $p<0.0001$ except hip vBMD $p=0.01$). Increased triceps skinfold thickness was positively associated with whole body BA ($\beta=3.9$, $p=0.02$), BMC ($\beta=5.7$, $p=0.001$), aBMD ($\beta=0.004$, $p=0.001$) and lumbar spine area ($\beta=0.2$, $p=0.04$); however a negative association was observed at the lumbar spine vBMD ($\beta=-0.1$, $p=0.003$).

Once childhood height and weight were added into the model, the main predictors of whole body BMC and BA were childhood height, weight, and grip strength. Triceps skinfold thickness was negatively associated with hip BA and BMC. Grip strength was also positively associated with vBMD at all three skeletal sites, however the child's height was negatively associated with whole body vBMD ($\beta=-2$, $p=0.007$). Vigorous activity remained positively associated with whole body aBMD ($\beta=0.0003$, $p=0.03$) and vBMD ($\beta=0.3$, $p=0.02$).

TABLE 26: Mutually independent childhood and maternal predictors of 6 year bone mineral at age 6 including the child's height and weight

	BA (cm^2)	BMC (g)	aBMD (g/cm^2)	vBMD (prentice g)
	Whole body			
R^2 for model, %	**62.1**	**72.6**	**69.7**	**33.6**
Maternal determinants				
Calcium (mg/day)	-13.6 (-45.9,18.8)	3.5 (-27.1,34.2)	0.01 (-0.008,0.03)	13.5 (-4.2,31.2)
Protein (g/day)	20.2 (-20.9,61.3)	-2.8 (-41.8,36.1)	-0.02 (-0.04,0.009)	-17.6 (-40.1,4.8)
LP Freq strenous activity	-0.1 (-1.4,1.1)	0.09 (-1.1,1.3)	0.0002 (-0.0006,0.0010)	0.2 (-0.5,0.9)
LP smoking	4.4 (-15.7,24.5)	11.4 (-7.6,30.4)	0.008 (-0.004,0.02)	8 (-3.0,19.0)
Log. Maternal weight (kg)	20.6 (-24.9,66.1)	17.9 (-25.2,61.0)	0.002 (-0.03,0.03)	3.5 (-21.4,28.3)
Maternal Height (cm)	0.1 (-1.1,1.4)	0.02 (-1.1,1.2)	-0.00009 (-0.0009,0.0007)	-0.3 (-0.9,0.4)
Childs determinants				
Child height (cm)	6.5 (3.9,9.1)***	3.4 (0.9,5.8)**	-0.0005 (-0.002,0.001)	-2 (-3.4,-0.6)**
Log childs weight	81 (-40.5,202.5)	258.5 (143.4,373.6)***	0.24 (0.2,0.3)***	50.6 (-15.8,117)
Vigorous activity mins/day	-0.04 (-0.5,0.4)	0.3 (-0.1,0.7)	0.0003 (0.00003,0.0006)*	0.3 (0.04,0.5)*
Milk intake pints/day	10.2 (1.3,19.0)*	7.3 (-1.1,15.7)	0.002 (-0.003,0.008)	-0.2 (-5.1,4.6)
Grip strength (kg)	3.7 (-0.05,7.5)*	7.2 (3.6,10.7)***	0.005 (0.003,0.008)***	4.3 (2.2,6.3)***
Triceps thickness (mm)	-0.07 (-3.6,3.5)	-1.8 (-5.2,1.6)	-0.002 (-0.004,0.00003)*	-1.9 (-3.8,0.09)
	Lumbar spine			
R^2 for model, %	**52.4**	**52**	**30.6**	**23.1**
Maternal determinants				
Calcium (mg/day)	0.2 (-1.4,1.9)	0.9 (-0.5,2.3)	0.02 (-0.01,0.06)	0.8 (-0.4,2.0)
Protein (g/day)	-0.2 (-2.3,1.9)	-1.3 (-3.1,0.6)	-0.03 (-0.08,0.010)	-1.2 (-2.7,0.3)
LP Freq strenous activity	-0.03 (-0.10,0.04)	-0.002 (-0.06,0.06)	0.0004 (-0.0010,0.002)	0.009 (-0.04,0.06)
LP smoking	0.1 (-0.9,1.1)	0.5 (-0.4,1.4)	0.01 (-0.01,0.03)	0.5 (-0.3,1.2)
Log. Maternal weight (kg)	0.3 (-2.1,2.7)	0.3 (-1.8,2.4)	-0.004 (-0.05,0.05)	0.3 (-1.5,2.0)
Maternal Height (cm)	0.01 (-0.05,0.08)	-0.03 (-0.09,0.03)	-0.001 (-0.002,0.0004)	-0.05 (-0.09,0.0009)*
Childs determinants				
Child height (cm)	0.3 (0.2,0.4)***	0.2 (0.05,0.3)**	0.0001 (-0.003,0.003)	-0.04 (-0.1,0.05)
Log childs weight	5.4 (-0.8,11.6)	6.8 (1.4,12.3)*	0.1 (-0.004,0.3)	0.5 (-3.9,4.9)
Vigorous activity mins/day	-0.003 (-0.03,0.02)	0.003 (-0.02,0.02)	0.0001 (-0.0003,0.0006)	0.005 (-0.01,0.02)
Milk intake pints/day	0.3 (-0.2,0.7)	0.1 (-0.3,0.5)	0.00009 (-0.010,0.010)	-0.003 (-0.3,0.3)
Grip strength (kg)	0.004 (-0.2,0.2)	0.2 (0.07,0.4)**	0.007 (0.003,0.01)**	0.2 (0.09,0.4)**
Triceps thickness (mm)	-0.02 (-0.2,0.2)	-0.2 (-0.3,-0.003)*	-0.005 (-0.008,-0.0007)*	-0.2 (-0.3,-0.02)*
	Hip			
R^2 for model, %	**48**	**42.4**	**20.8**	**15.8**
Maternal determinants				
Calcium (mg/day)	-0.5 (-1.7,0.8)	-0.04 (-1.3,1.2)	0.01 (-0.03,0.06)	0.3 (-0.4,1.0)
Protein (g/day)	0.5 (-1.1,2.2)	-0.5 (-2.1,1.2)	-0.05 (-0.1,0.01)	-0.9 (-1.8,0.05)
LP Freq strenous activity	0.02 (-0.03,0.07)	0.04 (-0.02,0.09)	0.001 (-0.0007,0.003)	0.02 (-0.009,0.05)
LP smoking	-0.08 (-0.9,0.7)	0.1 (-0.7,0.9)	0.008 (-0.02,0.04)	0.2 (-0.3,0.6)
Log. Maternal weight (kg)	1.4 (-0.5,3.3)	1.4 (-0.5,3.2)	0.02 (-0.05,0.08)	0.3 (-0.8,1.3)
Maternal Height (cm)	-0.03 (-0.08,0.02)	-0.05 (-0.10,0.002)	-0.002 (-0.003,0.00002)*	-0.03 (-0.06,-0.003)*
Childs determinants				
Child height (cm)	0.2 (0.09,0.3)***	0.1 (0.02,0.2)*	-0.0004 (-0.004,0.003)	-0.04 (-0.10,0.02)
Log childs weight	5.6 (0.7,10.5)*	6.4 (1.5,11.2)*	0.2 (-0.01,0.3)	1.1 (-1.7,3.8)
Vigorous activity mins/day	0.006 (-0.01,0.02)	0.009 (-0.008,0.03)	0.0003 (-0.0003,0.0009)	0.005 (-0.005,0.01)
Milk intake pints/day	0.1 (-0.2,0.5)	0.2 (-0.2,0.5)	0.004 (-0.009,0.02)	0.05 (-0.2,0.3)
Grip strength (kg)	-0.03 (-0.2,0.1)	0.07 (-0.08,0.2)	0.005 (0.0002,0.01)*	0.09 (0.005,0.2)*
Triceps thickness (mm)	-0.2 (-0.3,-0.04)*	-0.2 (-0.3,-0.04)*	-0.003 (-0.008,0.001)	-0.04 (-0.1,0.04)

table shows β and 95% CI; *p<0.05, **p<0.01, ***p<0.001

4.9 Discussion

The most robust association observed was the relationship between childhood height and weight and 6-year bone mineral. This represents the fact that taller and heavier children have increased measures of bone size but not volumetric density. In our data, there was evidence to suggest that the taller children had lower volumetric density, which may be consistent with the skeletal envelope being forced ahead of the capacity to mineralise.

Maternal calcium intake prior to pregnancy and childhood milk intake were independently associated with measures of bone size and areal not volumetric density. Whilst maternal calcium intake is strongly correlated with the child's milk intake, these relationships are consistent with the size of the skeletal envelope being influenced *in utero*, and with subsequent modification of bone mineralization by both childhood environmental and genetic factors.

Mothers that smoked during pregnancy appeared to have children with a higher areal and bone mineral apparent density (BMAD). Maternal smoking was also associated with a higher percentage fat mass and lower lean mass at aged 6, even once adjusted for maternal educational status and social class. The relationship was attenuated by the child's BMI implying that although smoking results in smaller neonatal bone mass there is rebound adiposity resulting in increased weight through the skeleton and hence increased density.

Grip strength is a good surrogate measure of muscle/lean body mass as well as muscle density. The results in this chapter show that even when adjusted for all maternal and childhood determinants, grip strength remains positively related to measures of both bone size and volumetric density at all three skeletal sites. Children with increased grip strength tended to drink more milk, were taller and had a lower percentage fat mass. Grip strength is an important determinant of disability and morbidity in later life.

Higher levels of strenuous activity in childhood appeared to be positively associated with measures of volumetric bone density at all three skeletal sites. However when

adjusted for all maternal and childhood factors only whole body vBMD remained significant. No interaction was seen with the child's milk intake and exercise levels. Although higher activity in the mother during late pregnancy was seen to be associated with increased bone mineral of the child's hip, the association was lost once adjusted for childhood factors. Mothers that exercised tended to have children that did higher intensities of exercise and had higher percentage lean mass.

Triceps skinfold thickness was strongly related to BMI ($r=0.58$, $p<0.0001$), therefore whilst it was positively associated with measures of bone mass, adjustment for maternal height and weight led to a negative association with bone size and density being unveiled.

5 MATERNAL AND CHILDHOOD DETERMINANTS OF VOLUMETRIC BONE MASS AND BONE STRENGTH

5.1 Aims

- To test the hypothesis that childhood lifestyle factors and body composition influence both childhood bone mineral structure and bone strength.
- To test the hypothesis that maternal lifestyle factors (diet, smoking, exercise) and body composition influence both childhood bone mineral structure and bone strength.

5.2 Methods

After the child's DXA scan, the parents were invited to attend for a further scan of the child's right tibia using a pQCT machine in order to provide additional information about the child's bone structure. The methods are described in detail in chapter 3.

5.3 Results

5.3.1 Descriptive statistics

All children that attended clinic visits after Sep 07 were given information about this part of the study. Of the 450 parents that were contacted 147 (32.6%) children attended for this further scan. 139 of these children had results available for their previous DXA scan. The main reason for non-participation in this phase of the study was that only a fixed number of appointments were available during the school holiday period, and once these appointments were filled no new appointments were made until the next holiday period. Children that had previously had DXA scan at birth and aged 4 years as well as children with a history of fracture were prioritised. In total 113 of the children had both 6 year and 4 year DXA scans available, whereas only 53 children had scans at all time points including birth. Tables 27 and 28 show the differences in both the maternal and childhood characteristics between the main group and the children that attended this second clinic visit. Hence mothers that brought their children to this second visit tended to be of higher social class ($p=0.03$) and drank slightly less alcohol ($p=0.05$).

TABLE 27: Maternal characteristics between those that attended or not for pQCT visit

		Responders	number	Non Responders	number	p value
Age at initial interview, years		28.6 (3.7)	139	28.4 (3.8)	391	0.76
Birthweight (mean, sd)		3196 (571)	126	3260 (486)	360	0.11
Percentage nulliparous		42.5	59	47.8	187	0.86
Qualifications %	None	1.4	2	2.1	8	
	CSE	7.2	10	9.7	38	
	O leve	21.6	30	32.5	127	
	A leve	36.7	51	25.6	100	
	HND	7.9	11	7.7	30	
	Degre	25.2	35	22.5	88	
			139		391	0.96
Social Class %	I	8.3	11	3.6	14	
	II	44.4	59	36.3	140	
	IIIN	27.8	37	40.4	156	
	IIIM	9	12	6.2	24	
	IV	10.5	14	11.7	45	
	V	0	0	1.8	7	
			133		386	0.027
Height, cm (mean, sd)		163.2 (6.4)	139	163.7 (6.5)	389	0.22
PP weight (Median, IQR)		65.7 (59.5-76.6)	138	65.7 (59.1-73.1)	389	0.51
PP BMI (Median, IQR)		24.6 (22.5-28.6)	138	24.3 (22.4-27.4)	388	0.34
PP triceps skinfold, mm (Median, IQR)		19.8 (15.7-26.6)	139	19.3 (14.6-24.9)	388	0.14
EP triceps skinfold, mm (Median, IQR)		19.2 (15.4-25.3)	122	19.4 (15.4-24.5)	300	0.81
LP triceps skinfold, mm (Median, IQR)		20.4 (16.7-25.3)	131	21.3 (15.5-26.1)	381	0.69
PP smoking,%		20.9	29	27.4	107	0.067
EP smoking, %		12.3	17	14.8	57	0.23
LP smoking, %		13	17	13.4	51	0.45
Units of alcohol per week		4.3 (1.5-7.6)	139	4.5 (1.5-10.7)	391	0.05
living with partner %		87.1	121	83.9	328	0.81

The characteristics of the children that came to this second appointment are shown in table 28. In general they had very similar anthropometry and lifestyle characteristics compared to the children that did not attend. However they had slightly higher aBMD at the hip ($p=0.05$) and they were more likely to have had a history of fracture ($p<0.0001$) as per the protocol design. Activity levels were only available for 49 children (scans completed by the end of 2008). The average age of the child at this visit was 6.7 (6.6-6.9) years.

There was no difference between boys and girls for any of the bone parameters measured with pQCT; however girls had significantly more subcutaneous fat at the 66% site compared to the boys ($p<0.0001$) (table 29). There were numerous statistically significant associations between pQCT bone parameters and age of the child hence all data was adjusted for age.

TABLE 28: A comparison of anthropometry and lifestyle characteristics between children that attended for pQCT and those that did not

	Responders	number	Non Responders	number	p value
Birth to age 1					
Gestational age	39.6 (2.0)	139	39.7 (1.7)	391	0.2
Birthweight (g)	3477 (561)	137	3430 (527)	387	0.8
Crown heel length birth(cm)	49.9 (2.2)	134	49.8 (2.1)	379	0.7
Weight at one year (kg)	10.1 (9.3-10.7)	139	9.9 (9.2- 10.8)	387	0.5
Crown heel length age one(cm)	75.4 (2.8)	136	75.5 (2.7)	384	0.3
Age 6 from DXA clinic visit					
Anthropometry					
Height at age 6, cm (mean sd)	119.9 (5)	130	120.1 (5.1)	362	0.3
Weight at age 6, kg (median IQR)	23.2 (21.4-25.5)	132	23.5 (21.5-26.2)	367	0.4
Grip Strength (max) kg (mean sd)	10.0 (2.4)	132	9.8 (2.5)	325	0.5
Triceps skinfold thickness, mm (median IQR)	10.1 (8.4-12.9)	107	9.5 (8.0-12.0)	305	0.06
Total BMC, g (mean sd)	533.2 (72.6)	136	529.8 (69.4)	375	0.6
Total BA, g/cm^2 (mean sd)	896.2 (66.5)	136	896.1 (63.1)	375	1
Total aBMD, g/cm^2 (mean sd)	(0.6) (0.05)	136	0.6 (0.05)	375	0.5
Spine BMC, g (mean sd)	18.0 2.7	139	17.8 (2.7)	387	0.6
Spine BA, g/cm2 (mean sd)	33.3 (3.3)	139	33.1 (3.0)	387	0.6
Spine aBMD, g/cm2 (mean sd)	0 .5 (0.06)	139	0 .5 (0.06	387	0.7
Hip BMC, g (mean sd)	11.5 (2.2)	139	11.1 (2.0)	387	0.06
Hip BA, g/cm2 (mean sd)	16.9 (2.2)	139	16.7 (2.1)	387	0.3
Hip aBMD, g/cm2 (mean sd)	0.67 (0.07)	139	0.66 (0.06)	387	0.05
Total lean, kg (mean sd)	17.3 (2.1)	132	17.2 (2.2)	367	0.8
Total fat kg, median IQR	5.3 (4.3-6.9)	132	5.2 (4.2-6.4)	367	0.4
Lifestyle					
% of children with fracture	25.2	34	5.2	20	<0.0001
Vigorous activity, mins per day (mean sd)	43.5 (17.5)	49	43.9 (19.8)	167	0.9
Sedentary activity, mins per day (mean sd)	855.7 (80.2)	49	879.2 (79.7)	167	0.07
Moderate activity, mins per day (mean sd)	34.6 (12.0)	49	36.9 (12.6)	167	0.3
Milk intake, pints/day (median IQR)	0.5 (0.35-0.75)	112	0.5 (0.33-0.75)	315	0.9

Of the 147 scans, a number were excluded due to movement artefact. The scans were only excluded if the cortex of the bone had been broken on the image obtained.

TABLE 29: Differences in pQCT parameters between boys and girls aged 6 years

Characteristic	Boys	n	Girls	n	P value
Trabecular content mg/mm (4%)	99.8 (26.5)	70	102.1 (22.6)	77	0.57
Trabecular density mg/mm3 (4%)	321.7 (59.3)	70	336.1 (51)	77	0.12
Trabecular content mg/mm (14%)	17.5 (5.1)	65	16.9 (4.7)	70	0.54
Trabecular density mg/mm3 (14%)	139.6 (38.4)	65	134.1 (35.6	70	0.39
Cortical content mg/mm (38%)	121 (17.7)	68	120.2 (17.1)	72	0.77
Cortical density mg/mm3 38%	1038 (34.5)	68	1038 (33.9)	72	0.92
Cortical thickness mm (38%)	2.8 (0.4)	68	2.7 (0.3)	72	0.16
Periosteal circumfernce mm 38%	51.2 (3.9)	68	51.9 (3.8)	72	0.25
Endosteal circumfernce mm 38%	33.9 (4.3)	68	35.2 (4.1)	72	0.07
Stress strain index 38%	443.5 (88.6)	68	453.6 (99.5)	72	0.53
Fracture load x (N)- 38%	952 (206)	68	983 (203)	72	0.38
Fracture load y (N) 38%	907(178)	68	916 (209)	72	0.79
Muscle area mm2 (66%)	2886 (414)	64	2941 (424)	73	0.44
Subcutaneous fat area mm2 (66%)	1269 (327)	64	1554 (448)	73	<0.0001

table shows mean and standard deviation

5.3.2 Childhood bone mass adjusted for 6 year anthropometry

Table 30 shows the relationship between childhood height, weight, BMI, triceps skinfold thickness and grip strength on the various bone parameters at 4%, 14%, 38% and 68%. Trabecular content is shown at both 4 % and 14%. This was necessary because at the 4% site a number of scans involved the epiphyseal growth plate, which makes the density artificially high. Overall the data showed that height and weight were important determinants of trabecular and cortical content, as well as being associated with increased cortical thickness, increased periosteal and endosteal circumference as well as increased bone strength as shown by SSI and fracture load (all $p<0.001$) (Figure 10). A similar relation was observed for BMI and grip strength, except there was no relationship between grip strength and endosteal circumference. Triceps skinfold thickness was positively associated with trabecular content (14%) cortical content, periosteal and endosteal circumference, as well as a small association with fracture load in the x and y axis ($p<0.05$). It was negatively associated with cortical density ($\beta=-2.4$, $p=0.03$).

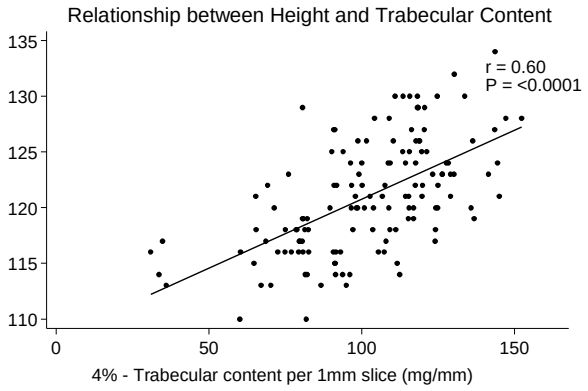
TABLE 30: Relationship between childhood anthropometry and lifestyle determinants of tibial structure and strength

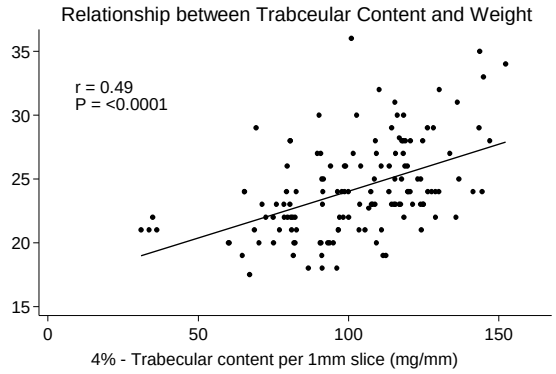
	Trabecular content 4% per mm slice	Trabecular density 4% mg/cm3	Trabecular content 14% per mm slice	Trabecular density 14% mg/cm3	Cortical content 38% per mm slice	Cortical density 38% mg/cm3
Anthropometry						
Height (cm)	2.8 (2.2,3.5)***	3 (1.3,4.7)***	0.3 (0.1,0.5)***	0.03 (-1.3,1.3)	1.8 (1.4,2.3)***	-0.3 (-1.5,0.8)
log: Weight (kg)	78.5 (54.5,102.6)***	58.2 (-1.5,117.8)	13.1 (7.9,18.4)***	29.6 (-13.1,72.3)	73.7 (59.7,87.7)***	-26.4 (-65.7,12.9)
BMI (kg/m2)	2.8 (0.6,5.0)*	0.2 (-4.7,5.1)	0.8 (0.4,1.3)***	3.2 (-0.2,6.5)	4.4 (3.1,5.7)***	-2 (-5.2,1.2)
Triceps skinfold (mm)	0.6 (-0.9,2.1)	-0.3 (-3.7,3.1)	0.3 (0.02,0.7)*	2 (-0.4,4.3)	1.6 (0.6,2.7)**	-2.4 (-4.5,-0.2)*
Grip strength (kg)	3.8 (2.1,5.5)**	3.8 (-0.3,7.8)	0.4 (0.04,0.8)*	0.2 (-2.7,3.1)	2.7 (1.6,3.8)***	-0.4 (-3.0,2.2)
Lifestyle						
Milk intake (pints /day)	1.1 (-11.7,13.9)	-2.7 (-30.8,25.5)	-1.8 (-5,1.4)	-8.9 (-32,14.2)	-3.1 (-12.1, 6)	2.2 (-16,20.4)
Vigorous activity (mins/day)	-0.08 (-0.5,0.3)	-0.2 (-1.1,0.7)	0.01 (-0.08,0.1)	0.008 (-0.7,0.7)	0.09 (-0.2,0.4)	0.6 (0.0006,1.2)*

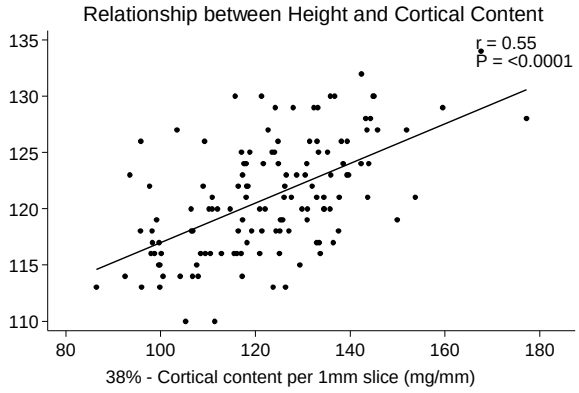
	Cortical thickness 38% (mm)	Periosteal circumference 38% (mm)	Endosteal circumference 38% (mm)	Stress Strain Index 38% mm^3	Fracture load x 38% N	Fracture load y 38% N
Anthropometry						
Height (cm)	0.02 (0.008,0.03)***	0.4 (0.3,0.5)***	0.3 (0.1,0.4)***	10.6 (8.1,13.0)***	23.9 (18.7,29.2)***	22.5 (17.4,27.7)***
log: Weight (kg)	0.8 (0.5,1.2)***	16.4 (13.0,19.8)***	11.2 (6.6,15.8)***	378.6 (297.3,459.9)***	920.7 (760.2,1081.1)***	810.7 (644.2,977.3)***
BMI (kg/m2)	0.05 (0.02,0.08)**	1 (0.6,1.3)***	0.6 (0.2,1.0)**	19.6 (11.9,27.3)***	51.3 (35.4,67.3)***	42.7 (26.7,58.7)***
Triceps skinfold (mm)	0.01 (-0.009,0.04)	0.5 (0.2,0.7)***	0.4 (0.1,0.7)**	4.6 (-1.5,10.6)	16.5 (3.8,29.3)*	12.8 (0.2,25.3)*
Grip strength (kg)	0.04 (0.01,0.06)**	0.5 (0.2,0.8)***	0.3 (-0.05,0.6)	15.7 (9.7,21.7)***	31.2 (17.8,44.6)***	31 (18.2,43.9)***
Lifestyle						
Milk intake (pints /day)	-0.8 (-0.3,0.1)	-0.1 (-2.2,2)	0.4 (-1.9,2.7)	-19.5 (-69.9,30.9)	-35.9 (-144.2,72.4)	59.7 (-164.9,45.4)
Vigorous activity (mins/day)	0.003 (-0.004,0.009)	-0.02 (-0.09,0.05)	-0.04 (0.1,0.04)	-0.2 (-1.7,1.3)	0.2 (-3.1,3.4)	-0.6 (-3.9,2.8)

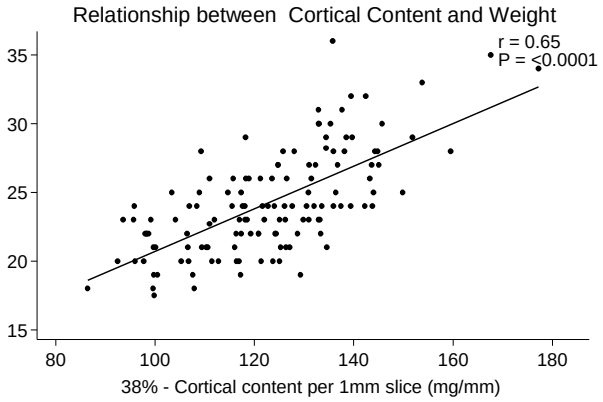
table shows β and 95% CI; *p<0.05, **p<0.01, ***p<0.001

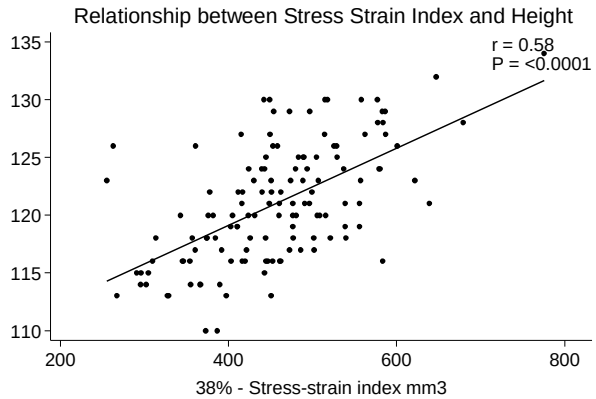
Figure 10: The relationship of height and weight on trabecular and cortical content and Stress Strain Index

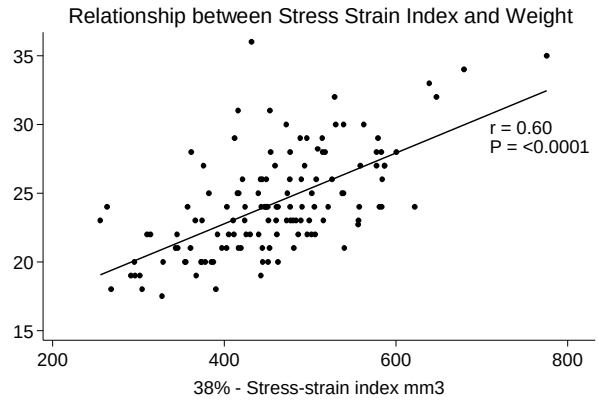












5.3.3 Childhood lifestyle factors

Milk intake was positively associated with muscle area (β=243.7, p=0.04) and vigorous and very vigorous activity was positively associated with cortical density (β=0.6, p=0.05) and negatively associated with subcutaneous fat area (β=-6.3, p=0.05). No other lifestyle characteristics predicted bone strength. (Factors studied included fruit and vegetable intake and history of fracture). Whilst there was a suggestion that other measures of vigorous or very vigorous activity were also related to cortical density this did not reach significance in this small sample.

5.4 Multivariate analysis of childhood predictors of bone mass using pQCT

Since height and weight were the largest predictors of tibial bone mass and strength using pQCT, when adding significant childhood determinants to a multivariate regression model, height and weight were excluded in the first model.

The relationships between triceps skinfold thickness, grip strength and vigorous activity are shown in table 31. Due to the low numbers of children that had measurements of physical activity, between 39-43 scans were included in each multivariate model.

Grip strength was a significant positive predictor for trabecular content at 4% and 14% site (β=5.7, p<0.001; β=1.4, p<0.001 respectively), cortical content (β=4.7, p<0.001), cortical thickness (β=0.08, p<0.01) and periosteal circumference at 38% site (β=0.7, p=0.01). It was additionally positively associated with the measures of bone strength (SSI: β=20.7, p<0.001; fracture load x: β= 42.3, p<0.001; fracture load y: β=44.8, p<0.001). Once height and weight were added to the model, the relationship was attenuated. However it remained positively associated with trabecular content at 4 and 14% site (β=4.5, p<0.01; β=0.8, p=0.04) cortical content (β=3.1, p<0.01), cortical thickness (β=0.06, p=0.02) stress strain index (β=11.7, p=0.04) and fracture load in the y axis (β=26.7, p=0.04).

TABLE 31: Mutually independent childhood determinants of tibial bone mass and strength at age 6 years

	Trabecular content 4% per mm slice	**Trabecular density 4% mg/cm^3**	**Trabecular content 14% per mm slice**	**Trabecular density 14% mg/cm^3**	**Cortical content 38% per mm slice**	**Cortical density 38% mg/cm3**
R^2	**32**	**10**	**29**	**11**	**43**	**14**
Triceps skinfold thickness	3.9 (-18.6,26.4)	3.3 (-54.8,61.3)	1.2 (-4.5,6.9)	19 (-27.1,65.1)	17.1 (1.4,32.9)*	-30.4 (-71.7,10.9)
Maximal grip strength (kg)	5.7 (3.0,8.5)***	7.4 (0.2,14.5)*	1.4 (0.6,2.1)***	5.6 (-0.3,11.5)	4.7 (2.8,6.7)***	-0.7 (-5.8,4.4)
Vigorous activity per day (mins)	-0.05 (-0.5,0.4)	-0.1 (-1.2,0.9)	0.03 (-0.07,0.1)	0.1 (-0.7,0.9)	0.3 (-0.005,0.6)	0.6 (-0.2,1.3)
Number	43	43	39	39	42	42
	Cortical thickness 38% (mm)	**Periosteal circumference 38% (mm)**	**Endosteal circumference 38% (mm)**	**Stress Strain Index 38% mm^3**	**Fracture load x 38% N**	**Fracture load y 38% N**
R^2	**24**	**22**	**5**	**28**	**29**	**28**
Triceps skinfold thickness	0.2 (-0.2,0.6)	4.5 (0.2,8.9)*	3.3 (-1.9,8.6)	31.9 (-56.8,120.6)	145.3 (-47.0,337.7)	82.4 (-113.1,277.8)
Maximal grip strength (kg)	0.08 (0.03,0.1)**	0.7 (0.2,1.2)*	0.2 (-0.4,0.9)	20.7 (9.7,31.6)***	42.3 (18.6,66.0)***	44.8 (20.7,68.9)***
Vigorous activity per day (mins)	0.003 (-0.004,0.01)	0.04 (-0.04,0.1)	0.02 (-0.08,0.1)	0.7 (-0.9,2.3)	2.5 (-1.0,6.0)	1.6 (-1.9,5.2)
Number	42	42	42	42	42	42

table shows β and 95% CI; *p<0.05, **p<0.01, ***p<0.001: PP-Pre pregnancy EP-Early pregnanacy, LP-late pregnancy

Triceps skinfold thickness was a positive predictor of cortical content (β=17.1, p=0.03) and periosteal circumference (β=4.5, p=0.04) only when adjusted for the determinants in table 31. Once height and weight were added to the models all associations were lost. If physical activity was excluded from the model to increase the numbers (92 participants per model) triceps skinfold thickness was additionally associated with endosteal circumference and fracture load in the x axis; however, once again, when height and weight were added all associations were attenuated. Vigorous activity did not predict any measure of tibial mass or strength when included in either multivariate model.

Weight predicted increased cortical content (β=58.5, p=0.04), stress strain index (β=334, p=0.04) and fracture load in the x axis (β=745, p=0.02). When physical activity was excluded from the model, weight was additionally associated with cortical thickness (β=1, p=0.02), periosteal circumference (β=12.9, p<0.01) and fracture load in the y axis (β=736, p<0.001). Height did not predict any measure of tibial mass when put into the full multivariate model, (if activity was excluded height still only predicted trabecular content at 4% site (β=2.3, p<0.001).

5.5 Maternal predictors of childhood bone pQCT bone parameters

Table 32 shows the relationship between maternal determinants (lifestyle and anthropometry) and the child's tibial mass as measured by pQCT.

There were no significant relationships between maternal social class, educational attainment and smoking status with the child's bone mineral and strength.

In the analysis concerning the mothers diet, macronutrient, micronutrient and dietary patterns were considered both before and during pregnancy.

The only macronutrient that showed a positive relationship was pre pregnancy protein intake, which was positively associated with both trabecular content and density at the 14% site (p=0.007, p=0.005), however there was no association with the tibia's strength. No association was seen between protein intake during pregnancy.

Whereas the micronutrient calcium was associated with whole body BMC (Chapter 4) there was no relationship seen with intake either prior or during pregnancy with any measure of tibial mass or strength. Vitamin C intake, was however positively associated with both trabecular content (r=0.26, p=0.004) and density (r=0.2, p=0.02) at the 14% site in early pregnancy, and trabecular density at the 4% site (r=0.18, p=0.04) during late pregnancy.

Analysing dietary patterns reduces problems of interactions and co linearity that can occur when analysing relations with single nutrients. One such pattern is the prudent diet score (a measure of healthy diet); in early pregnancy this was associated with trabecular content and density at 14% (r=0.2, p=0.03, r=0.22, p=0.01) whereas late pregnancy prudent dietary score was positively correlated with 4% trabecular content and density (r=0.17, p= 0.02; r=0.23, p=0.002) and reduced subcutaneous fat at 66% (β= -109.4, p=0.006).

Higher levels of maternal exercise prior to pregnancy were found to be positively associated with the periosteal circumference (r=0.19, p=0.03) and fracture load in the x axis (r=0.17, p=0.05) whereas faster maternal walking speed in late pregnancy was positively associated with trabecular content at the 4% site (r=0.17, p=0.05) cortical content and cortical thickness at 38% site (r=0.2, p=0.02; r=0.18, p=0.04) and fracture load in the y axis (r=0.18, p=0.05).

There were numerous relationships between maternal anthropometry and bone mass in the child (summarised in table 32). Maternal height was positively associated with trabecular content and density at 4% site, cortical content, periosteal circumference and measures of bone strength (SSI; r=0.31, p<0.001; fracture load x: r=27, p=0.002; fracture load y: r=0.25, p=0.003). Weight was positively associated with trabecular content at 4 and 14% sites, cortical content, cortical thickness, periosteal circumference and all measures of bone strength. BMI and biceps skinfold thickness were both associated with cortical content, cortical thickness and periosteal circumference. In addition BMI was weakly associated with increased fracture load in the x axis (r=0.19, p=0.03).

TABLE 32: Maternal anthropometric and lifestyle determinants of 6 year bone mineral and strength measured by pQCT

	Trabecular content 4% per mm slice	Trabecular density 4% mg/cm^3	Trabecular content 14% per mm slice	Trabecular density 14% mg/cm^3	Cortical content 38% per mm slice	Cortical density 38% mg/cm3
Anthropometry						
Logged Maternal weight (kg)	22.4 (1.7,43.0)*	23.1 (-23.4,69.6)	4.9 (0.6,9.2)*	22.2 (-10.3,54.6)	24.1 (10.3,37.8)***	-7.2 (-36.9,22.5)
Maternal height (cm)	1.2 (0.6,1.8)***	2.1 (0.7,3.5)***	0.06 (-0.07,0.2)	-0.3 (-1.3,0.7)	0.5 (0.08,0.9)*	0.4 (-0.5,1.3)
Log: Body mass index, kg/m2)	7.9 (-15.0,30.8)	-4.2 (-55.1,46.8)	4.8 (0.08,9.5)*	30.7 (-4.7,66.2)	20.9 (5.5,36.2)**	-15.8 (-48.4,16.7)
Biceps skinfold thickness, mm	1 (-6.7,8.7)	-2.7 (-19.9,14.4)	1.5 (-0.08,3.1)	10.7 (-1.1,22.5)	6.5 (1.3,11.6)*	-7.7 (-18.6,3.1)
lifestyle						
Late pregnancy walking speed	4.8 (0.03,9.5)*	10.3 (-0.6,21.2)	0.2 (-0.8,1.2)	0.6 (-7.0,8.1)	3.9 (0.5,7.2)*	-0.5 (-7.7,6.8)
strenous activity pre pregnancy	0.7 (-1.4,2.7)	-0.9 (-5.6,3.7)	0.4 (-0.06,0.8)	1.1 (-2.1,4.3)	0.9 (-0.6,2.3)	-0.08 (-3.0,2.9)
log PP Total protein (g/day)	5.4 (-10.5,21.3)	12.2 (-23.2,47.5)	4.9 (1.6,8.1)**	37.1 (13.1,61.0)**	5 (-6.3,16.3)	-8.1 (-31.4,15.3)
log EP Vit C intake mg/day	5.7 (-2.1,13.5)	8.8 (-8.6,26.2)	2.6 (1.0,4.2)**	15.8 (3.6,27.9)*	5.5 (0.2,10.8)*	1.3 (-9.6,12.3)
EP prudent diet score	3.6 (-1.0,8.2)	9.7 (-0.5,19.8)	1.1 (0.1,2.1)*	9.7 (2.5,16.9)**	1.2 (-2.1,4.4)	-2.7 (-9.2,3.8)
LP prident diet score	5.2 (0.9,9.5)*	15.3 (5.6,24.9)***	0.4 (-0.5,1.3)	4.5 (-2.3,11.3)	-1.6 (-4.6,1.5)	1.5 (-5.0,8.0)

	Cortical thickness 38% (mm)	Periosteal circumference 38% (mm)	Endosteal circumference 38% (mm)	Stress Strain Index 38% mm^3	Fracture load x 38% N	Fracture load y 38% N
Anthropometry						
Logged Maternal weight (kg)	0.4 (0.06,0.6)*	4.4 (1.2,7.6)**	2.2 (-1.6,5.9)	120.2 (43.9,196.5)**	284.1 (119.2,449.0)***	175.4 (13.9,336.9)*
Maternal height (cm)	0.005 (-0.005,0.01)	0.1 (-0.001,0.2)*	0.07 (-0.04,0.2)	4.3 (2.0,6.6)***	8.3 (3.2,13.4)**	7.3 (2.5,12.2)**
Log: Body mass index, kg/m2)	0.4 (0.03,0.7)*	3.7 (0.1,7.3)*	1.5 (-2.6,5.6)	77.2 (-8.7,163.1)	210.7 (25.1,396.4)*	95.5 (-84.4,275.4)
Biceps skinfold thickness, mm	0.1 (0.004,0.2)*	1.2 (-0.02,2.4)*	0.5 (-0.9,1.8)	14.9 (-14.0,43.7)	48 (-14.6,110.5)	14.5 (-45.8,74.7)
lifestyle						
Late pregnancy walking speed	0.07 (0.002,0.1)*	0.4 (-0.4,1.2)	-0.07 (-1.0,0.9)	14.9 (-4.2,34.0)	32.7 (-8.6,74.1)	39.2 (-0.2,78.5)*
strenous activity pre pregnancy	0.0001 (-0.03,0.03)	0.4 (0.04,0.7)*	0.4 (-0.008,0.7)	6 (-1.7,13.8)	16.5 (-0.3,33.3)*	10.2 (-5.9,26.3)
log PP Total protein (g/day)	0.06 (-0.2,0.3)	1.5 (-1.1,4.1)	1.1 (-1.9,4.0)	12.9 (-49.3,75.2)	34.6 (-100.8,170.1)	28.2 (-101.2,157.5)
log EP Vit C intake mg/day	0.1 (0.0001,0.2)*	0.6 (-0.6,1.8)	-0.1 (-1.6,1.3)	12.7 (-15.9,41.4)	34 (-29.2,97.3)	35.5 (-23.6,94.6)
EP prudent diet score	0.02 (-0.05,0.09)	0.2 (-0.5,0.9)	0.05 (-0.8,0.9)	-3.6 (-20.7,13.5)	-4 (-41.8,33.8)	1.2 (-34.3,36.6)
LP prident diet score	-0.03 (-0.09,0.04)	-0.4 (-1.1,0.4)	-0.2 (-1.0,0.6)	-10.8 (-27.9,6.3)	-27.8 (-64.8,9.1)	-11.5 (-47.1,24.1)

table shows β and 95% CI; *p<0.05, **p<0.01, ***p<0.001: PP-Pre pregnancy EP-Early pregnanacy, LP-late pregnancy

5.6 Multivariate analysis of maternal predictors of bone mass using pQCT

Since once again maternal height and weight were the largest determinants of childhood bone mass and strength, these were excluded in the first multivariate model.

Table 33 shows the independent relationships between biceps skinfold thickness, late pregnancy walking speed, strenuous activity pre pregnancy, pre pregnancy protein intake, early pregnancy vitamin C intake and prudent diet score in both early and late pregnancy.

Higher maternal activity was associated with enhanced bone mineral in her offspring. Maternal walking speed in late pregnancy was associated with trabecular content at 4% site, cortical content, cortical thickness, periosteal circumference, and all three measures of bone strength. Pre pregnancy strenuous exercise was associated with increased periosteal and endosteal circumference as well as SSI and fracture load in the x axis using the same model. When additionally adjusted for maternal height and weight, walking speed remained associated with trabecular content (β=5.8, p=0.03), cortical content (β=5.9, p=0.002), cortical thickness (β=0.1, p=0.02), and measures of bone strength (SSI: β=226, p=0.02; fracture load x: β=57.3, p=0.009; fracture load y: β=58.6, p=0.006). Pre pregnancy strenuous activity remained associated with increased periosteal and endosteal circumference (β=0.5, p=0.006; β=0.6, p=0.01 respectively) and fracture load in the x axis (β=21.1, p=0.03).

Maternal diet remained weakly associated with measures of trabecular content. Pre pregnancy protein intake was associated with trabecular content and density at the 14% site (β=3.7, p=0.03; β=27.4, p=0.04 respectively) whereas late pregnancy prudent diet score was associated with trabecular content and thickness at the 4% site (β=8.6, p=0.02; β=20.2, p=0.01 respectively). Once height and weight were added to the multivariate model pre pregnancy protein intake remained positively associated with trabecular content and density at 14% site (β=3.7, p=0.03; β=28.1, p=0.04) and late pregnancy prudent diet score remained associated with trabecular content and thickness at the 4% site (β=7, p=0.05; β=17, p=0.04).

TABLE 33: Mutually independent maternal anthropometric and lifestyle determinants of 6 year bone mineral and strength

	Trabecular content 4% per mm slice	Trabecular density 4% mg/cm^3	Trabecular content 14% per mm slice	Trabecular density 14% mg/cm^3	Cortical content 38% per mm slice	Cortical density 38% mg/cm3
R^2 as %	**13**	**11**	**15**	**12**	**17**	**3**
Anthropometry						
Biceps skinfold thickness, mm	6.6 (-2.1,15.3)	2 (-18.0,22.0)	1.3 (-0.6,3.1)	1.7 (-13.1,16.5)	7.7 (1.6,13.8)*	-3.2 (-17.1,10.7)
lifestyle						
Late pregnancy walking speed	6.2 (1.0,11.4)*	9.6 (-2.3,21.6)	0.5 (-0.6,1.6)	-1.1 (-9.6,7.5)	5.9 (2.3,9.5)**	-0.8 (-8.9,7.3)
strenous activity pre pregnancy	1.8 (-0.5,4.1)	0.08 (-5.2,5.4)	0.2 (-0.2,0.7)	-0.9 (-4.6,2.8)	1.2 (-0.4,2.8)	-0.5 (-4.1,3.1)
log PP Total protein (g/day)	4.3 (-11.6,20.3)	11.3 (-25.5,48.2)	3.7 (0.3,7.1)*	27.4 (0.9,53.9)*	1 (-10.4,12.3)	0.3 (-25.5,26.1)
log EP Vit C intake mg/day	-0.5 (-10.0,9.0)	0.4 (-21.6,22.4)	1.4 (-0.5,3.4)	10.6 (-4.8,25.9)	2.2 (-4.3,8.8)	6.3 (-8.6,21.1)
EP prudent diet score	-5.8 (-13.5,1.9)	-9.4 (-27.1,8.3)	0.009 (-1.6,1.6)	6.1 (-6.5,18.7)	0.4 (-5.0,5.7)	-9 (-21.2,3.2)
LP prudent diet score	8.6 (1.7,15.6)*	20.2 (4.1,36.3)*	0.1 (-1.3,1.6)	-1.4 (-12.8,10.1)	-3 (-7.9,1.9)	6.3 (-4.8,17.3)

	Cortical thickness 38% (mm)	Periosteal circumference 38% (mm)	Endosteal circumference 38% (mm)	Stress Strain Index 38% mm^3	Fracture load x 38% N	Fracture load y 38% N
R^2 as %	**11**	**14**	**8**	**11**	**15**	**10**
Anthropometry						
Biceps skinfold thickness, mm	0.1 (-0.04,0.2)	1.6 (0.09,3.0)*	0.9 (-0.9,2.7)	22.8 (-11.7,57.3)	69.1 (-5.2,143.5)	29.3 (-41.7,100.2)
lifestyle						
Late pregnancy walking speed	0.09 (0.01,0.2)*	0.9 (0.02,1.7)*	0.3 (-0.8,1.3)	25.5 (5.4,45.7)*	60.5 (17.1,103.9)**	59.4 (18.0,100.8)**
strenous activity pre pregnancy	-0.007 (-0.04,0.03)	0.6 (0.2,1.0)**	0.6 (0.2,1.1)**	9.7 (0.8,18.5)*	24.7 (5.6,43.8)*	17.2 (-1.0,35.4)
log PP Total protein (g/day)	-0.07 (-0.3,0.2)	1.5 (-1.3,4.2)	1.9 (-1.4,5.2)	7.1 (-57.1,71.3)	14.1 (-124.2,152.4)	10.3 (-121.7,142.2)
log EP Vit C intake mg/day	0.1 (-0.05,0.2)	-0.6 (-2.2,1.0)	-1.2 (-3.1,0.7)	-3.9 (-40.8,33.0)	-8.5 (-88.0,71.1)	-2.5 (-78.4,73.5)
EP prudent diet score	0.03 (-0.10,0.1)	0.2 (-1.1,1.5)	0.02 (-1.5,1.6)	-4.4 (-34.8,26.0)	-4.1 (-69.6,61.5)	-4.4 (-66.9,58.2)
LP prudent diet score	-0.08 (-0.2,0.03)	-0.5 (-1.6,0.7)	0.02 (-1.4,1.4)	-10.1 (-37.7,17.4)	-32.1 (-91.5,27.3)	-14.3 (-71.0,42.3)

table shows β and 95% CI; *p<0.05, **p<0.01, ***p<0.001: PP-Pre pregnancy EP-Early pregnanacy, LP-late pregnancy

Biceps skinfold thickness was associated with cortical content (β=7.7, p=0.01) and periosteal circumference (β=1.6, p=0.04) in the first model. When additionally adjusted for maternal height and weight the relationship was lost.

Although maternal height and weight predicted the offspring's bone mass on univariate analysis, once adjusted for the other maternal determinants (including height and weight) in the multivariate model all associations were lost.

5.7 Multivariate analysis of both childhood and maternal predictors of bone mass and strength using pQCT

When we include all maternal and childhood determinants (except child's height and weight) into a model only 38 subjects were included (Table 34). This is due mainly to the small number of activity records available for this analysis. Table 35 additionally shows the model including the child's height and weight.

Childhood grip strength was the largest independent determinant of tibial mass and strength. It was positively associated with trabecular content and density at the 4% site, trabecular content at 14%, cortical content, cortical thickness as well as all three measures of bone strength (SSI, and fracture load in the x and y axis). When additionally adjusting for the child's height and weight, 6 year grip strength remained positively associated with trabecular content at 4% (β=4.1, p=0.03) cortical content (β=4, p=0.004), cortical thickness (β=0.8, p=0.06) and SSI (β=14.6, p=0.04).

Childhood vigorous activity was positively associated with cortical density (β=1.2, p=0.02). This remained once additionally adjusted for childhood height and weight (β=1.3, p=0.02).

Of the maternal factors that remained significant, late walking speed was negatively associated with cortical density (β=-36.4, p=0.03), whereas strenuous activity was positively associated with endosteal circumference (β=1, p=0.04). When additionally adjusted for height and weight, late walking speed remained negatively

associated with cortical density (β=-21.8, p=0.03) and pre pregnancy strenuous activity was negatively associated with cortical thickness (β=-0.07, p=0.05).
Of the maternal dietary components, pre pregnancy protein intake was associated with decreased cortical thickness but increased endosteal circumference (β=-0.5, p=0.04; β=8.9, p=0.008). This remained after additionally adjusting for the child's height and weight.

To increase the number of subjects in the model, if we mutually adjusted excluding activity, triceps skinfold thickness was associated with increased cortical content (β=1.4, p=0.02), periosteal and endosteal circumference (β=0.4, p=0.003; β =0.4, p=0.02 respectively) and higher fracture load in the x and y axis (β=16.6, p=0.02; β 15.7, p=0.03 respectively). Higher maternal prudent diet score was associated with trabecular content and density at the 4% site (β=9, p=0.02; β=24.9, p=0.01). In general, adjustment for the child's height and weight led to attenuation of these relationships.

TABLE 34: Mutually independent maternal and childhood determinants of tibial bone structure and strength at age 6 (n=37)

	Trabecular content 4% per mm slice	Trabecular density 4% mg/cm^3	Trabecular content 14% per mm slice	Trabecular density 14% mg/cm^3	Cortical content 38% per mm slice	Cortical density 38% mg/cm3
R^2 as a %	**48**	**37**	**46**	**32**	**57**	**40**
Maternal determinants						
Logged Maternal weight (kg)	8.7 (-49.2,66.6)	55.3 (-91.1,201.6)	8.5 (-8.1,25.1)	91.6 (-40.6,223.7)	33.5 (-9.8,76.8)	33.4 (-81.7,148.5)
Maternal height (cm)	0.7 (-0.5,2.0)	0.8 (-2.4,4.1)	-0.1 (-0.5,0.2)	-2.1 (-4.7,0.5)	0.3 (-0.6,1.2)	0.2 (-2.2,2.7)
Biceps skinfold thickness, mm	6 (-18.8,30.9)	-18.8 (-81.7,44.0)	-1.9 (-8.7,4.9)	-17.8 (-71.8,36.3)	-8.4 (-26.4,9.5)	-36.4 (-84.0,11.3)*
Late pregnancy walking speed	4.5 (-5.2,14.2)	8.8 (-15.8,33.3)	-0.2 (-2.6,2.2)	-1.8 (-20.7,17.2)	-0.4 (-7.4,6.6)	-21.1 (-39.7,-2.4)
strenous activity pre pregnancy	2.6 (-1.6,6.9)	-1.2 (-11.9,9.5)	0.1 (-1.0,1.2)	-1.9 (-10.4,6.5)	-1 (-4.1,2.0)	-4.2 (-12.3,3.9)
log PP Total protein (g/day)	-11.9 (-45.4,21.6)	-30.7 (-115.3,54.0)	0.4 (-8.8,9.5)	-17.4 (-90.1,55.3)	-8.5 (-32.7,15.7)	-12.9 (-77.2,51.4)
log EP Vit C intake mg/day	-10.7 (-26.9,5.5)	-13.1 (-54.1,27.9)	0.9 (0.6,4.9)	10.9 (-20.8,42.6)	1.2 (-10.6,13.0)	17.8 (-13.6,49.2)
EP prudent diet score	0.4 (-12.7,13.5)	-1.2 (-34.4,31.9)	1.2 (-2.2,4.5)	12.4 (-14.1,38.9)	1 (-8.5,10.5)	-13.2 (-38.4,12.0)
LP prudent diet score	2.1 (-9.0,13.3)	14.3 (-14.0,42.5)	-0.2 (-3.0,2.6)	1.7 (-20.6,24.0)	-3.4 (-11.7,4.8)	16.3 (-5.6,38.2)
Childhood determinants						
Triceps skinfold thickness	0.2 (-3.1,3.6)	1 (-7.5,9.5)	-0.2 (-1.0,0.7)	-2.5 (-9.2,4.3)	2 (-0.5,4.4)	2.9 (-3.7,9.5)
Maximal grip strength (kg)	5 (1.5,8.6)**	9 (0.09,18.0)*	1.3 (0.4,2.2)***	6.3 (-0.7,13.4)	5.1 (2.6,7.7)***	4.4 (-2.4,11.2)
Vigorous activity per day (mins)	-0.2 (-0.7,0.3)	-0.3 (-1.6,1.0)	-0.02 (-0.1,0.1)	-0.3 (-1.3,0.7)	0.3 (-0.1,0.6)	1.2 (0.2,2.2)*

	Cortical thickness 38% (mm)	Periosteal circumference 38% (mm)	Endosteal circumference 38% (mm)	Stress Strain Index 38% mm^3	Fracture load x 38% N	Fracture load y 38% N
R^2 as a %	**54**	**55**	**59**	**48**	**47**	**36**
Maternal determinants						
Logged Maternal weight (kg)	0.9 (-0.03,1.8)	-1.5 (-12.3,9.4)	-7.2 (-18.6,4.2)	143.4 (-102.4,389.1)	193.3 (-338.0,724.6)	202.5 (-387.4,792.5)
Maternal height (cm)	-0.004 (-0.02,0.02)	0.1 (-0.10,0.4)	0.2 (-0.08,0.4)	3.5 (-1.8,8.8)	9.3 (-2.1,20.7)	3.7 (-9.0,16.3)
Log: Body mass index, kg/m2)	-1 (-54.9,52.8)	-36 (-656.7,584.6)	-29.5 (-684.5,625.5)	-7191.6 (-20966.9,6583.8)	-14152 (-44065.9,15761.9)	-5161.4 (-38990.4,28667.7)
Biceps skinfold thickness, mm	-0.2 (-0.6,0.2)	0.9 (-3.6,5.4)	2.1 (-2.6,6.8)	-24.5 (-126.2,77.3)	-3.6 (-223.6,216.5)	-119.2 (-363.6,125.1)
Late pregnancy walking speed	0.03 (-0.1,0.2)	0.5 (-1.2,2.3)	0.4 (-1.5,2.2)	-0.8 (-40.5,39.0)	14.6 (-71.4,100.5)	-4.5 (-99.9,91.0)
strenous activity pre pregnancy	-0.06 (-0.1,0.006)	0.6 (-0.1,1.4)	1 (0.2,1.8)*	-4.2 (-21.5,13.1)	3.2 (-34.2,40.6)	-10.5 (-52.0,31.0)
log PP Total protein (g/day)	-0.5 (-1.1,-0.02)*	5.4 (-0.6,11.5)	8.9 (2.5,15.2)**	-28.9 (-166.3,108.4)	19.1 (-277.9,316.1)	2.6 (-327.2,332.3)
log EP Vit C intake mg/day	0.04 (-0.2,0.3)	-0.4 (-3.3,2.5)	-0.6 (-3.7,2.5)	-25.9 (-92.9,41.1)	-37.2 (-182.0,107.6)	-16.5 (-177.3,144.3)
EP prudent diet score	0.04 (-0.2,0.2)	0.3 (-2.1,2.7)	0.04 (-2.5,2.5)	6.7 (-47.2,60.5)	1.9 (-114.4,118.2)	-12.8 (-142.0,116.4)
LP prudent diet score	-0.06 (-0.2,0.1)	-1.1 (-3.1,1.0)	-0.7 (-2.8,1.5)	-26.9 (-73.6,19.8)	-58.8 (-159.9,42.2)	-21.9 (-134.1,90.3)
Childhood determinants						
Triceps skinfold thickness	0.004 (-0.05,0.06)	0.5 (-0.1,1.1)	0.5 (-0.2,1.1)	4.4 (-9.7,18.5)	16.7 (-13.8,47.1)	21 (-12.7,54.8)
Maximal grip strength (kg)	0.1 (0.05,0.2)***	0.2 (-0.4,0.8)	-0.5 (-1.1,0.2)	22.2 (7.7,36.6)**	35.2 (3.9,66.6)*	46.8 (12.0,81.6)*
Vigorous activity per day (mins)	0.002 (-0.006,0.010)	0.02 (-0.08,0.1)	0.004 (-0.09,0.1)	0.8 (-1.3,2.9)	2.3 (-2.2,6.9)	2.2 (-2.9,7.2)

table shows β and 95% CI; *p<0.05, **p<0.01, ***p<0.001: PP-Pre pregnancy EP-Early pregnanacy, LP-late pregnancy

TABLE 35: Mutually independent maternal and childhood determinants of tibial bone structure and strength at age 6 including child's height and weight (n=37)

	Trabecular content 4% per mm slice	Trabecular density 4% mg/cm^3	Trabecular content 14% per mm slice	Trabecular density 14% mg/cm^3	Cortical content 38% per mm slice	Cortical density 38% mg/cm3
R^2 as a %	**54**	**39**	**53**	**37**	**67**	**44**
Maternal determinants						
Logged Maternal weight (kg)	4 (-57.4,65.4)	65.7 (-97.1,228.5)	3.6 (-14.1,21.4)	65.7 (-80.9,212.4)	23.1 (-19.3,65.5)	54.3 (-71.1,179.7)
Maternal height (cm)	0.4 (-0.9,1.8)	0.5 (-3.1,4.0)	-0.2 (-0.5,0.2)	-2.4 (-5.1,0.4)	0.09 (-0.8,1.0)	0.5 (-2.2,3.2)
Biceps skinfold thickness, mm	2.2 (-24.9,29.3)	-29.7 (-101.6,42.1)	-1.1 (-8.6,6.3)	-16.4 (-78.2,45.3)	-8.2 (-26.3,10.0)	-39.8 (-93.5,13.9)
Late pregnancy walking speed	4.4 (-5.2,14.1)	7.6 (-18.0,33.2)	-0.1 (-2.5,2.3)	-1.5 (-21.1,18.0)	0.3 (-6.2,6.7)	-21.8 (-40.9,-2.6)*
strenous activity pre pregnancy	1.3 (-3.4,5.9)	-3 (-15.3,9.3)	-0.02 (-1.2,1.2)	-3.3 (-13.1,6.5)	-1.8 (-4.9,1.3)	-3.3 (-12.5,5.9)
log PP Total protein (g/day)	-14.4 (-47.9,19.0)	-30.1 (-118.8,58.6)	-0.3 (-9.4,8.9)	-19.5 (-95.4,56.4)	-12.6 (-35.1,9.9)	-6.3 (-72.8,60.2)
log EP Vit C intake mg/day	-9.1 (-25.5,7.3)	-9.5 (-52.9,34.0)	0.9 (-3.1,4.9)	11.7 (-21.6,45.0)	1.7 (-9.4,12.8)	18.5 (-14.2,51.3)
EP prudent diet score	1.4 (-11.7,14.5)	0.7 (-34.1,35.5)	1 (-2.3,4.3)	11.6 (-15.6,38.8)	1.2 (-7.6,10.0)	-12.8 (-38.8,13.3)
LP prudent diet score	2.2 (-9.0,13.4)	13 (-16.8,42.7)	0.2 (-2.6,3.0)	3.8 (-19.3,26.9)	-2.5 (-10.2,5.2)	14.2 (-8.5,36.9)
Childhood determinants						
Childs height (cm)	1.1 (-1.5,3.7)	2.4 (-4.6,9.3)	-0.09 (-0.8,0.6)	0.3 (-5.9,6.4)	0.2 (-1.6,2.0)	0.2 (-5.0,5.4)
Child's weight (logged) kg	15.4 (-92.9,123.7)	-55.7 (-342.8,231.5)	18.1 (-12.3,48.6)	84.1 (-168.0,336.2)	54 (-18.6,126.5)	-90.8 (-305.3,123.8)
Triceps skinfold thickness	-1.3 (-44.7,42.1)	26.4 (-88.7,141.4)	-5.7 (-17.6,6.2)	-40.6 (-139.0,57.8)	6.1 (-23.7,35.9)	51.7 (-36.4,139.9)
Maximal grip strength (kg)	4.3 (0.4,8.1)*	9.3 (-1.0,19.5)	0.9 (-0.2,1.9)	4.1 (-4.4,12.6)	4.0 (1.4,6.6)**	6.3 (-1.4,14.0)
Vigorous activity per day (mins)	-0.2 (-0.7,0.4)	-0.1 (-1.5,1.3)	-0.03 (-0.2,0.1)	-0.3 (-1.4,0.8)	0.3 (-0.1,0.6)	1.3 (0.2,2.4)*

	Cortical thickness 38% (mm)	Periosteal circumference 38% (mm)	Endosteal circumference 38% (mm)	Stress Strain Index 38% mm^3	Fracture load x 38% N	Fracture load y 38% N
R^2 as a %	**63**	**60**	**59**	**62**	**61**	**59**
Maternal determinants						
Logged Maternal weight (kg)	0.6 (-0.3,1.6)	-2.8 (-14.3,8.6)	-6.8 (-19.5,6.0)	66.2 (-170.2,302.6)	57 (-456.1,570.2)	51.5 (-478.4,581.4)
Maternal height (cm)	-0.008 (-0.03,0.01)	0.09 (-0.2,0.3)	0.1 (-0.1,0.4)	2.3 (-2.7,7.4)	6.3 (-4.8,17.3)	-0.9 (-12.3,10.5)
Biceps skinfold thickness, mm	-0.1 (-0.5,0.3)	0.8 (-4.2,5.7)	1.6 (-3.8,7.1)	-13.1 (-114.3,88.1)	3.6 (-216.1,223.4)	-123 (-349.9,103.9)
Late pregnancy walking speed	0.04 (-0.1,0.2)	0.6 (-1.1,2.4)	0.4 (-1.6,2.3)	3.8 (-32.2,39.9)	23.5 (-54.7,101.8)	6.7 (-74.1,87.5)
strenous activity pre pregnancy	-0.07 (-0.1,-0.002)*	0.5 (-0.4,1.3)	0.9 (-0.007,1.9)	-8 (-25.4,9.4)	-7.5 (-45.2,30.3)	-27.1 (-66.0,11.9)
log PP Total protein (g/day)	-0.6 (-1.1,-0.1)*	4.8 (-1.3,10.9)	8.8* (2.1,15.6)	-55.3 (-180.7,70.1)	-33.4 (-305.5,238.8)	-61.5 (-342.5,219.6)
log EP Vit C intake mg/day	0.03 (-0.2,0.3)	-0.2 (-3.2,2.8)	-0.4 (-3.8,2.9)	-26.6 (-88.4,35.2)	-32.9 (-167.1,101.3)	-7.3 (-145.9,131.3)
EP prudent diet score	0.03 (-0.2,0.2)	0.4 (-2.0,2.7)	0.1 (-2.5,2.8)	6.4 (-42.8,55.5)	4.9 (-101.7,111.5)	-5.5 (-115.6,104.7)
LP prudent diet score	-0.03 (-0.2,0.1)	-1 (-3.0,1.1)	-0.7 (-3.0,1.6)	-19.7 (-62.5,23.1)	-46.8 (-139.8,46.1)	-9.2 (-105.1,86.8)
Childhood determinants						
Childs height (cm)	-0.004 (-0.04,0.03)	0.07 (-0.4,0.5)	0.1 (-0.4,0.6)	-0.5 (-10.3,9.4)	2.7 (-18.6,24.1)	7.3 (-14.7,29.3)
Child's weight (logged) kg	1.4 (-0.2,3.0)	7.7 (-12.0,27.3)	-0.9 (-22.7,20.9)	379.4 (-25.1,783.9)	692.9 (-185.1,1570.9)	783.2 (-123.4,1689.8)
Triceps skinfold thickness	-0.3 (-1.0,0.4)	3.2 (-4.9,11.2)	5.1 (-3.9,14.0)	-55.7 (-221.8,110.5)	-21.1 (-381.8,339.5)	-19.5 (-392.0,352.9)
Maximal grip strength (kg)	0.08 (0.02,0.1)**	0.05 (-0.7,0.7)	-0.5 (-1.2,0.3)	14.6 (0.1,29.1)*	20.2 (-11.2,51.6)	27.8 (-4.6,60.2)
Vigorous activity per day (mins)	0.001 (-0.007,0.009)	0.02 (-0.08,0.1)	0.01 (-0.10,0.1)	0.6 (-1.4,2.7)	2.2 (-2.2,6.6)	2.3 (-2.3,6.8)

table shows β and 95% CI; *p<0.05, **p<0.01, ***p<0.001: PP-Pre pregnancy EP-Early pregnanacy, LP-late pregnancy

5.8 Discussion

The most robust association observed was between childhood height and weight and measures of bone size and strength rather than volumetric bone density. When adjusted for the other maternal and childhood determinants it appeared that weight was the more important predictor, particularly of measures of bone strength. One might speculate this may represent the tibia adapting to increased loading; the data suggests that this is due to increased cortical thickness and content as a result of the increase in the periosteal circumference without a corresponding increase in the endosteal circumference. Whilst maternal height and weight also appeared to be positively associated with measures of bone size and strength, the relationship was no longer seen once adjusted for the child's height and weight implying that collinearity between maternal and childhood height may be operating.

Grip strength, which was associated with a higher muscle area, was also associated with measures of bone size and bone strength rather than volumetric density in a similar manner observed for the child's weight. However as this relationship was independent of the child's weight, it suggests that muscle and lean body mass are important in the remodelling and adaptation of the bone to the stresses placed upon them, resulting in an increase in bone size, altered geometry and an increase the amount of mass within the periosteal envelope, giving the bone an overall increased strength. Triceps skinfold thickness, a measure of fat mass, was associated with increased measures of bone size. There was also a suggestion that it was additionally associated with reduced cortical density resulting in a relatively under mineralised skeleton. However once the numbers included in the multivariate model reduced, this association was no longer seen.

It was difficult to ascertain the full relationship between childhood exercise and the outcomes observed due to the small number of subjects that had full data available. However the relationship between vigorous activity and cortical volumetric density remained after all adjustments. Increased time doing vigorous activity usually involves increased weight bearing exercise. The increased forces placed on the bone may explain the increased cortical density seen. Whilst there was no relationship between any measure of bone strength, maternal walking speed in late pregnancy, a

surrogate marker for the child's exercise intensity, resulted in an increase in bone size and strength when adjusted for all maternal determinants.

The relationship between diet and bone was less clear. Whilst maternal diet, in particular pre pregnancy protein intake, vitamin C consumption and dietary patterns during pregnancy resulted in higher trabecular content and volumetric density, the relationships were relatively weak particularly once adjusted for childhood height and weight. No relationship was seen between either maternal calcium intake or the child's milk intake and the child's bone size or density. Whilst dietary patterns and individual macronutrient and micronutrient intake are likely to be important to the offspring's bone health, power was limited in this study to examine these relationships.

6 DETERMINANTS OF HIP GEOMETRY AND STRENGTH

6.1 Aims

- To test the hypothesis that childhood lifestyle and body composition influence femoral neck structure and bone strength at age 6 years.
- To test the hypothesis that maternal lifestyle factors and body composition influence femoral neck structure and bone strength at age 6 years.

6.2 Methods

The femoral DXA scan images obtained from the 6 year children were analysed using an interactive computer program (hip structural analysis, HSA). This was used to derive a number of structural variables from the femoral DXA scans. Full methods are described in chapter 3.

6.3 Results

6.3.1 Descriptive statistics

The summary statistics of the children are shown in the descriptive statistics tables in chapter 4. The HSA program was unable to analyse all scans of the femoral neck. Although it was unclear why this was the case, one reason may be that the edge detection of the bone mineral in the hip was unclear in some scans, a known problem in scanning young children. Table 36 compares all children who had scans of the femoral neck according to whether HSA analysis was possible. Of the 530 total scans, 478 could be used in further analysis, which left 52 excluded. The children for whom the program was unable to interpret the hip tended to be shorter (p=0.0009) and lighter (p=0.01). These children also had a lower grip strength, lower whole body and lumbar BMC, BA and aBMD, lower hip BA and lower lean mass. There was no difference between total fat mass and activity levels in these children. However they appeared to drink slightly more milk (p=0.006).

There was no difference between any maternal anthropometric measures, including height and weight, or any other maternal dietary or lifestyle determinants in the children whose scans were excluded.

TABLE 36: Differences in the characteristics of the children whose femoral neck scans were analysable/non-analysable

	Children with suitable scans	number	Children with unsuitable scans	number	p value
Birth to age 1					
Gestational age	39.7 (1.8)	478	39.5 (2)	52	0.4
Birthweight (g)	3449 (518)	472	3378 (682)	52	0.4
Crown heel length birth(cm)	49.9 (2.1)	462	49.5 (2.6)	51	0.2
Weight at one year (kg)	10.1 (9.3-10.8)	475	9.6 (8.8,10.2)	51	0.01
Crown heel length age one(cm)	75.6 (2.7)	468	74.5 (3)	52	0.007
Age 6 from DXA clinic visit					
Anthropometry					
Height at age 6, cm (mean sd)	120.3 (5)	444	117.8 (5.4)	48	0.0009
Weight at age 6, kg (median IQR)	23.4 (21.5,25.7)	451	21.9 (19.8,24.7)	48	0.01
Grip Strength (max) kg (mean sd)	10 (2.4)	411	8.9 (2.3)	41	0.008
Triceps skinfold thickness, mm (median IQR)	9.8 (8.1-12.1)	370	9.7 (7.9,11.6)	40	0.5
Total BMC, g (mean sd)	533.9 (67.7)	461	501 (85.4)	50	0.002
Total BA, g/cm^2 (mean sd)	899 (62.5)	461	869(71.1)	50	0.002
Total aBMD, g/cm^2 (mean sd)	0.59 (0.04)	461	0.57 (0.06)	50	0.006
Spine BMC, g (mean sd)	18 (2.7)	478	16.8 (3)	48	0.004
Spine BA, g/cm2 (mean sd)	33.3 (3)	478	32.1 (3.7)	48	0.009
Spine aBMD, g/cm2 (mean sd)	0.54 (0.06)	478	0.52 (0.06)	48	0.05
Hip BMC, g (mean sd)	11.2 (2)	478	10.7 (2.6)	48	0.08
Hip BA, g/cm2 (mean sd)	16.8 (2.1)	478	15.9 (2.6)	48	0.005
Hip aBMD, g/cm2 (mean sd)	0.66 (0.06)	478	0.66 (0.07)	48	0.9
Total lean, kg (mean sd)	17.3 (2.1)		16.7 (2.8)		0.05
Total fat kg, median IQR	5.2 (4.3,6.6)	451	5.1 (4.1,6.2)	48	0.21
Lifestyle					
% of children with fracture	12.4	51	6.3	3	0.006
Vigorous activity, mins per day (mean sd)	44.1 (19.6)	196	38.1 (11.3)	19	0.7
Sedentary activity, mins per day (mean sd)	875 (80.4)		858 (84.6)	19	0.4
Moderate activity, mins per day (mean sd)	36.3 (12.6)		38.1(11.3)	19	0.5
Milk intake, pints/day (median IQR)	0.5 (0.25,0.5)	384	0.5 (0.35,0.75)	41	0.006

BMC: bone mineral content; BA: bone area; aBMD: areal bone mineral density

Table 37 shows the differences between summary values in boys and girls. Boys had significantly greater narrow neck BMD, cross sectional area (CSA), cortical thickness and had a higher section modulus (Z). At the intertrochanteric site, whilst the boys had a higher BMD and cortical thickness, the girls had a higher sub periosteal width and buckling ratio with no statistical difference in Z modulus. Boys had slightly greater BMD and cortical thickness at the femoral shaft. An increase in hip axis length, CSA and Z modulus were also seen with increasing age of the child at the time of scan in both sexes, hence all analyses in this chapter were adjusted for age and sex.

TABLE 37: Differences between hip geometry and strength between boys and girls

Characteristic	Boys		Girls		P value
		n		n	
Hip axis length (mm)	77.6 (5.5)	269	75.8 (5.5)	251	0.0002
Shaft neck angle	131.2 (5.6)	148	133.4 (5.4)	225	<0.0001
Narrow neck BMD (g/cm^2)	0.77 (0.08)	249	0.71 (0.08)	229	<0.0001
Narrow neck cross section area (cm^2)	1.67 (0.26)	249	1.55 (0.22)	229	<0.0001
Narrow neck sub periosteal width (cm)	2.27 (0.18)	249	2.27 (0.18)	229	0.81
Narrow neck average cortical thickness (cm)	0.15 (0.02)	249	0.14 (0.02)	229	<0.0001
Narrow neck section of modulus (cm^4)	0.58 (0.13)	249	0.54 (0.12)	229	0.0002
Narrow neck buckling ratio	8.13 (1.02)	249	8.82 (1.3)	229	<0.0001
Intertrochanter BMD (g/cm^2)	0.76 (0.09)	248	0.69 (0.09)	225	<0.0001
Intertrochanter cross section area (cm^2)	2.18 (0.33)	248	2.13 (0.32)	225	0.12
Intertrochanter sub-periosteal width (cm)	3.01 (0.27)	248	3.24 (0.3)	225	<0.0001
Intertrochanter cortical thickness (cm)	0.28 (0.04)	248	0.27 (0.04)	225	0.008
Intertrochanter section of modulus (cm^4)	1 (0.2)	248	1 (0.2)	225	0.72
Intertrochanter buckling ratio	5.9 (0.9)	248	6.7 (1.1)	225	<0.0001
Femur shaft BMD (g/cm^2)	0.96 (0.09)	248	0.94 (0.09)	225	0.02
Femur shaft cross section area (cm^2)	1.72 (0.23)	248	1.69 (0.22)	225	0.09
Femur shaft sub-periosteal width (cm)	1.89 (0.15)	248	1.9 (0.16)	225	0.85
Femur shaft average cortical thickness (cm)	0.36 (0.05)	248	0.35 (0.05)	225	0.03
Femur shaft section of modulus (cm^4)	0.6 (0.12)	248	0.59 (0.12)	225	0.27
Femur shaft section buckling ratio	2.77 (0.5)	248	2.85 (0.5)	225	0.07

table shows mean and standard deviation, n:number

6.3.2 Childhood hip structure adjusted for 6 year anthropometry

Table 38 shows that height, weight and maximum grip strength were all positively associated with femoral neck, intertrochanter and femoral shaft BMD, CSA, sub-periosteal width and Z modulus ($p<0.001$) and narrow neck and intertrochanter cortical thickness ($p<0.01$). Figure 11 shows scatter graphs of the relationship between the structure and strength at the three sites and the child's current height. Triceps skinfold thickness was associated with increased sub-periosteal width at all three sites ($p<0.01$). It was also associated with increased intertrochanteric and femoral shaft Z modulus, increased femoral shaft CSA and increased intertrochanteric and femoral shaft buckling ratios ($p<0.05$). In addition height ($r=0.51$, $p<0.001$), weight ($r=0.47$, $p<0.001$), grip strength ($r=0.29$, $p<0.001$), triceps skinfold thickness ($r=0.11$, $p=0.02$) and BMI ($r=0.2$, $p<0.001$) were all positively correlated with increased hip axis length. Finally there was a small negative association between height and shaft neck angle ($r=-0.1$, $p=0.04$).

Figure 11: Relationship between child's height and hip structure at age 6 years

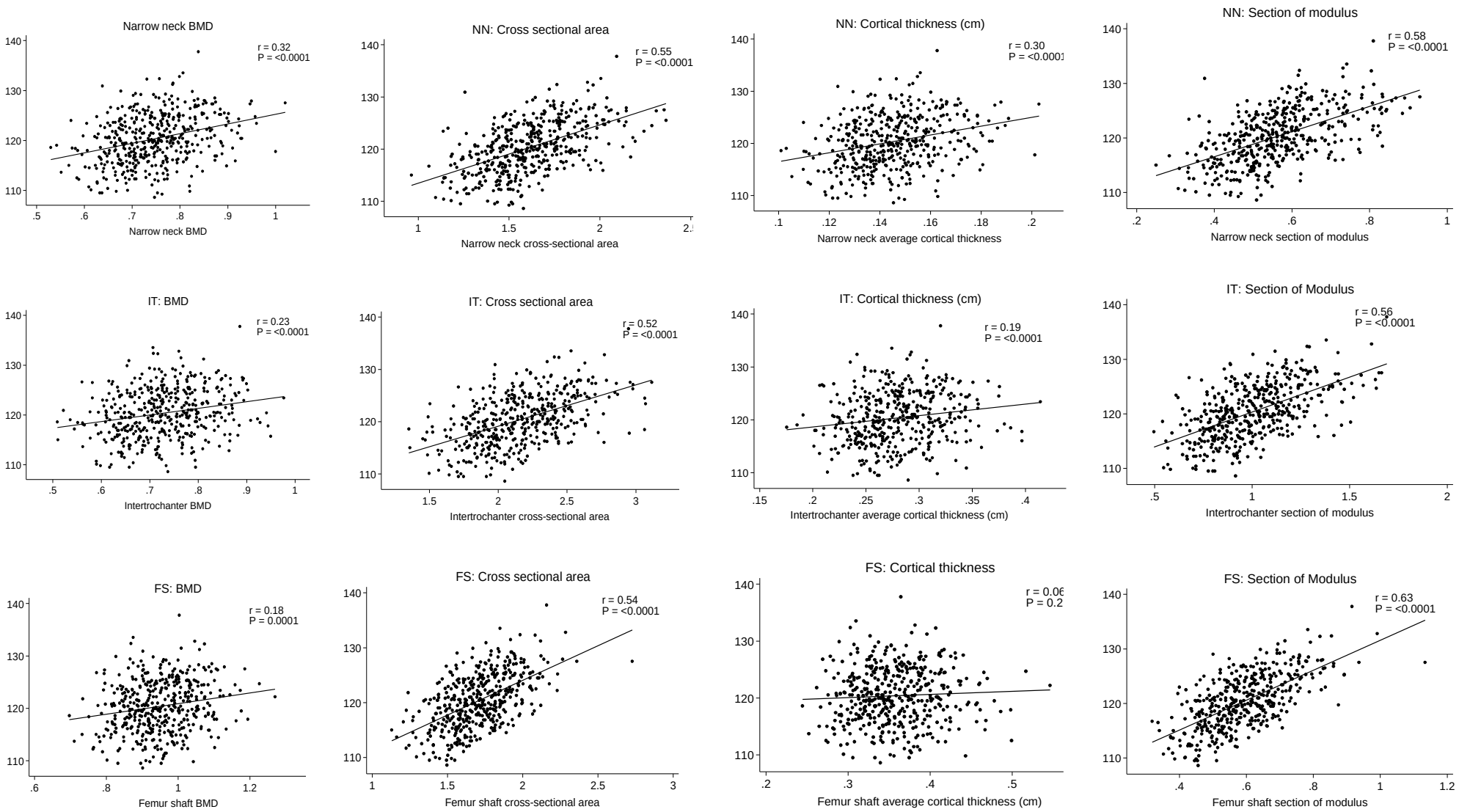


TABLE 38: Childhood anthropometry as determinants of childhood hip structure at age 6 years using univariate regression analysis

	Narrow Neck					
	BMD (g/cm^2)	**Cross sectional area (cm^2)**	**Sub periosteal width (cm)**	**Cortical thickness (cm)**	**Section of modulus (cm^4)**	**Buckling ratio**
Maximum grip strength (kg)	0.008 (0.005,0.01)***	0.03 (0.03,0.04)***	0.02 (0.02,0.03)***	0.002 (0.0010,0.002)***	0.02 (0.01,0.02)***	-0.009 (-0.05,0.04)
log triceps skinfold thickness (mm)	-0.007 (-0.04,0.02)	0.05 (-0.03,0.1)	0.09 (0.03,0.2)**	-0.002 (-0.008,0.004)	0.03 (-0.010,0.07)	0.5 (0.1,0.9)
log. Weight (kg)	0.2 (0.1,0.2)***	0.9 (0.7,1.0)***	0.7 (0.6,0.8)***	0.03 (0.02,0.04)***	0.5 (0.4,0.5)***	1.1 (0.3,1.8)**
Height (cm)	0.005 (0.004,0.007)***	0.03 (0.02,0.03)***	0.02 (0.02,0.02)***	0.001 (0.0007,0.001)***	0.01 (0.01,0.02)***	0.03 (0.004,0.05)*
BMI,kg/m2	0.006 (0.002,0.01)**	0.03 (0.02,0.05)***	0.03 (0.02,0.04)***	0.001 (0.0002,0.002)*	0.02 (0.01,0.02)***	0.07 (0.003,0.1)*
	Intertrochanter					
	BMD (g/cm^2)	**Cross sectional area (cm^2)**	**Sub periosteal width (cm)**	**Cortical thickness (cm)**	**Section of modulus (cm^4)**	**Buckling ratio**
Maximum grip strength (kg)	0.006 (0.003,0.010)***	0.05 (0.04,0.06)***	0.04 (0.03,0.05)***	0.003 (0.001,0.004)***	0.03 (0.02,0.04)***	0.02 (-0.01,0.06)
log triceps skinfold thickness (mm)	-0.02 (-0.04,0.01)	0.08 (-0.03,0.2)	0.2 (0.08,0.3)***	-0.01 (-0.02,0.003)	0.08 (0.004,0.2)*	0.7 (0.4,1.0)***
log. Weight (kg)	0.1 (0.06,0.2)***	1.2 (1.0,1.3)***	1.2 (1.0,1.3)***	0.04 (0.02,0.07)**	0.9 (0.8,1.0)***	1.7 (1.0,2.3)***
Height (cm)	0.004 (0.002,0.005)***	0.03 (0.03,0.04)***	0.03 (0.03,0.04)***	0.001 (0.0006,0.002)***	0.02 (0.02,0.03)***	0.04 (0.02,0.06)***
BMI,kg/m2	0.004 (-0.0004,0.009)	0.05 (0.04,0.07)***	0.06 (0.04,0.07)***	0.002 (-0.0003,0.004)	0.04 (0.03,0.05)***	0.09 (0.04,0.1)***
	Femur shaft					
	BMD (g/cm^2)	**Cross sectional area (cm^2)**	**Sub periosteal width (cm)**	**Cortical thickness (cm)**	**Section of modulus (cm^4)**	**Buckling ratio**
Maximum grip strength (kg)	0.007 (0.003,0.01)***	0.03 (0.03,0.04)***	0.04 (0.03,0.05)***	0.002 (-0.0002,0.004)	0.02 (0.02,0.02)***	0.02 (0.0009,0.04)*
log triceps skinfold thickness (mm)	0.01 (-0.02,0.04)	0.1 (0.05,0.2)***	0.2 (0.08,0.3)***	-0.0004 (-0.02,0.02)	0.08 (0.04,0.1)***	0.2 (0.03,0.4)*
log. Weight (kg)	0.2 (0.1,0.2)***	0.9 (0.8,1.1)***	1.2 (1.0,1.3)***	0.05 (0.01,0.08)**	0.6 (0.5,0.6)***	0.7 (0.4,1.0)***
Height (cm)	0.003 (0.002,0.005)***	0.02 (0.02,0.03)***	0.03 (0.03,0.04)***	0.0005 (-0.0004,0.001)	0.02 (0.01,0.02)***	0.03 (0.02,0.04)***
BMI,kg/m2	0.01 (0.007,0.02)***	0.05 (0.04,0.06)***	0.06 (0.04,0.07)***	0.004 (0.002,0.007)**	0.03 (0.02,0.03)***	0.02 (-0.010,0.04)

table shows β and 95% CI; *p<0.05, **p<0.01, ***p<0.001

6.3.3 Childhood lifestyle determinants

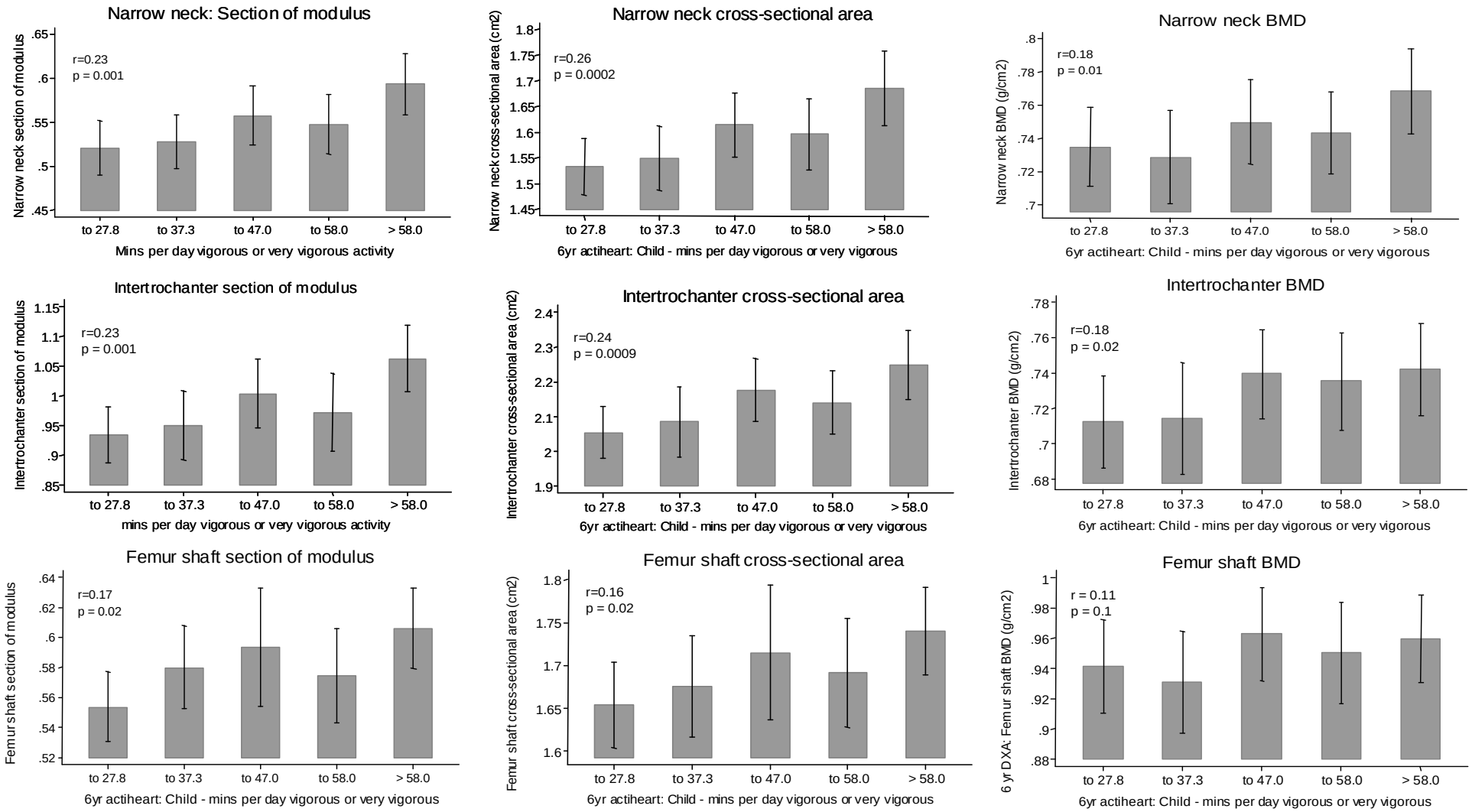
It was noted that children that had higher intensities of physical activity tended to be both smaller (r=-0.17, p=0.01) and lighter (r=-0.16, p=0.03) than the other children. Since height was such a large predictor of bone mass and geometry in the hip and hence a major confounder for childhood activity, the following results were additionally adjusted for childhood height.

Table 39 shows the child's lifestyle as determinants of hip structure adjusted for the child's height. In addition Figure 12 shows barcharts to show the relationship between vigorous and very vigorous activity and the child's hip structure and strength.

Very vigorous activity levels in the child were associated with increased narrow neck and intertrochanteric BMD (both $p<0.05$), CSA (both $p<0.001$), sub periosteal width (both $p<0.05$) and Z modulus (both $p<0.01$). In addition it was also associated with increased CSA and Z modulus at the femoral shaft (both p=0.02). Increased time spent in sedentary activity was negatively associated with narrow neck BMD and cortical thickness (both p =0.05). Whilst there was a suggestion that sedentary activity was also associated with decreased Z modulus at the narrow neck, this did not reach significance (p=0.1).

Increased milk intake was positively associated with narrow neck and femoral shaft BMD and cortical thickness and femoral shaft CSA (all $p<0.05$). In addition milk intake was negatively associated with the narrow neck buckling ratio (r=-0.14, p=0.008).

Figure 12: Barcharts to show the relationship between vigorous/very vigorous activity and section of modulus, cross sectional area and bone mineral density at the three sites measured in the femoral neck



Values are mean (95% CIs)

TABLE 39: Childhood lifestyle as a determinant of childhood hip structure using univariate regression analysis

	Narrow Neck BMD (g/cm^2)	Cross sectional area (cm^2)	Sub periosteal width (cm)	Cortical thickness (cm)	Section of modulus (cm^4)	Buckling ratio
Sedentary activity	-0.0001 (-0.0003,-0.00000	-0.0003 (-0.0007,0.00002)	-0.00008 (-0.0003,0.0002)	-0.00003 (-0.00006,0.00000	-0.0001 (-0.0003,0.00007)	0.001 (-0.0008,0.003)
Light activity (mins per day)	0.0001 (-0.00006,0.0003)	0.0002 (-0.0003,0.0006)	-0.00005 (-0.0004,0.0003)	0.00002 (-0.00001,0.00006)	0.00002 (-0.0002,0.0002)	-0.001 (-0.004,0.001)
Moderate activity (mins per day)	0.0009 (0.00006,0.002)*	0.003 (0.0004,0.005)*	0.0009 (-0.0008,0.003)	0.0002 (0.0000009,0.0004)*	0.0009 (-0.0003,0.002)	-0.008 (-0.02,0.005)
Vigorous activity (mins per day)	0.0008 (-0.00003,0.002)	0.003 (0.001,0.005)**	0.002 (0.0008,0.004)**	0.0001 (-0.00003,0.0003)	0.002 (0.0006,0.003)**	-0.001 (-0.01,0.01)
Very vigorous activity (mins per day)	0.0007 (0.0002,0.001)**	0.003 (0.001,0.004)***	0.001 (0.0004,0.003)**	0.0001 (0.00002,0.0003)*	0.001 (0.0005,0.002)**	-0.003 (-0.01,0.005)
Total milk intake (pints/day)	0.009 (0.00002,0.02)*	0.01 (-0.01,0.03)	-0.01 (-0.03,0.004)	0.002 (0.00006,0.004)*	0.002 (-0.010,0.01)	-0.2 (-0.3,-0.05)*
	Intertrochanter BMD (g/cm^2)	**Cross sectional area (cm^2)**	**Sub periosteal width (cm)**	**Cortical thickness (cm)**	**Section of modulus (cm^4)**	**Buckling ratio**
Sedentary activity	-0.0001 (-0.0003,0.00004)	-0.0004 (-0.0009,0.00007)	-0.0002 (-0.0006,0.0002)	-0.00004 (-0.0001,0.00003)	-0.0002 (-0.0005,0.0001)	0.0005 (-0.001,0.002)
Light activity (mins per day)	0.00006 (-0.0001,0.0002)	0.0002 (-0.0004,0.0008)	0.00003 (-0.0004,0.0005)	0.000007 (-0.00008,0.00009)	0.0000007 (-0.0004,0.0004)	-0.00004 (-0.002,0.002)
Moderate activity (mins per day)	0.001 (0.00003,0.002)*	0.004 (0.0010,0.007)*	0.002 (-0.0007,0.004)	0.0004 (-0.00003,0.0008)	0.002 (0.0002,0.004)*	-0.005 (-0.02,0.006)
Vigorous activity (mins per day)	0.0008 (-0.00005,0.002)	0.004 (0.001,0.007)**	0.002 (0.0002,0.005)*	0.0005 (0.00006,0.0009)*	0.003 (0.0010,0.005)**	-0.006 (-0.02,0.004)
Very vigorous activity (mins per day)	0.0007 (0.0001,0.001)*	0.003 (0.001,0.005)***	0.002 (0.00009,0.003)*	0.0004 (0.00009,0.0006)**	0.002 (0.0008,0.003)**	-0.005 (-0.01,0.002)
Total milk intake (pints/day)	0.004 (-0.005,0.01)	0.02 (-0.01,0.05)	0.01 (-0.01,0.04)	0.003 (-0.002,0.007)	0.007 (-0.01,0.03)	-0.03 (-0.1,0.07)
	Femur shaft BMD (g/cm^2)	**Cross sectional area (cm^2)**	**Sub periosteal width (cm)**	**Cortical thickness (cm)**	**Section of modulus (cm^4)**	**Buckling ratio**
Sedentary activity	-0.00002 (-0.0002,0.0002)	-0.0002 (-0.0005,0.0002)	-0.0001 (-0.0004,0.00007)	0.000008 (-0.00008,0.0001)	-0.00007 (-0.0002,0.00009)	-0.0002 (-0.001,0.0006)
Light activity (mins per day)	-0.00005 (-0.0003,0.0002)	0.00003 (-0.0004,0.0004)	0.0001 (-0.0001,0.0004)	-0.00004 (-0.0001,0.00007)	-0.000005 (-0.0002,0.0002)	0.0005 (-0.0006,0.002)
Moderate activity (mins per day)	0.0008 (-0.0003,0.002)	0.002 (0.0003,0.004)*	0.001 (-0.0003,0.002)	0.0003 (-0.0003,0.0008)	0.001 (0.00008,0.002)*	-0.001 (-0.007,0.004)
Vigorous activity (mins per day)	0.0004 (-0.0006,0.001)	0.001 (-0.0005,0.003)	0.0007 (-0.0005,0.002)	0.0002 (-0.0004,0.0007)	0.0008 (-0.0001,0.002)	-0.0006 (-0.006,0.004)
Very vigorous activity (mins per day)	0.0005 (-0.0002,0.001)	0.002 (0.0002,0.003)*	0.0006 (-0.0002,0.001)	0.0002 (-0.0002,0.0006)	0.0008 (0.0001,0.001)*	-0.001 (-0.005,0.002)
Total milk intake (pints/day)	0.01 (0.0008,0.02)*	0.02 (0.002,0.04)*	0.002 (-0.01,0.02)	0.005 (-0.00009,0.01)*	0.007 (-0.003,0.02)	-0.04 (-0.09,0.02)

table shows β and 95% CI; *p<0.05, **p<0.01, ***p<0.001

6.3.4 Independent childhood determinants of hip structure

Table 40 shows the mutually adjusted childhood determinants of hip structure at 6 years when childhood height, weight, grip strength, vigorous activity and milk intake were included in the multivariate model.

Height was a significant independent predictor of increased sub-periosteal width at all three sites ($p<0.001$), increased narrow neck and intertrochanteric CSA ($\beta=0.02$, $p=0.002$; $\beta=0.02$, $p=0.03$) and Z modulus ($\beta=0.01$, $p<0.0001$; $\beta=0.02$, $p=0.001$). In addition height was positively associated with both the femoral shaft Z modulus ($\beta=0.008$, $p<0.001$) and buckling ratio ($\beta=0.04$, $p=0.003$) and increased hip axis length ($\beta=0.5$, $p<0.001$).

Weight was independently associated with narrow neck CSA ($\beta=0.4$, $p=0.03$) and intertrochanteric and femoral shaft CSA ($\beta=0.7$, $p=0.02$; $\beta=0.7$, $p<0.001$), sub-periosteal width ($\beta=0.6$, $p=0.006$; $\beta=0.4$, $p=0.002$) and Z modulus ($\beta=0.02$, $p<0.001$; $\beta=0.008$, $p<0.001$). In addition it was also positively associated with increased BMD at the femoral shaft ($\beta=0.2$, $p=0.03$) and hip axis length ($\beta=10.2$, $p=0.04$).
Grip strength was positively associated with narrow neck, intertrochanteric and femoral shaft BMD ($\beta=0.01$, $p=0.001$; $\beta=0.01$, $p=0.004$; $\beta=0.008$, $p=0.04$ respectively), CSA ($\beta=0.02$, $p=0.002$; $\beta=0.04$, $p=0.001$; $\beta=0.02$, $p<0.001$ respectively, and Z modulus ($\beta=0.01$, $p=0.008$; $\beta=0.02$, $p=0.01$; $\beta=0.01$, $p<0.001$). It was also positively associated with cortical thickness at the narrow neck ($\beta=0.002$, $p<0.001$) and intertrochanteric sites ($\beta=0.004$, $p=0.01$).

Total milk intake showed a negative association between narrow neck buckling ratio ($\beta=-0.3$, $p=0.03$) and an increased hip axis length ($\beta=1$, $p=0.04$).
Since the various subtypes in intensities of physical activity were closely related, vigorous and very vigorous activity was chosen to put into this multivariate model. This was positively associated with narrow neck and intertrochanteric CSA ($\beta=0.003$, $p=0.01$; $\beta=0.004$, $p=0.02$) sub-periosteal width ($\beta=0.003$, $p=0.06$; $\beta=0.003$, $p=0.04$) and Z modulus ($\beta=0.003$, $p=0.01$; $\beta=0.003$, $p=0.01$).

When triceps thickness was added to the multivariate model, the number of children's scans available for analysis reduced to 142. Triceps skinfold thickness was negatively associated with narrow neck CSA (β=-0.02, p=0.05) and Z modulus (β=-0.01, p=0.03), Intertrochanteric CSA (=-0.02, p= 0.03), sub-periosteal width (β=-0.2, p=0.02) cortical thickness (β =-0.003, p=0.04) and Z modulus (β =-0.02, p=0.006) and femoral shaft BMD (β =-0.008 p=0.03) CSA (β =-0.01, p=0.04) and cortical thickness (β =-0.005, p=0.03). The relationship between grip strength and hip structure was significantly weakened after triceps skinfold was added to the model and the association between height only remained for narrow neck section modulus (β=0.007, p=0.01). The only association seen between physical activity and hip structure was for sub-periosteal width at the narrow neck (β=0.002, p=0.02); the associations between narrow neck and intertrochanteric CSA and z modulus did not quite reach significance but a trend remained (all $p<0.1$). It was noted that there was a significant negative association between triceps skinfold thickness and vigorous activity (r=-0.31, $p<0.0001$) suggesting that those with higher skinfold thickness did less vigorous activity.

When looking at the multivariate model, without measures of physical activity, the number of participants included in the model increased to 293. Height was once again positively associated with narrow neck CSA (β=0.01, p=0.006) and Z modulus (β=0.006, p=0.001), intertrochanteric Z modulus (β=0.008, p=0.02) and femoral shaft z modulus (β=0.005, p=0.001) and buckling ratio β=0.04, $p<0.001$). Triceps skinfold thickness was negatively associated with all measures of hip structure and strength (except buckling ratio) at all three sites of the hip (all $p<0.05$). Grip strength was only associated with femoral shaft Z modulus (β=0.006, p=0.007) and total milk intake was positively associated with femoral shaft CSA (β=0.02, p=0.03) and negatively associated with narrow neck buckling ratio (β=0.02, p=0.03).

TABLE 40: Mutually adjusted childhood determinants of childhood hip structure at age 6 years

	Narrow Neck BMD (g/cm^2)	Cross sectional area (cm^2)	Sub periosteal width (cm)	Cortical thickness (cm)	Section of modulus (cm^4)	Buckling ratio
R^2 as a %	**20**	**39**	**41**	**18**	**41**	**9**
Childs height (cm)	0.001 (-0.003,0.005)	0.02 (0.006,0.02)**	0.02 (0.01,0.03)***	0.00008 (-0.0007,0.0008)	0.01 (0.006,0.02)***	0.05 (-0.005,0.1)
log. Child's weight (kg)	0.1 (-0.02,0.3)	0.4 (0.05,0.8)*	0.2 (-0.08,0.5)	0.03 (-0.004,0.06)	0.2 (-0.03,0.4)	-0.01 (-2.2,2.2)
Maximum grip strength (kg)	0.01 (0.005,0.02)***	0.02 (0.009,0.04)**	0.004 (-0.008,0.02)	0.002 (0.0009,0.003)***	0.01 (0.003,0.02)**	-0.1 (-0.2,-0.02)*
Very vigorous activity (mins/day)	0.0005 (-0.0004,0.001)	0.003 (0.0007,0.006)*	0.003 (0.0008,0.005)**	0.0001 (-0.0001,0.0003)	0.002 (0.0004,0.003)**	0.003 (-0.01,0.02)
Total milk intake (pints/day)	0.01 (-0.005,0.03)	0.003 (-0.04,0.04)	-0.03 (-0.06,0.005)	0.002 (-0.0010,0.005)	-0.004 (-0.02,0.02)	-0.3 (-0.5,-0.03)*
	Intertrochanter BMD (g/cm^2)	**Cross sectional area (cm^2)**	**Sub periosteal width (cm)**	**Cortical thickness (cm)**	**Section of modulus (cm^4)**	**Buckling ratio**
R^2 as a %	**11**	**36**	**39**	**8**	**41**	**9**
Childs height (cm)	0.0006 (-0.004,0.005)	0.02 (0.002,0.03)*	0.02 (0.009,0.03)***	-0.0003 (-0.002,0.002)	0.02 (0.007,0.02)***	0.04 (-0.009,0.09)
log. Child's weight (kg)	0.05 (-0.1,0.2)	0.7 (0.1,1.2)*	0.6 (0.2,1.1)**	0.02 (-0.05,0.1)	0.4 (0.07,0.8)*	1.2 (-0.8,3.1)
Maximum grip strength (kg)	0.010 (0.003,0.02)**	0.04 (0.02,0.06)***	0.01 (-0.004,0.03)	0.004 (0.0008,0.007)*	0.02 (0.004,0.03)*	-0.06 (-0.1,0.01)
Very vigorous activity (mins/day)	0.0006 (-0.0005,0.002)	0.004* (0.0006,0.007)	0.003 (0.0001,0.006)*	0.0004 (-0.0001,0.0008)	0.003 (0.0007,0.005)*	-0.002 (-0.01,0.010)
Total milk intake (pints/day)	0.003 (-0.01,0.02)	0.03 (-0.03,0.08)	0.03 (-0.02,0.07)	0.002 (-0.006,0.010)	0.01 (-0.02,0.05)	-0.02 (-0.2,0.2)
	Femoral shaft BMD (g/cm^2)	**Cross sectional area (cm^2)**	**Sub periosteal width (cm)**	**Cortical thickness (cm)**	**Section of modulus (cm^4)**	**Buckling ratio**
R^2 as a %	**10**	**45**	**49**	**4**	**54**	**11**
Childs height (cm)	-0.002 (-0.007,0.003)	0.008 (-0.0002,0.02)	0.01 (0.008,0.02)***	-0.002 (-0.005,0.0005)	0.008 (0.004,0.01)***	0.04 (0.01,0.06)**
log. Child's weight (kg)	0.2 (0.02,0.4)*	0.7 (0.4,1.0)***	0.4 (0.1,0.6)**	0.08 (-0.02,0.2)	0.3 (0.2,0.5)***	-0.06 (-1.0,0.9)
Maximum grip strength (kg)	0.008 (0.0004,0.01)*	0.02 (0.010,0.04)***	0.009 (-0.0007,0.02)	0.003 (-0.001,0.007)	0.01 (0.006,0.02)***	-0.02 (-0.06,0.02)
Very vigorous activity (mins/day)	0.00004 (-0.001,0.001)	0.001 (-0.001,0.003)	0.001 (-0.0004,0.003)	-0.00004 (-0.0007,0.0006)	0.0009 (-0.0002,0.002)	0.001 (-0.005,0.008)
Total milk intake (pints/day)	0.005 (-0.01,0.02)	0.02 (-0.01,0.06)	0.01 (-0.01,0.04)	0.0008 (-0.009,0.01)	0.01 (-0.005,0.03)	0.003 (-0.09,0.1)

table shows β and 95% CI; *p<0.05, **p<0.01, ***p<0.001

6.3.5 Maternal determinants of childhood hip structure

Table 41 shows the relationship between various anthropometric and lifestyle determinants of hip structure at 6 years.

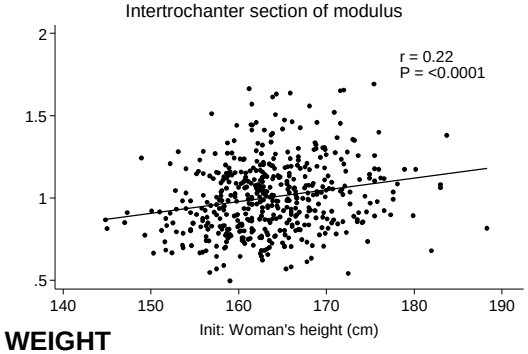
Figure 13 shows scattergraphs of the relationships between maternal height, weight and BMI and intertrochanteric section of modulus, cross sectional area and sub periosteal width. Maternal height was positively associated with narrow neck, intertrochanteric and femoral shaft CSA, sub-periosteal width and Z modulus (all $p<0.001$). In addition it was also positively associated with intertrochanteric and femoral shaft buckling ratio ($p<0.01$). Weight and BMI were positively associated with narrow neck, intertrochanteric and femoral shaft BMD, CSA and Z modulus. They were also associated with cortical thickness at the narrow neck and femoral shaft. In addition BMI was also associated with cortical thickness at the intertrochanteric region. Both height and weight were associated with the hip axis length ($r=0.29$, $p<0.001$, $r=0.2$, $p<0.001$ respectively). However there was no relationship seen with the shaft angle.

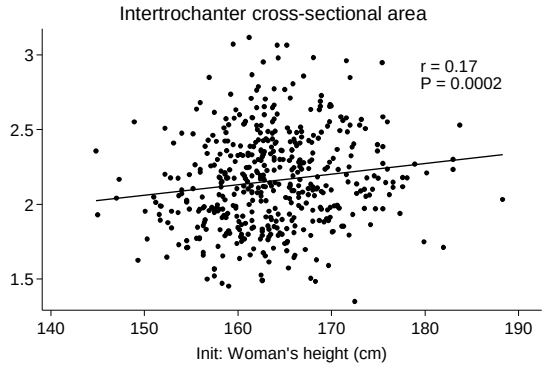
There was no association between maternal social class, educational attainment and smoking status and any measure of hip structure in the child. The largest lifestyle predictor was the frequency of strenuous exercise in late pregnancy which was positively associated with narrow neck and intertrochanteric BMD, CSA and cortical thickness (all $p<0.05$), intertrochanteric Z modulus ($r=0.12$, $p=0.009$) and negatively associated with intertrochanteric buckling ratio ($r=-0.07$, $p=0.02$). The relatively few mothers that undertook regular strenuous activity during this part of their pregnancy appeared to drive this relationship.

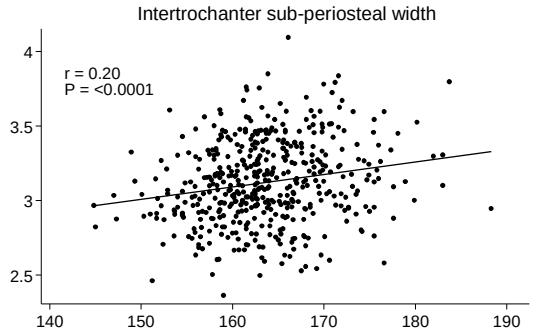
Pre pregnancy calcium and vitamin B12 intake were both positively associated with hip axis length ($r=0.14$, $p<0.001$, $r=0.13$, $p=0.004$). When looking at the other parameters of hip structure there was a suggestion that both these nutrients were associated with CSA and Z modulus at all three sites, however this did not quite reach significance (all $p<0.1$). In addition pre pregnancy energy intake ($\beta=2.1$, $p=0.01$), protein ($\beta=2.2$, $p=0.01$) and vitamin D intake ($\beta=0.8$, $p=0.03$) were all associated with hip axis length.

Figure 13: Effect of maternal pre pregnancy height, weight and BMI on intertrochanteric section modulus, cross sectional area, and sub-periosteal width

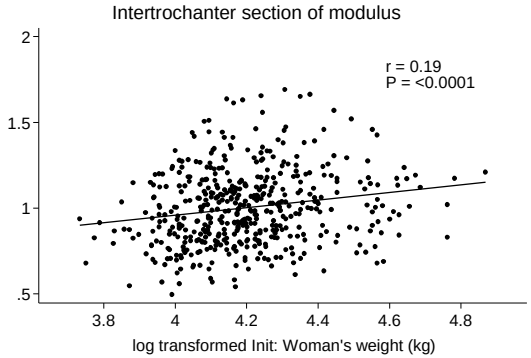
(A) HEIGHT

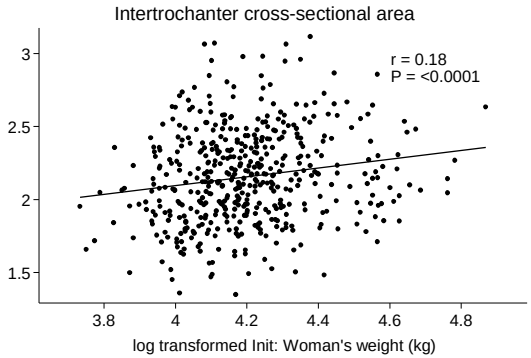


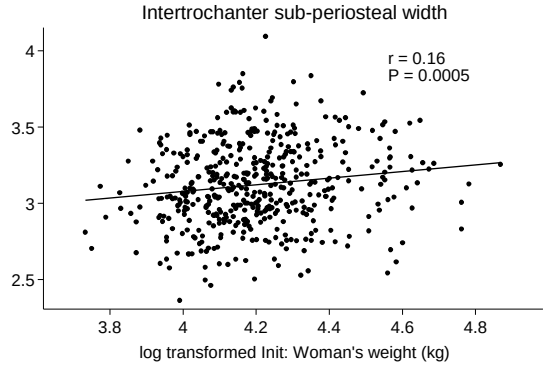




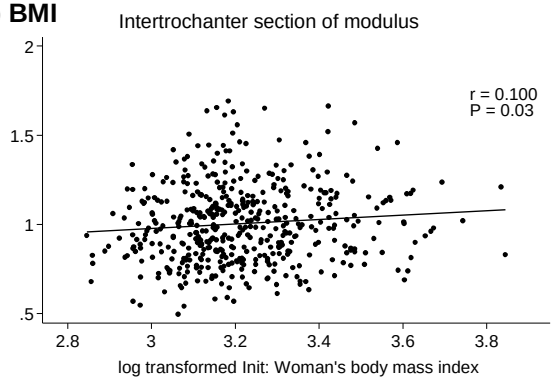
(B) WEIGHT

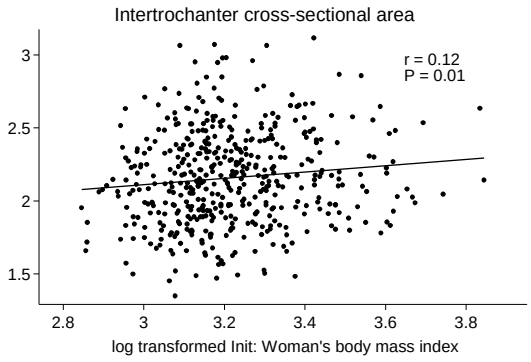






(C) BMI





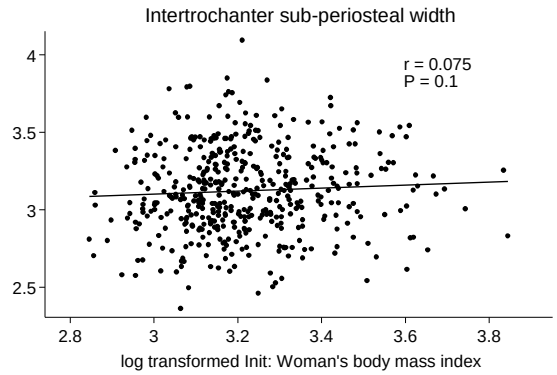


TABLE 41: Maternal determinants of child's hip structure at age 6 years in univariate regression analysis

	Narrow Neck BMD (g/cm^2)	Cross sectional area (cm^2)	Sub periosteal width (cm)	Cortical thickness (cm)	Section of modulus (cm^4)	Buckling ratio
Anthropometry						
Woman's height (cm)	0.001 (-0.000009,0.002)*	0.007 (0.004,0.01)***	0.006 (0.004,0.009)***	0.0002 (-0.00003,0.0004)	0.004 (0.002,0.006)***	0.01 (-0.006,0.03)
log: Woman's weight (kg)	0.07 (0.03,0.1)***	0.3 (0.2,0.4)***	0.2 (0.08,0.3)***	0.01 (0.006,0.02)**	0.1 (0.08,0.2)***	-0.2 (-0.8,0.4)
log: Woman's body mass index	0.06 (0.02,0.1)**	0.2 (0.06,0.3)**	0.08 (-0.02,0.2)	0.01 (0.004,0.02)*	0.09 (0.03,0.2)**	-0.4 (-1.0,0.2)
Lifestyle						
log: PP Total calcium (mg/day)	0.009 (-0.01,0.03)	0.05 (-0.02,0.1)	0.04 (-0.01,0.08)	0.002 (-0.003,0.006)	0.03 (-0.006,0.06)	0.04 (-0.3,0.3)
log PP :Total B12(mcg/day)	0.01 (-0.001,0.03)	0.04 (-0.002,0.08)	0.02 (-0.01,0.05)	0.003 (-0.0003,0.006)	0.02 (-0.0009,0.04)	-0.1 (-0.3,0.1)
LP: Strenuous exercise per week (hr)	0.002 (0.0005,0.004)*	0.006 (0.0007,0.01)*	0.0008 (-0.003,0.005)	0.0005 (0.0001,0.0008)*	0.002 (-0.0005,0.005)	-0.02 (-0.04,0.005)
	Intertrochanter BMD (g/cm^2)	**Cross sectional area (cm^2)**	**Sub periosteal width (cm)**	**Cortical thickness (cm)**	**Section of modulus (cm^4)**	**Buckling ratio**
Anthropometry						
Woman's height (cm)	0.0005 (-0.0007,0.002)	0.007 (0.003,0.01)**	0.008 (0.005,0.01)***	-0.0002 (-0.0007,0.0004)	0.007 (0.004,0.01)***	0.02 (0.007,0.03)**
log: Woman's weight (kg)	0.05 (0.009,0.09)*	0.3 (0.1,0.5)***	0.2 (0.08,0.4)**	0.02 (-0.0009,0.04)	0.2 (0.1,0.3)***	0.01 (-0.5,0.5)
log: Woman's body mass index	0.05 (0.002,0.09)*	0.2 (0.05,0.4)*	0.1 (-0.05,0.2)	0.02 (0.003,0.04)*	0.1 (0.008,0.2)*	-0.3 (-0.9,0.2)
Lifestyle						
log: PP Total calcium (mg/day)	0.007 (-0.02,0.03)	0.07 (-0.02,0.2)	0.06 (-0.01,0.1)	0.005 (-0.006,0.01)	0.05 (-0.008,0.1)	0.02 (-0.2,0.3)
log PP :Total B12(mcg/day)	0.01 (-0.003,0.03)	0.06 (0.002,0.1)*	0.04 (-0.01,0.08)	0.006 (-0.001,0.01)	0.03 (-0.006,0.07)	-0.04 (-0.2,0.1)
LP: Strenuous exercise per week (hr)	0.003 (0.001,0.005)**	0.01 (0.005,0.02)**	0.003 (-0.003,0.009)	0.002 (0.0008,0.002)***	0.006 (0.002,0.01)**	-0.02 (-0.04,-0.002)*
	Femur shaft BMD (g/cm^2)	**Cross sectional area (cm^2)**	**Sub periosteal width (cm)**	**Cortical thickness (cm)**	**Section of modulus (cm^4)**	**Buckling ratio**
Anthropometry						
Woman's height (cm)	-0.00001 (-0.001,0.001)	0.006 (0.003,0.009)***	0.007 (0.005,0.009)***	-0.0004 (-0.001,0.0002)	0.005 (0.003,0.006)***	0.01 (0.008,0.02)***
log: Woman's weight (kg)	0.07 (0.02,0.1)**	0.3 (0.1,0.4)***	0.1 (0.07,0.2)***	0.03 (0.003,0.05)*	0.1 (0.07,0.2)***	0.07 (-0.2,0.3)
log: Woman's body mass index	0.08 (0.03,0.1)**	0.2 (0.07,0.3)**	0.05 (-0.03,0.1)	0.04 (0.01,0.06)**	0.07 (0.005,0.1)*	-0.2 (-0.4,0.09)
Lifestyle						
log: PP Total calcium (mg/day)	0.01 (-0.01,0.04)	0.05 (-0.008,0.1)	0.03 (-0.02,0.07)	0.006 (-0.006,0.02)	0.03 (-0.005,0.06)	-0.004 (-0.1,0.1)
log PP :Total B12(mcg/day)	0.005 (-0.01,0.02)	0.02 (-0.01,0.06)	0.02 (-0.01,0.04)	0.001 (-0.007,0.010)	0.02 (-0.003,0.04)	0.003 (-0.08,0.09)
LP: Strenuous exercise per week (hr)	0.001 (-0.0005,0.003)	0.002 (-0.002,0.007)	-0.0003 (-0.003,0.003)	0.0007 (-0.0003,0.002)	0.0007 (-0.002,0.003)	-0.006 (-0.02,0.004)

table shows β and 95% CI; *p<0.05, **p<0.01, ***p<0.001

6.3.6 Independent maternal predictors of hip strength

Maternal factors, which showed a significant association with the child's hip structure, were added to a multivariate model. When looking at whether any of the maternal macro or micronutrients should be included, the nutrients that were shown to be related to hip axis length in univariate regression were adjusted for each other using multivariate regression (pre pregnancy total energy, protein, vitamin B12, vitamin D and calcium). Whilst no relationship was observed for hip axis length, calcium was seen to be positively associated with femoral shaft CSA (β=0.1, p=0.02) and Z modulus (β=0.05, p=0.04). Vitamin B12 was additionally added to the model following the weak associations seen in the univariate analysis.

Table 42 shows the independent relationship between maternal height, weight, pre pregnancy vitamin B12, pre pregnancy calcium intake and strenuous activity in late pregnancy and childhood hip structure at age 6 years. Whilst overall the R^2 for the models are small, the largest predictor was weight followed by height. Weight was positively associated with narrow neck, intertrochanteric and femoral shaft BMD, CSA, cortical thickness, Z modulus (all p<0.05). Maternal height was associated with narrow neck CSA (β=0.004, p=0.02), sub-periosteal width (β=0.005, p<0.001) and Z modulus (β=0.003, p=0.003), intertrochanteric sub-periosteal width (β=0.007, p=0.001) Z modulus (β=0.005, p=0.001) and buckling ratio (β=0.03, p=0.001) and femoral shaft CSA (β=0.003 p=0.04) sub-periosteal width (β=0.006, p<0.001), cortical thickness (β=-0.001, p=0.005), Z modulus (0.004, p<0.001) and buckling ratio (β=0.02, p<0.001). No relationship was observed between either pre pregnancy calcium, or vitamin B12 intake and hip structure. Strenuous activity in late pregnancy however remained significantly associated with narrow neck and intertrochanteric BMD (β=0.002, p=0.009; β=0.003, p=0.002), CSA (β=0.006,p=0.01; β=0.01, p=0.001) and cortical thickness (β=0.0005, p=0.009; β=0.002, p<0.001) in addition to a positive association between intertrochanteric z modulus (β=0.007, p=0.005) and a negative buckling ratio (β=-0.02, p=0.04).

TABLE 42: Mutually adjusted maternal determinants of childhood hip structure at age 6 years

	Narrow Neck BMD (g/cm^2)	**Cross sectional area (cm^2)**	**Sub periosteal width (cm)**	**Cortical thickness (cm)**	**Section of modulus (cm^4)**	**Buckling ratio**
R^2 as a %	**5**	**8**	**7**	**4**	**8**	**2**
Woman's height (cm)	0.0003 (-0.0010,0.002)	0.004* (0.0007,0.008)	0.005 (0.003,0.008)***	0.00003 (-0.0002,0.0003)	0.003 (0.0009,0.004)**	0.02 (-0.002,0.03)
log: Woman's weight (kg)	0.07(0.02,0.1)**	0.2(0.10,0.3)***	0.1 (0.01,0.2)*	0.01 (0.005,0.02)*	0.1 (0.05,0.2)***	-0.4 (-1.0,0.3)
log: PP Total calcium (mg/day)	-0.002 (-0.03,0.02)	0.009 (-0.07,0.08)	0.02 (-0.04,0.07)	-0.0005 (-0.006,0.005)	0.005 (-0.03,0.04)	0.1 (-0.3,0.5)
log PP :Total B12(mcg/day)	0.01 (-0.005,0.03)	0.03 (-0.02,0.08)	0.006 (-0.03,0.04)	0.003 (-0.0010,0.006)	0.01 (-0.01,0.04)	-0.2 (-0.4,0.10)
LP: Strenuous exercise (hr/week)	0.002(0.0006,0.004)**	0.006* (0.001,0.01)	0.001 (-0.003,0.005)	0.0005 (0.0001,0.0008)**	0.002 (-0.0002,0.005)	-0.02 (-0.04,0.006)
	Intertrochanter BMD (g/cm^2)	**Cross sectional area (cm^2)**	**Sub periosteal width (cm)**	**Cortical thickness (cm)**	**Section of modulus (cm^4)**	**Buckling ratio**
R^2 as a %	**4**	**7**	**5**	**5**	**8**	**4**
Woman's height (cm)	-0.0001 (-0.001,0.001)	0.004 (-0.0007,0.009)	0.007 (0.003,0.01)**	-0.0005 (-0.001,0.00008)	0.005 (0.002,0.009)**	0.03 (0.01,0.04)***
log: Woman's weight (kg)	0.05 (0.004,0.10)*	0.2 (0.08,0.4)**	0.1 (-0.01,0.3)	0.02 (0.004,0.04)*	0.2 (0.04,0.3)**	-0.3 (-0.8,0.2)
log: PP Total calcium (mg/day)	-0.004 (-0.03,0.02)	0.01 (-0.09,0.1)	0.03 (-0.06,0.1)	0.0005 (-0.01,0.01)	0.02 (-0.05,0.09)	0.04 (-0.3,0.4)
log PP :Total B12(mcg/day)	0.01 (-0.005,0.03)	0.05 (-0.02,0.1)	0.02 (-0.04,0.07)	0.006 (-0.002,0.01)	0.02 (-0.03,0.06)	-0.08 (-0.3,0.1)
LP: Strenuous exercise (hr/week)	0.003 (0.001,0.005)**	0.01 (0.005,0.02)***	0.003 (-0.002,0.009)	0.002 (0.0008,0.002)***	0.007(0.002,0.01)**	-0.02 (-0.04,-0.0007)*
	Femur shaft BMD (g/cm^2)	**Cross sectional area (cm^2)**	**Sub periosteal width (cm)**	**Cortical thickness (cm)**	**Section of modulus (cm^4)**	**Buckling ratio**
R^2 as a %	**3**	**6**	**10**	**3**	**8**	**5**
Woman's height (cm)	-0.001 (-0.003,0.0002)	0.003 (0.0001,0.007)*	0.006 (0.004,0.009)***	-0.001 (-0.002,-0.0003)**	0.004 (0.002,0.005)***	0.02 (0.01,0.02)***
log: Woman's weight (kg)	0.08 (0.03,0.1)***	0.2 (0.10,0.3)***	0.07 (-0.010,0.1)	0.04 (0.01,0.07)**	0.08 (0.02,0.1)**	-0.1 (-0.4,0.1)
log: PP Total calcium (mg/day)	0.02 (-0.01,0.05)	0.03 (-0.04,0.1)	0.003 (-0.04,0.05)	0.009 (-0.006,0.02)	0.006 (-0.03,0.04)	-0.04 (-0.2,0.1)
log PP :Total B12(mcg/day)	0.0009 (-0.02,0.02)	0.008 (-0.04,0.05)	0.008 (-0.02,0.04)	-0.0008 (-0.01,0.009)	0.01 (-0.01,0.03)	-0.0003 (-0.1,0.10)
LP: Strenuous exercise (hr/week)	0.002 (-0.0004,0.003)	0.003 (-0.002,0.007)	0.00004 (-0.003,0.003)	0.0007 (-0.0003,0.002)	0.0009 (-0.001,0.003)	-0.006 (-0.02,0.004)

table shows β and 95% CI; *p<0.05, **p<0.01, ***p<0.001

6.3.7 Independent childhood and maternal predictors of childhood hip strength

The first multivariate model that was created included all childhood and maternal determinates described in tables 40 and 42 (maternal and childhood height and weight, maternal pre pregnancy calcium and vitamin B12 intake, strenuous activity in late pregnancy and the child's grip strength, total milk intake and amount of very vigorous activity). The results are shown in table 43. The total number of participants in this model was 135.

The relationship between maternal height and weight and the child's hip structure is much weaker, in that height only negatively predicts intertrochanteric and femoral shaft cortical thickness (β=-0.001, p=0.01 β=-0.002, p=0.005) and positively predicts buckling strength ratio (β=0.03, p=0.04; β =0.02, p=0.002). Pre pregnancy vitamin B12 intake was negatively associated with femoral shaft BMD, cortical thickness and positively associated with an increased buckling strength ratio. Strenuous activity in late pregnancy remained positively associated with intertrochanteric CSA (β=0.01, p=0.008), cortical thickness (β=0.001, p=0.01) and Z modulus (β=0.008, p=0.02).

Childhood height was associated with narrow neck, intertrochanteric and femoral shaft Z modulus, in addition to narrow neck CSA, whereas childhood weight was independently associated with intertrochanteric and femoral shaft CSA and Z modulus only. The child's grip strength was positively associated with narrow neck, intertrochanteric and femoral shaft BMD, CSA and Z modulus in addition to increased cortical thickness at the narrow neck and intertrochanteric sites. Milk intake was only associated with a reduced bucking strength ratio at the narrow neck (β=-0.3, p=0.03).

Increased time spent doing vigorous or very vigorous activity was positively associated with increased narrow neck, intertrochanteric and femoral shaft Z modulus (β=0.002, p=0.008; β=0.003, p=0.005; β=0.001, p=0.04) in addition to increased CSA at the narrow neck and intertrochanteric sites (β=0.004, p=0.009; β=0.005, p=0.008 respectively).

When triceps skinfold thickness was additionally added to the model the only difference in maternal factors was that pre pregnancy calcium intake was associated with increased cortical thickness (β=0.03, p=0.04) and a reduced buckling strength ratio (β=-0.3, p=0.05) at the femoral shaft. Triceps skinfold thickness remained an important negative independent predictor of narrow neck and intertrochanteric CSA (β =-0.02, p=0.04; β –0.03, p=0.02 respectively) and Z modulus (β =-0.01, p=0.02; β=-0.02, p=0.006 respectively), intertrochanteric cortical thickness (β=-0.004, p=0.03) and femoral shaft BMD (β=-0.008, p=0.04) and cortical thickness (β=-0.004, p=0.04). The relationship with the child's height was only significant for narrow neck Z modulus, femoral shaft buckling ratio and hip axis length, however weight was positively associated with narrow neck, intertrochanteric and femoral shaft CSA and Z modulus (all $p<0.01$).The relationship between grip strength was once again significantly weaker. However an association remained between narrow neck BMD (β=0.009, p=0.01), CSA (β =0.02, p=0.04) and buckling ratio (β=-0.1, p=0.05), intertrochanteric BMD (β=0.008, p=0.03) and CSA (β=0.03, p=0.01) and femoral shaft CSA (β=0.02, p=0.006) and Z modulus (β=0.01, p=0.001). The relationship between vigorous activity was also significantly weakened, only narrow neck and intertrochanteric CSA remained significant (β=0.003, p=0.05; β=0.004, p=0.05). However the Z modulus at these sites showed a trend towards significance (p=0.08, p=0.06 respectively).

To increase the numbers in the model, if physical activity was excluded, 282 participants were included. The negative relationship between triceps skinfold thickness became stronger in that it was now associated with all measures of hip structure and strength (except buckling ratio) at all three sites. The relationship between maternal determinants remained very similar, as did childhood height and weight however grip strength was only associated with femoral shaft CSA (β=0.01, p=0.04). Milk intake was associated with increased narrow neck BMD (β=0.01, p=0.05), cortical thickness (β=0.002, p=0.05) and reduced buckling strength ratio (β=-0.2, p=0.004) and CSA at the femoral shaft (β=0.02, p=0.03).

TABLE 43: Mutually adjusted maternal and childhood determinants of childhood hip structure

	Narrow Neck BMD (g/cm^2)	Cross sectional area (cm^2)	Sub periosteal width (cm)	Cortical thickness (cm)	Section of modulus (cm^4)	Buckling ratio
R^2 as a %	**22**	**40**	**42**	**20**	**42**	**11**
Woman's height (cm)	-0.001 (-0.003,0.001)	-0.002 (-0.008,0.004)	0.0003 (-0.004,0.005)	-0.0002 (-0.0007,0.0003)	-0.0009 (-0.004,0.002)	0.01 (-0.02,0.05)
log: Woman's weight (kg)	0.007 (-0.07,0.09)	0.06 (-0.2,0.3)	0.06 (-0.10,0.2)	0.002 (-0.02,0.02)	0.05 (-0.06,0.2)	0.2 (-1.0,1.5)
log: PP Total calcium (mg/day)	-0.01 (-0.06,0.03)	-0.06 (-0.2,0.06)	-0.05 (-0.1,0.04)	-0.002 (-0.01,0.007)	-0.04 (-0.10,0.02)	0.1 (-0.6,0.8)
log PP :Total B12(mcg/day)	-0.005 (-0.04,0.03)	0.001 (-0.08,0.08)	0.02 (-0.04,0.09)	-0.001 (-0.008,0.006)	0.008 (-0.04,0.05)	0.1 (-0.3,0.6)
LP: Strenuous exercise (hr/week)	0.002 (-0.0002,0.004)	0.004 (-0.002,0.010)	-0.0005 (-0.005,0.004)	0.0004 (-0.00005,0.0009)	0.001 (-0.002,0.004)	-0.02 (-0.06,0.01)
Childs height (cm)	0.001 (-0.003,0.005)	0.02 (0.004,0.03)**	0.02 (0.010,0.03)***	0.0001 (-0.0008,0.0010)	0.01 (0.005,0.02)***	0.05 (-0.02,0.1)
log. Child's weight (kg)	0.1 (-0.03,0.3)	0.4 (-0.004,0.9)	0.2 (-0.1,0.5)	0.03 (-0.006,0.06)	0.2 (-0.06,0.4)	-0.3 (-2.9,2.2)
Maximum grip strength (kg)	0.01 (0.004,0.02)***	0.03 (0.010,0.04)**	0.005 (-0.007,0.02)	0.002 (0.0009,0.003)**	0.01 (0.003,0.02)**	-0.1 (-0.2,-0.02)*
Total milk intake (pints/day)	0.01 (-0.005,0.03)	0.006 (-0.04,0.05)	-0.02 (-0.05,0.008)	0.002 (-0.001,0.006)	-0.002 (-0.02,0.02)	-0.3 (-0.5,-0.03)*
Very vigorous activity (mins/day)	0.0007 (-0.0003,0.002)	0.004 (0.0010,0.006)**	0.003 (0.0008,0.005)**	0.0001 (-0.00009,0.0003)	0.002 (0.0005,0.003)**	0.0009 (-0.01,0.02)
	Intertrochanter BMD (g/cm^2)	**Cross sectional area (cm^2)**	**Sub periosteal width (cm)**	**Cortical thickness (cm)**	**Section of modulus (cm^4)**	**Buckling ratio**
R^2 as a %	**14**	**4**	**41**	**16**	**44**	**15**
Woman's height (cm)	-0.001 (-0.004,0.0010)	-0.004 (-0.01,0.003)	0.0002 (-0.006,0.007)	-0.001 (-0.003,-0.0004)**	0.0003 (-0.005,0.005)	0.03 (0.002,0.06)*
log: Woman's weight (kg)	0.03 (-0.06,0.1)	0.04 (-0.2,0.3)	-0.08 (-0.3,0.2)	0.01 (-0.03,0.05)	0.03 (-0.2,0.2)	-0.5 (-1.6,0.5)
log: PP Total calcium (mg/day)	-0.003 (-0.05,0.05)	-0.06 (-0.2,0.09)	-0.08 (-0.2,0.05)	0.003 (-0.02,0.03)	-0.05 (-0.1,0.05)	-0.2 (-0.8,0.4)
log PP :Total B12(mcg/day)	-0.005 (-0.04,0.03)	0.01 (-0.10,0.1)	0.04 (-0.05,0.1)	-0.004 (-0.02,0.01)	-0.0004 (-0.07,0.07)	0.2 (-0.2,0.6)
LP: Strenuous exercise (hr/week)	0.002 (-0.0001,0.005)	0.01 (0.003,0.02)**	0.004 (-0.003,0.01)	0.001 (0.0003,0.003)*	0.008 (0.003,0.01)**	-0.02 (-0.05,0.01)
Childs height (cm)	0.0005 (-0.004,0.005)	0.01 (-0.001,0.03)	0.02 (0.006,0.03)**	0.0002 (-0.002,0.002)	0.01 (0.003,0.02)*	0.03 (-0.02,0.08)
log. Child's weight (kg)	0.05 (-0.1,0.2)	0.7 (0.1,1.3)*	0.8 (0.3,1.3)**	0.03 (-0.06,0.1)	0.5 (0.09,0.8)*	1.5 (-0.6,3.6)
Maximum grip strength (kg)	0.01 (0.003,0.02)**	0.04 (0.02,0.06)***	0.01 (-0.007,0.03)	0.004 (0.0008,0.007)*	0.02 (0.006,0.03)**	-0.08 (-0.2,0.002)
Total milk intake (pints/day)	0.003 (-0.01,0.02)	0.03 (-0.03,0.08)	0.02 (-0.02,0.07)	0.001 (-0.007,0.009)	0.02 (-0.02,0.05)	-0.03 (-0.2,0.2)
Very vigorous activity (mins/day)	0.0007 (-0.0004,0.002)	0.005 (0.001,0.008)**	0.004 (0.0007,0.007)*	0.0004 (-0.0001,0.0009)	0.003 (0.0010,0.006)**	-0.002 (-0.01,0.01)
	Femur shaft BMD (g/cm^2)	**Cross sectional area (cm^2)**	**Sub periosteal width (cm)**	**Cortical thickness (cm)**	**Section of modulus (cm^4)**	**Buckling ratio**
R^2 as a %	**18**	**45**	**52**	**14**	**56**	**20**
Woman's height (cm)	-0.003 (-0.006,-0.0008)*	-0.002 (-0.007,0.003)	0.005 (0.002,0.008)**	-0.002 (-0.004,-0.0006)**	0.002 (-0.0002,0.005)	0.02 (0.009,0.04)**
log: Woman's weight (kg)	0.03 (-0.07,0.1)	0.01 (-0.2,0.2)	-0.04 (-0.2,0.09)	0.02 (-0.03,0.07)	-0.03 (-0.1,0.06)	-0.1 (-0.6,0.4)
log: PP Total calcium (mg/day)	0.04 (-0.01,0.09)	0.008 (-0.09,0.1)	-0.07 (-0.1,0.0005)	0.03 (-0.002,0.06)	-0.04 (-0.09,0.01)	-0.3 (-0.5,0.02)
log PP :Total B12(mcg/day)	-0.04 (-0.08,-0.006)*	-0.05 (-0.1,0.02)	0.03 (-0.02,0.08)	-0.03* (-0.05,-0.005)	0.006 (-0.03,0.04)	0.2 (0.02,0.4)*
LP: Strenuous exercise (hr/week)	0.0009 (-0.002,0.004)	0.002 (-0.003,0.007)	0.0002 (-0.003,0.004)	0.0004 (-0.001,0.002)	0.0004 (-0.002,0.003)	-0.004 (-0.02,0.010)
Childs height (cm)	-0.00008 (-0.005,0.005)	0.009 (-0.001,0.02)	0.010 (0.003,0.02)**	-0.0008 (-0.004,0.002)	0.006 (0.001,0.01)*	0.02 (-0.003,0.05)
log. Child's weight (kg)	0.2 (-0.005,0.4)	0.7 (0.4,1.1)***	0.4 (0.2,0.7)**	0.08 (-0.03,0.2)	0.4 (0.2,0.6)***	0.03 (-1.0,1.1)
Maximum grip strength (kg)	0.008 (0.0002,0.02)*	0.02 (0.009,0.04)**	0.009 (-0.0007,0.02)	0.003 (-0.001,0.007)	0.01 (0.006,0.02)***	-0.02 (-0.06,0.02)
Total milk intake (pints/day)	0.004 (-0.01,0.02)	0.02 (-0.01,0.06)	0.02 (-0.007,0.04)	0.0003 (-0.01,0.01)	0.01 (-0.003,0.03)	0.01 (-0.08,0.1)
Very vigorous activity (mins/day)	0.0001 (-0.001,0.001)	0.002 (-0.0008,0.004)	0.001 (-0.00009,0.003)	-0.00005 (-0.0007,0.0006)	0.001 (0.00008,0.002)*	0.002 (-0.004,0.008)

table shows β and 95% CI; *p<0.05, **p<0.01, ***p<0.001

6.4 Discussion

The most robust associations observed with the child's hip structure and strength, when all determinants were adjusted for, were childhood weight and triceps skinfold thickness. However whilst weight was a strong positive predictor, explained by the increased loads and forces placed through the growing hip, in contrast triceps skinfold thickness was associated with reduced strength, BMD and cross sectional area. Children with higher skinfold thickness tend to do less vigorous activity but are taller and heavier. The results seen suggest that we are identifying this subgroup of children.

When excluding triceps skinfold thickness the importance of increased vigorous activity and grip strength become apparent. The activity monitors used in this study measure vertical movement (which tends to correspond to weight bearing activity). It is not surprising that increased amount of vigorous activity are associated with increased forces through the hip axis and bone adaptation resulting in increased strength. Grip strength, which is a good surrogate for muscle function and lean mass, was associated with increased BMD, cross sectional area and strength. One explanation for this is that the increased muscle gives advantageous loads onto the hip axis, supporting its growth.

Maternal factors did not seem as important in determining hip structure although maternal height and weight were strongly related to the child's height and weight. The small numbers of mothers that did strenuous activity in late pregnancy had children with increased hip structure and strength, however much of this relationship can be explained by the association with increased intensities of activity in their offspring. Whilst weak associations were seen with maternal calcium and vitamin B12 intake, much of the relationship was lost once adjusted for childhood milk intake suggesting that the child's diet is more important. Milk intake was associated with increased BMD and cortical thickness at the femoral neck and a reduced buckling strength ratio, although these relationships were not particularly strong it emphasises the importance of a diet rich in calcium in order to maximise osteogenic adaptation.

7 PATTERN OF GROWTH AND BONE MASS OF THE CHILD AT AGE 6 YEARS

7.1 Aims

- To determine maternal and childhood factors which influence childhood growth relative to peers
- To explore the relationships between growth in childhood and bone size and density at 6 years
- To determine how intra uterine growth predicts bone mineral accrual and bone strength at aged 6 years

7.2 Statistical analysis

Changes in body size at various time points are likely to be correlated, for example growth in height from 12-24 months is likely to predict growth from 24 to 36 months.

Conditional regression modelling derives uncorrelated measures of change over the total time period, thus reducing the problem of collinearity between measures. The first component is the birth z score (height or weight), next the residuals of the regression of the z score at age12 months on z score at birth are obtained. This represents the amount by which either the height or weight exceeds that which would have been predicted from the z score at birth. This is called the conditional gain from 0-12 months. Next the residuals of the regression of the z score at age 2 years on both the z score at birth and z score at age 12 months simultaneously are obtained. These residuals are uncorrelated with both the z score at birth and at age 12 months and are called the conditional gain from 12 months to 2 years. This method was additionally used to obtain conditional growth scores between the ages of 2-3 years, 3-4 years and 4-6 years using the anthropometric data that had been previously collected at these ages.

7.3 Results

7.3.1 Birthweight and 6 year bone mineral

Birthweight was associated with whole body BA (β=0.03, p<0.0001) and BMC (β=0.03, p<0.0001) but not vBMD. Similar results were observed for lumbar spine and hip bone mass. In addition birthweight was associated with increased cortical content (β=0.006, p=0.02), periosteal circumference (β=0.002, p=0.003), endosteal circumference (β=0.001, p=0.03) and measures of bone strength (SSI: β=0.03, p=0.02; fracture load x: β= 0.07, 0=0.02; fracture load y; β= 0.08, p=0.006). It was also associated with increased CSA, periosteal width and section modulus at the narrow neck, intertrochanteric and femoral shaft regions of the femoral neck (all p<0.01).

When birthweight was divided into quartiles, there was a step increase in 6 year height, weight and BMI from the lowest to highest quartile (table 44). Thus babies in the lowest quartile remained shorter, lighter and thinner compared to babies in the highest quartile.

TABLE 44: Birthweight and 6 year height, weight and BMI

	6 year height (cm) mean sd	**6 year weight (kg)** median IQR	**6 year BMI (kg/m^2)** median IQR
Mean Birthweight gp1	117.3 (5)	21.8 (19.8-24.3)	15.4 (14.5-16.8)
Mean Birthweight gp2	118.6 (5.2)	22.4 (20.8-25)	15.5 (14.8-16.5)
Mean Birthweight gp3	119.9 (4.7)	23.2 (21.5-25.8)	15.9 (15.3-16.8)
Mean Birthweight gp4	120.4 (4.9)	23.8 (22.1-26)	16.1 (15.3-17)
P value for trend	**<0.0001**	**0.0001**	**0.002**

7.3.2 Predictors of childhood growth

The predictors of the greatest gain in height and weight from birth to age 12 months were greater maternal height, being first born and being formula fed (table 45). There was no association with maternal class, however mothers that had higher educational attainment had infants with a reduced height gain compared to their peers (β=-0.07, p=0.05).

TABLE 45: Predictors of conditional change in growth between birth and aged 12 months

	Weight 0-12 months (sd)		Height 0-12 months (sd)	
	β (CI)	p value	β (CI)	p value
log: Woman's weight (kg)	-0.01 (-0.9,0.8)	0.978	-1.0 (-1.8,-0.1)	0.027
Woman's height (cm)	0.03 (0.02,0.05)	<0.0001	0.05 (0.03,0.07)	<0.0001
log Woman's triceps skinfold (mm)	0.09 (-0.3,0.5)	0.671	0.5 (0.1,0.9)	0.013
Parity, two groups	-0.2 (-0.4,-0.04)	0.016	-0.2 (-0.4,-0.04)	0.014
Currently smoke (EP)	0.2 (-0.03,0.5)	0.078	0.1 (-0.1,0.4)	0.321
Months completed breastfeeding	-0.03(-0.04,-0.01)	0.001	-0.03 (-0.05,-0.02)	<0.0001
R^2 as %	**9**		**13**	

EP = Early pregnancy

For the other age ranges children who grew at a faster rate than their peers between 1-2 years had been been breastfed for longer (weight: β=0.02, p=0.007 height: β=0.02, p=0.06). Furthermore children who grew faster between the ages of 3-4 years had mothers with a higher educational attainment (height: β=0.1, p=0.002) . Childhood milk intake at age 3 years also predicted increased height and weight velocity between the ages of 2-3 years (weight: β=0.2, p=0.001; height: β=0.1, p=0.002), however there was no further effect of either 4 or 6 year milk on subsequent growth velocity. Mothers that smoked during pregnancy had children with more rapid weight gain between the ages of 2-3 years (β=1.1, p<0.001), however smoking did not predict weight gain at any other age.

7.3.3 Childhood growth and whole body, lumbar spine and hip bone mineral accrual

Of the children that had DXA scans at aged 6 years 316 of them had previous measurements for height and weight at all time points (birth, 12 months, 2, 3, 4 and 6 years).

Table 46 shows the independent relationships between the relative gain in height through childhood. Greater growth in height relative to peers was associated with increased whole body BA, BMC and aBMD (p<0.001) but not vBMD (at all time points during the first 6 years of life)(shown in figure 14). A similar pattern was observed for lumbar spine and hip BA and BMC (p<0.01). Whilst growth was important at all stages, the largest increases in BA and BMC were seen if there was increased growth during 0-12 months (β=27.7, p<0.001; β= 29.8, p<0.001) and 1-2 years (β=21.4, p<0.001; β=26.1, p<0.001).

TABLE 46: Mutually adjusted relationships between conditional change in height from birth to 6 years and whole body, lumbar spine and hip bone mass

Conditional change in height (z score)	Whole body BA (cm^2)	BMC (g)	BMD (g/cm^2)	vBMD (g)
0-12m	27.7 (22.5,32.9)***	29.8 (24.3,35.4)***	0.01 (0.01,0.02)***	-2.4 (-5.2,0.4)
1-2yr	21.4 (16.1,26.7)***	26.1 (20.4,31.7)***	0.01 (0.01,0.02)***	2.6 (-0.3,5.4)
2-3yr	15.1 (9.8,20.3)***	19.0 (13.4,24.6)***	0.01 (0.008,0.02)***	0.6 (-2.3,3.4)
3-4yr	13.5 (8.2,18.8)***	15.4 (9.8,21.1)***	0.008 (0.004,0.01)***	0.3 (-2.5,3.2)
4-6yr	13.1 (7.8,18.4)***	16.2 (10.5,21.9)***	0.009 (0.005,0.01)***	-0.2 (-3.1,2.7)
R^2 as a %	**47**	**51**	**37**	**2**
	Lumbar spine			
	BA (cm^2)	**BMC (g)**	**BMD (g/cm^2)**	**vBMD (g)**
0-12m	1.0 (0.8,1.3)***	0.9 (0.7,1.1)***	0.01 (0.004,0.02)***	-0.1 (-0.3,0.08)
1-2yr	0.9 (0.7,1.2)***	1.0 (0.7,1.2)***	0.01 (0.008,0.02)***	0.2 (0.02,0.4)*
2-3yr	0.4 (0.1,0.7)**	0.5 (0.3,0.8)***	0.010 (0.004,0.02)**	0.02 (-0.2,0.2)
3-4yr	0.4 (0.1,0.7)**	0.4 (0.1,0.6)**	0.005 (-0.0010,0.01)	-0.03 (-0.2,0.2)
4-6yr	0.7 (0.4,1.0)***	0.5 (0.2,0.7)***	0.004 (-0.002,0.010)	-0.1 (-0.3,0.08)
R^2 as a %	**32**	**35**	**14**	**2**
	Hip			
	BA (cm^2)	**BMC (g)**	**BMD (g/cm^2)**	**vBMD (g)**
0-12m	0.8 (0.6,1.0)***	0.6*** (0.4,0.8)	0.003 (-0.004,0.010)	-0.1 (-0.2,-0.02)*
1-2yr	0.8 (0.6,0.9)***	0.7*** (0.5,0.9)	0.01 (0.005,0.02)***	0.07 (-0.04,0.2)
2-3yr	0.5 (0.3,0.7)***	0.4*** (0.2,0.6)	0.007 (0.0005,0.01)*	0.005 (-0.1,0.1)
3-4yr	0.3 (0.1,0.5)**	0.3*** (0.1,0.5)	0.008 (0.001,0.01)*	0.06 (-0.04,0.2)
4-6yr	0.4 (0.2,0.6)***	0.3*** (0.1,0.5)	0.005 (-0.001,0.01)	-0.005 (-0.1,0.1)
R^2 as a %	**38**	**32**	**8**	**2**

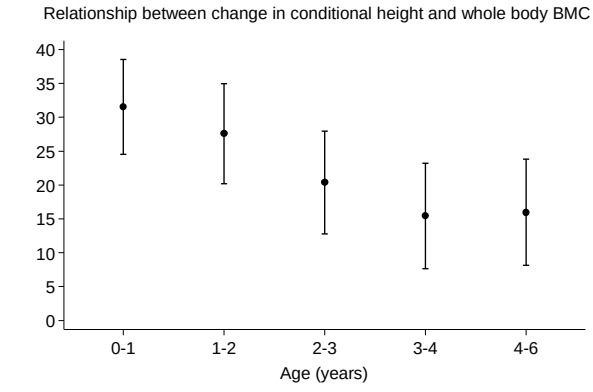
table shows β and CI; *p<0.05, **p<0.01, ***p<0.001

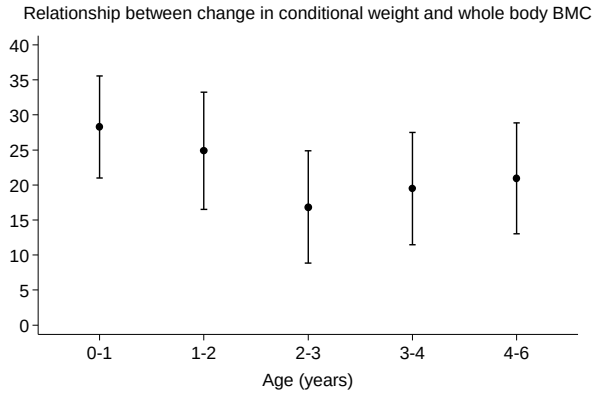
TABLE 47: Mutually adjusted relationships between conditional change in weight from birth to 6 years and whole body, lumbar spine and hip bone mass

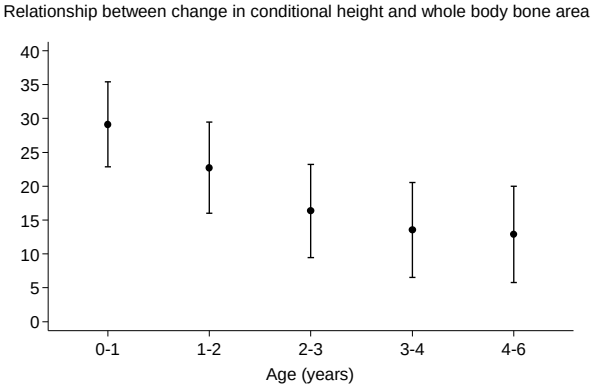
Conditional change in weight (z score)	Whole body BA (cm^2)	BMC (g)	BMD (g/cm^2)	vBMD (g)
0-12m	24.9 (19.1,30.8)***	30.4 (24.9,36.0)***	0.02 (0.01,0.02)***	-3.5 (-6.6,-0.4)*
1-2yr	16.2 (9.7,22.6)***	25.0 (18.9,31.2)***	0.02 (0.01,0.02)***	0.9 (-2.5,4.4)
2-3yr	9.9 (3.9,15.9)**	16.7 (11.0,22.4)***	0.01 (0.008,0.02)***	2 (-1.2,5.2)
3-4yr	13.5 (7.5,19.5)***	20.0 (14.3,25.7)***	0.01 (0.01,0.02)***	4.0 (0.7,7.2)*
4-6yr	13.3 (7.3,19.3)***	20.9 (15.2,26.6)***	0.01 (0.01,0.02)***	1.1 (-2.1,4.4)
R^2 as a %	**35**	**54**	**55**	**5**
	Lumbar spine			
	BA (cm^2)	**BMC (g)**	**BMD (g/cm^2)**	**vBMD (g)**
0-12m	1.2 (0.9,1.5)***	0.9 (0.7,1.2)***	0.009 (0.003,0.02)**	-0.2 (-0.4,0.01)
1-2yr	0.7 (0.4,1.0)***	0.9 (0.6,1.2)***	0.02 (0.009,0.02)***	0.1 (-0.09,0.4)
2-3yr	0.3 (-0.05,0.6)	0.4 (0.2,0.7)**	0.009 (0.002,0.02)**	0.05 (-0.2,0.3)
3-4yr	0.6 (0.3,0.9)***	0.9 (0.7,1.2)***	0.02 (0.01,0.03)***	0.4 (0.2,0.7)***
4-6yr	0.3 (0.02,0.7)*	0.3 (0.04,0.6)*	0.004 (-0.003,0.01)	-0.2 (-0.4,0.06)
R^2 as a %	**26**	**36**	**20**	**8**
	Hip			
	BA (cm^2)	**BMC (g)**	**BMD (g/cm^2)**	**vBMD (g)**
0-12m	0.7 (0.5,0.9)***	0.5 (0.3,0.7)***	0.003 (-0.004,0.010)	-0.1 (-0.3,-0.03)*
1-2yr	0.6 (0.4,0.8)***	0.5 (0.3,0.8)***	0.008 (-0.0002,0.02)	-0.02 (-0.2,0.1)
2-3yr	0.4 (0.2,0.6)***	0.4 (0.2,0.6)***	0.009 (0.002,0.02)*	0.04 (-0.08,0.2)
3-4yr	0.3 (0.09,0.5)**	0.5 (0.3,0.7)***	0.02 (0.008,0.02)***	0.2 (0.05,0.3)**
4-6yr	0.4 (0.2,0.6)***	0.4 (0.2,0.6)***	0.008 (0.0007,0.02)*	-0.0002 (-0.1,0.1)
R^2 as a %	**27**	**26**	**11**	**5**

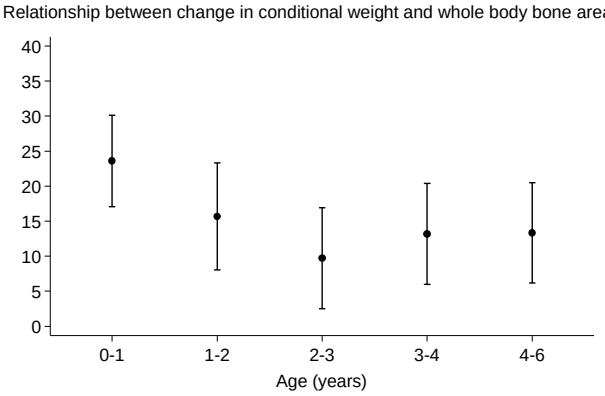
table shows β and CI; *p<0.05, **p<0.01, ***p<0.001

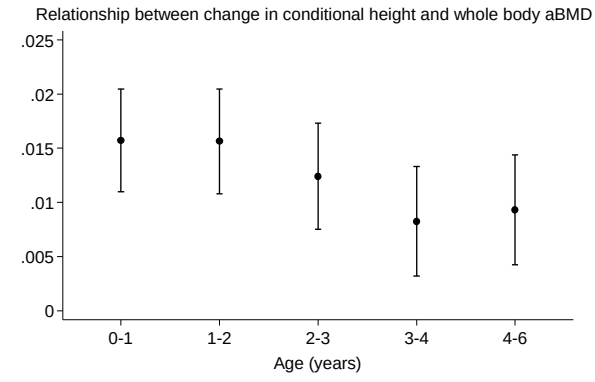
Figure 14: Graphs to show the relationship between change in conditional growth and whole body BMC, BA. aBMD and vBMD

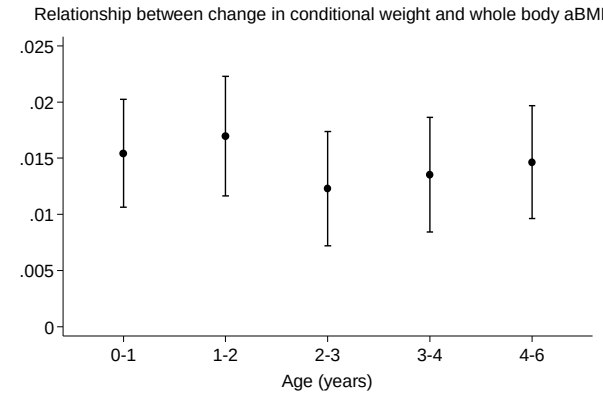


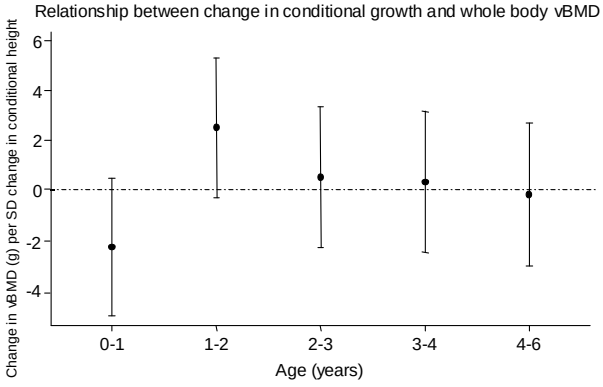


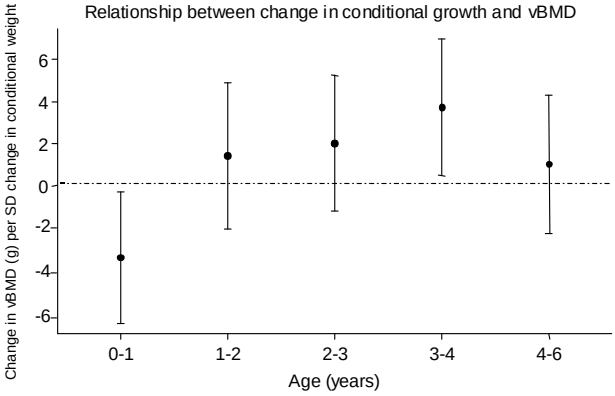












Graphs show mean and 95% confidence intervals
BMC: bone mineral content; BA: bone area; aBMD; areal bone mineral density;
vBMD estimated volumetric bone mineral density

Table 47 shows the independent relationships between the relative gain in weight through childhood and the bone mass of the child aged 6 years. Greater increases in weight relative to peers at all childhood timepoints were independently associated with increased whole body BA, BMC and a BMD (all $p<0.01$). A similar pattern was observed for the lumbar spine and hip. Increased weight gain during the ages 3-4 years appears to be associated with increased vBMD at whole body ($\beta=4.0$, $p=0.02$), lumbar spine ($\beta=0.4$, $p<0.001$) and hip ($\beta=0.2$, $p=0.008$). Weight gain during 0-12 months and 1-2 years were once again associated with the highest increases in BA and BMC at age 6 years.

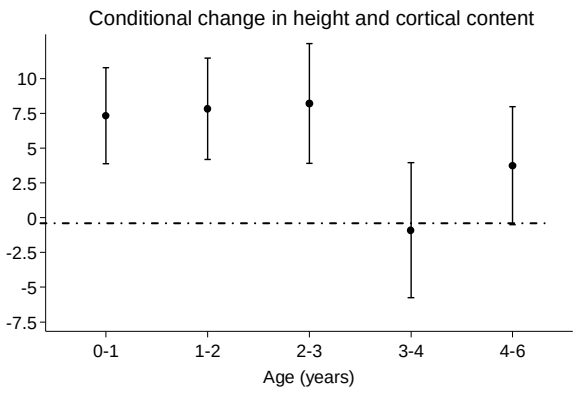
7.3.4 Childhood growth as a determinant of tibial bone structure and strength at age 6 years

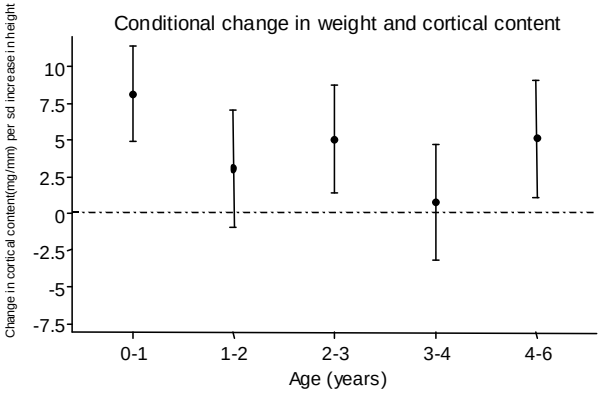
Of the children who underwent a pQCT scan at age 6 years, 79 had previous measurements of height and weight at all time points (birth, 12 months, 2, 3, 4 and 6 years).

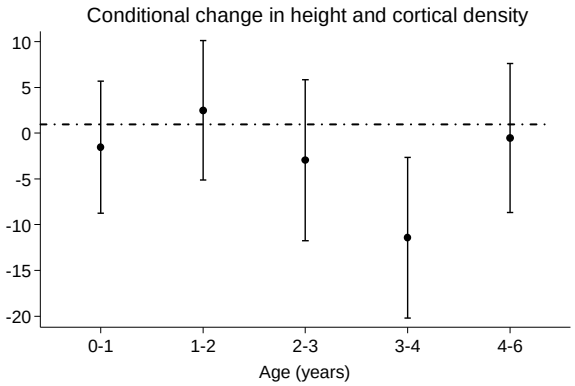
Table 48 shows the independent relationships between the relative gain in childhood height and weight and the tibial bone structure and strength of the child aged 6 years. Greater growth in height relative to peers, was independently associated with increased trabecular and cortical content, cortical thickness as well as measures of bone strength (stress strain index and fracture load in the x and y axis) at all increments of increasing age (except the gain between 3-4 years). There was no significant pattern observed for measures of true volumetric trabecular or cortical bone density. The relationship between bone size and strength appear to be greater for the relative gains seen at earlier ages. The results are shown graphically in figure 15.

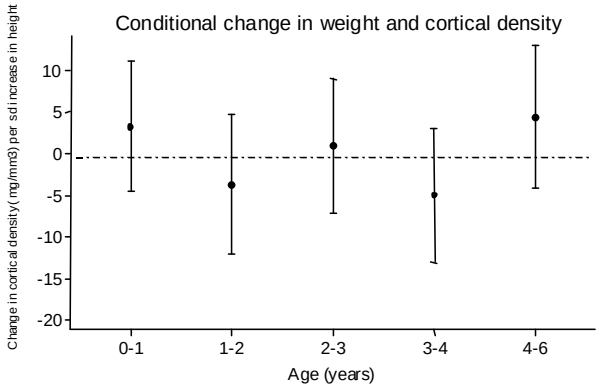
The relative increases in weight at ages 0-12 months, 1-2 and 4-6 years were independently associated with increased measures of cortical content ($p<0.001$), cortical thickness ($p<0.05$), stress strain index ($p<0.01$) and fracture load in the X and Y axis ($p<0.01$). In addition, increased weight gain between ages 2-3 years were associated with increased cortical content and fracture load in the X axis (both $p<0.001$). No relationship was observed between weight gain and either trabecular or cortical volumetric density. Once again the relationship between bone size and strength appear to be greater for the relative weight gains seen at earlier ages; however the difference was not as great as that observed for change in conditional height.

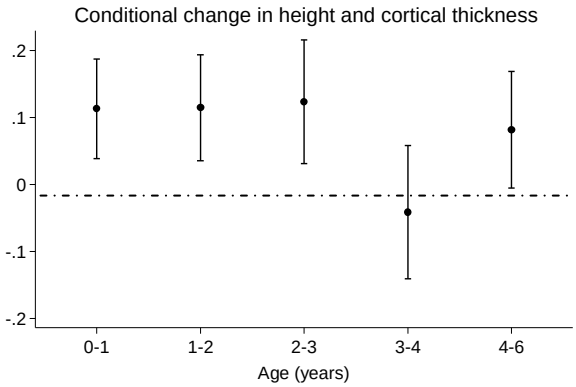
Figure 15: Conditional growth in height and weight (per sd increase) and cortical content, density and thickness and the subsequent bending strength measured by pQCT at 6 years

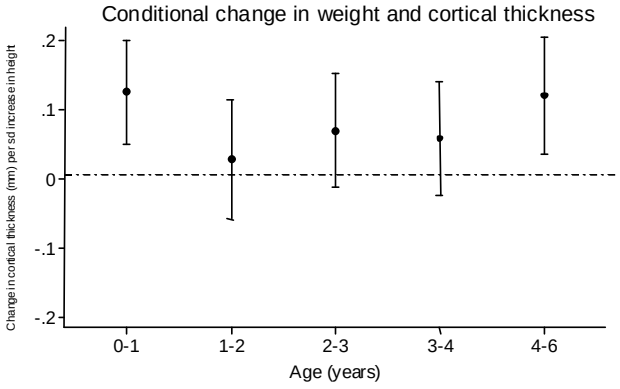


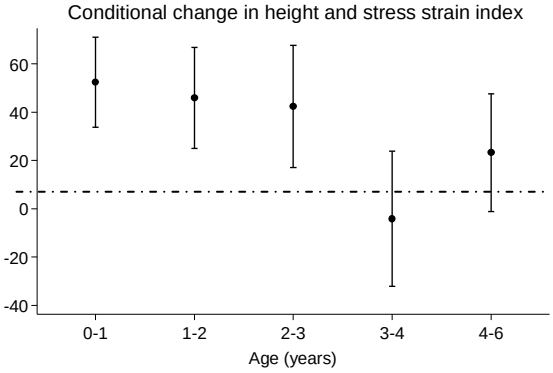












Graphs show mean and 95% confidence intervals

TABLE 48: Mutually independent relationship between conditional change in height and weight (per sd increase) from birth to aged 6 years and tibial bone structure and strength at age 6 years (n=79)

Conditional change in height (z score)	Trabecular content 4% per mm slice	Trabecular density 4% mg/cm^3	Trabecular content 14% per mm slice	Trabecular density 14% mg/cm^3	Cortical content 38% per mm slice	Cortical density 38% mg/cm3
0-12m	5.0 (1.0,9.0)*	-3.8 (-13.9,6.3)	-0.07 (-0.9,0.8)	-8.1 (-15.0,-1.1)*	5.5 (2.6,8.4)***	-1.4 (-8.6,5.9)
1-2yr	10.3 (6.3,14.2)***	19.1 (9.2,29.1)***	1.5 (0.6,2.4)**	5.9 (-1.3,13.0)	6.5 (3.4,9.6)***	3.5 (-4.1,11.1)
2-3yr	6.1 (1.4,10.7)*	6.1 (-5.6,17.8)	1.3 (0.3,2.3)*	8.3 (0.04,16.5)*	6.9 (3.4,10.5)***	-1.9 (-10.7,6.8)
3-4yr	-1.8 (-6.9,3.3)	-0.5 (-13.4,12.5)	-0.3 (-1.4,0.9)	5.8 (-3.5,15.1)	-3.4 (-7.1,0.3)	-11.6 (-20.8,-2.5)*
4-6yr	5.9 (1.4,10.5)*	11.5 (0.1,23.0)*	0.6 (-0.5,1.6)	-0.7 (-9.2,7.8)	3.5 (0.3,6.8)*	1.4 (-6.7,9.6)
R^2 as a %	**42**	**21**	**22**	**14**	**48**	**9**

	Cortical thickness 38% (mm)	Periosteal circumference 38% (mm)	Endosteal circumference 38% (mm)	Stress Strain Index 38% mm^3	Fracture load x 38% N	Fracture load y 38% N
0-12m	0.09 (0.02,0.2)*	0.8 (-0.02,1.6)	0.2 (-0.7,1.2)	42.8 (27.0,58.6)***	85.2 (48.6,121.9)***	91.3 (58.2,124.4)***
1-2yr	0.10 (0.02,0.2)*	0.9 (0.008,1.7)*	0.3 (-0.8,1.3)	36.4 (19.7,53.0)***	71.3 (32.7,109.8)***	73.0 (38.2,107.8)***
2-3yr	0.1 (0.02,0.2)*	1.3 (0.3,2.2)*	0.6 (-0.6,1.8)	33.3 (14.1,52.4)***	83.0 (38.7,127.3)***	69.6 (29.6,109.6)***
3-4yr	-0.08 (-0.2,0.004)	0.4 (-0.6,1.4)	0.9 (-0.3,2.2)	-18.9 (-38.8,1.1)	-39.1 (-85.4,7.2)	-38.3 (-80.1,3.5)
4-6yr	0.08 (0.005,0.2)*	0.1 (-0.8,1.0)	-0.4 (-1.5,0.7)	22.0 (4.3,39.6)*	45.1 (4.2,86.1)*	44.3 (7.2,81.3)*
R^2 as a %	**30**	**22**	**6**	**54**	**49**	**54**

table shows β and CI; *p<0.05, **p<0.01, ***p<0.001

Conditional change in weight (z score)	Trabecular content 4% per mm slice	Trabecular density 4% mg/cm^3	Trabecular content 14% per mm slice	Trabecular density 14% mg/cm^3	Cortical content 38% per mm slice	Cortical density 38% mg/cm3
0-12m	7.0 (2.1,12.0)**	3.4 (-8.2,15.0)	-0.1 (-1.2,1.0)	-8 (-16.4,0.4)	9.5 (6.8,12.2)***	2.6 (-5.6,10.8)
1-2yr	2.7 (-2.7,8.1)	-3.8 (-16.5,8.9)	0.7 (-0.5,2.0)	-1.6 (-11.2,7.9)	6.1 (3.1,9.1)***	-3.8 (-13.0,5.3)
2-3yr	4.9 (-0.3,10.1)	10.2 (-2.1,22.4)	1.2 (-0.006,2.3)	8.3 (-0.7,17.3)	3.8 (0.9,6.6)**	-0.4 (-8.9,8.2)
3-4yr	-1 (-6.3,4.3)	-2 (-14.5,10.5)	0.8 (-0.3,2.0)	6.4 (-2.4,15.2)	1.9 (-1.0,4.8)	-7 (-15.7,1.7)
4-6yr	6.2 (0.9,11.6)*	9.7 (-2.8,22.2)	1.3 (0.2,2.5)*	5 (-3.9,13.9)	6.0 (3.0,8.9)***	5.4 (-3.5,14.3)
R^2 as a %	**21**	**8**	**18**	**15**	**56**	**7**

	Cortical thickness 38% (mm)	Periosteal circumference 38% (mm)	Endosteal circumference 38% (mm)	Stress Strain Index 38% mm^3	Fracture load x 38% N	Fracture load y 38% N
0-12m	0.1 (0.07,0.2)***	1.2 (0.4,1.9)**	0.3 (-0.7,1.2)	56.6 (40.7,72.4)***	128.4 (95.4,161.4)***	110.7 (77.7,143.6)***
1-2yr	0.09 (0.010,0.2)*	1.2 (0.4,2.1)**	0.7 (-0.4,1.8)	29.5 (11.7,47.2)**	73.7 (36.9,110.5)***	58.1 (21.4,94.9)**
2-3yr	0.06 (-0.010,0.1)	0.6 (-0.2,1.4)	0.2 (-0.8,1.2)	15 (-1.5,31.6)	37.9 (3.5,72.3)***	32.1 (-2.3,66.5)
3-4yr	0.07 (-0.005,0.1)	0.1 (-0.7,0.9)	-0.3 (-1.3,0.7)	5.4 (-11.5,22.3)	11.3 (-23.7,46.4)	13.9 (-21.1,48.9)
4-6yr	0.1 (0.05,0.2)**	0.2 (-0.6,1.0)	-0.5 (-1.6,0.5)	25.1 (7.9,42.3)**	56.9 (21.2,92.6)**	53.2 (17.5,88.9)**
R^2 as a %	**36**	**24**	**7**	**51**	**56**	**49**

table shows β and CI; *p<0.05, **p<0.01, ***p<0.001

7.3.5 Childhood growth as a determinant of hip structure and strength at age 6 years

Of the children that had a successful hip structural analysis on their femoral neck DXA scan at aged 6 years, 250 had previous height and weight measurements at all time points (birth, 12 months, 2, 3, 4 and 6 years).

Table 49 shows the independent relationships between the relative gain in height at all childhood timepoints. Greater growth in height relative to peers was associated with hip structure; in particular cross sectional area, sub-periosteal width and Z modulus at all three sites measured in the hip (except narrow neck sub periosteal width and Z modulus at 3-4 years). Increased height gain between ages 1-2 years were associated with increased narrow neck and intertrochanteric BMD ($p<0.01$) and cortical thickness ($p<0.05$) in addition to increased femoral shaft BMD. A small increase in the buckling strength ratio was observed at the intertrochanteric and femoral shaft sites with increased gain between 0-12 months ($\beta=0.2$, $p<0.001$; $\beta=0.1$, $p<0.001$ respectively).

Table 50 shows the independent relationships between relative gain in weight and the hip structure and strength of the child aged 6 years. At all childhood timepoints, greater weight gain relative to peers was associated with increased cross sectional area, sub periosteal width and Z modulus at all three sites measured in the hip. Weight gain between the ages of 3-4 years was associated with increased BMD and cortical thickness at all three sites. Early gain in weight (0-12months and 1-2 years) was associated with increased buckling strength ratios ($p<0.01$).

The relationships between conditional height and weight gain and narrow neck aBMD, CSA, periosteal width and Z modulus are shown in figure 16.

TABLE 49: Mutually independent relationships between conditional change in height (per sd increase) from birth to age 6 years and hip structure and strength at aged 6 years

Conditional change in height (z score)	Narrow Neck BMD (g/cm^2)	Cross sectional area (cm^2)	Sub periosteal width (cm)	Cortical thickness (cm)	Section of modulus (cm^4)	Buckling ratio
0-12m	0.01 (0.002,0.02)*	0.07 (0.05,0.09)***	0.07*** (0.05,0.08)	0.002 (0.0001,0.004)*	0.04 (0.03,0.05)***	0.1 (-0.002,0.3)
1-2yr	0.02 (0.009,0.03)***	0.08 (0.06,0.1)***	0.06*** (0.04,0.08)	0.003 (0.002,0.005)***	0.04 (0.03,0.05)***	0.05 (-0.08,0.2)
2-3yr	0.006 (-0.003,0.01)	0.05 (0.03,0.07)***	0.05*** (0.03,0.07)	0.0009 (-0.0009,0.003)	0.03 (0.02,0.04)***	0.1 (-0.02,0.3)
3-4yr	0.008 (-0.0006,0.02)	0.03 (0.003,0.05)*	0.01 (-0.006,0.03)	0.002 (-0.0001,0.004)	0.008 (-0.003,0.02)	-0.03 (-0.2,0.1)
4-6yr	0.009 (-0.00009,0.02)	0.03 (0.009,0.06)**	0.02* (0.0010,0.04)	0.002 (-0.0001,0.004)	0.02 (0.004,0.03)**	-0.04 (-0.2,0.1)
R^2 as a %	**10**	**29**	**34**	**8**	**32**	**3**
	Intertrochanter BMD (g/cm^2)	**Cross sectional area (cm^2)**	**Sub periosteal width (cm)**	**Cortical thickness (cm)**	**Section of modulus (cm^4)**	**Buckling ratio**
0-12m	0.003 (-0.007,0.01)	0.08 (0.05,0.1)***	0.1*** (0.08,0.1)	0.001 (-0.004,0.005)	0.07 (0.05,0.10)***	0.2 (0.09,0.3)***
1-2yr	0.02 (0.006,0.02)**	0.1 (0.07,0.1)***	0.08*** (0.06,0.1)	0.005 (0.0008,0.009)*	0.07 (0.05,0.09)***	0.06 (-0.04,0.2)
2-3yr	0.007 (-0.002,0.02)	0.06 (0.03,0.09)***	0.06*** (0.03,0.08)	0.003 (-0.002,0.007)	0.04 (0.02,0.06)***	0.07 (-0.04,0.2)
3-4yr	0.006 (-0.003,0.02)	0.05 (0.02,0.08)**	0.04** (0.02,0.07)	0.003 (-0.002,0.007)	0.03 (0.003,0.05)*	0.05 (-0.06,0.2)
4-6yr	0.005 (-0.004,0.01)	0.05 (0.02,0.09)**	0.05*** (0.02,0.08)	0.002 (-0.002,0.007)	0.04 (0.01,0.06)**	0.07 (-0.04,0.2)
R^2 as a %	**5**	**25**	**32**	**3**	**28**	**6**
	Femur shaft BMD (g/cm^2)	**Cross sectional area (cm^2)**	**Sub periosteal width (cm)**	**Cortical thickness (cm)**	**Section of modulus (cm^4)**	**Buckling ratio**
0-12m	0.003 (-0.008,0.01)	0.06 (0.04,0.08)***	0.06*** (0.04,0.07)	-0.003 (-0.008,0.003)	0.04 (0.03,0.05)***	0.1 (0.06,0.2)***
1-2yr	0.01 (0.002,0.02)*	0.07 (0.05,0.10)***	0.06*** (0.04,0.07)	0.002 (-0.003,0.008)	0.04 (0.03,0.05)***	0.06 (0.009,0.1)*
2-3yr	0.009 (-0.001,0.02)	0.05 (0.02,0.07)***	0.03*** (0.02,0.05)	0.002 (-0.003,0.008)	0.03 (0.02,0.04)***	0.03 (-0.02,0.08)
3-4yr	0.003 (-0.007,0.01)	0.03 (0.005,0.05)*	0.02** (0.009,0.04)	0.0003 (-0.005,0.006)	0.02 (0.004,0.03)**	0.04 (-0.02,0.09)
4-6yr	0.002 (-0.009,0.01)	0.03 (0.006,0.05)*	0.03*** (0.01,0.04)	-0.001 (-0.007,0.005)	0.02 (0.009,0.03)***	0.05 (-0.009,0.1)
R^2 as a %	**3**	**25**	**36**	**1**	**33**	**9**

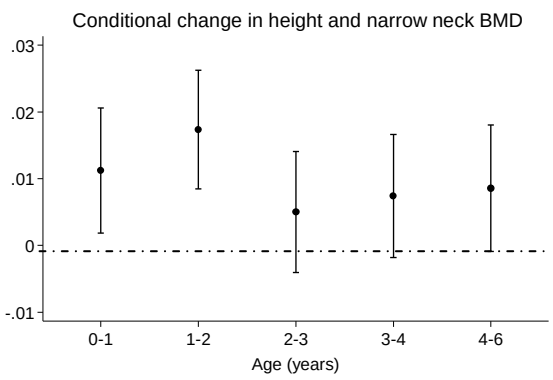
table shows β and 95% CI; *p<0.05, **p<0.01, ***p<0.001

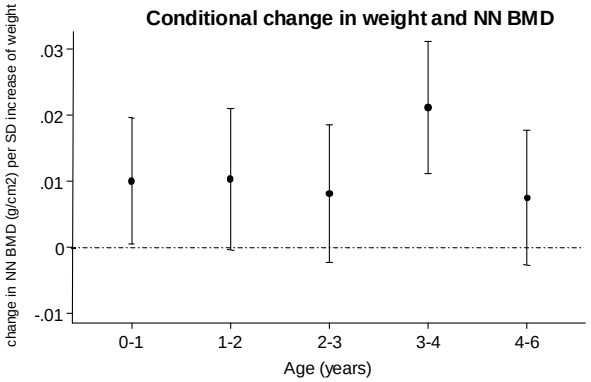
TABLE 50: Mutually independent relationships between conditional change in weight from birth to age 6 years and hip structure and strength at age 6 years

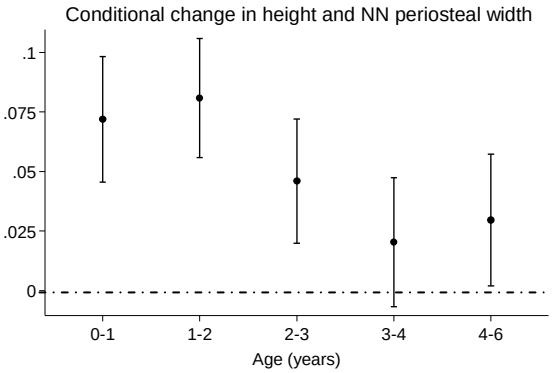
Conditional change in weight (z score)	Narrow Neck BMD (g/cm^2)	Cross sectional area (cm^2)	Sub periosteal width (cm)	Cortical thickness (cm)	Section of modulus (cm^4)	Buckling ratio
0-12m	0.01 (0.003,0.02)**	0.08 (0.06,0.1)***	0.08*** (0.06,0.10)	0.002 (0.0003,0.004)*	0.05 (0.03,0.06)***	0.2 (0.07,0.3)**
1-2yr	0.01 (0.0008,0.02)*	0.08 (0.05,0.1)***	0.08*** (0.06,0.10)	0.002 (-0.0002,0.004)	0.04 (0.03,0.06)***	0.2 (0.06,0.4)**
2-3yr	0.007 (-0.003,0.02)	0.04 (0.01,0.07)**	0.04*** (0.02,0.05)	0.001 (-0.0008,0.003)	0.02 (0.008,0.03)**	0.06 (-0.09,0.2)
3-4yr	0.02 (0.01,0.03)***	0.08 (0.06,0.1)***	0.05*** (0.03,0.07)	0.004 (0.002,0.006)***	0.04 (0.03,0.05)***	-0.06 (-0.2,0.08)
4-6yr	0.009 (-0.0008,0.02)	0.05 (0.02,0.07)***	0.04*** (0.02,0.05)	0.002 (-0.0003,0.004)	0.02 (0.01,0.04)***	0.04 (-0.1,0.2)
R^2 as a %	**12**	**35**	**45**	**10**	**37**	**7**
	Intertrochanter BMD (g/cm^2)	**Cross sectional area (cm^2)**	**Sub periosteal width (cm)**	**Cortical thickness (cm)**	**Section of modulus (cm^4)**	**Buckling ratio**
0-12m	0.004 (-0.006,0.01)	0.09 (0.05,0.1)***	0.1*** (0.08,0.1)	-0.001 (-0.006,0.003)	0.08 (0.06,0.1)***	0.3 (0.1,0.4)***
1-2yr	0.01 (0.00009,0.02)*	0.1 (0.07,0.1)***	0.1*** (0.08,0.1)	0.003 (-0.002,0.008)	0.08 (0.05,0.1)***	0.2 (0.06,0.3)**
2-3yr	-0.002 (-0.01,0.009)	0.04 (0.003,0.08)*	0.07*** (0.04,0.10)	0.001 (-0.004,0.006)	0.04 (0.01,0.06)**	0.1 (0.02,0.3)*
3-4yr	0.02 (0.008,0.03)***	0.1 (0.06,0.1)***	0.07*** (0.04,0.10)	0.010 (0.005,0.01)***	0.06 (0.03,0.08)***	-0.1 (-0.2,0.02)
4-6yr	0.004 (-0.006,0.01)	0.07 (0.04,0.1)***	0.09*** (0.06,0.1)	0.003 (-0.002,0.008)	0.06 (0.03,0.08)***	0.1 (0.008,0.3)*
R^2 as a %	**6**	**28**	**42**	**7**	**33**	**14**
	Femur shaft BMD (g/cm^2)	**Cross sectional area (cm^2)**	**Sub periosteal width (cm)**	**Cortical thickness (cm)**	**Section of modulus (cm^4)**	**Buckling ratio**
0-12m	0.01 (-0.001,0.02)	0.09 (0.07,0.1)***	0.08*** (0.06,0.09)	-0.0002 (-0.006,0.006)	0.06 (0.05,0.07)***	0.1 (0.05,0.2)***
1-2yr	0.006 (-0.007,0.02)	0.08 (0.05,0.1)***	0.07*** (0.06,0.09)	-0.001 (-0.008,0.005)	0.05 (0.04,0.06)***	0.1 (0.06,0.2)***
2-3yr	0.009 (-0.004,0.02)	0.04 (0.02,0.06)**	0.03*** (0.01,0.04)	0.003 (-0.003,0.010)	0.02 (0.010,0.03)***	0.02 (-0.04,0.09)
3-4yr	0.02 (0.006,0.03)**	0.05 (0.03,0.08)***	0.02** (0.009,0.04)	0.007 (0.0006,0.01)*	0.03 (0.01,0.04)***	-0.03 (-0.09,0.04)
4-6yr	0.007 (-0.005,0.02)	0.05 (0.03,0.07)***	0.04*** (0.03,0.06)	0.001 (-0.005,0.008)	0.03 (0.02,0.04)***	0.06 (-0.002,0.1)
R^2 as a %	**6**	**38**	**48**	**2**	**49**	**11**

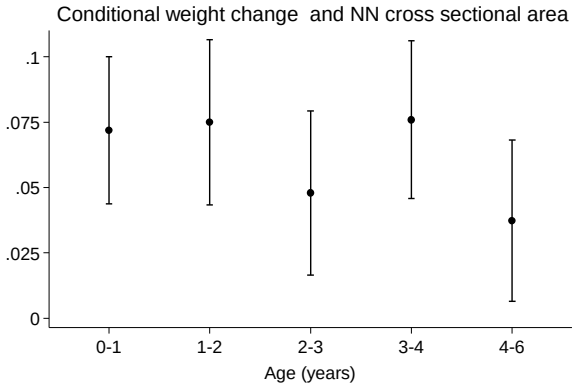
table shows β and 95% CI; *p<0.05, **p<0.01, ***p<0.001

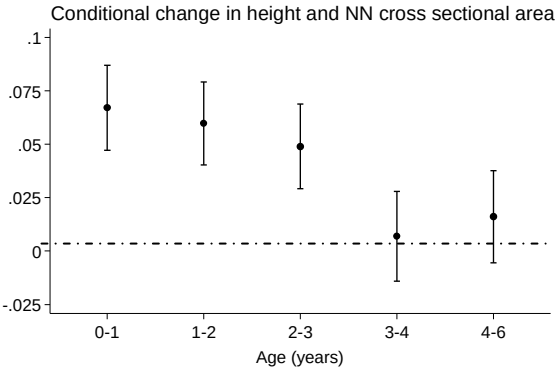
Figure 16: Graphs to show the relationship of conditional change in growth and Narrow neck BMD, CSA, periosteal width and section modulus

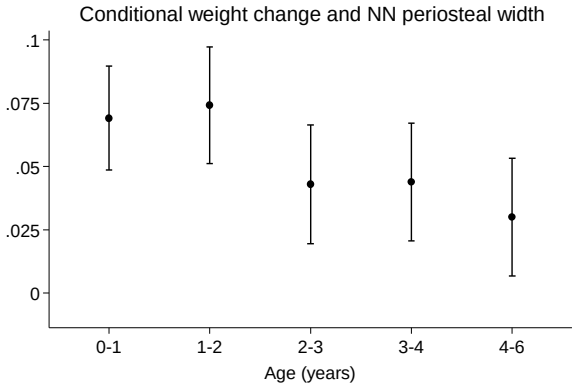


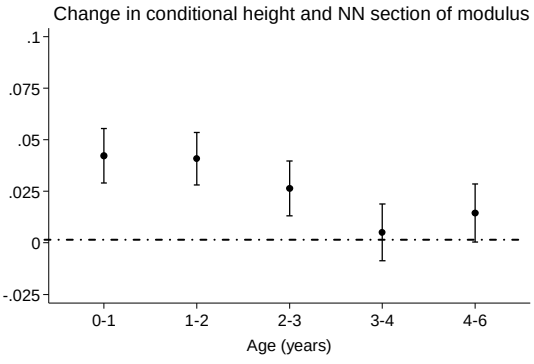


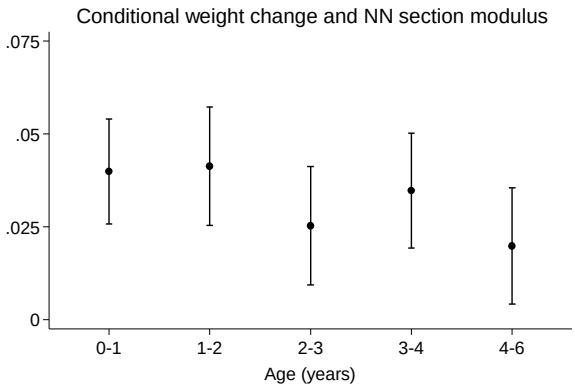












BMD-bone mineral density, CSA- cross sectional area, NN- narrow neck

7.3.6 Foetal growth and bone mass of the child at 6 years

Previous doctoral work by Dr Pam Mahon utilised 3D ultrasound techniques to measure the femur length and abdominal circumference, in the foetus at 19 and 34 weeks gestation. In this final part of this anlysis we explored the relationships between these foetal measurements and bone mineral accrual in the child at aged 6 years.

In order to perform these analyses Royston models were fitted to foetal measurements of femur length and abdominal circumference at 19 and 34 weeks gestation to create Z scores for size and conditional growth[168]. Correlation and linear regression models were used to explore the relationship between the foetal ultrasound measurements and bone size and density at aged 6 years using whole body, lumbar spine and hip DXA, pQCT of the tibia and hip structural analysis.

7.3.6.1 *Foetal growth and whole body, lumbar spine and hip bone mass*

After mothers who were uncertain of their late menstrual cycle were excluded, there was 205 mother child pairs with complete 11, 19 and 34 week data available; 327 mother child pairs had data available for 19 and 34 weeks.

Absolute femoral length and abdominal circumference were associated with whole body BA, BMC and aBMD at 19 and 34 weeks (table 51). Associations were generally stronger at 34 than 19 weeks. Figure 17 show scatterplots between conditional change in femoral length and abdominal circumference and whole body BA, BMC and vBMD. There were strong statistically significant correlations between conditional change in femur length between 19-34 weeks and indices of skeletal size, but not volumetric density (BA: $r=0.31$, $p<0.001$; BMC: $r=0.32$, $p<0.001$; vBMD $r=0.01$, $p=0.82$).

TABLE 51: Relationships between absolute and conditional foetal abdominal circumference and femoral length during pregnancy and whole body BA, BMC, aBMD and estimated vBMD

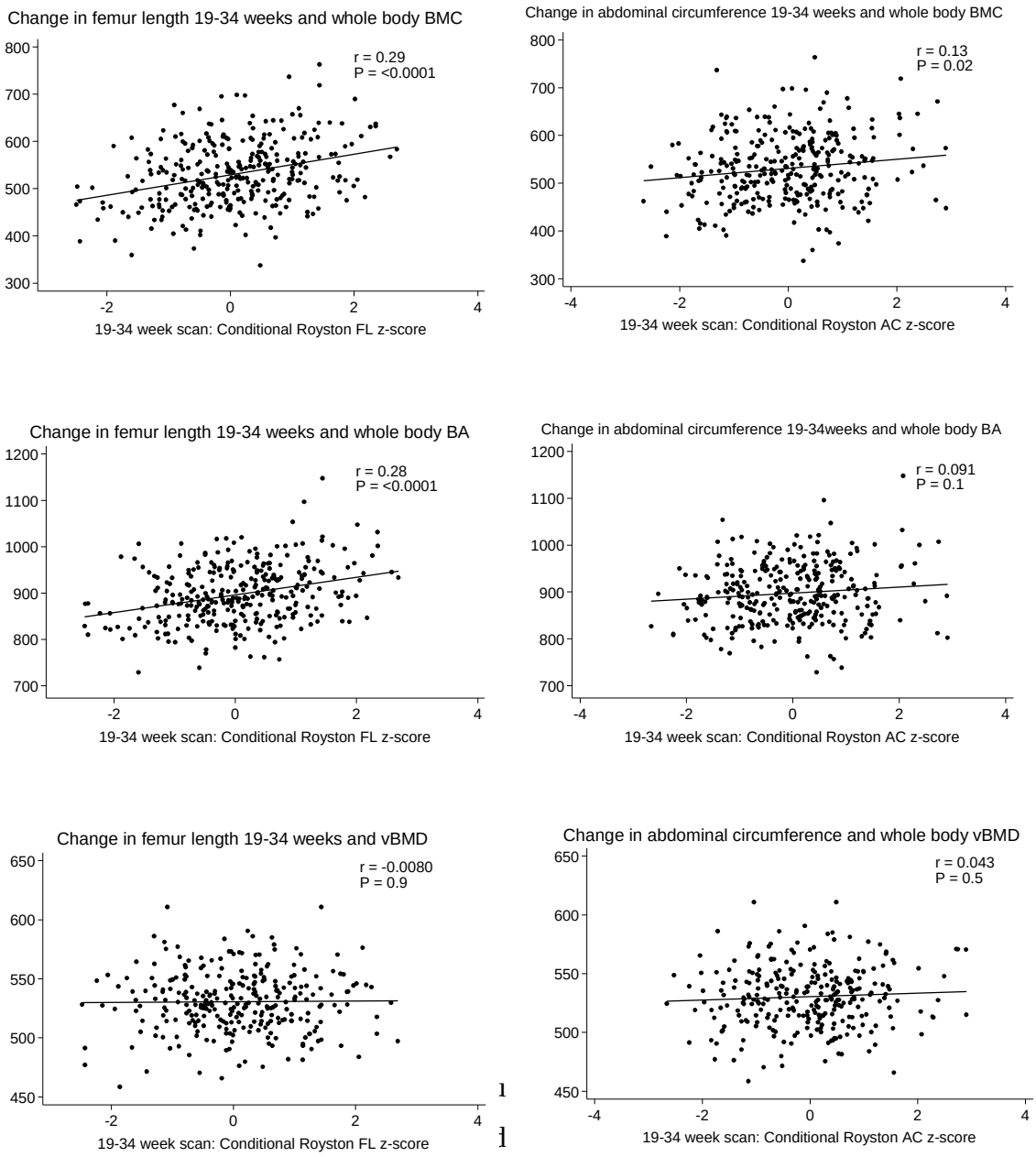
	Whole body BA (cm^2)	BMC (g)	BMD (g/cm^2)	vBMD (g)
Abdominal circumferenence (z score)				
11 weeks	5.3 (-4.0,14.5)	6.3 (-4.1,16.6)	0.003 (-0.003,0.01)	-0.003 (-4.1,4.1)
19 weeks	7.7 (0.4,15.0)*	10.1 (2.0,18.1)*	0.006 (0.001,0.01)*	-0.5 (-3.6,2.7)
34 weeks	11.1 (3.9,18.4)**	15.8 (7.8,23.8)***	0.01 (0.005,0.02)***	1.2 (-2.0,4.5)
∆ 11-19 weeks	8.5 (-2.5,19.5)	12.2 (-0.04,24.5)*	0.008 (0.0002,0.02)*	1.6 (-3.3,6.5)
∆ 19-34 weeks	6.5 (-0.2,13.1)	9.7 (2.3,17.0)*	0.006 (0.002,0.01)**	1.5 (-1.4,4.3)
Femoral length (z score)				
19 weeks	9.8 (2.5,17.1)**	10.7 (2.6,18.9)*	0.006 (0.0008,0.01)*	-1.2 (-4.4,2.1)
34 weeks	21.6 (14.9,28.3)***	23.6 (16.2,31.0)***	0.01 (0.007,0.02)***	-0.5 (-3.6,2.5)
∆ 19-34 weeks	19.1 (12.5,25.8)***	21.8 (14.5,29.1)***	0.01 (0.007,0.02)***	0.3 (-2.7,3.3)

table shows β and CI; *p<0.05, **p<0.01, ***p<0.001

A weak association was seen for change in abdominal circumference between 19-34 weeks for skeletal size (BMC: r=0.15, p=0.01). However once additionally adjusted for change in femoral length using multivariate regression modelling, only femoral length remained a significant determinant (BA: β=18.8, p<0.001; BMC: β=20.1, p<0.001). No association was seen for absolute abdominal circumference at 11 weeks; however, a weak association was seen for change in abdominal circumference between 11-19 weeks for BMC (r=0.14, p=0.05) and between femoral length and lumbar spine BA and BMC (p<0.05) at 19 weeks.

Abdominal circumference at 19 weeks was associated with lumbar spine BMC only (p=0.02). There were strong statistically significant correlations between conditional change in femur length between 19-34 weeks and indices of lumbar spine and hip skeletal size, but not volumetric density (Lumbar spine BA: r=0.25, p<0.001; BMC: r=0.27, p<0.001; vBMD r=0.03, p=0.58: Hip BA: r=0.22, p<0.001; BMC: r=0.26, p<0.001; vBMD r=0.08, p=0.16). A weaker association was seen between change in abdominal circumference between 19-34 weeks for lumbar spine and hip bone size (p<0.05) however once additionally adjusted for change in femoral length at 19-34 weeks using multivariate regression modelling, only femoral length remained a significant determinant (Lumbar spine BA: β=0.7, p<0.001; BMC: β=0.6, p<0.001: Hip BA: β=0.4, p<0.001; BMC: β=0.5, p<0.001). A weak association was seen between changes in abdominal circumference between 11-19 weeks for lumbar spine BMC only (r=16, p=0.02).

Figure 17: Scatterplots to show the relationship between change in femoral length and abdominal circumference between 19-34 weeks and whole body BMC, BA and estimated vBMD



7.3.6.2 *Foetal growth and tibial bone structure and strength at age 6 years*

After mothers that were uncertain of their late menstrual cycle were excluded there was 54 mother child pairs with complete 11,19 and 34 week data available; 86 mother child pairs had data available for 19 and 34 weeks.

Absolute femoral length at 34 weeks was associated with increased cortical content, periosteal circumference, stress strain index and fracture load in the x and y axis as shown in table 52 ($p<0.001$). A weak association was seen for absolute abdominal circumference at 34 weeks and increased periosteal circumference, endosteal circumference, stress strain index and fracture load in the x and y axis ($p<0.05$). No overall pattern was observed for the relationships between either trabecular or cortical density. There were strong statistically significant correlations between conditional change in femur length between 19-34 weeks and indices of skeletal size (cortical content: $r=0.37$, $p<0.001$; cortical thickness: $r=0.23$, $p=0.04$; periosteal circumference: $r=0.3$, $p=0.006$; stress strain index: $r=0.44$, $p<0.001$; fracture load x: $r=0.43$, $p<0.001$; fracture load y: $r=0.39$, $p<0.001$). The scatterplots showing these relationships are displayed overleaf (figure 18).

TABLE 52: Relationships between absolute and conditional foetal abdominal circumference and femoral length during pregnancy and measures of tibial structure and strength at age 6 years

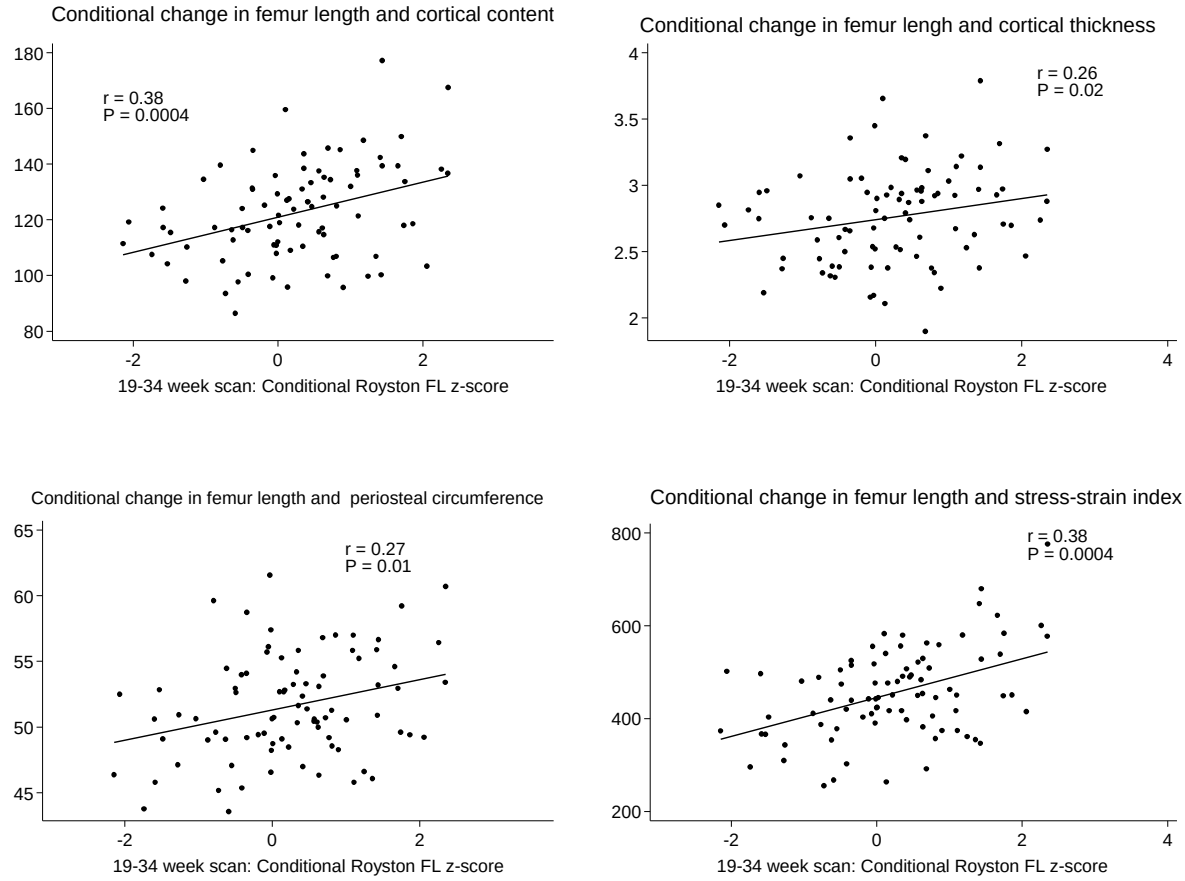
	Trabecular content 4% per mm slice	Trabecular density 4% mg/cm^3	Trabecular content 14% per mm slice	Trabecular density 14% mg/cm^3	Cortical content 38% per mm slice	Cortical density 38% mg/cm3
Abdominal circumferenence						
11 weeks	3.2 (-5.5,11.8)	-1.5 (-19.6,16.6)	0.08 (-1.9,2.1)	-5.6 (-20.1,9.0)	3 (-2.9,9.0)	0.8 (-9.3,10.9)
19 weeks	4.9 (-0.1,10.0)	1.8 (-9.5,13.1)	0.7 (-0.5,1.8)	-1.1 (-9.9,7.8)	1.4 (-2.5,5.2)	-7.7 (-15.1,-0.3)*
34 weeks	3.7 (-1.5,8.9)	-2.1 (-13.8,9.5)	0.7 (-0.4,1.8)	-0.5 (-9.1,8.1)	2.8 (-1.0,6.6)	-9.6 (-17.2,-2.1)*
Δ 11-19 weeks	12 (3.3,20.7)**	18.5 (-0.2,37.2)*	1.6 (-0.5,3.7)	5.5 (-10.1,21.2)	4.1 (-2.2,10.5)	-1.6 (-12.5,9.2)
Δ 19-34 weeks	0.3 (-4.5,5.1)	-4.6 (-15.2,6.0)	0.4 (-0.6,1.4)	0.4 (-7.5,8.4)	2.1 (-1.4,5.6)	-5.4 (-12.5,1.8)
Femoral length						
19 weeks	3.9 (-1.2,9.0)	-1.3 (-12.5,10.0)	-0.4 (-1.5,0.8)	-9 (-17.5,-0.6)*	1.2 (-2.5,5.0)	-3.7 (-11.1,3.8)
34 weeks	5.5 (0.5,10.4)*	0.2 (-11.0,11.3)	0.2 (-0.9,1.3)	-8.2 (-16.3,-0.08)*	5.9 (2.4,9.4)***	-2.8 (-10.3,4.8)
Δ 19-34 weeks	4.2 (-0.8,9.1)	0.8 (-10.3,11.8)	0.6 (-0.5,1.6)	-3.7 (-11.8,4.5)	6.3 (2.9,9.7)***	-0.6 (-8.2,6.9)

	Cortical thickness 38% (mm)	Periosteal circumference 38% (mm)	Endosteal circumference 38% (mm)	Stress Strain Index 38% mm^3	Fracture load x 38% N	Fracture load y 38% N
Abdominal circumferenence						
11 weeks	0.06 (-0.06,0.2)	0.3 (-1.0,1.6)	-0.1 (-1.5,1.3)	16.2 (-15.1,47.5)	26.4 (-46.0,98.8)	48.6 (-13.1,110.3)
19 weeks	-0.04 (-0.1,0.04)	1.2 (0.4,2.1)**	1.5 (0.6,2.4)**	17.2 (-3.5,37.9)	42.3 (-3.5,88.2)	45.1 (3.9,86.3)*
34 weeks	0.02 (-0.06,0.10)	1.2 (0.3,2.0)**	1.1 (0.1,2.0)*	21.9 (1.0,42.9)*	48.4 (2.4,94.5)*	49.5 (7.6,91.4)*
Δ 11-19 weeks	0.04 (-0.08,0.2)	1.1 (-0.3,2.5)	0.8 (-0.6,2.3)	24 (-9.5,57.4)	54.3 (-22.7,131.3)	47.1 (-19.7,113.9)
Δ 19-34 weeks	0.03 (-0.04,0.1)	0.5 (-0.3,1.3)	0.3 (-0.6,1.2)	10.6 (-9.0,30.3)	23.6 (-19.7,66.9)	19.7 (-19.9,59.2)
Femoral length						
19 weeks	-0.03 (-0.1,0.04)	1 (0.1,1.8)*	1.2 (0.3,2.1)*	15 (-5.6,35.5)	41.2 (-4.0,86.5)	38.6 (-2.4,79.6)
34 weeks	0.05 (-0.03,0.1)	1.5 (0.7,2.3)***	1.2 (0.3,2.1)*	44.1 (25.7,62.5)***	98.7 (58.4,139.1)***	83.4 (45.7,121.1)***
Δ 19-34 weeks	0.08 (0.004,0.2)*	1.2 (0.3,2.0)**	0.7 (-0.3,1.6)	41.8 (23.4,60.2)***	89.2 (48.3,130.1)***	74.5 (36.4,112.5)***

table shows β and CI; *p<0.05, **p<0.01, ***p<0.001

There were no relationships between change in abdominal circumference between 19-34 weeks and any measure of bone structure or strength. Once adjusted for change in abdominal circumference, change in femur length remained associated with cortical content (β=6.3, p=0.001), periosteal circumference (β=1.1, p=0.01), stress strain index (β=43.6, p<0.001) and fracture load in the x and y axis (β=92.9, p<0.001; β=77.2, p<0.001).

Figure 18: Scatterplots to show the relationship between conditional change in femur length between 19-34 weeks pregnancy and cortical content, cortical thickness, periosteal circumference and stress strain index at age 6years



7.3.6.3 *Foetal growth and hip structure and strength at age 6 years*

After mothers that were uncertain of their late menstrual cycle were excluded there was 188 mother child pairs with complete 11,19 and 34 week data available; 297 mother child pairs had data available for 19 and 34 weeks.

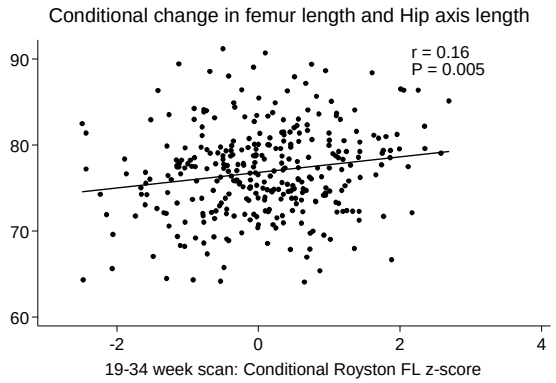
Absolute femoral length and abdominal circumference at 34 weeks were positively associated with aBMD, CSA, cortical thickness and Z modulus at the narrow neck, intertrochanteric and femoral shaft regions (table 53). The relationships between hip axis length, narrow neck BMD, CSA and Z modulus are shown in scatterplots in figure 19. There were strong statistically significant correlations between conditional change in femur length between 19-34 weeks and measures of both hip structure at strength at all three sites (narrow neck: BMD r=0.26, p<0.001; CSA: r=0.3, p<0.001; subperiosteal width: r=0.18, p=0.002; cortical thickness: r=0.25, p<0.001; Z modulus r=0.3, p<0.001; intertrochanteric BMD: r=0.21, p<0.001; CSA: r=0.29, p<0.001; sub-periosteal width: r=0.2, p<0.001; cortical thickness r=0.16, p=0.005; Z modulus r=0.28, p<0.001; femoral shaft BMD: r=0.15, p=0.01; CSA r=0.28, p<0.001; sub-periosteal width r=0.27, p<0.001; Z modulus r=0.31, p<0.001). Weaker but still significant positive associations between conditional change in abdominal circumference between 19-34 weeks were seen for most measures of hip structure and strength (see table 54), however once additionally adjusted for change in femoral length using a multivariate model, only femoral length remained associated with narrow neck BMD (β=0.02, p<0.001) CSA (β=0.06, p<0.001) cortical thickness (β=0.004, p<0.001) Z modulus (β=0.03, p<0.001) intertrochanteric BMD (β=0.2, p=0.003) CSA (β=0.08, p<0.001), sub-periosteal width (β=0.06, p=0.007) cortical thickness (β=0.005, p=0.03) Z modulus (β=0.05, p<0.001) femoral shaft BMD (β=0.01, p=0.05) CSA (β=0.05, p<0.001) sub-periosteal width (β=0.03, p=0.004) and z modulus (β=0.03, p<0.001).

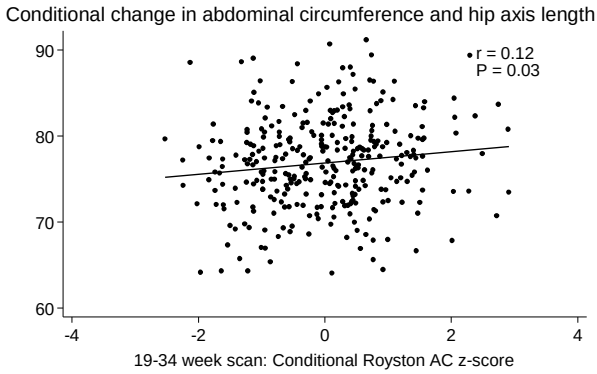
TABLE 53: Relationships between absolute and conditional foetal abdominal circumference and femoral length during pregnancy and measures of hip structure and strength at age 6 years

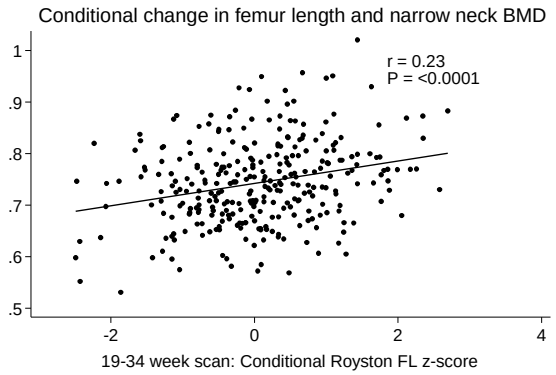
	Narrow Neck BMD (g/cm^2)	Cross sectional area (cm^2)	Sub periosteal width (cm)	Cortical thickness (cm)	Section of modulus (cm^4)	Buckling ratio
Abdominal circumferenence						
11 weeks	0.004 (-0.009,0.02)	-0.0008 (-0.04,0.04)	-0.01 (-0.04,0.01)	0.0008 (-0.002,0.003)	-0.005 (-0.02,0.01)	-0.09 (-0.3,0.09)
19 weeks	0.005 (-0.005,0.01)	0.02 (-0.005,0.05)	0.02 (-0.003,0.04)	0.0009 (-0.001,0.003)	0.01 (-0.002,0.03)	0.01 (-0.1,0.2)
34 weeks	0.01 (0.003,0.02)*	0.05 (0.02,0.08)***	0.03 (0.010,0.05)**	0.003 (0.0004,0.005)*	0.02 (0.010,0.04)**	-0.04 (-0.2,0.1)
Δ 11-19 weeks	0.007 (-0.008,0.02)	0.04 (0.002,0.09)*	0.04 (0.01,0.08)**	0.001 (-0.002,0.004)	0.03 (0.006,0.05)*	0.1 (-0.1,0.3)
Δ 19-34 weeks	0.01 (0.0009,0.02)*	0.04 (0.01,0.06)**	0.02 (0.002,0.04)*	0.002 (0.00008,0.004)*	0.02 (0.004,0.03)*	-0.04 (-0.2,0.09)
Femoral length						
19 weeks	0.004 (-0.006,0.01)	0.02 (-0.007,0.05)	0.02 (-0.0005,0.04)	0.0006 (-0.001,0.003)	0.01 (-0.005,0.02)	0.05 (-0.09,0.2)
34 weeks	0.02 (0.01,0.03)***	0.07 (0.05,0.1)***	0.04 (0.02,0.06)***	0.004 (0.002,0.006)***	0.04 (0.02,0.05)***	-0.1 (-0.3,0.02)
Δ 19-34 weeks	0.02 (0.01,0.03)***	0.07 (0.05,0.10)***	0.03 (0.01,0.05)**	0.004 (0.002,0.006)***	0.04 (0.02,0.05)***	-0.2 (-0.3,-0.02)*
	Intertrochanter BMD (g/cm^2)	**Cross sectional area (cm^2)**	**Sub periosteal width (cm)**	**Cortical thickness (cm)**	**Section of modulus (cm^4)**	**Buckling ratio**
Abdominal circumferenence						
11 weeks	0.01 (-0.003,0.02)	0.02 (-0.03,0.07)	-0.01 (-0.05,0.03)	0.002 (-0.003,0.008)	-0.004 (-0.04,0.03)	-0.03 (-0.2,0.1)
19 weeks	0.01 (0.003,0.02)*	0.05 (0.01,0.09)*	0.02 (-0.02,0.05)	0.004 (-0.0002,0.009)	0.03 (0.004,0.06)*	-0.04 (-0.2,0.08)
34 weeks	0.02 (0.006,0.03)**	0.08 (0.04,0.1)***	0.04 (0.01,0.08)**	0.007 (0.002,0.01)**	0.05 (0.03,0.08)***	-0.06 (-0.2,0.06)
Δ 11-19 weeks	0.01 (-0.0004,0.03)	0.05 (-0.003,0.1)	0.02 (-0.03,0.07)	0.007 (0.0007,0.01)*	0.03 (-0.006,0.07)	-0.1 (-0.3,0.06)
Δ 19-34 weeks	0.009 (-0.0008,0.02)	0.05 (0.02,0.09)**	0.04 (0.007,0.06)*	0.004 (0.0003,0.009)*	0.04 (0.01,0.06)**	-0.04 (-0.1,0.07)
Femoral length						
19 weeks	0.01 (-0.0001,0.02)*	0.05 (0.01,0.09)*	0.03 (-0.004,0.06)	0.003 (-0.002,0.007)	0.03 (0.003,0.06)*	0.03 (-0.09,0.2)
34 weeks	0.02 (0.01,0.03)***	0.1 (0.07,0.1)***	0.06 (0.03,0.09)***	0.006 (0.002,0.01)**	0.07 (0.04,0.09)***	-0.01 (-0.1,0.1)
Δ 19-34 weeks	0.02 (0.008,0.03)***	0.09 (0.06,0.1)***	0.05 (0.02,0.08)***	0.006 (0.002,0.01)**	0.06 (0.04,0.08)***	-0.04 (-0.2,0.08)
	Femur shaft BMD (g/cm^2)	**Cross sectional area (cm^2)**	**Sub periosteal width (cm)**	**Cortical thickness (cm)**	**Section of modulus (cm^4)**	**Buckling ratio**
Abdominal circumferenence						
11 weeks	0.009 (-0.005,0.02)	0.02 (-0.01,0.05)	0.005 (-0.02,0.03)	0.004 (-0.003,0.01)	0.005 (-0.01,0.02)	-0.02 (-0.09,0.05)
19 weeks	0.005 (-0.006,0.02)	0.03 (-0.001,0.05)	0.02 (-0.002,0.04)	0.001 (-0.005,0.007)	0.01 (-0.0009,0.03)	0.02 (-0.04,0.08)
34 weeks	0.01 (0.003,0.03)*	0.05 (0.02,0.08)***	0.03 (0.008,0.04)**	0.006 (0.00004,0.01)*	0.03 (0.01,0.04)***	0.000004 (-0.06,0.06)
Δ 11-19 weeks	0.009 (-0.007,0.03)	0.02 (-0.02,0.06)	0.005 (-0.02,0.03)	0.004 (-0.004,0.01)	0.01 (-0.01,0.03)	-0.03 (-0.1,0.05)
Δ 19-34 weeks	0.01 (0.0006,0.02)*	0.03 (0.010,0.06)**	0.02 (-0.00009,0.03)*	0.005 (-0.0003,0.01)	0.02 (0.005,0.03)**	-0.008 (-0.06,0.05)
Femoral length						
19 weeks	0.0004 (-0.01,0.01)	0.02 (-0.003,0.05)	0.03 (0.008,0.05)**	-0.002 (-0.008,0.004)	0.02 (0.0008,0.03)*	0.06 (0.002,0.1)*
34 weeks	0.01 (0.0005,0.02)*	0.06 (0.04,0.09)***	0.05 (0.03,0.07)***	0.003 (-0.003,0.009)	0.04 (0.03,0.05)***	0.06 (0.002,0.1)*
Δ 19-34 weeks	0.01 (0.003,0.03)*	0.06 (0.04,0.09)***	0.04 (0.02,0.06)***	0.005 (-0.0007,0.01)	0.04 (0.02,0.05)***	0.03 (-0.03,0.09)

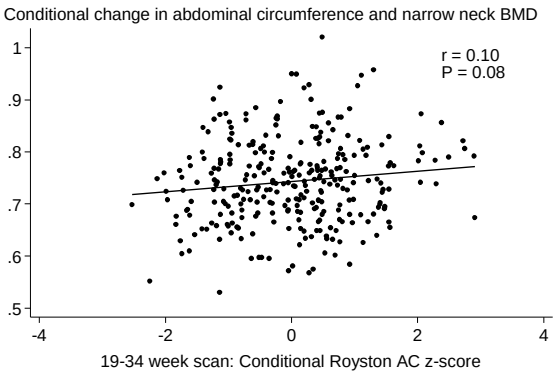
table shows β and 95% CI; *p<0.05, **p<0.01, ***p<0.001

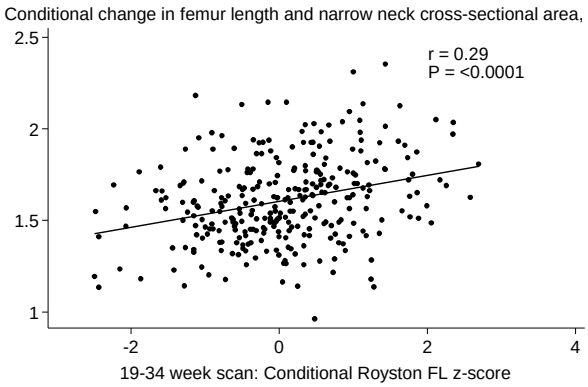
Figure 19: Scatterplots to show the relationship of conditional growth of femur length and abdominal circumference between 19-34 weeks gestation and hip axis length, narrow neck BMD, cross sectional area, and section modulus

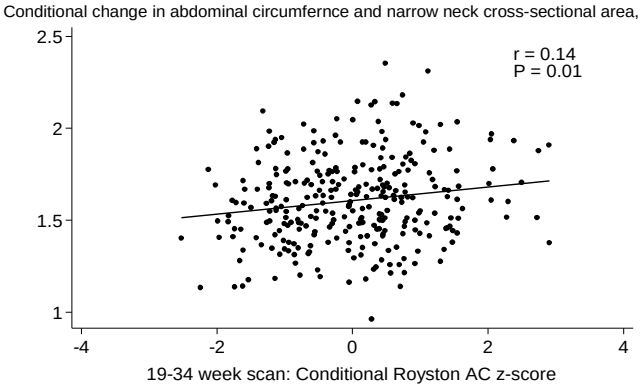


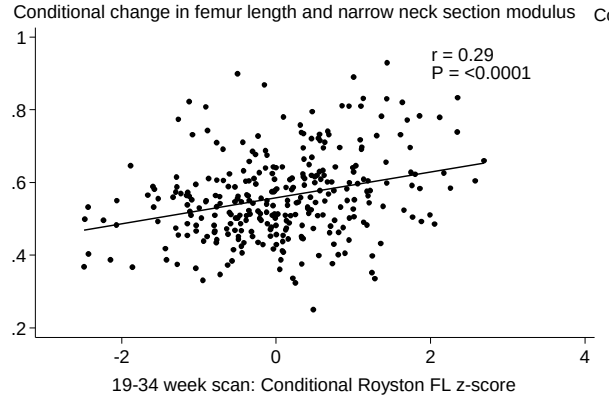


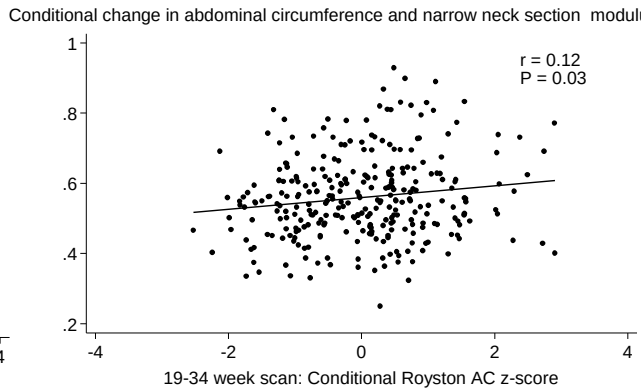












7.4 Discussion

Despite relative catch up in small babies and catch down in large ones, the general pattern overall was that small babies became small 6 year olds whereas large babies became large 6 year olds. Greater catch up growth was associated with maternal height, however it is likely the height is a marker of an inherited drive towards tallness. Increased milk intake during early childhood may help the accrual of calcium in the skeleton and hence growth. Although catch down growth was observed in children born to mothers with a higher educational attainment and in those that were breastfed, catch up growth was observed later in infancy so that at aged 6 there was no difference between the height and weight of children that were breastfed/not breastfed.

Increases in height and weight during childhood that exceeded that expected were associated with increased bone size (BA and BMC) rather than volumetric density. This was supported by the information gained from pQCT of the tibia and HSA of the femoral neck. The pQCT study showed that the increase in bone strength was as a result of increased trabecular and cortical content, increased periosteal circumference and cortical thickness. Similarly the increased strength observed at the femoral neck after the HSA analysis resulted from an increase in periosteal expansion and hence cross sectional area, rather than an increase in density although it should be noted that the created variables were for areal not volumetric density.

Change in femur length during 19-34 weeks gestation was associated with increased whole body BA, BMC and aBMD but not volumetric density. This remained after adjustment for abdominal circumference. The increased strength observed in both the pQCT scan of the tibia and HSA scan of the femoral neck confirm that the increased strength observed with increased growth of the femur during this pre natal period was as a result of increased cross sectional area, periosteal circumference and cortical thickness rather than an increase in trabecular or cortical density. This is consistent with previous data suggesting that late intrauterine growth may have persisting effects on postnatal skeletal development.

8 GRAND DISCUSSION

8.1 Principal findings

- Maternal height, pre pregnancy calcium intake and time spend in strenuous activity in late pregnancy were associated with 6 year bone mineral accrual in the offspring; however the association was removed by adjustment for childhood lifestyle determinants.
- Increased growth of the femur during late pregnancy was associated with enhanced skeletal size at age 6 years
- Breastfeeding was associated with reduced growth during the first year of life but enhanced growth during the second year, hence no difference was seen in bone mass at age 6 years.
- Children born small remained smaller, lighter and thinner at age 6 years
- Increased growth relative to peers was associated with increased maternal height and increased milk drank during early childhood. Maternal smoking was associated with increased weight gain.
- Catch up growth was important at all stages of early childhood growth and determined 6 year bone mass and strength. However maximal increases were seen when height velocity was greatest.
- Children with increased total fat mass had evidence of increased skeletal size but reduced volumetric density.
- Children with increased grip strength had evidence of increased skeletal size, volumetric density and increased bone strength at all sites measured.
- Children with increased triceps skinfold thickness had evidence of increased whole body skeletal size, but reduced volumetric density.
- Childhood milk intake was associated with increased skeletal size, independent of maternal calcium intake.
- Increased time spent doing vigorous activity was associated with increased volumetric density at all sites measured. At the tibia an increase in cortical density was observed and at the femoral neck there was an increase in the bones bending strength.

8.2 Maternal predictors of childhood bone mass

8.2.1 Maternal height

Consistent with previous studies, maternal height was associated with increased skeletal size in the offspring at age 6 years. Maternal and childhood height were positively correlated, reflecting the fact that taller mothers have taller children. However, there was also evidence in this study to suggest that this increase in height was associated with reduced volumetric density. Tall maternal height has previously been documented as an independent risk factor for hip fracture. Whether this reflects the skeletal envelope being pushed beyond its capacity to mineralise, or whether it is due to these children having a longer hip axis length is unclear. Our data also reported an increase in the buckling ratio (ratio to the outer radius to the cortical thickness) in children born to taller mothers; While a large buckling ratio may help to preserve strength with increased periosteal expansion on aging, a ratio of over 10 can result in a precipitous loss of strength as a result of cortical instability and buckling can occur on the compressive surface.

8.2.2 Maternal adiposity

Whilst maternal weight was associated with increased skeletal size much of this association was a result of co linearity with maternal height, rather than fat mass per se. Since any association was removed once adjusted for childhood BMI it is likely that this can be explained by the fact that mothers with an increased BMI have children with increased BMI at age 6. They also have children with increased triceps skinfold thickness and increased percentage fat mass with proportionally less lean mass and bone mineral. Whilst it is important to have a normal adipose fat stores during pregnancy in order to potentiate intrauterine skeletal growth, increased fatness may lead to higher fat mass at the expense of lean and bone mass in the offspring which may have implications for other aspects of health, especially taking into account the current epidemic of childhood obesity.

8.2.3 Maternal diet

Isolated epidemiological studies have reported that greater childhood skeletal size and mineral density might be associated with higher maternal intakes of calcium[169], magnesium, potassium and folate[170].Whilst we found higher consumptions of both

milk and calcium prior to becoming pregnant were associated with increased skeletal size in the offspring, the effect was attenuated by the child's milk intake at age 6 years, implying mothers that drink more milk have children who also drink more milk. We found no effect with either magnesium or folate intake at any time point during pregnancy. Since many nutrients are collinear and single nutrients may potentiate or attenuate the effects of other dietary patterns, principal component analysis was used as an alternative way of assessing maternal diet. We have previously shown in an earlier mother cohort study that healthier patterns of eating during pregnancy are associated with increased bone mass of the offspring at age 9 years[74]. Whilst we did not find these strong associations in this cohort, a higher dietary score, consistent with healthy eating, was associated with increased lean mass, decreased fat mass and increased trabecular content and density at age 6 years.

8.2.4 Maternal smoking

It is well known that maternal smoking during pregnancy is associated with reductions in both birth weight and length and increased risk of intrauterine growth retardation[133]. Whilst we were able to show that mothers who smoked during pregnancy had children with higher BMC adjusted for bone area, the relationship was attenuated once adjusted for height and weight. At birth the children in our study born to mothers that smoked in late pregnancy had reduced fat stores. However these children had increased catch up in terms of weight gain, but not corresponding height during early childhood resulting in 6 year olds with increased percentage fat and reduced percentage lean and bone mass. Maternal smoking and increased risk of obesity has been widely reported[171]. Whether this rebound adiposity is a result of being small for gestational age or whether it is due directly to the effects of cigarette smoking is unclear but is probably a combination of the two since the results were the same once adjusted for birthweight.

Possible explanations for the physiological effects of cigarette smoking during pregnancy are nicotine, which is transported across the placenta, and carbon monoxide which may influence placental vascular function and cause foetal hypoxia. Nicotine acts centrally and peripherally to reduce appetite and body weight; withdrawal can result in hyperphagia and weight gain[172]. Children of smokers also tend to be less physically active and have poorer diets[173]. Investigations of other

mediators for example insulin like growth hormone, growth hormone and leptin have not been studied.

8.2.5 Maternal physical activity

Strenuous activity and increased walking speed in late pregnancy have previously been associated with reduced neonatal bone mass[130;174]. We found that at age 6 years the reverse was true. Maternal constraint during late intrauterine life was thought to be the reason for the reduced birthweight and bone mass. Mothers that exercise, particularly during late pregnancy, had children that participated in higher amounts of vigorous activity on a daily basis. Hence once the results were adjusted for this, the relationship was attenuated. It may be reassuring that any detrimental effects of late pregnancy strenuous activity are no longer seen at age 6 years.

8.2.6 Maternal parity, social class and education

Whilst increasing maternal parity was associated with increased birthweight and neonatal bone mass in line with previous studies[174], decreased growth relative to peers during the first two years of life resulted in no effect on any measure on bone mass or body composition at aged 6 years. Mothers with a higher level of education had children with reduced growth during the first year of life. However these children subsequently caught up by the age of four. Mothers with higher educational attainment were more likely to breastfeed; increased duration of breastfeeding determined early childhood growth. Mothers of higher educational attainment were also more likely to feed their children healthy diets,[175] since they themselves may eat healthier diets. This results in reduced fat mass and increased lean mass at age 6. Whilst there was no effect on bone mass at age 6 years it is important that young mothers are given the appropriate information in order to choose healthy choices for themselves and family in order to reduce the increased burden of obesity.

8.3 Childhood predictors of bone mass

8.3.1 Infant and childhood diet

By the age of 6 years we were unable to detect any influence in pattern of infant feeding and the child's bone mass. Children who were exclusively bottle-fed appeared to have accelerated weight gain during early infancy. This is well recognized and compared to formula feed infants, breast-fed term infants grow slower during the first few months of life and then have an accelerated growth, such that by the age of 6 years there was no overall measurable difference in height. However the duration of breastfeeding was associated with reduced fat mass in the child at age 6 in line with previous studies[176]. Energy intakes have previously been shown to be higher in formula fed infants[177] compared to breast fed babies, it has also been suggested that breastfed babies are better at self regulating their total energy intake by reducing their milk intake when solids are introduced. These early feeding patterns may explain why children who were formula fed are at more risk of obesity in later life.

The association between 6 year total daily milk intake and increased skeletal bone mass supports previous studies suggesting that calcium and milk intake are important for skeletal growth. However unlike previous studies we were unable to find an association with height. Greater bone mineral gains were seen at cortical skeletal sites, in particular the femoral shaft compared to the narrow neck and intertrochanteric regions of the femoral neck[62]. This increase in cortical thickness reduces the buckling ratio, an indicator of cortical instability and risk of buckling on the compressive surface. Milk intake during childhood has previously shown persisting beneficial effects during adulthood[68]. Low intakes are associated with an increased risk of both childhood[111] and adult fracture[68]. Whilst we were only able to study milk intake as a surrogate for calcium, recent evidence suggests that it is milk rather than other dairy products or food rich in calcium that results in increased skeletal size[178]. Milk contains calories, protein, and calcium, among other nutrients, and bioactive components such as insulin-like growth factor-I (IGF-I), all of which may facilitate bone growth.

Individual nutrient data was not available. However dietary patterns were assessed using principal component analysis at ages 6, 12 and 3 years. Children with a high score (high consumption of fruit, vegetables, wholemeal bread, rice and pasta) had an increased skeletal size once adjusted for the weight of the child. Whilst dietary patterns and bone mass have not previously been studied in children, previous literature may support our findings. In particular recent work has studied dietary scores of the mother during pregnancy and bone mass of her offspring[74]and other studies have shown that increased fruit and vegetable intakes are associated with increased bone mass[71;72]. Whilst micronutrient interventions, such as calcium might be effective in improving skeletal health to some degree, the alteration of both maternal and child choice and behaviour to a healthier eating pattern, might yield greater health dividends.

8.3.2 Physical activity

The beneficial effects of exercise on bone mass have been well documented. Bone adapts to increased loading in order to maintain structural and functional support to the skeleton without injury or fracture. There are two ways in which the skeleton can adapt. The first is by increasingly the size of the skeleton through periosteal expansion and the second is to increase the amount of mass within the periosteal envelope by increasing the density of the bone mineral.

We found that in our cross sectional study of habitual exercise, children that participated in high amounts of daily vigorous activity had increased whole body, lumbar spine and hip volumetric density (using method of Prentice), but no increase in bone area. In contrast children that spent more time in sedentary activity had lower volumetric density. Tibial bone mass was only associated with increased cortical density, while there was no evidence of periosteal expansion, the numbers in this part of the study were very small and it is difficult to draw negative conclusions. When measuring the femoral neck, increased cross sectional area, sub-periosteal width and bending strength were only present once adjusted for the child's height. Together these results suggest that habitual vigorous activity increases bone mass by increasing density. At sites where loading is higher (for example the femoral neck), increases in skeletal size are relative to the overall size of the child. Children that participated in increased amount of activity also had higher percentage lean mass and

a corresponding decrease in fat mass. Whilst children that participated in increased activity at aged 4 years also participated in more activity at aged 6, there was no effect of 4 year exercise on 6 year bone mass. This may be due to higher levels of habitual exercise in 4 year olds compared to 6 year old children who are now in school.

8.3.3 Obesity

Children with increased fat mass had a larger overall skeletal size. However the data from both DXA and pQCT suggests that these bones are under mineralised. For a given weight, children with increased adiposity had a relatively smaller and weaker skeleton. Whilst children that are overweight have an increased total lean mass, one of the strongest determinants of bone mass throughout life, this is not enough to compensate for the increased adiposity. The mechanostat model proposed by Harold Frost in the 1960s suggests that the growing skeleton is sensitive to mechanical strain and responds by increasing periosteal apposition. This results in wider bones and increased trabecular bone mass[179].With a reduction in total body BA relative to body size and BMC relative to lean mass in obese children, obesity appears to impair the normal response of the growing skeleton to mechanical loading, effectively resulting in an intrinsic bone abnormality This may explain the increased risk of fracture reported in the literature, although we did not have the power to show this in our study.

Several potential mechanisms have been proposed to explain the complex relationship between fat and bone mass. Studies of adipocyte function have revealed that adipose tissue is not just an inert organ for energy storage. It expresses and secretes a variety of biologically active molecules, such as oestrogen, resistin, leptin, adiponectin, and interleukin-6 (IL-6). These molecules affect human energy homeostasis and may be involved in bone metabolism, which may contribute to the complex relationship between fat mass and bone. The secretion of bone-active hormones from the pancreas (including insulin, amylin, and preptin) may also explain part of the relationship between fat mass and bone mass. Finally, adipocytes and osteoblasts originate from a common progenitor, the pluripotential mesenchymal stem cell[180]. These stem cells display an equal propensity for differentiation into adipocytes or osteoblasts, and the balance of the differentiation is regulated by

several interacting pathways that may contribute to the final effect of fat mass on bone[181]. Further work is needed to elucidate these complex mechanisms.

8.3.4 Lean mass and muscle strength

Studies investigating the relationship between growth in early life and muscle mass have demonstrated consistent findings linking low birthweight with reduced muscle mass[182]. An association between low birthweight and reduced muscle strength was first reported in the Hertfordshire aging study[183]. The association was replicated in a younger Hertfordshire cohort[184] and in a national birth cohort of middle aged men and women born in 1946 and participating in the national survey of health and development[185]. More recent work has demonstrated a similar effect size of birthweight on adult muscle strength in young women aged 20-34 years, taking part in the Southampton Women's Survey, suggesting an association between early size and peak muscle strength rather than decline[186]. Grip strength is a simple measure of muscle function, but is a powerful predictor of disability and morbidity[187]. It is highly correlated with muscle mass[188] and reflects a complex mixture of contractions between hand and forearm muscles.

In this study, we were able to confirm that increased birthweight was associated with increased grip strength at age 6 years and that grip strength was independently related to both increase in size and density of the skeleton at age 6 years. The only other study to examine the relationship between grip strength and bone mineral density in children was one performed in Hong Kong by Chan et al on 10-12 year old girls and boys. In this study prediction models by grip strength and weight explained about 60% and 40% of the variations in BMC of different sites and in BMD of hip and spine respectively[189]. Our own data extends this work; the pQCT analysis might suggest that there was a differential effect with increased density at trabecular rather than cortical sites. However overall bone strength was increased at all sites among children with higher grip strength.

8.3.5 Childhood growth

Growth appears to follow a predetermined path, probably set out by genetic factors, which may be temporally or permanently modified by environmental influence. The

tracking of skeletal development begins in the pre natal period. Hence factors that influence growth during this period have lasting effects on skeletal growth. Only maternal and paternal height predicted the conditional gain in femur length during 19-34 weeks in contrast other maternal influences such as smoking status, fat stores and walking speed affected the conditional gain in abdominal circumference during the same period. This suggests that intrauterine growth may differentially influence postnatal skeletal size in keeping with other studies[134].

Whilst there is a tendency for an individual to stay in the same position relative to peers over the growth period in the distribution of bone mineral, factors such as physical activity[78;80] and milk intake[68;69]have been shown to permanently alter bone mineral accrual postnatally and lead to higher bone mass in later life. Since the genetic component to peak bone mass around 60% of the variance is explained by inheritance[190], it is not surprising that other environmental factors are important in determining skeletal growth. This supports the phenomenon termed "programming" in which persisting changes in structure and function result from environmental influences at critical stages of early development.

Unlike some of the previous literature, we found that children born light (lowest quartile of birthweight) remained light at age 6 years and did not appear to under go catch up growth relative to their peers. However, most of the literature relates to children that were born small for gestational age. This definition terms SGA as neonates whose weight at birth is below 2 standard deviations from the mean for the infants gestational age[191]. Among our group, of the 99 children in the lowest quartile for birthweight, only 15 of them fall into the category of SGA. Since maternal height was a strong predictor of catch up growth, and the observation that the children in the lowest quartile had smaller mothers, the findings in our study may just reflect the genetic influences of body size. Whilst we found no effect of catch up weight, in the children born smaller we did see relative catch up growth in terms of height during the first year. In contrast the children born in the highest quartile had relative catch down growth in terms of both height and weight; however they remained larger than their peers at aged 6 years.

Increases in height and weight during childhood that exceeded the expected rate were associated with increased bone size rather than volumetric density. There were

corresponding increases in bone strength. The data from this study suggests that the increased bone mass and strength was through periosteal expansion, increased cortical thickness and increased trabecular and cortical content.

Whilst increased peak bone mass is an important predictor of the risk of osteoporotic fracture in later life, both tall maternal height and poor growth has previously been associated with the risk of hip fracture[118]. Taller adults have an increased risk of fracture[192] possibly because they have a longer femoral neck length, or a greater tendency to fall, despite overall greater skeletal mass. Thus children with tall mothers who grow quickly may end up with relatively undermineralised bones and thus an increased risk of fracture. This may suggest that the cause of catch up growth is important, as if it is genetically driven by a taller mother, in the absence of adequate nutrition, poorer skeletal mineralization may result. In contrast, if the catch up is driven by nutrition, then healthier bones may result.

8.4 Limitations

This study utilised a prospective cohort, with comprehensive assessment of mothers before and during pregnancy and follow up of the children from birth. However, there are a number of limitations during the stages of this study.

8.4.1 Interpretation of multiple analyses and exposures

This thesis has used multiple testing due to the multiple outcomes and exposures. As a result there is a higher risk of incorrectly rejecting the null hypothesis and getting high false positive rates. There are several methods that have been developed to deal with the problem of multiple testing. A commonly used method is the Bonferroni correction, which multiplies the p value by the number of tests performed. However this method can be too conservative and results in an inflation of false negatives. For this reason the data in this thesis has not been corrected statistically for multiple testing. Instead,our strategy on interpreting multiple analyses was to give weight for a priori hypotheses and overall patterns of association for bone size, density or strength.

8.4.2 Parental data

Self report of maternal lifestyle factors such as alcohol intake, smoking and exercise may have been influenced by women tending to under report behaviour known to be associated with poorer health outcomes and over report beneficial habits.

Food frequency questionnaires (FFQ) were used to assess diet over the preceding 3 month period. Whilst there could have been significant recall bias, nutrient intakes assessed by FFQ have previously been validated against prospective 4 day diaries early in the second trimester.[193]

Intrauterine ultrasound measurements are a standard part of the care pregnant women receive. The measurements we used in this study (abdominal circumference and femur length) were standard measurements, in order to reduce error we used two experienced operators whose repeatability was good. The coefficient of variation for triplicate measurements of femur length was 0.6% at 19 weeks and 0.9% at 34 weeks.

8.4.3 Anthropometry

The anthropometic measurements taken of the mother and of her offspring at birth, aged 6 months, 12 months, 2, 3, 4 and 6 years were performed by trained research nurses. These nurses underwent regular training in anthropometric measurements in order to optimise accuracy and precision and minimise measurement bias. Accuracy is the degree of closeness of the measurement to its actual value whilst precision is the degree of reproducibility. To maximise accuracy, staff were trained to measure from specific landmarks and record the results appropriately. Maximising the sample size in this study improved precision. Precision was improved further by repeating all measurements three times and averages were used in the analyses.

8.4.4 6 year follow up

The study cohort was a subset of the Southampton women's survey. Whilst attempts were made to contact all parents that had initially taken part in the study and whose child was now 6 years, mothers whose children underwent DXA scanning were on average more educated, of higher social class, were less likely to smoke and were

taller and heavier. However our results are based on internal comparisons, so will not have been biased by these differences.

8.4.4.1 *Dietary data*

Although diet was assessed using a FFQ that assessed 100 foods or food groups which will ultimately allow us to look at nutrient derivations and dietary patterns, the only information available at the time of writing was the consumption of various food items. Although there is concern that FFQs can be prone to measurement error[194], they have been shown to identify similar patterns of diet as other dietary methods, and dietary pattern scores determined using different dietary methods are highly correlated[195].

Milk intake was used as a surrogate of calcium intake. However other dairy products or foods containing calcium were not included. Whilst it would have been useful to look at total calcium intake and bone mass, milk intake per se is important due to the additional nutrients, and bioactive components such as insulin-like growth factor-I (IGF-I), all of which may facilitate bone growth.

8.4.4.2 *Actiheart*

The actiheart monitor has previously been validated, showing high linearity with acceleration and agreement within 5 beats per minute of ECG monitoring during rest and treadmill exercise[196].

Only the accelerometery data was available for analysis, (Since the devices also measure heart rate it should be possible to calculate daily total expenditure). Mathematical algorithims are currently being developed at the MRC Epidemiology Unit in Cambridge to optimally clean the data in order to account for times when the data was lost. Actiheart measurements were not performed on all children and at the time of writing only a small proportion of children with PQCT data had clean data available for use. This was due in part to the high frequency of skin rashes with the electrodes used. If the child was unable to wear the device for less than 4 days the data was excluded.

8.4.4.3 *DXA measurements*

DXA is highly reproducible, easy to perform and uses minimum radiation. Although this technique has been well validated in adults, it is beset by technical limitations when used in children. These can be broadly classified as difficulties in scan acquisition due to the limitations in the bone edge detection software in children with low bone mass[197], inadequacy of paediatric reference data across maturational stages, ethnic groups and gender in healthy children and difficulties in the interpretation of DXA in children with impaired growth.

The reduced amounts of bone mineral lead to increased proportional error[198]; in particular any artefact such as movement or foreign objects result in disproportionately large discrepancies. Whilst movement was not a major problem at aged 6 years, with the majority of children being able to lie still, all study movement was graded and any child with excessive movement or those with visible foreign objects on the scan were excluded. Edge detection of bones is more difficult in smaller children due to the lower absolute BMD. However specific paediatric software was used with increased sensitivity for edge detection. The DXA measures of bone mass have been shown to correlate well with whole body calcium content in ashing studies of piglets and DXA lean and fat mass validated against the chemical lean and fat contents[199]. Another known problem is variability between the proportions of intraosseous marrow fat and that in lean tissue; in osteopenic individuals, accuracy errors in estimation in BMC could be as much as much as 20%[200]. DXA calculates aBMD from 2D images. However whilst this is suitable for use in adult populations, in children as the child grows so does the volume of bone. Whilst adjustments can be made for body size in order to calculate estimated volumetric density, all incorrectly assume the bone to be cylindrical in shape. pQCT which uses 3D images is therefore more appropriate for assessing true volumetric density and was thus incorporated into our study methodology.

Finally DXA imaging could be improved with further refinement of the algorithms used for its body composition modeling. It is not able to differentiate muscle from other lean tissues, such as liver, spleen and other organ tissue, nor can it distinguish adipose tissue from bone marrow fat or fat within solid viscera. Regional fat mass analysis with DXA does not give a reliable assessment of visceral fat, particularly in

smaller children. However, despite these limitations DXA is widely available and has the largest body of research and clinical data associated with it.

8.4.4.4 *Peripheral quantitative tomography*

Few studies have investigated factors influencing bone geometric measurements in young children. However it has been validated in children as young as 3 years[94]. In our study whilst movement occurred frequently; good positioning , tibial restraint and distraction of the child using television significantly all reduced movement. Scans where the cortex was interrupted were excluded, but this was only a small proportion of the total scans done (4.6% at 38% site). Whilst it would have been beneficial to have also had radial scans, movement artefact was so high at this site the procedure was abandoned from the protocol to concentrate on the tibia.

In children of this age the growth plate is still visible. Therefore the reference line should be positioned to bisect the medial border of the distal dense metaphysis. Our reference line was positioned to bisect the medial border of the articular surface of the tibia. Hence for a number of scans, the 4% site went through the growth plate, giving artificially high density. For this reason trabecular content and density was presented from both the 4% and 14% site. Accurate and consistent positioning accurate and positioning of this reference line is essential in any longitudinal or multi-centre studies for comparable results[201].

At the tibia the most common sites scanned include 4%, 38%, 50% and between 60% and 68% regions[202]. We used 4%, 14%, 38% and 66%, which were the machine's preset values. Both the 4% and 14% were used to measure the trabecular bone whilst the 38% site used to measure cortical bone and 66% the muscle bone unit. This variety and inconsistency of sites scanned, particularly in children, make comparison of results between studies problematic. However since our results were based on internal comparisons this was not a major problem.

As with other bone densitometry techniques pQCT require skilled and dedicated technical staff to perform the scans with optimal precision. The technical staff and nurses using this machine had regular training from experts in the field.

8.4.4.5 *Hip structural analysis*

Though the HSA program is commonly used in adults and more recently in children, however there are limitations to its use[203].

In particular, bending strength indices are measured only in the plane of the scan image; bending strength differences in other directions may exist; however, they cannot be determined by this method[204]. Cortical thickness measurements were made after making assumptions regarding the shape and symmetry of the bone in cross section. It is not always clear what assumptions are made and hence the results should be treated with caution.

In addition, the HSA algorithm assumes average mineralization of 1.05 g/cm^3 which is appropriate for adults[205], but lower mineralization densities would be expected in children and, therefore, a systematic underestimation of (absolute) CSA and Z modulus is assumed. We found edge detection in the smaller less mineralised hips to be a major problem. The HSA software was unable to analyse these images and despite discussion with the program designer 52 images were excluded. Although the children that were excluded were smaller than the rest of the cohort, their lifestyle characteristics were similar and hence it is unlikely that the results would have been biased by these differences.

Inconsistent positioning in sequential scans can change projected dimensions so that it can be difficult to distinguish dimensional changes from positional area. The scans were therefore obtained by trained technicians in paediatric densitometry and the scans analysed using dedicated technical staff.

It should be noted that whilst HSA has previously been validated in adults against quantitative CT of the hip[206] there are no such validation studies in children of this age.

8.5 Further work

There are two main aspects to future work. Firstly a greater understanding of the mechanisms behind the results obtained is required. Secondly, there needs to be translation of the observations seen into clinical practice. In particular interventional trials, which improve the lifestyle of both mothers and children, might be envisaged. To this end we are currently planning a study to improve the self-efficacy and perceived control of women attending Sure Start Children's Centres in Southampton by training the Children's Centre staff in holding 'healthy conversations' with their clients. In the first pilot we aim to assess dietary quality, physical activity levels and emotional well-being of the women in the women in both control and the interventional arms. In the longer term we plan to extend this trial out to other primary health care trusts and follow up women who become pregnant.

Furthermore, as the dataset is enhanced using data obtained during this fellowship, there are many potential areas of investigation.

- The detailed dietary data at aged 6 years might be further explored to determine the relationships between both nutrient intake and dietary patterns. This could be further explored using the previous dietary data to see whether there is an optimum age for dietary effects and to look at the effect on longitudinal growth.
- More detailed analysis of our physical activity data, looking at the relationships with maternal physical activity and the total energy expenditure in both mother and child might be planned. This could be explored further by comparing the results against the dietary data, in particular looking at how the diet matches up with the energy expenditure and the effect on body composition and also any individual nutrient interactions.
- Hip structural analysis is currently being performed on the 4 year hip scans. Longitudinal analysis has never been carried out in children this young. Hence it would be very interesting to see the predictors of relative hip geometry in various subgroups, particularly the very active and the overweight children.

- Further scans using pQCT are being obtained. The relationship between physical activity needs to be explored further and additional analysis to see how the results match with the results seen for hip structural analysis performed.
- Detailed body composition data is available for this cohort using the DXA images. Whilst we showed a relationship between total fat mass, the role of regional fat and lean mass needs to be further explored.
- Further work to look at the difference between children that do and don't fracture is needed. We did not have enough power in this current study to detect this but further data collection is underway.

Finally, as the children get older we plan to reassess them using DXA at aged 8 and 10 years. This will give further opportunities to look at longitudinal growth. It will also give us information about the importance of puberty and how this relates to both current and previous body composition. This work will be linked to a follow up of the mothers and fathers, in which DXA and pQCT measurement will be obtained. This will help us understand the role of the genetic influences which determine bone mass.

8.6 Conclusions

In summary, our study showed that the childhood physical activity, milk intake and diet and body composition were all important predictors of bone mass at aged 6 years. Maternal height and smoking were associated with variation in the childhood growth trajectory relative to their peers from birth to aged 6 years. Maternal lifestyle and educational attainment in turn predicted childhood lifestyle determinants in particular diet and activity. These observations suggest that a lifestyle approach starting from preconception is appropriate to increase bone mass in the offspring and reduce the burden of osteoporotic fracture in later life.

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APPENDIX

APPENDIX A: SWS parent home visit information leaflet

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 British Lung Foundation and Food Standards Agency. The study is being 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. The study has also been reviewed by the British Lung Foundation.

Will I be asked to do anything else?
We are very interested in obtaining detailed assessment of the way in which your child's lungs have developed and how he/she has grown. We would like to do more detailed measurements in a clinic at the hospital. The nurse who visits you will bring you another leaflet that describes this, so that you can think about whether you wish to do that part of the study

THANK YOU FOR READING THIS BOOKLET
If you have any further questions please feel free to ask.
Contact us on 0800 783 4503

Southampton Women's Survey
MRC Epidemiology Resource Centre
Southampton General Hospital
Tremona Road
Southampton SO16 6YD

SOUTHAMPTON Women's SURVEY

Southampton Women's Survey

Growth and Asthma:

HOME VISIT

to 6 year old children

Parent information booklet

LREC NO: 06/Q1702/104
v2 03/07

Part 1

Southampton Women's Survey
Developmental influences on childhood respiratory health

As a member of the Southampton Women's Survey (SWS) you have already given a huge amount of time to support the research study. We are extremely grateful for this. You and your child are now being invited to take part in the next stage of SWS. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take the time to read this carefully and discuss it with anyone you wish. Ask us if there is anything that is not clear or if you would like more information. Thank you for reading this.

The study is trying to find out how children grow and why some develop asthma. If you agree, a research nurse will come to your home to measure your child and ask some questions We would like to do some breathing tests to see how their lungs work, and we will also do some allergy skin tests, as we did at a previous visit. We are also interested in finding out how active they are. It is very important that we have healthy children involved in this study as well as children with health problems.

Do we have to take part?
It is up to you and your child to decide whether or not to take part. If you do decide to take part, you will both be asked to sign consent forms, 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 or your child may want to do some parts of the study but not others. That is fine.

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 children's research team, and aim never to cause harm to your child. As outlined in Part 1, the planned investigations are considered safe. In the very unlikely event that something does go wrong and you or your child is 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 our taking part in this study be kept confidential?
All information collected about your child and family during the course of the research will be kept strictly confidential. If we discover information that may be useful for your family doctor (eg. allergies or asthma that were previously unrecognised), with your permission we will contact your doctor.

What will happen to the results of the research study?
The results of the study will be published in medical journals so that doctors and health professionals all over the world can understand what increases the likelihood of illness in children. We will also arrange for local papers (e.g. The Echo) to write about the study results so that you know what we have found.

Activity monitoring: We will ask you and your child to wear a small Actiheart monitor for up to a week. The monitor is a small plastic instrument about the same size as a 50p piece. It will be stuck to your chest using adhesive pads and it records your activity and heart rate while you are wearing it. This activity monitor is waterproof and very safe. Very occasionally the adhesive pads cause some temporary itchiness.

Skin prick tests (allergy tests): As in previous visits, the nurse will test for various allergic reactions in your child. These tests are very safe. The most common effect is mild redness and itching at the site of testing. A rare side effect is a more widespread rash. The nurse will have antihistamine medication to use if necessary.

Trained staff will be present throughout the tests. The investigations have all been performed in very large studies of adults and children and are all considered safe.

What do I have to do?

If your child takes an antihistamine (eg. Piriton, Cetirizine, Zirtec®), please try to avoid them taking it for 7 days before the visit because it interferes with the allergy tests. If your child needs to take an antihistamine in this time, please let them and telephone the study team before the visit because it may be necessary to change the appointment.

If your child uses asthma medication please try not to use their reliever medication or long acting beta agonist *(blue inhaler, Ventolin®, Salbutamol, Bricanyl® green or purple inhaler Serevent®, Seretide® white and red Symbicort®)* for at least 12 hours before the appointment, unless you feel it's necessary

Please use your child's preventer medication *(inhaled steroid, Flixotide®, Becotide®, Pulmicort®, Singulair®)* as usual. **If in doubt, please contact us before the day of the appointment.**

We also ask that your child does not have any tea, coffee, fizzy drinks or chocolate, on the day of the test as this can affect the lung function results.

What are the possible benefits of taking part?
The main benefit is knowing that you and your child are part of a unique study that will help identify risks for diseases in childhood. By the end of the visit your child will have learnt lots of facts about their body and how it works.

In addition, some children may be helped by obtaining information about their lung function and potential allergies. Some children may not have been diagnosed as asthmatic or as having allergies. With your permission, we will inform your family doctor of any unexpected results.

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. The detailed information on this is given in Part 2.

Will my taking part in the study be kept confidential?
All information about your participation in this study will be kept confidential. The details are included in Part 2.

Contact Details:
If you have any questions about this study, 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.

What will happen if we take part? As with previous SWS visits a research nurse will come to your home and ask you questions and measure your child.

Questionnaire: The nurse will ask you about infections, allergies, asthma and other health problems your child may have had. She will also ask questions about your child's lifestyle, for example, about the foods they eat and the sports they do.

Growth measurements: The nurse will make similar measurements as those obtained before, such as height, weight and skinfold thickness.

Lung tests: We will ask your child to blow hard a few times, into a tube that is connected to a computer. This will give information about how your child's lungs have grown, whether they have any inflammation in their lungs and whether they are likely to be asthmatic. Because the test involves forced, rapid breathing, occasionally people experience temporary shortness of breath or mild wheeze.

Genetics testing: We would like to look for genes that may be important for asthma, allergy and growth. This will involve taking a painless swab from the inside of the cheek. The results of any genetics tests will be linked by a unique ID number with information about your child's growth and asthmatic status on the SWS computer, which also uses ID numbers and not names. Therefore the results of any genetics tests (or any other information or investigations) will remain completely anonymous and will not be linked to your name or your child's. We will therefore not be able to feedback the results of these tests to you.

Appendix B: SWS child's home information leaflet

Do you have allergies?

You probably had allergy tests as part of the SWS study when you were a baby and young child. We would like to do the tests again to see if your allergies have changed. If you are allergic, the skin tests might make your arm a bit itchy afterwards. Luckily the nurse has soothing cream that takes away the itch!

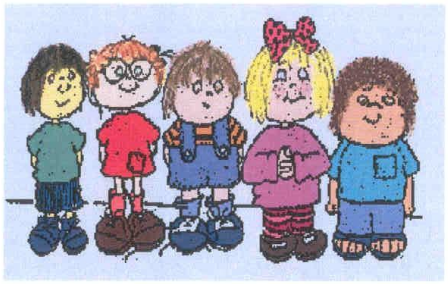
If you would like to join in with the study, a nurse will visit you at home. You'll learn lots about your body and how it works.

If you have any questions, show this leaflet to your Mum or Dad who may be able to help you. If you still have questions the SWS nurse will be happy to answer them when she visits your house.

Contact Details:
Southampton Women's Survey
MRC Epidemiology Resource Centre
Southampton General Hospital
Tremona Road
Southampton SO16 6YD
Phone: 0800 783 4503

LREC 06/Q1702/104
v2 03/07

SOUTHAMPTON Women's SURVEY



Six year Follow-Up

HOME VISIT

Child's Information Leaflet

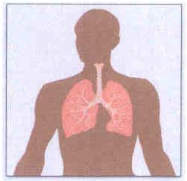
Since before you were born, you and your family have been helping with a very special project that is helping doctors understand how to help children to be healthier. It's called the **Southampton Women's Survey** (or **SWS** for short). It's a long time since we've seen the children on our study, and we are hoping that you will be able to help us again. We want to find out how you have grown. We also want to see how your lungs work and whether you have any allergies.

Our bodies are made up of **fat**, **muscle**, **bone** and **water**. How much we have of each is very important for health and fitness.

A SWS nurse will visit you at home. She will measure things like your height, weight, arms and head.

How healthy are your lungs?

To find out how your lungs are working, we will ask you to blow very hard into a tube that is connected to a computer. If you blow really hard you might be able to blow out the candles on the computer screen!



How active are you?

We want to find out whether you are someone who sits still ..... or whether you move around lots. We have special monitors, about the same size as a 50p piece, that you and your Mum can wear for a week. This will tell us how active you both are.

The nurse will show you both how to wear it and will lend them to you when she visits your home.

Developmental influences on childhood respiratory health

6 years assessment Protocol

Developmental influences on childhood respiratory health

Protocol v 4 June 2007
LREC 06/Q1702/104

Developmental influences on childhood respiratory health

SWS cohort study at age 6 years

Protocol

A. Research questions and hypotheses

The study has four main research questions.

1. What are the links between asthma and obesity in childhood? Is the link:

- Common antenatal environmental exposures?
- Common postnatal environmental exposures?
- Common genetics?

2. Do maternal genotype and phenotype impact on the child's phenotype independent of infant genotype.

3. Does pre- and post-natal nutrition affect the development of asthma and other wheezing illnesses in the child?

4. Is there a link between asthma and obesity in childhood and impaired bone growth

The research questions will be addressed by investigating the following hypotheses:

1. The association between asthma and obesity is the result of particular *prenatal* environmental influences (maternal high fat mass, low energy intake and smoking during pregnancy) that increase the risk of both disorders.

2. The association between asthma and obesity is the result of particular *postnatal* environmental influences (high infant weight gain and low childhood physical activity) that increase the risk of both disorders.

3. The association between asthma and obesity is the result of polymorphisms in particular candidate genes that increase the risk of both disorders.

4. Maternal genotype and phenotype determine obesity and asthma in the child independent of the child's genotype.

5. Impaired maternal nutrition during pregnancy (specifically low maternal fat and/or muscle mass and low intakes of vitamins A and/or C) is associated with impaired lung function (defined by spirometry) at 6 years of age

6. High maternal fat mass, high vitamin D status and low maternal vitamin E intake in pregnancy are associated with atopy (positive skin prick test) at 6 years of age

7. Maternal nutrition and faltering of fetal growth in late gestation relate to each of the childhood wheeze syndromes (transient viral induced wheeze, atopic asthma and non-atopic asthma).

8. The association of asthma and obesity with the increased risk of childhood fractures is the result of postnatal environmental influences such as low childhood activity which impairs the growth of bone.

B. Background

The prevalence of asthma and obesity increased in parallel during the 1980s and 90s, and mounting evidence suggests a link between obesity and the development of asthma(Wannamethee, Shaper, and Whincup;Weiss and Shore). It has been proposed that environmental or genetic factors common to both disorders are responsible(Schaub and von Mutius). The proposed study will investigate whether particular aspects of the pre-natal environment (maternal high fat mass, low energy intake and smoking during pregnancy) and/or postnatal environment (high infant weight gain and low physical activity) are associated with asthma and obesity at 6 years of age. It will also investigate the genetic influences that determine asthma and obesity. It has been suggested that polymorphisms of the beta-2-adrenergic receptor (ADRB2), ADAM33, IL6, leptin, TNFA and PPARG genes may contribute to both asthma and obesity, but there is currently little evidence to support or refute a role for these candidate genetic influences.

The proposed study will use prospectively collected, longitudinal growth and respiratory data in 950 6 - 7-year olds enrolled in the Southampton Women's Survey(Inskip et al.). The children's mothers were extensively characterised before and during pregnancy; body composition (detailed anthropometry), dietary intakes (food frequency questionnaires and food diaries), physical activity, atopic disorders and smoking were recorded. Longitudinal fetal growth measurements were collected by ultrasound at 11, 19 and 34 weeks. Children have been monitored for growth and features of respiratory morbidity and atopy at 6 months and 1, 2 and 3 years. Additionally, 131 of the cohort had lung function measured in early infancy, showing impaired lung development in infants that had had lower rates of fetal growth and higher weight gain in the first weeks after birth(Lucas et al.). The assessment at 6 years will allow us to collect a detailed dataset that combines information on asthma, body composition and physical activity in childhood. To add to information that is being collected on lung function and respiratory symptoms, we will measure adiposity and regional body composition (anthropometry, densitometry and DXA scanning), to collect objective physical activity data using a combined accelerometer and heart rate monitor, and to characterize the genetic variation of particular candidate genes linked with asthma and/or obesity. For 6 candidate genes, haplotype tagging sets of Single Nucleotide Polymorphisms (SNPs) will be selected based on information available from the Seattle SNPs variation discovery resource (http://pga.mbt.washington.edu/) (ADRB2, IL6, PPARG, TNFA and leptin) and from our own data (TNFA and ADAM33). These SNPs together with putative functional SNPs (e.g. PPARG Pro12Ala and IL-6 -174) will be typed in child and parental DNA by a combination of methods.

Collection of these data will allow us to relate pre-natal (maternal high fat mass, low energy intake and smoking during pregnancy) and postnatal (high infant weight gain and low physical activity) exposures and genotypes to respiratory outcomes and adiposity at age 6 years, and to examine whether maternal genotype and phenotype impact on the child's phenotype independent of infant

genotype. We hope that understanding the links between these disorders will enable us to develop strategies to reduce the chance of individuals developing asthma.

C. Study design and methodology

The Southampton Women's Survey

The Southampton Women's Survey (SWS) was started in 1998. It is a study of a population sample of non-pregnant women aged 20 to 34 years resident in the city of Southampton, UK. They are representative of the British population in terms of ethnicity and deprivation. From this group, 1477 of those who have become pregnant and delivered infants would be eligible for the assessment of the children at age 6 years proposed in this study. We conservatively estimate that 65% will participate, giving 950 children.

Existing data from the SWS cohort

Maternal nutritional data

Uniquely, maternal body composition and diet have been assessed before and during pregnancy. Body composition is assessed by 4-site skinfold thicknesses and other anthropometric measures, allowing estimation of fat and muscle mass. Diet is assessed using a 100-item administered food frequency questionnaire to record the average frequency of consumption over a 3-month period preceding the interview. Although such questionnaires can be subject to bias, validation using 4-day food diaries and measurement of maternal micronutrient concentrations has indicated that our questionnaire gives an assessment of diet that can be used to rank the nutrient intakes of individuals. Dietary supplement use is assessed in detail over the same period, allowing us to derive maternal intakes of vitamins A, C, D and E during pregnancy. Vitamin intakes in a previous cohort of Southampton pregnancies showed marked variability. Maternal 25-OH vitamin D concentrations in early and late pregnancy are being measured and are combined with information on ultraviolet B exposure calculated from the hours of sunshine from a local Meteorological Office weather station with an adjustment for seasonal energy variation in ultraviolet B radiation (http://www.soda-is.com/index.html); in our previous study, the correlation coefficient between this measure of ultraviolet B exposure and maternal 25-OH vitamin D concentration in late pregnancy was 0.60, $P<0.0001$ (unpublished data).

Fetal growth data

Longitudinal fetal growth measurements have been collected by ultrasound at 11, 19 and 34 weeks, together with detailed neonatal anthropometry. Using the method of Royston, we will generate Z-scores of crown-rump length, head and abdominal circumferences adjusted for duration of gestation in early, mid and late pregnancy and at birth, and calculate the velocities of growth unconditional and conditional upon the initial measurement of size. We will use longitudinal ultrasound measurements of fetal anthropometry at 11 and 19 weeks gestation to define the velocity of the initial trajectory of growth, and the change in abdominal measurements between 34 weeks and delivery to describe growth faltering in late pregnancy.

Other information available about this cohort from birth to 4 years of age

At 6, 12, 24 and 36 month visits, the principal carer has been questioned about the child's illnesses since the previous visit. These questions focused on respiratory, allergic and gastrointestinal symptoms and illness. Specifically, the questionnaire asked about episodes of wheezing or whistling in the chest. We also have prospectively collected data about other important exposures, including environmental cigarette smoke, pets and childcare. Skin prick testing has been undertaken at 1 and 3 years of age at a time when subjects had not taken any anti-histamine for at least 72 hours. Testing to cat, dog, grass pollens, house dust mite, milk and egg allergens (ALK, Horsholm, Denmark) was undertaken with a single headed lancet. Weal diameters were measured and a positive result defined as one that is at least 3mm in diameter in the presence of valid controls. The controls are valid if the negative (saline) control was zero and positive control (histamine) is at least 3mm. Additionally, we have DNA stored for each subject. Premorbid infant lung function data, domestic dust samples and urinary cotinine measurements are available from a subset of 150 participants. At aged 4 years 650 children underwent body composition measurement by DXA. A number of these children have also had their physical activity measured using an actiheart monitor.

Respiratory and growth data to be collected at age 6 years

Recruitment

All families are already recruits of SWS. Families currently enrolled with the SWS, whose child is between 6 and 7 years during the period of recruitment will be identified from the SWS database.

Some families will have moved house and not informed the SWS of their change of contact details. We will therefore have a press release aimed at local media to inform them of the aims of the 6 year old assessment, and to encourage families to contact the team if they need to update contact information.

Families who are enrolled with SWS will be contacted by post to inform them of the 6 year follow-up. A member of the research team will then contact the family by telephone or email to ask if they would like to participate in this part of the study. This follows the format of previous SWS contacts and appointment making. No undue pressure will be placed on families to participate.

Children will be recruited between the ages of six and seven years. They will have a home visit from a SWS nurse and will be invited for further investigations to the Wellcome Trust Clinical Research Facility (WTCRF), Southampton, with paediatric facilities for more detailed respiratory and body composition investigations.

A number of parents and children will be invited back for an additional study at the osteoporosis centre. These children will be given the relevant information at the initial clinic visit and contacted by telephone at a later date to arrange this further visit.

Inclusion criteria

All children enrolled on the SWS who will be between 6 and 7 years old during the study period.

Exclusion criteria
All carers will be invited to complete the questionnaire.

Exclusions for specific investigations are as follows:

- Methacholine challenge: Baseline FEV_1< 75% predicted; unstable asthma; current respiratory infection.
- Skin testing: antihistamine use within 72 hours.

Consent

Consent will be taken by a nurse or doctor who has a detailed understanding of the study protocol. Prior to consent, the child and parent will have received age-appropriate information sheets (appendix) at least a week before the appointment. The person taking consent will ensure that the parent and child understand the aims and procedures. The parent and child will have as much time as is necessary to ask questions. If both the child and parent are in agreement that the research should proceed we will ask the parent to sign a consent form (appendix).

Where will the studies take place?

Home	**WTCRF**
Questionnaire	DXA
Height, Weight, skin folds	Methacholine breathing test OR reversibility studies using salbutamol
Simple spirometry	Exhaled nitric oxide
Buccal brushing (genetics)	Blood pressure and heart rate
Actiheart	Allergy tests if food allergic
Allergy tests (at WTCRF if food allergic)	Grip Strength
	Peripheral quantitative CT scan (pQCT) at separate visit

Questionnaire

- The questionnaire will be administered by a member of the research team to the child's carer at home.
- If carers live outside the Southampton area, the questionnaire will be administered over the telephone.
- The questionnaire is attached in the appendix. It is primarily designed to assess the child's respiratory and atopic status. It also includes questions to assess current diet and activity.

Body composition

- Measurements of height, weight and skinfold thicknesses, will be made by a trained nurse or doctor.

- DXA scan. This will be performed by technicians trained and experienced in its use. Approximately 500 of the children have had previous DXA measurements in the SWS.
- Measurement of grip strength using a dynamometer . The child will be asked to grip this meter very tightly in each hand separately three times in order to register the best score
- PQCT scan. This will be performed as an extra optional procedure in up to 250 children This visit takes about 30 minutes and involves a short scan of the forearm and lower leg. The procedure is completely painless but does involve the child sitting very still for 5 minutes whilst each scan is being done.

Activity

- Physical activity over a 5-7 day period using an Actiheart combined accelerometer and heart rate monitor; we have successfully used these in over 50 SWS children at age 4 years.
- The Actiheart will be applied by small stickers to the child's torso during the home visit and instructions describing reapplication will be given. The child will be asked to wear the monitor for up to a week, during which time they should pursue their normal activities. They will be provided with a pre-paid package to return the Actiheart to the research team for analysis, or it can be returned when they attend the WTCRF for a visit.
- The Actiheart will be accompanied by a questionnaire about activity and exercise for the parent/carer to complete and return in the pre-paid package with the Actiheart monitor

Atopy

- Skin prick testing to house dust mite, cat, dog, mixed grass pollen, mixed tree pollen, egg and milk. Subjects with a test result ≥3mm with a negative saline control will be defined as atopic. Up to 3 additional allergens will be tested if clinically indicated.

Lung Function

- Lung function, including flow volume loops will be measured using Koko incentive software.
- Within the WTCRF, children will be invited to have a more detailed assessment of their lung function by either (a) methacholine challenge or (b) reversibility with salbutamol. All children with a history of wheeze and approx 100 children without wheeze will be invited to have a methacholine challenge. Those who decline, and all other patients will be invited to have reversibility studied using salbutamol.
- Children prescribed 6 puffs of salbutamol to be administered via metered-dose-inhaler (MDI) and spacer (100mcg per puff) to access reversibility of airway obstruction. Lung function measurement will be repeated 20 minutes after the salbutamol dose.
- Methacholine challenge will be used to document bronchial hyperresponsiveness as an objective marker for asthma. In this test, the patient inhales an aerosol of one or more concentrations of methacholine. Results of lung function tests (e.g. FEV_1) performed before and

after the inhalations are used to quantify the response. A positive test is defined as a decrease from the baseline forced expiratory volume in the first second (FEV_1) or of the post-diluent FEV_1 value of at least 20%.

Exhaled nitric oxide

- Exhaled NO, as a non-invasive marker of airway inflammation will be measured using the single expiratory breath method with a chemiluminescence analyser (Niox desktop system, Aerocrine, Solna, Sweden) set at a rate of 50 ml/s. Measurements are repeated until two consecutive results within 10% were obtained; this generally requires 2–4 attempts. All measurements will be undertaken before spirometric testing. Exhaled NO values will be discarded if the ambient level was above100 ppb.

Clinical Samples

- DNA buccal swabs will be taken from children and parents who consent.
- Samples will be collected by trained nurses and doctors.
- Samples will be labelled with the child's unique SWS identification number for subsequent linking with information in the SWS database. The results will not be linked to individual names.
- The samples will be stored in the SWS freezers in the MRC Epidemiology Resource Centre, SGH. They will be stored until all analyses are completed. Prof Cyrus Cooper, Director of the Centre, and subsequent Directors will have custodial responsibility.
- Stored cord blood and parental DNA (LREC 340/97; 307/97; 018/99) as well as newly collected DNA specimens will be analysed for asthma and obesity genotyping. Stored linked-anony[1]mised samples will be analysed for all eligible children, whether or not they are recruited for this 6 year assessment. This will allow linking of the genotype data to respiratory and growth outcomes in early life as well as at 6 years.

Analysis

Primary outcome measures will be current wheeze at age 6-years, estimated fat mass and distribution, bone mass and strength, atopy and FEV_1, together with FEF_{50-75} (more sensitive to small airway disease, although less reproducible). Controlling for potential confounders, binary outcomes (wheeze, atopy) will be analysed by logistic regression, and continuous outcomes (FEV_1, FEF_{50-75}) by linear regression after transformation to normalize them as necessary. As secondary outcomes, we will investigate clinical wheeze phenotypes defined as (1) transient viral induced wheeze: presence of wheeze only with viral upper respiratory tract infections within the first 5 years of life; (2) atopic asthma: wheeze between viral upper respiratory tract infections or with exercise that responds to a bronchodilator in an atopic child; (3) non-atopic asthma: as for atopic asthma but in a child who is not atopic.

Hypothesis 1

Primary *prenatal* exposure variables will be maternal pre-pregnancy fat mass, energy intake in pregnancy and smoking. To investigate the secondary exposures of low early trajectories of fetal growth and faltering of growth in late gestation, we will generate Z-scores of fetal size adjusted for duration of gestation in early, mid and late pregnancy and at birth, and calculate velocities of growth unconditional and conditional upon the earlier measurement of size(Royston). In those with infant lung function data[10] we will explore whether any relationship between maternal influences and childhood asthma was already apparent in early postnatal life.

Hypothesis 2

Primary *postnatal* exposure variables will be rapid weight gain in infancy (change in Z-score of weight for height, conditional and unconditional upon size at birth) and lower physical activity at age 6 years. Secondary postnatal exposures will be infant feeding mode, duration of breast-feeding and postnatal smoke exposure. Cord blood leptin will be used as a measure of adiposity at birth, to examine whether any associations truly reflect postnatal influences.

Hypotheses 3 & 4

We will relate genotypes directly to outcomes, and analyse associations between the environmental exposures and outcomes, stratifying for category of genotype. We will also utilise the parental DNA to undertake family-based analyses of association that avoids potential confounding by population stratification using FBAT methodology.

Hypothesis 5

FEV_1 and FEF_{50-75} will be transformed to normalize them as necessary. Multiple regression analysis will be used to investigate whether they are influenced by low maternal fat and/or muscle mass and low maternal intake of vitamins A and C. We will explore whether the children whose fetal growth faltered in late gestation (as measured by serial ultrasound scans) are those whose impaired maternal nutrition during pregnancy most affects their childhood lung function, and whether there is an interaction between vitamin C intake and smoking. Lastly, we plan to use the infant lung function data, available for a subgroup, to allow us to explore whether any relationship between maternal nutrition in pregnancy and childhood lung function is already apparent in the first few weeks of life.

Hypothesis 6

Logistic regression will be used to investigate the effects of high maternal fat mass and high vitamin D status, and of low maternal intake of vitamin E during pregnancy on atopic status at age 6 years. Atopy will be defined as at least one positive skin prick test.

Hypothesis 7

The clinical wheeze phenotypes will be defined as (1) transient viral induced wheeze: presence of wheeze only with viral upper respiratory tract infections within the first 5 years of life; (2) atopic asthma: wheeze between viral upper respiratory tract infections or with exercise that responds to a bronchodilator in an atopic child; (3) non-atopic asthma: as for atopic asthma but in a child who is not atopic. Children with history of wheeze will be assigned to one of these categories according to the timing of symptoms and the presence of atopy. An exploratory analysis will be undertaken to examine how maternal nutrition (as defined by body composition, vitamin D status and vitamin A, E and C intakes) and fetal growth (as measured by serial ultrasound scans) differ between these three wheeze phenotypes and atopic and non-atopic children who have no history of wheeze. This will allow us to explore whether impaired maternal nutrition during pregnancy and impaired fetal growth are important in the development of each of these wheeze phenotypes. Lastly, the infant lung function data, available for a subgroup, will allow us to explore how impaired lung function develops in each of these childhood wheeze phenotypes.

Hypothesis 8

The inital analysis will focus on differences in bone mass and strength between asthmatic and non asthmatic children. regression will be used to confirm an association between bone mass, density and bone strength in children with asthma and obesity. Multiple regression analysis will be used to investigate whether this in influenced by postnatal exposure variables (physical activity levels, muscle and fat mass, childhood diet and use of inhaled steroids). We will then explore prenatal exposure variables such as pre-pregnancy maternal fat mass, smoking and activity levels to look at how the growth trajectory of bone is set during early life.

Sample size and power calculations

Assuming a 65% follow-up of the 1477 children gives a sample size of about 950. Our experience of similar longitudinal cohorts indicates that we may well achieve a higher follow-up, giving greater statistical power than shown here. Assuming a 5% level of significance, for objectives 1 and 2, we have 97% power to detect a difference of 0.25 SDs in the continuous outcomes of FEV_1 and estimated fat mass, between the top and bottom halves of the distribution of each continuous exposure variable (maternal fat mass, energy intake, infant weight gain and physical activity). This falls to 90% power for a 1% level of significance. Analysis of the continuous outcomes without dichotomisation will provide greater power. For objectives 1, 3 and 4, in relation to the dichotomous exposure variables of smoking and genetic polymorphisms, the table below gives the power to detect a difference of 0.25 SDs in the same continuous outcomes for various different prevalences of the exposure variable. We anticipate that the prevalence of the genetic polymorphisms ranges from 50%-10%; the prevalence of smoking in pregnancy in this population is 17%:

Frequency of exposure	Statistical power
50%	97%
40%	97%
30%	94%
20%	87%
17%	83%
15%	79%
10%	64%

For hypothesis 5, we have 87% power to detect a difference of 0.2 standard deviations (SDs) in FEV_1 between the top half and the bottom half of the distribution of each exposure variable. Defining impaired fetal growth or impaired maternal nutrition as those in the lowest 20% of the distribution, we have 87% power to detect a difference of 0.25 SDs in FEV_1 between these groups and the remaining children.

For hypothesis 6, for any normally distributed exposure measurement we have 81% power to detect a difference of 0.25 SDs between the exposure of those with atopy (assuming a 16% prevalence of atopy at age 6 years) and those without. Dichotomising the exposure gives 80% power to detect a relative risk of atopy of 1.55 for those in the bottom half of the exposure distribution compared with the top half.

For hypothesis 7, we will perform an exploratory analysis. As an example of our power, if we measure lung function at six years in 70 children with infant lung function data and the prevalence of wheezing at six years is 20%, we will have 80% power to identify a 20% difference in infant $FEV_{0.4}$ between those who wheeze at six years of age and those who do not.

For hypothesis 8 we have 90% power to detect a difference of 5% in whole body bone mineral content between the highest and lowest quartiles of the distribution for each exposure variable.

D. Key Milestones

Respiratory and body composition assessments at 6 years of age will occur during the initial 27 months. During year 1 the whole cohort will be genotyped. A final report, and drafts of publications will be submitted to LREC by October 2011.

E. The research team

Drs Lucas, Roberts and Holloway are academic researchers within the Infection, Inflammation and Repair (IIR) Division of Southampton School of Medicine. Professors Godfrey and Cooper and Dr Inskip work within the MRC Epidemiology Resource Centre. The applicants have a track record of successful collaboration(Lucas et al.). Dr Lucas is a respiratory paediatrician with a research interest in lung development. Professor Godfrey's research within SWS is characterizing the interactions between prenatal, postnatal and genetic influences on health outcome. Dr Holloway

heads the Asthma Genetics Group and recently reported ADAM33 as an asthma-susceptibility gene. Dr Inskip, a statistician/epidemiologist, coordinates the SWS, studying the effects of pre-conceptional factors on fetal and postnatal growth. Dr Roberts is a respiratory paediatrician, with an expertise in epidemiology. Professor Cooper is Director of the MRC Epidemiology Resource Centre and has expertise in developmental influences on body composition.

The team will include nurses from the WTCRF who are experienced in research with children. Training will be provided to the nurses in any aspects of the protocol, as necessary. Home visits will generally be conducted by SWS nurses who have been involved in earlier visits to SWS families and have developed and nurtured relationships between the participants and the Survey.

We have employed Dr Katy Pike as a Clinical Research Fellow to assist in the clinical investigation of the cohort and the analysis of the data, under the supervision of the PI. Dr Pike is a Paediatric SpR with an interest in paediatric respiratory medicine. Dr Zoe Cole, clinical research fellow and rheumatology SpR will be working with the team assisting with DXA, pQCT assessment and activity monitoring, Her PhD will focus on the developmental influences of body composition. Training in research governance, ethics, child protection, respiratory physiology etc will be provided.

Appendices

1. Questionnaire
2. Parent information sheets

a. Home visit

b. WTCRF visit

3. Children information sheets

a. Home Visit

b. WTCRF visit

4. Consent/ Assesnt forms
5. Peripheral Quantitaive Computed Tomography Optional Study Protocol

a. Parent information sheet

b. Child information Sheet

c. Consent form

d. Ionising radiation form

Investigators

University Child Health, IIR/ DOHaD

Dr Jane Lucas, Senior Lecturer/ Honorary Respiratory Paediatrician

Dr Graham Roberts, Senior Lecturer/ Honorary Respiratory Paediatrician

Dr Katy Pike Clincal Research Fellow

MRC Epidemiology Resource Centre

Dr Hazel Inskip, Statistician/ Senior Lecturer

Professor Keith Godfrey, Professor in Epidemiology & Human Development

Dr Sian Robinson, Senior Research Fellow, Nutritionist

Dr Zoe Cole Clinical Research Fellow

Professor Cyrus Cooper, Director, MRC Epidemiology Resource Centre.

Human Genetics Division, IIR

Dr John Holloway, Lecturer in Pharmacology

Reference List

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SWS Label or:
SWS ID number:

6 Year QUESTIONNAIRE HOME VISIT

Part 1

Mother's forename only: _____

Child's forename only: _____

[Nurse to refer to six-year visit record card to ensure child's name is correct, and record any changes thereon. Also to request additional telephone numbers, email addresses etc, for tracing purposes if family move]

Child's date of birth d d m m y y

Sex M=Male F=Female

Date of interview d d m m y y

Interviewer

Discuss the visit with the mother and child and obtain completed consent and assent forms

To be completed by the nurse if the mother was not the person interviewed:

1. *Why was the mother not available?*
2. *Has left the family home*
3. *Still lives in family home, but was unavailable for interview*
4. *Has died*
5. *Is ill or in hospital*
6. *Other, specify* _____
7. *Don't know*

Who was interviewed?

1. *Study child's father*
2. *Mother's partner (if not father)*
3. *Study child's grandparent*
4. *Other family member*
5. *Mother "figure" (eg father's partner/step-mother)*
6. *Family friend*
7. *Other, specify* _____

Food frequency

Now I am going to ask you about the foods your child has eaten, and the drinks they have had in the **past 3 months**. I will ask you how often your child has had certain foods and drinks. Please include meals and snacks eaten away from home if possible, including school meals. *(Define the 3 month period)*

	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		
BREAD, CRACKERS AND CEREALS													
1	**white bread**	0	0.3	0.5	1	2	3	4	5	6	7	8	☐
2	**brown & wholemeal bread**	0	0.3	0.5	1	2	3	4	5	6	7	8	☐
3	**savoury biscuits**	0	0.3	0.5	1	2	3	4	5	6	7	8	☐
4	Breakfast cereals and porridge	0	0.3	0.5	1	2	3	4	5	6	7	8	☐
POTATOES, RICE & PASTA													
5	**boiled & baked potatoes**	0	0.3	0.5	1	2	3	4	5	6	7	8	☐
6	**chips, waffles and potato shapes**	0	0.3	0.5	1	2	3	4	5	6	7	8	☐
7	**roast potatoes**	0	0.3	0.5	1	2	3	4	5	6	7	8	☐
8	**tinned pasta and instant noodles**	0	0.3	0.5	1	2	3	4	5	6	7	8	☐
9	**pasta and noodles – fresh and dried**	0	0.3	0.5	1	2	3	4	5	6	7	8	☐
10	**rice** – white & brown	0	0.3	0.5	1	2	3	4	5	6	7	8	☐
MEAT													
11	**chicken & turkey** in breadcrumbs/batter	0	0.3	0.5	1	2	3	4	5	6	7	8	☐
12	**chicken and turkey** roast meats	0	0.3	0.5	1	2	3	4	5	6	7	8	☐
13	**chicken and turkey** casseroles & curries	0	0.3	0.5	1	2	3	4	5	6	7	8	☐
14	**beef, pork & lamb -** roast meats	0	0.3	0.5	1	2	3	4	5	6	7	8	☐
15	**beef, pork & lamb** casseroles & curries	0	0.3	0.5	1	2	3	4	5	6	7	8	☐
16	**beefburgers**	0	0.3	0.5	1	2	3	4	5	6	7	8	☐
17	**bacon & gammon**	0	0.3	0.5	1	2	3	4	5	6	7	8	☐
18	**sausages**	0	0.3	0.5	1	2	3	4	5	6	7	8	☐
19	**liver, kidney & faggots**	0	0.3	0.5	1	2	3	4	5	6	7	8	☐

	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		
20	**meat pies and sausage rolls**	0	0.3	0.5	1	2	3	4	5	6	7	8	☐
21	**ham & processed cold meats**	0	0.3	0.5	1	2	3	4	5	6	7	8	☐
FISH													
22	**fish in batter or breadcrumbs**	0	0.3	0.5	1	2	3	4	5	6	7	8	☐
23	**other white fish**	0	0.3	0.5	1	2	3	4	5	6	7	8	☐
24	**tuna fish**	0	0.3	0.5	1	2	3	4	5	6	7	8	☐
25	**oily fish**	0	0.3	0.5	1	2	3	4	5	6	7	8	☐
OTHER MEAL ITEMS													
26	**quiche & savoury flans**	0	0.3	0.5	1	2	3	4	5	6	7	8	☐
27	**pizza**	0	0.3	0.5	1	2	3	4	5	6	7	8	☐
28	**processed meat replacements**	0	0.3	0.5	1	2	3	4	5	6	7	8	☐
29	**quorn and soya casseroles & mince**	0	0.3	0.5	1	2	3	4	5	6	7	8	☐
30	**eggs**	0	0.3	0.5	1	2	3	4	5	6	7	8	☐
31	**cottage cheese**	0	0.3	0.5	1	2	3	4	5	6	7	8	☐
32	**cheese**	0	0.3	0.5	1	2	3	4	5	6	7	8	☐
33	**soup**	0	0.3	0.5	1	2	3	4	5	6	7	8	☐
34	**savoury white sauce**	0	0.3	0.5	1	2	3	4	5	6	7	8	☐
35	**tomato pasta sauce**	0	0.3	0.5	1	2	3	4	5	6	7	8	☐
VEGETABLES													
36	**tinned vegetables**	0	0.3	0.5	1	2	3	4	5	6	7	8	☐
37	**carrots**	0	0.3	0.5	1	2	3	4	5	6	7	8	☐
38	**peas & green beans**	0	0.3	0.5	1	2	3	4	5	6	7	8	☐
39	**Sweetcorn, mush-rooms & mixed veg**	0	0.3	0.5	1	2	3	4	5	6	7	8	☐
40	**broccoli,cauliflower courgettes, marrow**	0	0.3	0.5	1	2	3	4	5	6	7	8	☐
41	**green leafy vegetables**	0	0.3	0.5	1	2	3	4	5	6	7	8	☐

	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		
42	**parsnips, turnip and swede**	0	0.3	0.5	1	2	3	4	5	6	7	8	
43	**tomatoes**	0	0.3	0.5	1	2	3	4	5	6	7	8	
44	**salad**	0	0.3	0.5	1	2	3	4	5	6	7	8	
45	**baked beans**	0	0.3	0.5	1	2	3	4	5	6	7	8	
46	**other beans and pulses**	0	0.3	0.5	1	2	3	4	5	6	7	8	
FRUIT													
47	**tinned fruit**	0	0.3	0.5	1	2	3	4	5	6	7	8	
48	**apples & pears**	0	0.3	0.5	1	2	3	4	5	6	7	8	
49	**bananas**	0	0.3	0.5	1	2	3	4	5	6	7	8	
50	**oranges, satsumas and grapefruit**	0	0.3	0.5	1	2	3	4	5	6	7	8	
51	**peaches, nectarines and melon**	0	0.3	0.5	1	2	3	4	5	6	7	8	
52	**berry fruit and tropical fruit**	0	0.3	0.5	1	2	3	4	5	6	7	8	
53	**plums, cherries & grapes**	0	0.3	0.5	1	2	3	4	5	6	7	8	
54	**dried fruit**	0	0.3	0.5	1	2	3	4	5	6	7	8	
55	**cooked/stewed fruit**	0	0.3	0.5	1	2	3	4	5	6	7	8	
56	**nuts**	0	0.3	0.5	1	2	3	4	5	6	7	8	
DESSERTS													
57	**yoghurt & fromage frais**	0	0.3	0.5	1	2	3	4	5	6	7	8	
58	**other ready made desserts in pots**	0	0.3	0.5	1	2	3	4	5	6	7	8	
59	**ice-cream**	0	0.3	0.5	1	2	3	4	5	6	7	8	
60	**ice lollies**	0	0.3	0.5	1	2	3	4	5	6	7	8	
61	**custard, sweet white sauce & instant whip**	0	0.3	0.5	1	2	3	4	5	6	7	8	
62	**other puddings**	0	0.3	0.5	1	2	3	4	5	6	7	8	

Now I would like to ask in more detail about some specific foods

1.2 * Which types of milk has your child used regularly in drinks and added to breakfast cereals over the past 3 months? *(list up to 3 below)*

0 None
1 Whole pasteurised
2 Semi-skimmed pasteurised
3 Skimmed pasteurised
4 Whole UHT
5 Semi-skimmed UHT
6 Skimmed UHT
7 Whole organic
8 Semi-skimmed organic
9 Skimmed organic
10 whole omega 3
11 Semi-skimmed omega 3
12 Other

Milk 1 ☐ If "Other", *specify* _____

Milk 2 ☐ If "Other", *specify* _____

Milk 3 ☐ If "Other", *specify* _____

1.3 * On average over the last 3 months how much of each milk has he/she consumed per day?
(1 average cup = 0.35 pints; 1 pint = 20oz; 1 cup milkshake per wk – liquid = 0.05, powder = 0.01)

Milk 1 ☐☐☐ pints

Milk 2 ☐☐☐ pints

Milk 3 ☐☐☐ pints

1.4 Does your child have sugar added to his/her breakfast cereals, tea & coffee, etc ? ☐

0. No go *to 1.6*
1. Yes

1.5 Approximately how many teaspoons of sugar are added to his/her food and drinks each day? ☐☐

1.6 ***How often does your child eat organic foods (not including milk or fat spreads)?
(Use food frequency categories, 0, 0.3, 0.5, 1 - 8)

Freq 0 – 8 .

Freq >1/d ☐☐

1.7 ***Just thinking about the past week, how many servings did your child have of vegetables and vegetable-containing dishes? *(Including pulses, baked beans and salad but not potatoes)*

☐☐

1.8 *Just thinking about the past week, how many servings did your child have of fruit?
(Including fresh, cooked and tinned but not dried fruit)

☐☐

1.9 *In an average week over the past 3 months, how many **meals per week** did your child have away from home (do not include packed lunches provided by you, or snacks, such as biscuits or crisps, etc)?

0. None *go to 1.11*

No. of times ☐☐

1.10 * How many of these **meals** away from home were you able to include in the previous questions?
0. None
1. Some
2. Most
3. All

☐

1.11 During the past 3 months have you given him/her any vitamins or minerals, including iron and fluoride drops?
No *go to section 2*
Yes

☐

1.12 Please state which:

Supplement Name	Code	How many days in the last 90?	If not a tablet or capsule what is the dose	No. of tablets or stated doses per day
	☐☐☐☐			
	☐☐☐☐			
	☐☐☐☐			
	☐☐☐☐			
	☐☐☐☐			
	☐☐☐☐			

2. NEONATAL HISTORY

Now I'm going to ask you some questions about what happened to your child around the time of birth.

2.1 Was your child admitted to a Special Care Baby Unit?

0. No *go to section 3*
1. Yes

☐

2.3 Was he/she admitted for breathing problems?

0. No
1. Yes
9. Don't know

☐

2.3 How long was your child in the Special Care Baby Unit? ☐ mths ☐ wks ☐ days

2.4 Did he/she need any help with his/her breathing (ventilator / life-support machine / CPAP)?

0. No *go to section 3*
1. Yes

☐

2.5 Did he/she require invasive ventilation (tube into lungs) or non-invasive (e.g.CPAP)?

0. Non- invasive (e.g. CPAP)
1. Invasive (e.g. tube into lungs)
2. Both

☐

2.6 For how long was he/she ventilated? ☐ mths ☐ wks ☐ days

(Note if ventilated both non-invasively and invasively, give combined time here)

3 FAMILY HISTORY

3.1 *Have you or any other members of the child's family (mother, father, siblings or half-siblings) ever been diagnosed **by a doctor** with any of the disorders on the list?

0. No *go to section 4*
1. Yes ☐

Complete each box with a 0 for No or a 1 for Yes)

IF ANY ANSWERS TO 3.2 OR 3.3 ARE 'YES' PLEASE ADD A RED DOT TO THE CARD

	Mother	Father	Sibling	Half - sibling
3.2 Asthma				
3.3 Wheezing				
3.4 Eczema				
3.5 Hayfever				
3.6 Food allergy				
3.7 Drug allergy				
3.8 Bee or wasp sting allergy				
3.9 Cystic Fibrosis				

Prompts
Asthma*: wheeze or whistling in the chest with exercise or other triggers that is rapidly relieved with a reliever inhaler. Only if doctor diagnosed.*
Wheeze*: whistling in the chest when breathing out.*
Eczema: *A skin condition resulting in dry, itchy, red skin. If it is infected the skin may become wet. (Doctor diagnosed only).*
Hayfever: runny*, itchy eyes or/and nose in the spring or summer, not caused by a cold.*

Note: Only record 'Yes' if the person has definitely had the problem. If the person has, for example, never been stung by a bee or a wasp then the answer is 'No'.

4 ASTHMA

I would now like to ask a few questions about illnesses your child has had

4.1. Has your child **ever** had **asthma**? ☐
- 0. No *go to section 5*
- 1. Yes *ADD RED DOT TO CARD*

4.2 Was the asthma diagnosed by a doctor? ☐
- 0. No *go to section 5*
- 1. Yes

4.3 How old was he/she when he/she was first diagnosed? ☐ yrs ☐☐ mths ☐☐ wks

4.4 Has he/she ever been admitted to hospital for asthma? ☐
- 0. No
- 1. Yes

4.5 Has he/she received inhalers or other medication for asthma prescribed by a doctor **in the past 12 months**? ☐
- 0. No
- 1. Yes

5 OTHER RESPIRATORY ILLNESSES AND SYMPTOMS

5.1. Has he/she **ever** been diagnosed as having **bronchiolitis** by a doctor? ☐
- 0. No *go to 5.4*
- 1. Yes

5.2 How old was he/she when he/she was first diagnosed? ☐ yrs ☐☐ mths ☐☐ wks

5.3 Has he/she ever been admitted to hospital for this? ☐
- 0. No
- 1. Yes

5.4 Has he/she **ever** been diagnosed as having **pneumonia or a chest infection** by a doctor? ☐
- 0. No *go to 5.8*
- 1. Yes

5.5 Has he/she ever been admitted to hospital for this? ☐
- 0. No
- 1. Yes

5.6 Has he/she been diagnosed as having **pneumonia or a chest infection** by a doctor **in the past 12 months**? ☐
- 0. No *go to 5.8*
- 1. Yes

5.7 Has he/she been admitted to hospital for **pneumonia or a chest infection in the past 12 months**? ☐
- 0. No
- 1. Yes

5.8 Has he/she **ever** had a **persistent cough** every day for more than 3 weeks?

0. No *go to 5.12*
1. Yes

5.9 Has he/she **ever** been admitted to hospital for this?

0. No
1. Yes

5.10 Has he/she had a **persistent cough** every day for more than 3 weeks **in the past 12 months**?

0. No *go to 5.12*
1. Yes

5.11 Has he/she been admitted to hospital for a persistent cough **in the past 12 months**?

0. No
1. Yes

5.12 Does your child have any other respiratory problems (eg cystic fibrosis)?

0. No
1. Yes *if yes specify*_____

5.13 Has your child regularly snored at night (3 nights a week or more) for at least 6 months over the past year?

0. No
1. Yes

5.14 *****Has your child had his/her adenoids or tonsils removed?

0. No
1. Adenoids only
2. Tonsils only
3. Adenoids and tonsils

6 FURTHER QUESTIONS ABOUT ASTHMA AND WHEEZE

(based on core ISAAC questions and proposed standardised BPRS questionnaire)

6.1 Has your child **ever** had wheezing or whistling in the chest at any time in the past? ☐

0. No *go to 6.13*
1. Yes *ADD RED DOT TO CARD*

6.2 Were these wheezy or whistling episodes associated with colds? ☐

0. No *go to 6.4*
1. Yes

6.3 Has he/she **ever** wheezed or whistled in the chest between colds? ☐

0. No
1. Yes

6.4 Has your child had wheezing or whistling in the chest **in the last 12 months**? ☐

0. No *go to 6.12*
1. Yes

6.5 ***How many attacks of wheezing has your child had **in the last 12 months**? ☐

0. None
1. 1-3
2. 4-12
3. more than 12

6.6 ***In the last 12 months**, how often, on average, has your child's sleep been disturbed due to wheezing? ☐

0. Never woken with wheeze
1. Woken less than one night per week
2. One or more nights per week

6.7 **In the last 12 months**, has your child's chest sounded wheezy during or after exercise? ☐

0. No
1. Yes

6.8 **In the last 12 months** has wheezing ever been severe enough to limit your child's speech to only one or two words at a time between breaths? ☐

0. No
1. Yes

6.9 *Does your child wheeze? *(please put 0 for No or 1 for Yes in each box)*

In winter	
In spring	
In summer	
In autumn	

6.10 *What else makes him/her wheeze? *(please put 0 for No or 1 for Yes in each box)*

Change of weather	
Emotion (eg. excited / upset)	
Smoky rooms	
Exercise	
Pollen Season	
During vacuum cleaning or bed making	
Perfume	
Certain foods (*specify):*	
Moulds	
Hairy / furry animals *(specify):*	
Other *(specify):*	

6.11 ***In the last 12 months** how many of the following has your child had? *(please complete with 0s if none have occurred)*

Hospital admissions with asthma/wheeze	
Visits to Casualty Dept with asthma/wheeze	
Visits to GP or 'out of hours' doctor with asthma/ wheeze	
Days off school due to asthma/wheeze	
Nights woken with asthma / wheeze (with or without colds) – approximate number	

Go to 6.13

6.12 At what age did your child last wheeze? ☐ yrs ☐☐ mths

6.13 **In the last 12 months,** has your child had a cough at night, apart from a cough associated with a cold or chest infection? ☐

0. No
1. Yes

6.14 Has your child **ever** been prescribed an asthma reliever inhaler**?** ☐

0. No *go to section 7*
1. Yes *ADD RED DOT TO CARD*

6.15 Did it help his/her breathing (wheezing or coughing) to improve? ☐

0. No
1. Yes
2. Never Used

7 ECZEMA

7.1 Has he/she **ever** had an itchy skin condition - by itchy we mean scratching or rubbing the skin a lot ? *(exclude chicken pox, if asked to clarify "itchy skin condition" then ask "Has he/she had any episodes lasting more than 2 weeks when he/she scratched or rubbed his/her skin a lot")*

0. No *go to 7.3*
1. Yes ☐

(Note if the woman says 'No' to this, you will not need to ask questions 7.6-7.8 when you come to them)

7.2 How old was he/she when the rash **first** appeared ? ☐ yrs ☐☐ mths ☐ wks

7.3 *Has he/she **ever** had a **scaly**, **or red and weeping** skin rash affecting any of the following areas:

A) the scalp or behind the ears (including "cradle cap")
0. No
1. Yes ☐

B) around the neck
0. No
1. Yes ☐

C) the cheeks or forehead
0. No
1. Yes ☐

D) either the folds of the elbows or behind the knees
0. No
1. Yes ☐

E) the forearms, wrists, shins or ankles
0. No
1. Yes ☐

F) the shoulders, chest, tummy or back
0. No
1. Yes ☐

G) in the armpits
0. No
1. Yes ☐

H) the nappy area (including nappy rash)
0. No
1. Yes ☐

7.4 Has he/she **ever** suffered from a generally dry skin ?
0. No *go to 7.6 (but see note above question 7.6)*
1. Yes
8. To a minor degree ☐

7.5 In **the past twelve months**, has he/she suffered from a generally dry skin ?
0. No
1. Yes
8. To a minor degree ☐

¤¤

(If the answer to question 7.1 was 'No' – ie the child has never had an itchy skin condition – then go to section 8)

¤¤

7.6 In **the past twelve months,** has he/she suffered from an itchy skin condition? (exclude chicken pox)

0. No *go to section 8*
1. Yes

☐

7.7 ***In the last 12 months** how often, on average has your child been kept awake at night by this itchy rash?

0. Never in the last 12 months
1. Less than one night per week
2. One or more nights per week

☐

7.8 Has this skin condition affected **the cheeks**, **the outer arms or legs**, or the **skin creases** in **the past twelve months -** by skin creases we mean the folds of the elbows, behind the knees, the fronts of the ankles, or around the eyes ?

0. No
1. Yes

☐

8 RHINITIS/HAYFEVER (Core ISAAC questions)

I'm now going to ask some questions about problems which occur when your child does **not** have a cold or 'flu.

8.1 Has your child ever had a problem with sneezing, or a runny, or blocked nose when he/she did not have a cold or the 'flu?

0. No *go to 8.8*
1. Yes ☐

8.2 **In the past 12 months**, has your child had a problem with sneezing, or a runny, or blocked nose when he/she did not have a cold or the 'flu?

0. No *go to 8.8*
1. Yes ☐

8.3 **In the past 12 months** was this nose problem accompanied by itchy-watery eyes?

0. No
1. Yes ☐

8.4 *In which of the past 12 months did this nose problem occur?
(*For each month record 0 for No or 1 for Yes)*

January		July	
February		August	
March		September	
April		October	
May		November	
June		December	

8.5 *In the past 12 months, how much did this nose problem interfere with your child's daily activities?

0. Not at all
1. A little
2. A moderate amount
3. A lot ☐

8.6 Is there any particular time of day that sneezing and nasal symptoms occur?

0. No *go to 8.8*
1. Yes ☐

8.7 At which times do they occur? (*more than one box can have the answer yes, code 0 for No and 1 for Yes)*

Mornings	
Afternoons	
Evenings	
Night	

8.8 Has your child **ever** had hayfever? (*Prompt: **Hayfever: runny**, itchy eyes or/and nose in the spring or summer, not caused by a cold).*

0. No
1. Yes ☐

9 FOOD ALLERGY

9.1 Has your child **ever** had a reaction to particular foods?

0. No *go to section 10*
1. Yes *ADD RED DOT TO CARD*

☐

9.2 *What sort of problems has he/she had? (*Code 0 for No and 1 for Yes for each problem)*

Food that always makes him/her vomit	
Swelling of the face, lips or throat when eating certain food(s)	
Tingling of the mouth	
Rashes with a certain food	
Wheeze with a certain food	
Breathing difficulties caused by foods	
Collapse/faint with certain food	
Other symptoms (*specify)*	

9.3 *Which foods have caused these problems? *(0 for No, 1 for Yes for each food)*

01	Cows milk	
02	Egg	
03	Peanuts	
04	Tree nuts	
05	Wheat	
06	Seeds	

07	Kiwi fruit	
08	Fish	
09	Shellfish	
10	Other *(specify)*	
11	Other *(specify)*	
12	Other (*specify)*	

The following questions ask about the reaction to up to three foods. If the child reacts to more than three foods ask which three give the most severe problems and answer the questions in relation to those three.

9.4 Food 1 (*Give code as in table above)* ☐☐

9.5 ***Does the reaction always happen when he/she eats <**food 1 – *name the food***>?

1. Yes, it always happens
2. No, he/she is sometimes OK
3. He/She used to have problems but has now outgrown them
4. He/She never now eats the food

☐

9.6 How long after he/she is first in contact with <**food 1 – *name the food***> does he/she start to get symptoms?

Immediately? ☐ 0. No *give hours and/or minutes below*
1. Yes

Hours ☐☐ Minutes ☐☐

9.7 Food 2 (*Give code as in table above)* ☐☐

9.8 *Does the reaction always happen when he/she eats <**food 2 – *name the food***>? ☐

1. Yes, it always happens
2. No, he/she is sometimes OK
3. He/She used to have problems but has now outgrown them
4. He/She never now eats the food

9.9 How long after he/she is/was first in contact with <**food 2 – *name the food***> does/did he/she start to get symptoms?

Immediately ☐ 0. No *give hours and/or minutes below*
1. Yes

Hours ☐☐ Minutes ☐☐

9.10 Food 3 (*Give code as in table above)* ☐☐

9.11 * Does the reaction always happen when he/she eats <**food 3 – *name the food***>? ☐

1. Yes, it always happens
2. No, he/she is sometimes OK
3. He/She used to have problems but has now outgrown them
4. He/She never now eats the food

9.12 How long after he/she is/was first in contact with <**food 3 – *name the food***> does/did he/she start to get symptoms?

Immediately ☐ 0. No *give hours and/or minutes below*
1. Yes

Hours ☐☐ Minutes ☐☐

10 MEDICATION

Now I would like to ask about medicines and other treatments your child has taken

Oral steroids

10.1 Has he/she **<u>ever</u>** taken Oral steroids for any condition? (eg Prednisolone) ☐

0. No *go to 10.5*
1. Yes

10.2 How many courses has he/she ever taken? ☐☐

10.3 How many courses has he/she taken in the last 12 months? ☐☐

10.4 How long ago did the last course finish? ☐ years ☐☐ months ☐ weeks

(Complete all 4 boxes above with 8s if the course is still on-going)

Antihistamines

10.5 Has he/she taken antihistamines in the last 12 months?
(e.g. Ketotifen, Loratidine, Piriton, Zirtek etc.)
0. No *go to 10.7*
1. Yes

☐

10.6 How often does he/she use these ?
1. All the time?
2. During hayfever season only?
3. Only occasionally?

☐

Current/recent asthma or medication

10.7 In the past three months has he/she used any inhalers or antihistamines, or taken any medicines for asthma, or any chest symptoms
0. No *go to 10.9*
1. Yes

☐

10.8 *Please ask the mother/carer for all those medicines that the child has taken and ask to see them if possible. Then fill in the table below, using the FFQ codes for how often they have been taken*

Name of medicine	**Medicine Code**	**Number of puffs/spoons/ tablets/etc taken for each dose**	**How often does he/she take this dose?** **FFQ code 1-8**	**Number of times per day, if more than once a day**
	☐☐☐☐	☐☐	☐ . ☐	☐☐
	☐☐☐☐	☐☐	☐ . ☐	☐☐
	☐☐☐☐	☐☐	☐ . ☐	☐☐
	☐☐☐☐	☐☐	☐ . ☐	☐☐
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	☐☐☐☐	☐☐	☐ . ☐	☐☐
	☐☐☐☐	☐☐	☐ . ☐	☐☐

10.9 Has your child taken any other medications in the past three months? Please include **both** prescribed medicines and those bought over the counter. (*Note: do not include vitamins or food supplements, but do include cough remedies, paracetamol etc).*

0 No *go to section 11*
1 Yes ☐

10.10 What medicines has he/she taken? *(please specify)*

Medicine 1_____

Medicine 2_____

Medicine 3_____

Medicine 4_____

Medicine 5_____

Medicine 6_____

Medicine 7_____

Medicine 8_____

11 SMOKING

11.1 Are you/*child's main carer* currently smoking? ☐
0. No *go to 11.5*
1. Yes

11.2 If yes, and offered, is it: ☐
1. Only in a separate room?
2. Only outside the house?

11.3 How many per day? ☐☐

11.4 What is your current brand? _____

11.5 Does anyone else smoke in the home, or is he/she ever looked after more than once a week by anyone who smokes? ☐
0. No *go to 11.8*
1. Yes

11.6 If yes, and offered, is it: ☐
1. Only in a separate room
2. Only outside the house

11.7 How many smokers live in the same house as the child? ☐

11.8 Is your child regularly exposed to non-household smoking? ☐
0. No
1. Yes

11.9 Has he/she been exposed to smoke in the last 24 hours?

0. No *go to section 12*
1. Yes

11.10 *Where? *(please enter 0 for no and 1 for yes)*

Family home	
Car	
Relative/friends' house	
Public place	
Other (*specify)* ----------	

12 ANIMAL EXPOSURE DURING PREGNANCY

Now I'm going to ask you about pets and animals at home when you were pregnant with this child.

12.1 Did you have any pets at home at that time?

0. No *go to section 13*
1. Yes

12.2 How many of each of the pets on the list did you have at the time?

Cats	
Dogs	
Birds	
Other *(specify)* ----------	

12.3 *Please tell me where these pets were allowed:

	Your bedroom	Living room	Kitchen	Garden
Cats				
Dogs				
Birds				
Other				

Please score through lines for pets that the woman did not have. For pets she has, put 0 for No and 1 for Yes. If she had more than one 'other' pet, please put 1 if any of these pets is allowed in the area.

13 PETS AND ANIMALS NOW

Now I'd like to move on to ask about pets and animals in your house now

13.1 Do you have any pets at home now?

0. No *go to 13.4*
1. Yes

13.2 How many of each of the pets on the list do you have?

Cats	
Dogs	
Birds	
Other *(specify)* ----------	

13.3 *Please tell me where these pets are allowed:

	Child's bedroom	Living room	Kitchen	Garden	Other
Cats					
Dogs					
Birds					
Other					

Please score through the lines for pets that they do not have. For pets they do have, put 0 for No and 1 for Yes. If they have more than one 'other' pet, please put 1 if any of these pets is allowed in the area.

13.4 Does your child have regular (ie. more than once a week) contact with pets in other people's homes?

0 No *go to section 14*
1 Yes

13.5 What pets is he/she in contact with? (*please enter 0 for No and 1 for Yes for each type of pet)*

Dogs	
Cats	
Birds	
Other *(specify)* ----------	

14 RESPIRATORY SYMPTOMS ON DAY OF SPIROMETRY

14.1 Has your child had a cold in the last 3 weeks?
0. No *go to 14.4*
1. Yes

14.2 Does he/she still have symptoms of the cold?
0. No
1. Yes *go to 14.4*

14.3 How many days is it since he/she last had symptoms of the cold? ☐☐

14.4 Has your child coughed in the last 7 days?
0. No *go to 14.6*
1. Yes

14.5 ***What type of cough was it?
1. A cough that produced sputum
2. A cough that sounded "wet" but didn't produce sputum
3. A cough that sounded dry

(may need to explain that we mean coughing something up from the chest)

14.6 Has your child wheezed in the last 7 days?
0. No
1. Yes

14.7 Has your child used a bronchodilator (eg. ventolin, bricanyl, salbutamol, terbutaline) in the last 12 hours? *(Nurse: please note that many mothers will have said that their children do not use such medication in their answers to section 10. Be aware of this but nonetheless please confirm prior to spirometry that there has been no bronchodilator use).*

0. No *go to section 15*
1. Yes

14.8 How long ago was it used? ☐☐ hours ☐☐ minutes

(If less than four hours ago, do not do spirometry and go to section 16)

15 SPIROMETRY

Please record the room temperature ☐☐ . ☐ ^{o}C

Please record the child's ethnic group by asking the mother/carer which ethnic group the child belongs to:

* 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)_____

☐☐

Perform the spirometry on the laptop using the Koko incentive software.

APPENDIX E: Actiheart instruction sheet

Mother ID: Child ID:

Actiheart N°: Actiheart N°:

MRC | Epidemiology Unit

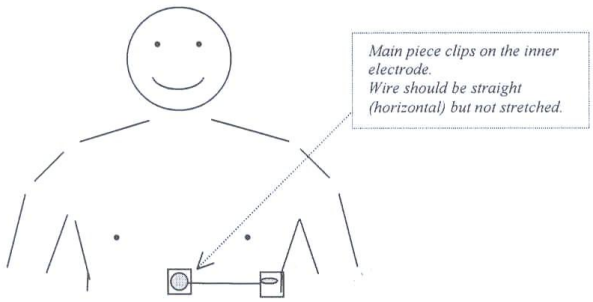
Study: Physical Activity monitoring in 6 yr old Children & their mothers

Actiheart Instruction Sheet

Please try and have you and your son/daughter wear the Actiheart sensor from leaving the study centre for 7 days and nights. During this time, please carry on with all your normal activities as usual in your daily environment.

Description: The Actiheart sensor is a combined heart rate and movement sensor. From the main piece, a wire runs to a little button. These two pieces clip on to two ECG electrodes. The sensor and the electrodes are waterproof, so it can be worn all the time, including during showering, bathing and swimming.

Placement: The sensor is held to the skin by two sticky electrodes. These will be placed on the lower left side of your son/daughter's stomach during your visit but should they for any reason become detached, please place new electrodes in the same place, as shown in the diagram below. Before application of new electrodes, the skin must be prepared in the following manner: Clean the skin and dry thoroughly with a clean towel or soft tissue. Please do not use skin lotion where the electrodes are placed. To attach the main piece and the small piece, you will need to press the little tabs on the edges of the pieces towards the centre and place it on the electrode.



Replacement: **PLEASE CHANGE THE ELECTRODES EVERY TWO DAYS**. When you want to replace the electrodes, remove the old ones. Follow the skin prep procedure described above, before you apply a new set of electrodes.

Please record on the diary overleaf the times if the monitor is removed for any reason.

Please post the monitor within 4 days of completion of measurement to ensure that the data is not lost.

If you have any problems or any queries, please call Freephone: 0800 7834503

Many thanks for your help in this study

Version 1.1. 24/02/2006

Diary for wearing the ActiHeart monitor:

Child:

	day	day	day	day	day	day	day	Finished Measurement
Time taken off								
Time put back on								
Other issues:								

Mother:

	day	day	day	day	day	day	day	Finished Measurement
Time taken off								
Time put back on								
Other issues:								

APPENDIX F: SWS Parent clinic information sheet

- ***If your child uses asthma medication*** please try not to use their reliever medication or long acting beta agonist (blue inhaler, *Ventolin®, Salbutamol, Bricanyl®* green or purple inhaler *Serevent®, Seretide®* white and red *Symbicort®*) for at least 12 hours before the appointment. Please use your child's preventer medication (inhaled steroid, *Flixotide®, Becotide®, Pulmicort®*, Singulair®) as usual. ***If in doubt, please contact us before the day of the appointment.***

What are the possible benefits of taking part?
The main benefit is knowing that you and your child are taking part in a unique study that will help identify risks for diseases in childhood. By the end of the visit your child will have learnt lots of facts about their body and how it works.

In addition, some children may be helped by obtaining information about their lung function and potential allergies. Also if there are any problems with your child's bones identified from the DXA scan 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 your child might suffer will be addressed as described in the previous home visit leaflet.

Will my taking part in the study be kept confidential?
As always, all information about your participation in this study will be kept confidential.

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.

Contact Details:
Southampton Women's Survey
MRC Epidemiology Resource Centre
Southampton General Hospital
Tremona Road
Southampton SO16 6YD

Freephone: 0800 783 4503

SOUTHAMPTON Women's SURVEY

Southampton Women's Survey

Growth and Asthma:

CLINIC VISIT

for 6 year old children

Parent information booklet

LREC NO: 06/Q1702/104
v2 03/07

6 year SWS follow-up – what next?

When the nurse visited you and your child at home she discussed the possibility of a clinic visit. We would be grateful if you and your child could come to the Osteoporosis Centre and then across the corridor to the Wellcome Trust Clinical Research Facility (WTCRF) at Southampton General Hospital.

If your child is happy to have all the tests, your visit will last 1½- 2 hours. You will both be looked after by a team of children's doctors and nurses who are very experienced in research with children.

What measurements will you be taking?

The nurses will discuss all the following tests with you in more detail at the clinic. Firstly, your child's height and weight will be measured.

Bone scan: In the Osteoporosis Centre, your child will have a scan of their skeleton using a DXA machine. This will also tell us how much muscle and fat your child has. This scan takes approximately 5-10 minutes to perform. Your child will lie on a table and a small scanning arm will pass overhead, about 2 feet in the air. It does not touch your child. 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. Your child will be given a picture of his / her skeleton to take home with them.

Lung function: In the WTCRF we would like to do two further tests of your child's lungs.

Test 1: We will use a special machine which measures nitric oxide in the lungs. Your child will simply be asked to gently breathe in and out through a tube.

Test 2: There are two variations of this test. Your child will be asked to do one of them. The technique is similar to the breathing and blowing test they did at the home visit:-

a) The first test investigates whether your child's lungs tend to tighten up (as with asthma) and how easily this happens. Under close supervision, they will be asked to breathe in a mild histamine-like substance *(methacholine)* that causes tightening of the airways in some people. We start with a very small amount, only increasing the amount if your child's lungs show no change. As soon as they show symptoms similar to mild asthma (eg slight shortness of breath or coughing), we will stop. Your child will be closely monitored throughout. As soon as the test is finished, they can be given a very safe asthma inhaler (*salbutamol*) which speeds up the recovery of their lungs back to their normal state within a few minutes.

b) The second test assesses how much the airways expand using a bronchodilator. After doing the same lung function tests as in the home visit, your child will be given 5 puffs of *salbutamol* via a special child-friendly inhaler. The lung function tests will then be repeated 20 minutes later.

Other measurements: Also in the WTCRF, your child will have their blood pressure taken. If skin prick allergy testing was not done in the home for any reason, your child will be invited to have it done in the clinic.

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. If you live more than 10 miles from the hospital, travel costs for car use will be reimbursed at the current rate recommended by the University of Southampton.

What do we need to do?

- It is important that your child does NOT eat *chocolate* or drink *tea, coffee, or fizzy drinks (especially coca-cola)* on the day of the visit.
- Your child can wear their normal clothes, but the DXA scan does not work if your child is wearing any metal objects (eg. belts, zips, buttons, hair bobbles).
- You can bring your child's favourite DVD to watch whilst they are lying still for the DXA scan.
- ***If your child takes an antihistamine*** (eg. *Piriton, Cetirizine, Zirtec®*), please try to avoid them taking it for 7 days before the visit because it interferes with the allergy tests. If your child needs to take an antihistamine in this time, please let them and telephone the study team before the visit because it may be necessary to change the appointment.

APPENDIX G: SWS Child's clinic information sheet

When you come to see us we would like you to wear your normal clothes *BUT no metal belts or zips because they upset the scan machine!* It is important that you ***don't*** eat *chocolate* or drink *tea, coffee, or fizzy drinks (especially coca-cola)* on the day of your visit.

If you have any questions please ask the nurse, or your Mum or Dad.

We are looking forward to seeing you soon

LREC 06/Q1702/104
v2 03/07

SOUTHAMPTON Women's SURVEY

Children's Follow-Up CLINIC VISIT

Child's Information Leaflet

Thank you for helping us with our study.

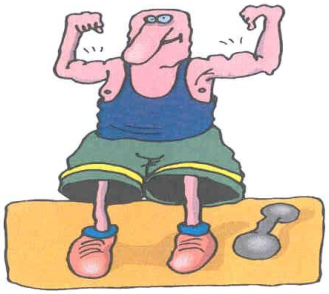
The information you gave the SWS nurse when she visited your home, and the measurements she took will help us to understand how to help children to be healthier. If you would like to, you can help us some more by visiting us at our clinic.

Our bodies are made up of **fat**, **muscle**, **bone** and **water**.

At our clinic the nurses will measure your **bones** and **lungs** using some very special equipment.

How big are your bones?

Your **bones** are measured by the **DXA scan machine**. This machine tells us the size of your skeleton, and how much **fat** and **muscle** you have. The **scan machine** uses **x-rays** to measure the amount of **bone** you have AND it takes a picture of your skeleton. You have to lie very still while the camera passes over you or the picture will be *fuzzy*. You can bring along your favourite DVD to watch whilst you are lying very still. AND you can take the picture of your skeleton home to show your friends!



How healthy are your lungs?

We want to learn more about your lungs. We have a special machine that you blow into. It measures a gas from your lungs called **nitric oxide**. This gas can tell us other things about your lungs and how they work.

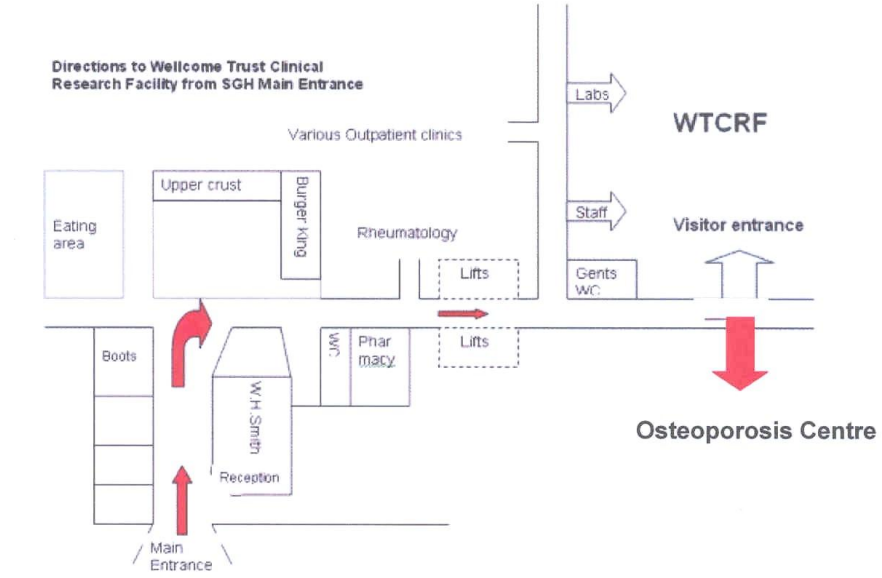
We will ask you to do the same breathing and blowing that you did at home, but this time the nurse will ask you to breathe in some special air. The nurse will tell you and your Mum or Dad more about this.

If you come to the clinic to do some of these clever things you'll learn lots about *your body* and how it works.

APPENDIX H: Directions to osteoporosis centre

SOUTHAMPTON Women's SURVEY

SOUTHAMPTON WOMEN'S SURVEY
6 YEAR CLINIC APPOINTMENT
ZOE'S BONE STRENGTH STUDY



SWS ID..... **Child's Name**.....

Your Appointment is on : Date..... **Time**.....

Place.....

The Osteoporosis Centre is on C level. Enter by the main entrance and walk towards Burger King. When you get to the corridors leading off to the right and left, take the corridor to the right and go past the Pharmacy on the right. Walk past the stairs and lifts and continue down the corridor ahead. Take the first corridor on the right sign-posted Osteoporosis Centre.
The reception is near the end of the corridor on the right.

IF YOU ARE UNABLE TO KEEP THIS APPOINTMENT PLEASE TELEPHONE OUR FREEPHONE NUMBER-: 0800 783 4503

APPENDIX I: DXA Consent form

SOUTHAMPTON WOMEN'S SURVEY
MRC Epidemiology Resource Centre
(University of Southampton)
Southampton General Hospital
Southampton S016 6YD

FREEPHONE: 0800 7834503

SWS serial no:

Determinants of skeletal growth in early life: a longitudinal study

CONSENT FORM – SIX YEAR CHILD BONE MASS MEASUREMENT

Please initial box:

1) I confirm that I have read and understand the information given in the parent information booklet for the above study and have had the opportunity to ask questions ☐

2) I understand that my child's participation is voluntary and that I am free to withdraw my child at any time, without giving any reason, and without our medical care or legal rights being affected ☐

3) I agree for my child to take part in DXA component of the 6year old study. ☐

Name of child.....

.....	.....	.....
Name of parent giving consent	Date	Signature
.....	.....	.....
Name of person taking consent	Date	Signature
.....	.....	.....
Name of researcher	Date	Signature

Local Research Ethics Committee no. 06/Q1702/104 v 2 03/07

APPENDIX J: Bone questionnaire and grip strength measurement

SOUTHAMPTON Women's SURVEY

MRC Epidemiology Resource Centre
University of Southampton
Southampton General Hospital
Southampton SO16 6YD

SWS No

Bone Questions for 6 year old Questionnaire

Has your child ever broken a bone Yes/No

When and how did your child break a bone or bones, and which bones were broken?

Date	Bones Broken	What Happened?

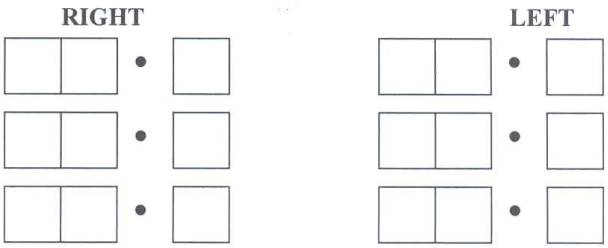
Is there a family history of low trauma fractures? Yes/No

Which Family Members, which bones. Please state which family members broke which bones, and how old they were when they first started to fracture

Family member	Bones Broken	Age of first fracture

Local Research Ethics Committee no. 06/Q1702/104 v 2 03/07

GRIP STRENGTH



Which hand does your child use to write with?

- Left ☐
- Right ☐
- Both ☐

APPENDIX K: Parent information sheet for pQCT study

What are the possible benefits of taking part?

Should you take part in this study, your child will have an assessment of bone strength. We will of course provide necessary advice if the bone strength values are found to be low. The information we get from this study may help us find ways of preventing osteoporosis and broken bones in future generations.

Will my taking part in this study be kept confidential?

Your name/ address and all the information collected during the study will be kept strictly confidential and only made available to researchers in the study.

What will happen to the results of the research?

Together with all the previous information you have given us we will look at which factors affect bone strength. These findings will be published in medical literature. We will also summarise them on the SWS website. We may pass on the results to the local and national press. You will not be identified in these reports/ publications in any way.

Contact for further information

For further information please contact Professor C Cooper or Dr Zoë Cole at the Medical Research Council Epidemiology Resource Centre at Southampton on 023 8077 7624
This information sheet is for you to keep and you will also be given a copy of your signed consent form should you agree to take part.

Thank you for reading this

Prof C Cooper MA DM FRCP FmedSci
Professor of Rheumatology

Dr Hazel Inskip BSc MSc PhD
Coordinator, SWS

SOUTHAMPTON Women's SURVEY

Parent Information Sheet

A study to identify what makes bones strong

LREC NO 06/Q1702/104
Version 3 April 07

Parent Information Sheet

A Study to help identify what makes bones strong

Both you and your child are being invited to take part in a special sub study of the Southampton Women's Survey. Before you decide whether you are willing to take part, it is important for you that you understand why the research is being done and what it will involve. Please take time to read the information carefully and discuss it with anyone you wish. Ask us if there is anything that is not clear or if you would like more information. Thank you for reading this

What is the purpose of this study?

This study is trying to find out how a child's arms and legs grow, what gives the bones their strength and what may alter the risk of fracture in later life. Some of these factors are inherited from the parents. However recent studies have suggested that factors, such as a women's diet and body build during pregnancy, may affect the growth of her child's bones. There is also evidence, even at this age to suggest some groups of children have a higher risk of fracture.
Together with the information you have previously provided we hope to understand these interactions and bone strength.

Why have I been chosen?

As part of the Southampton Women's Survey you and your child have provided much useful information about your pregnancy and your child's growth. Now that your child is 6-7 years old we would like to perform a special scan of your child's arm and leg, it is called a peripheral quantitative computed tomography scan (pQCT). This scan will give us important 3D images of your child's bone and muscle giving us information on how strong your child's bones have become since birth.

Do I have to take part?

It is up to you to decide whether or not to take part. If you decide to take part you are still free to withdraw at any time and without giving a reason. This will not affect the standard of care you receive.

What will happen to me if I take part?

We will contact you, and if you decide to take part, we will arrange a single appointment at Southampton General Hospital Osteoporosis centre. The appointment will last 30 minutes. During this time we will measure your child's height and weight and perform the pQCT scan on his/her lower leg and forearm

What are the possible disadvantages and risks in taking part?

The pQCT scan involves your child sitting on a chair and putting his/her limb into an open metal tube; it does not touch your child. The dose of X rays is equivalent to less than half a day natural sunlight. The scan will not cause any pain or harm. It does involve keeping still for about 5 minutes whilst each scan is being done.

APPENDIX L: Child's information leaflet on pQCT study

SOUTHAMPTON Women's SURVEY

Healthy bones in children

Child's Information leaflet

Child's Information leaflet

Since before you were born, you and your family have been helping with a very special project that is helping doctors understand how to get children to lead healthier lives. It is called the Southampton's Women survey (or SWS for short). We are hoping that you will be able to help us again.

Our bodies are made up of fat, muscle, bone and water. How much we have of each is important for heath and fitness. The SWS nurse will have already visited you and taken measurements of how much fat and muscle you have.

How healthy are your bones?

We want to find out how strong your bones are, to try and stop you breaking them in the future. You may have already had a scan to look at all the bones in your body, we would like to look in more detail at how strong your arms and legs are. We do a special test at the hospital where you put either your arm or leg through a tube whilst it takes pictures using X rays. This doesn't hurt but you will need to keep still whilst the pictures are being taken. We can give you a copy of these to take home.

Together with all the information you and your family have given us in the past we can use this to look at how best to grow healthy strong bones.

LREC NO 06/Q1702/104
Version 3 April 07

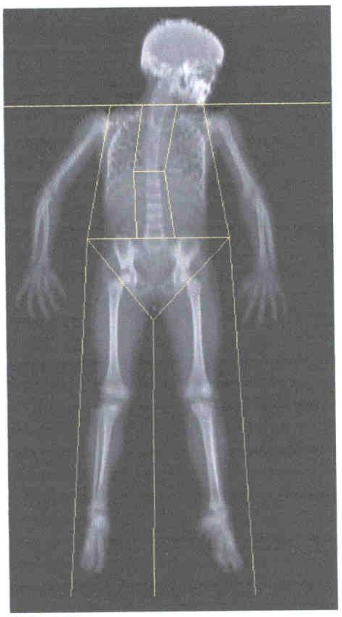
APPENDIX M: Copy of the DXA results given to child

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

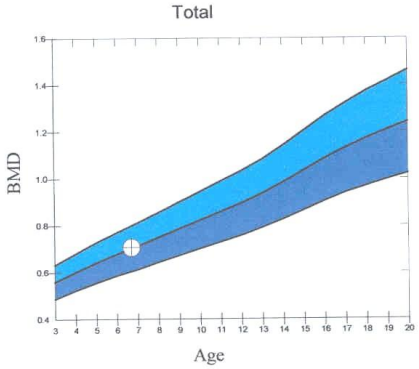
Telephone: 02380794696 E-Mail: pat.taylor@suht.swest.nhs.uk Fax: 02380798995

Name: 6 year study	Sex: Male	Height: 131.5 cm
Patient ID:	Ethnicity: Pediatric	Weight: 26.6 kg
DOB: 01 August 2003		Age: 6

Referring Physician: Cole



318 x 118



Scan Information:

Scan Date: 14 April 2010 ID: A0414100A
Scan Type: a Whole Body
Analysis: 14 April 2010 10:23 Version 13.0
Auto Whole Body
Operator: lj
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	109.82	54.88	0.500				
R Arm	102.42	51.12	0.499				
L Ribs	52.96	25.41	0.480				
R Ribs	50.24	27.91	0.555				
T Spine	40.11	17.91	0.446				
L Spine	48.68	27.12	0.557				
Pelvis	118.78	87.36	0.735				
L Leg	211.08	154.44	0.732				
R Leg	202.12	137.34	0.679				
Subtotal	936.23	583.49	0.623				
Head	267.55	268.64	1.004				
Total	**1203.78**	**852.13**	**0.708**		**57**	**0.1**	**101**

Total BMD CV 1.0%

Physician's Comment:

T-score vs. Pediatric Male; Z-score vs. Pediatric Male. Source:Hologic, 2005

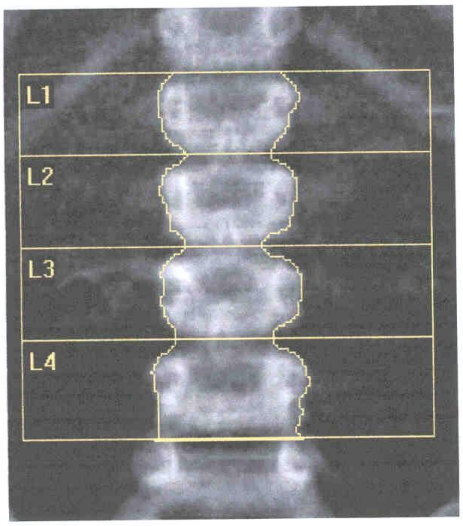
HOLOGIC

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

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

Name: 6 year study	Sex: Male	Height: 131.5 cm
Patient ID:	Ethnicity: Pediatric	Weight: 26.6 kg
DOB: 01 August 2003		Age: 6

Referring Physician: Cole



116 x 104

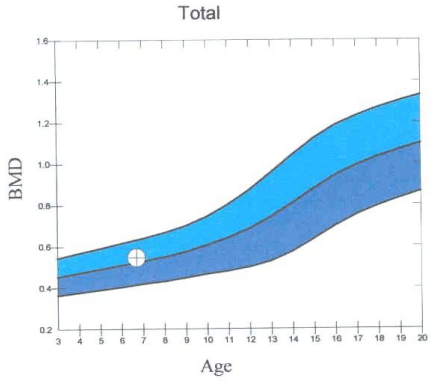
Scan Information:

Scan Date: 14 April 2010 ID: A0414100B
Scan Type: x Lumbar Spine
Analysis: 14 April 2010 10:22 Version 13.0
Lumbar Spine (auto low density)
Operator: lj
Model: Discovery W (S/N 80019)
Comment:

DXA Results Summary:

Region	Area (cm^2)	BMC (g)	BMD (g/cm^2)	T - score	PR (%)	Z - score	AM (%)
L1	8.11	3.70	0.457		44	-0.2	97
L2	8.66	5.02	0.579		52	0.9	110
L3	9.13	5.35	0.586		52	0.6	107
L4	11.55	6.44	0.558		50	0.3	104
Total	**37.44**	**20.50**	**0.548**		**50**	**0.4**	**105**

Total BMD CV 1.0%



Physician's Comment:

T-score vs. Pediatric Male; Z-score vs. Pediatric Male. Source:Hologic, 2005

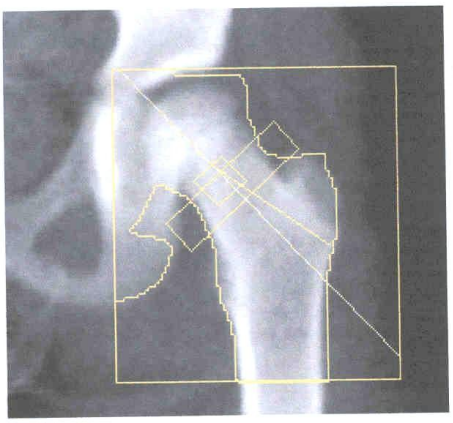
HOLOGIC

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

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

Name: 6 year study	Sex: Male	Height: 131.5 cm
Patient ID:	Ethnicity: Pediatric	Weight: 26.6 kg
DOB: 01 August 2003		Age: 6

Referring Physician: Cole



82 x 91
NECK: 44 x 12
HAL: 87 mm

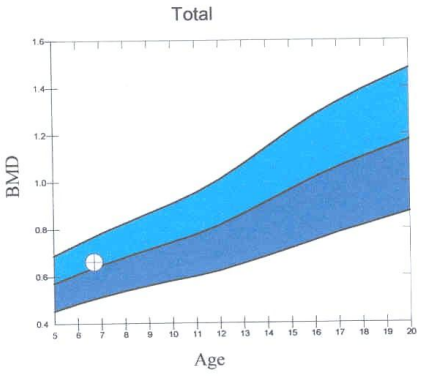
Scan Information:

Scan Date: 14 April 2010 ID: A0414100C
Scan Type: x Left Hip
Analysis: 14 April 2010 10:24 Version 13.0
Left Hip (low density)
Operator: lj
Model: Discovery W (S/N 80019)
Comment:

DXA Results Summary:

Region	Area (cm^2)	BMC (g)	BMD (g/cm^2)	T - score	PR (%)	Z - score	AM (%)
Total	21.86	14.50	0.663		56	0.4	104

Total BMD CV 1.0%



T-score vs. Pediatric Male; Z-score vs. Pediatric Male. Source:Hologic, 2005

Physician's Comment:

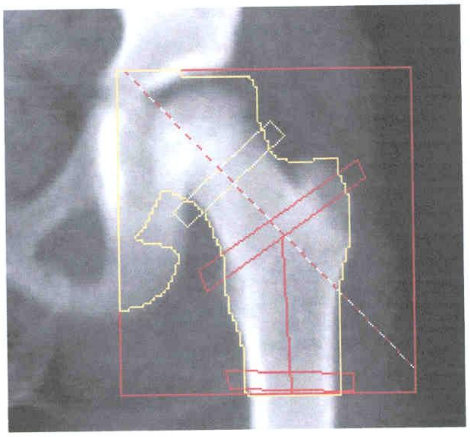
HOLOGIC

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

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

Name: 6 year study	Sex: Male	Height: 131.5 cm
Patient ID:	Ethnicity: Pediatric	Weight: 26.6 kg
DOB: 01 August 2003		Age: 6

Referring Physician: Cole



82 x 91

Scan Information:

Scan Date: 14 April 2010 ID: A0414100C
Scan Type: x Left Hip
Analysis: 14 April 2010 10:24 Version 13.0
Left Hip (low density)
Operator: lj
Model: Discovery W (S/N 80019)
Comment:

HSA™ Results Summary:

Region	Sub Peri. Width(cm)	Endo Cort. Width(cm)	CSA (cm^2)	CSMI (cm^4)	Z (cm^3)	Cort. Thick (cm)	BR
NN	3.08	2.83	1.90	0.83	0.46	0.12	14.6
IT	3.68	3.11	2.69	2.77	1.41	0.29	6.8
FS	2.26	1.59	2.04	1.02	0.83	0.34	3.6

Neck Shaft Angle: 139°
HAL: 87 mm

HOLOGIC

APPENDIX N: Certificate of achievement for DXA scan

APPENDIX O: Consent for pQCT scan

MRC Epidemiology Resource Centre
University of Southampton
Southampton General Hospital
Southampton SO16 6YD

A study to determine what makes bones strong

SWS ID

CONSENT FORM

For completion by Parent or guardian

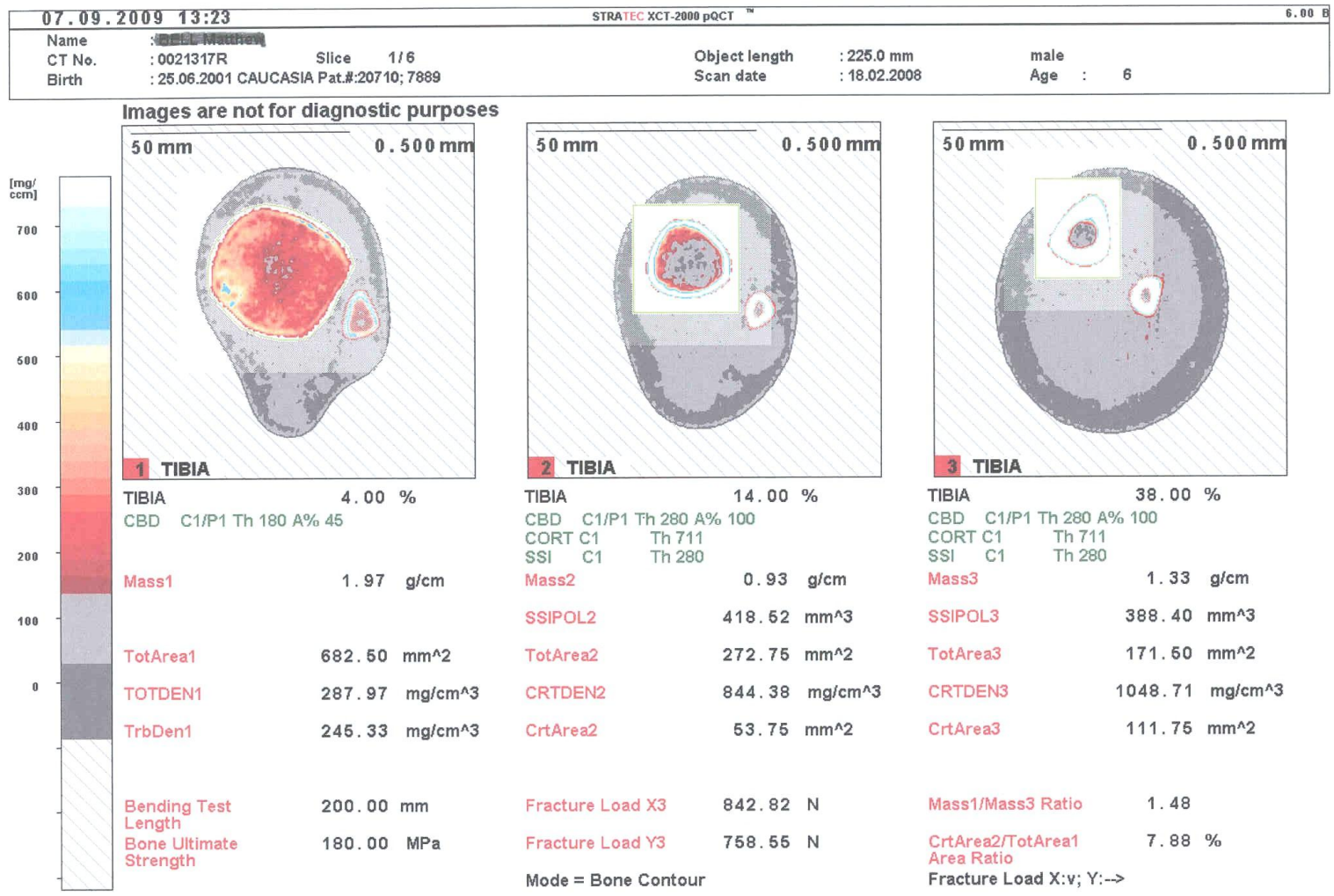
Please initial box

1. I confirm that I have read and understand the information sheet dated April 07 for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐
2. I understand that my child's participation is voluntary and that they are free to withdraw at any time, without giving reason and without medical care or legal rights being affected. ☐
3. I understand that data collected during the study, may be looked at by responsible individuals involved in research and the children's doctors who are involved in the study. ☐
4. I agree to my child..... taking part in the above study. ☐

Name of Parent/guardian	Signature	Date
Name of person taking consent	Signature	Date

When completed, 1 copy for patient; 1copy for researcher site file; 1 original to be kept in medical notes

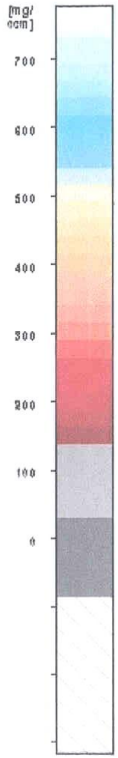
APPENDIX P: pQCT images given to parents

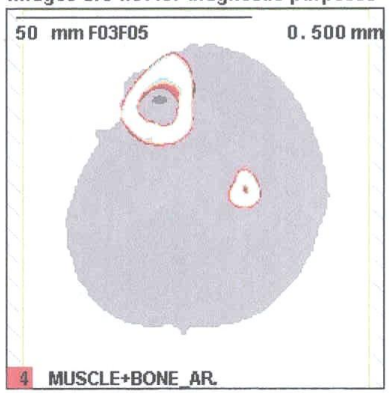


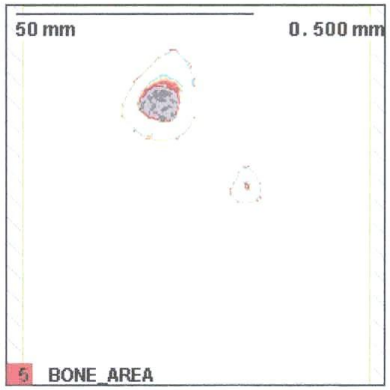
18. 02. 2008 16:16 STRATEC XCT-2000 pQCT ™ 6.00 B

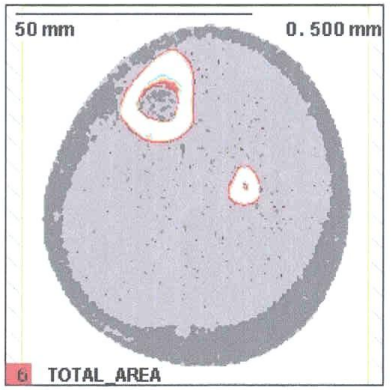
Name : [redacted]
CT No. : 0021317R Slice 4/6 Object length : 225.0 mm male
Birth : 25.06.2001 CAUCASIA Pat.#20710; Scan date : 18.02.2008 Age : 6

Images are not for diagnostic purposes









MUSCLE+BONE_AR.	66.00 %	BONE_AREA	66.00 %	TOTAL_AREA	66.00 %
CBD C3/P1 Th 40 A% 100		CBD C1/P2 Th 280 100 A% - CORT C1 Th 711		CBD C3/P1 Th -53 A% 100	
		Mass2	1.82 g/cm		
TotArea1	2566.75 mm^2	TotArea2	259.00 mm^2	TotArea3	3688.75 mm^2
		CRTDEN2	1032.87 mg/cm^3		
		CrtArea2 (=Bone Area)	149.00 mm^2		
Muscle Density	77.69 mg/cm^3	Marrow Density	35.25 mg/cm^3	Fat+Muscle Density	54.97 mg/cm^3
Muscle Area	2307.75 mm^2	Marrow Area	44.50 mm^2	Bone/Muscle Area Ratio	6.46 %
Fat Area	1122.00 mm^2			Fat/Muscle Area Ratio	48.62 %
		Mode = Muscle Contour		Fracture Load X:↔; Y:-->	

APPENDIX Q: Certificate of achievement for pQCT study

APPENDIX R: Ethics and R&D approval

COPY for SWS.

JOS/sta

16 August 2006

SOUTHAMPTON & SOUTH WEST HAMPSHIRE
RESEARCH ETHICS COMMITTEES (A)
1ST Floor, Regents Park Surgery
Park Street, Shirley
Southampton
Hampshire
SO16 4RJ

Tel: 023 8036 2466
023 8036 3462
Fax: 023 8036 4110

Email: GM.E.hio-au.SWHRECA@nhs.net

Dr Jane Lucas
Senior Lecturer/ Consultant Respiratory Paediatrician
University of Southampton
MP 803, IIR,
Academic Block, F Level, Southampton General Hospital
Tremona Road, Southampton
SO16 6YD

Dear Dr Lucas

Full title of study: **Developmental influences on childhood respiratory health. SWS cohort study at age 6 years**
REC reference number: **06/Q1702/104**

The Research Ethics Committee reviewed the above application at the meeting held on 08 August 2006. Thank you for attending to discuss the study.

Ethical opinion

Note: It may be necessary for non-negligent harm to be in place due to the nature of some of the tests being carried out.

The members of the Committee present gave a favourable ethical opinion of the above research on the basis described in the application form, protocol and supporting documentation.

Ethical review of research sites

The favourable opinion applies to the research sites listed on the attached form.

Conditions of approval

The favourable opinion is given provided that you comply with the conditions set out in the attached document. You are advised to study the conditions carefully.

Approved documents

The documents reviewed and approved at the meeting were:

Document	*Version*	*Date*
Application		11 July 2006
Investigator CV for Dr J Lucas		
Protocol	1	01 June 2006
Covering Letter		11 July 2005
Letter from Sponsor		23 June 2006
Questionnaire: 6 Year Physical Activity	1	01 June 2006
Questionnaire: 6 Year Clinic Visit	1	01 June 2006
Questionnaire: 6 Year Home Visit	1	01 June 2006
Letter of invitation to participant	1	01 June 2006

National Research Ethics Service
SOUTHAMPTON & SOUTH WEST HAMPSHIRE
RESEARCH ETHICS COMMITTEE (A)
1ST Floor, Regents Park Surgery
Park Street, Shirley
Southampton
Hampshire
SO16 4RJ

Tel: 023 8036 2466
023 8036 2870
Fax: 023 8036 4110

Email: scsha.SWHRECA@nhs.net

VY/sta

27 June 2007

Dr. Jane Lucas
Senior Lecturer and Honorary Consultant in Child Health
University Child Health,
Mailpoint 803, F level, South Block.
Southampton General Hospital
Southampton
SO16 6YD

Dear Dr. Lucas

Study title: **Developmental influences on childhood respiratory health. SWS cohort study at age 6 years**

REC reference: **06/Q1702/104**
Amendment number: **Protocol 2**
Amendment date: **01 March 2007**

Thank you for submitting the above amendment, which was received on 25 June 2007. It is noted that this is a modification of an amendment previously rejected by the Committee (our letter of 16 May 2007 refers).

The modified amendment has been considered on behalf of the Committee by the Vice-Chair.

Ethical opinion

I am pleased to confirm that the Committee has given a favourable ethical opinion of the modified amendment on the basis described in the notice of amendment form and supporting documentation.

Approved documents

The documents reviewed and approved are:

Document	Version	Date
Protocol	4	01 June 2007
Participant Information Sheet: Child	3	01 April 2007
Participant Information Sheet: Parent	3	01 April 2007
Participant Consent Form: Parent/Guardian	4	01 June 2007
Detrimental Effect Risk from Diagnostic Medical Exposure		12 April 2007
Ionising Radiation Form - Section B, Part B		
Modified Amendment	Protocol 2	01 March 2007
Covering Letter		21 June 2007

Southampton & South West Hampshire REC (A)

LIST OF SITES WITH A FAVOURABLE ETHICAL OPINION

For all studies requiring site-specific assessment, this form is issued by the main REC to the Chief Investigator and sponsor with the favourable opinion letter and following subsequent notifications from site assessors. For issue 2 onwards, all sites with a favourable opinion are listed, adding the new sites approved.

REC reference number:	06/Q1702/104	**Issue number:**	1	**Date of issue:**	16 August 2006
Chief Investigator:	Dr Jane Lucas				
Full title of study:	Developmental influences on childhood respiratory health.SWS cohort study at age 6 years				

This study was given a favourable ethical opinion by Southampton & South West Hampshire REC (A) on 08 August 2006. The favourable opinion is extended to each of the sites listed below. The research may commence at each NHS site when management approval from the relevant NHS care organisation has been confirmed.

Principal Investigator	*Post*	*Research site*	*Site assessor*	*Date of favourable opinion for this site*	*Notes* [1]
Dr Jane Lucas	**Senior Lecturer/ Consultant Respiratory Paediatrician**	**Southampton General Hospital**	**Southampton & South West Hampshire REC (A)**	**16/08/2006**	

Approved by the Chair on behalf of the REC:

..... (Signature of Chair/Administrator)
(delete as applicable)

Mrs Jane Ogden-Swift