MATERNAL NUTRITION, MATERNAL BODY COMPOSITION DURING PREGNANCY AND NEONATAL BONE MASS

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ABSTRACT

AIMS: To determine the maternal lifestyle and anthropometric factors before and during pregnancy that influence *in utero* and childhood bone accrual. In addition, to characterize the environmental predictors of changes in maternal bone mass, as measured by quantitative ultrasound of the calcaneus (QUS), during pregnancy.

METHODS: A cohort of healthy women was assessed before and during pregnancy and their offspring underwent anthropometric assessment, including whole body DXA, in the neonatal period. A second, older, birth cohort, now aged nine years, with records of their mother's lifestyle and anthropometry during pregnancy, had anthropometric assessment including whole body and lumbar spine DXA.

RESULTS: Maternal fat stores, smoking in late pregnancy and parental height independently predicted neonatal whole body bone mass. Of these factors, maternal fat stores and height had persisting effects on childhood bone mass. In addition, there was a significant decline in maternal calcaneal QUS during pregnancy; greater loss was predicted by reduced triceps skinfold thickness, nulliparity, low milk intake in the pre-pregnancy period and being pregnant over the winter months. After adjustment for maternal size, greater SOS decline was associated with greater neonatal bone area and mineral content. Of the predictors of childhood anthropometry, birth weight and size predicted bone and lean mass at age nine years but not fat mass. Maternal height and cord blood calcium were independent determinants of bone mineral content at age nine years.

CONCLUSION: We have demonstrated that maternal body build and lifestyle influence bone mineral accrual in the developing foetus and have persistent effects on post-natal growth, supporting the programming of skeletal growth by the maternal environment. The mechanism may involve maternal effects on foetal calcium homeostasis.

TABLE OF CONTENTS

FIGURES

TABLES

ABBREVIATIONS

aBMD	Areal bone mineral density
ALP	Alkaline phosphatase
BA	Bone area
BMC	Bone mineral content
BMAD	Bone mineral apparent density
BMI	Body mass index
BUA	Broadband ultrasound attenuation
COCA	Calcium concentration corrected for albumin concentration
CRHL	Crown rump heel length
DXA	Dual energy X-ray absorptiometry

FN	Femoral neck
IGF-1	Insulin-like-growth factor 1
IGF-2	Insulin-like-growth factor 2
LS	Lumbar spine
MUAC	Mid upper arm circumference
PTH	Parathyroid hormone
PTHrP	Parathyroid hormone related peptide
QUS	Quantitative ultrasound
ROI	Region of interest
SD	Standard deviation
SOS	Speed of sound
vBMD	volumetric bone mineral density
V_D	Vitamin D
WB	whole body

1 INTRODUCTION

1.1 SUMMARY

Osteoporosis is a common skeletal condition that leads to significant morbidity, mortality and economic burden through associated fragility fractures. Bone strength is determined by both peak bone mass accrued by early adult life and bone loss in later years. While most treatment strategies for osteoporosis have been targeted at retarding bone loss, optimizing peak bone mass remains an equally effective preventative strategy. However, the major interventions studied to optimize peak bone mass to date, dietary supplementation and exercise during late childhood and puberty, have led to transient increases in bone mass with little sustained benefit. This suggests that the trajectory of bone growth is relatively insensitive to such interventions in childhood

A child's growth is the result of interaction between genetic potential and the environment. When the trajectory is set and whether there are critical windows when environmental influences can lead to permanent changes is not known. Epidemiological studies have suggested that the environmental programming of skeletal growth may occur *in utero* or during infancy. For this reason, an appraisal of the physiology of normal bone growth during early life will be presented.

The aim of this investigation is to characterize the environmental factors in early life that may influence an individual's future risk of osteoporotic fracture by the modulation of peak bone mass accrual.

1.2 OSTEOPOROSIS

1.2.1 DEFINITION

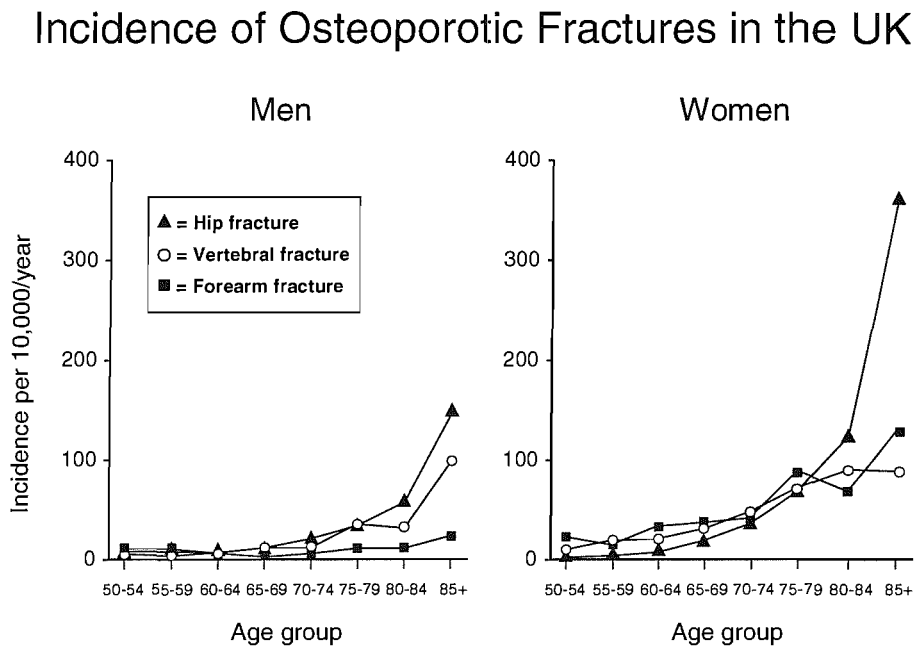
Osteoporosis is defined as a disease characterized by low bone mass, microarchitectural deterioration of bone tissue or both, leading to skeletal fragility." (1).

1.2.2 EPIDEMIOLOGY

While the incidence of fracture with age is bimodal, being higher in young people and in the elderly, the incidence of fragility fracture increases in those over 50 years (Figure 1). The peak in youth typically follows substantial trauma whilst those in the elderly follow only minor trauma; both are associated with reduced bone strength.

The common sites for osteoporotic fracture are the vertebral body, distal forearm and proximal femur; other sites include the proximal humerus, ribs. At age 50 years, the lifetime risk of a fracture of the hip, vertebral body, or distal forearm, approaches 40% among white women and 13% among white men (2). The most frequent site of fracture is the thoraco-lumbar spine, with prevalence rates of morphometric vertebral deformities of 25% among US white women aged 50 years and over (3), (4), (5). However, two thirds of morphometric vertebral deformities do not reach clinical attention acutely.

FIGURE 1 AGE AND SEX-SPECIFIC INCIDENCE RATES FOR FRAGILITY FRACTURES



Adapted from (40)

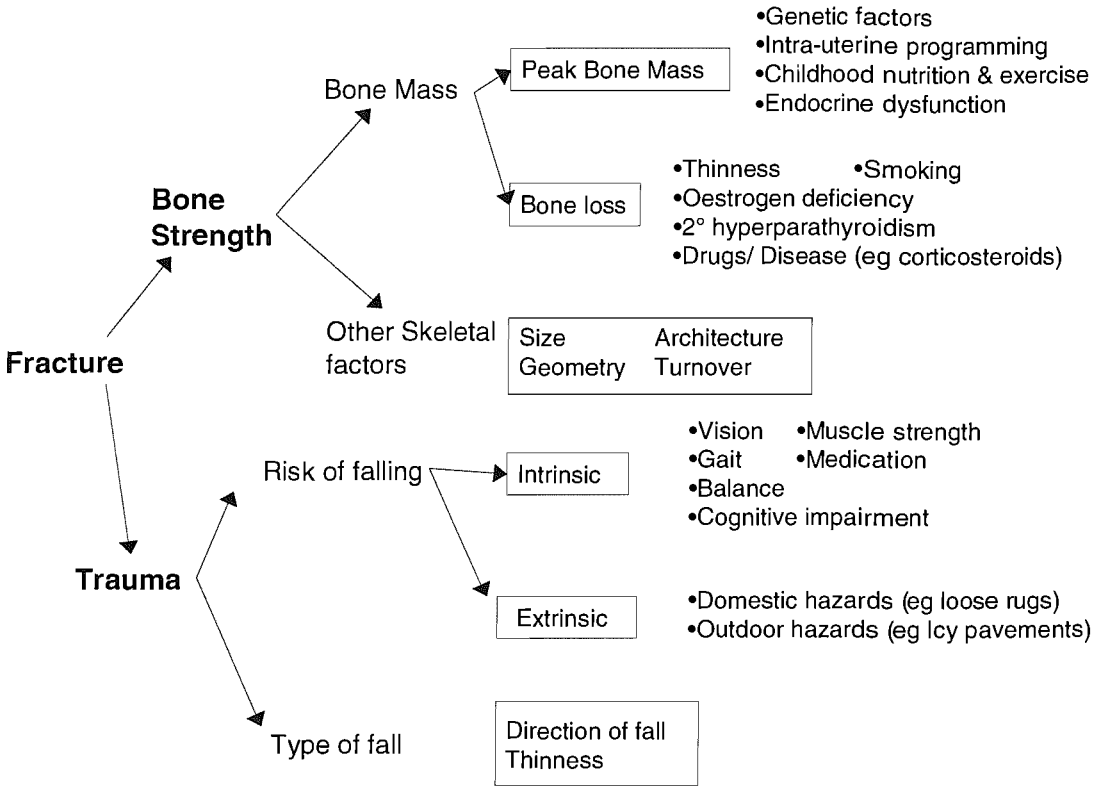
Whilst fragility fractures of the proximal femur occur less frequently (lifetime risk 18% among women aged 50 years), the mortality and morbidity associated with fractures at this site is considerably greater than that associated with vertebral deformity. Hip fractures invariably require hospitalization and one year following fracture 27% of patients have entered a nursing home for the first time, 40% are unable to walk independently, 60% have difficulty with at least one essential activity of daily living, and 80% are restricted in other activities such as driving and shopping. Mortality rates are increased among subjects with both hip and vertebral fractures; reductions in survival of 20% have been reported during the five years following fracture at both of these sites. Both clinically apparent and asymptomatic morphometric vertebral deformities are associated with an excess mortality.

The global economic burden of fragility fractures is considerable. In the United States, the care of these fractures costs around US $20 billion each year; in the United Kingdom, the amount is £1.5 billion. The most expensive fracture is hip fracture, and around half of hip fracture costs arise from care required after departure from hospital. In the UK, patients with a hip fracture occupy 20% of all orthopaedic beds, and 19% of patients require long-term nursing care.

1.2.3 PATHOPHYSIOLOGY

Fractures occur when the load applied to bone exceeds its material strength and so depend on two factors: bone strength and trauma; the multifactorial aetiology of fracture is illustrated in Figure 2

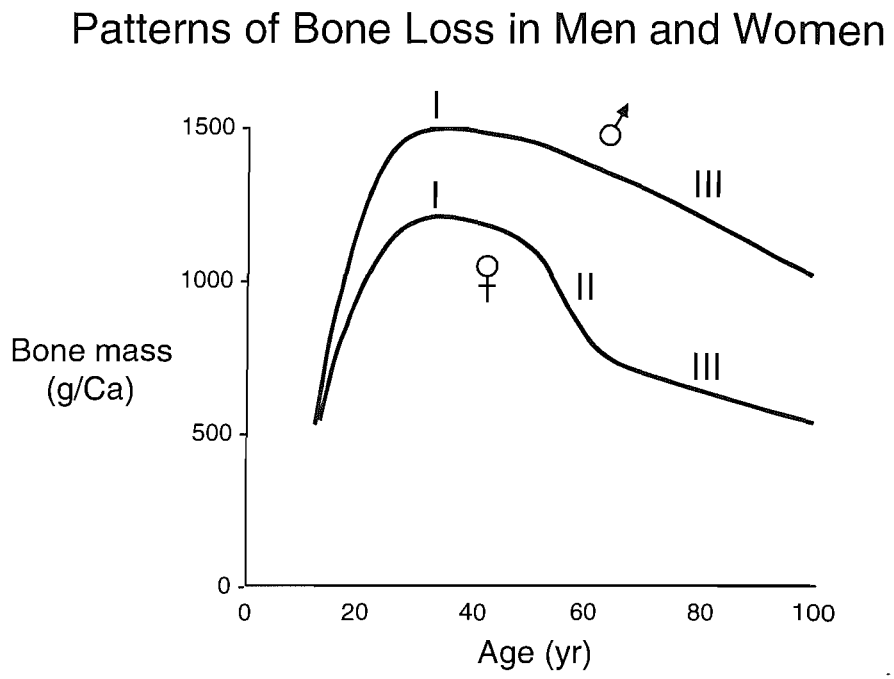
FIGURE 2 PATHOPHYSIOLOGY OF FRACTURE.



During the first three decades of life, fractures are considered to arise from higher energy trauma compared with fractures occurring in later life. After 65 years of age, 90% of all fractures result from a fall from standing height or lower (6;7) and reduced bone strength is an important determinant of fracture risk in the elderly. Bone mineral density is a major determinant of bone strength; dual energy X-ray absorptiometry (DXA) measurements of bone mineral density account for 75-90% of the variance in bone strength observed during in vitro and in vivo studies (8). Bone density in later life is a function of both the peak bone mass attained during early adulthood and the subsequent rate of bone loss (Figure 3 and it is estimated that 60% of the variation in adult bone mass is due to variation in peak bone mass (9)).

Other aspects of bone structure that determine bone strength include: geometry, micro-architecture and turnover.

FIGURE 3 BONE MASS THROUGH LIFE



Adapted from (40)

1.3 ENDOCRINOLOGY OF SKELETAL GROWTH

1.3.1 CALCIUM METABOLISM

In the non-pregnant state, parathyroid hormone (PTH), vitamin D and calcitonin regulate the circulatory concentration of free calcium. In adults, the skeleton stores over 99% of the 1 kg of total body calcium. The main site of calcium absorption is the ileum and the colon absorbs a smaller fraction (10). Intestinal calcium absorption is 80% passive and the fraction of calcium absorbed increases with lower dietary calcium intake, slower gut transit time and in the presence of lactose. The remaining 20% of absorption is active, under the influence of vitamin D dependent calcium binding proteins such as calbindin D5.

A high calcium intake not only reduces the fractional calcium absorption from the gut, but by complexing with phosphate in the food, the fractional absorption of phosphate is also reduced (11). While the Western diet is typically rich in phosphate; at the extremes of age phosphate content in the diet maybe low and so phosphate insufficiency may occur.

Calcium is principally lost in the urine with renal calcium reabsorption regulated by PTH. Faecal losses due to insoluble complexes are higher when the diet is rich in phosphates, carbonates, oxalate, fatty acids and fibre. Approximately 20mg/d of calcium is lost in sweat.

1.3.1.1 VITAMIN D (V_D)

Vitamin D is a key hormone for the regulation of bone growth and mineralization during life. Although a vitamin, it is not an essential requirement from the diet as it can be synthesized from the skin to form vitamin D_3. In the diet, the major sources of vitamin D are oily fish, eggs, fortified margarine/fat spreads, and fortified breakfast cereals (12). Vitamin D molecule is metabolized by the hepatic and renal parenchyma to form 1, 25 dihydroxyvitamin D_3, the most active moiety. The key rate-limiting enzyme, 1 α hydroxylase, is inhibited by 1, 25 dihydroxyvitamin D_3 and stimulated by hypocalcaemia, hypophosphataemia, hypomagnesaemia, parathyroid hormone (PTH), oestrogen, growth hormone, prolactin and insulin. 25-hydroxyvitamin D_3 can also be metabolized by 24 α hydroxylase into 24, 25 dihydroxyvitamin D_3, which may have additional effects on the chondrocytes and PTH gland. This enzyme is stimulated by 1, 25 dihydroxyvitamin D_3 and inhibited by PTH. As part of the feedback control, 1, 25 dihydroxyvitamin D_3 is a potent inhibitor of PTH secretion.

Vitamin D_3 increases serum calcium and phosphate concentration through actions on bone and the gut. In bone, 1, 25 dihydroxyvitamin D_3 stimulates the differentiation of macrophages into osteoclasts and enhances the mobilization of osteoclasts to promote bone resorption and release of calcium. In the gut, it stimulates calcium absorption by increasing duodenal and jejunal transcription of calcium binding protein. 1, 25 dihydroxyvitamin D_3 also enhances jejunal phosphate absorption.

1.3.1.1.1 VITAMIN D_3 CHANGES DURING PREGNANCY

During pregnancy, there is a significant decline in maternal 25 hydroxyvitamin D_3 with an increase in vitamin D binding protein, driven by oestrogen. Levels of 1, 25 dihydroxyvitamin D_3 progressively rise in the maternal serum during pregnancy with a rise in free 1, 25 dihydroxyvitamin D_3 in the last trimester of gestation (13). While maternal renal parenchyma is the prime site of 1, 25 dihydroxyvitamin D_3 synthesis, 1α hydroxylase is also present in placenta and the uterine decidual tissue and the role of the increase synthesis of 1, 25 dihydroxyvitamin D_3. may be to stimulation by PTHrP, oestrogen, placental lactogen or calcitonin, which are increased during pregnancy. While 25 hydroxyvitamin D_3 can cross the placenta, 1, 25, dihydroxyvitamin D_3 cannot, hence changes in maternal 1, 25 dihydroxyvitamin D_3 concentration do not directly influence 1, 25 dihydroxyvitamin D_3 levels in the foetus.

The increase in maternal 1, 25 dihydroxyvitamin D_3 leads to enhanced absorption of calcium from the gut (13) and inhibits the secretion of PTH. Vitamin D also increases placental transfer of calcium to the foetus. In animal studies, pharmacological administration of vitamin D to the mother increased both placental calcium transfer and foetal calcium content.

Both the foetal renal parenchyma and the placenta synthesize and secrete 1,25 vitamin D_3 into the foetal circulation; As well as regulating calcium homeostasis, 1,25 vitamin D_3 promotes maturation of prehypertrophic chondrocytes to hypertrophic chondrocytes in the growth plate (14). Whether placental calcium-binding-protein synthesis is influenced by vitamin D_3 has not yet been established.

1.3.1.1.2 EFFECTS OF SEASON ON VITAMIN D_3 DURING PREGNANCY

While severe vitamin D deficiency leads to growth impairment and the phenotypic features of rickets, the effect of seasonal variation of sunlight on vitamin D concentrations is unclear with observational studies finding variable effects (15). In a Korean cohort, winter born infants had an eight percent lower whole body BMC, with increased bone resorption, as measured by bone markers. This effect was reversed in US cohorts, with summer born babies having the lower BMC. The difference by population was suggested to be due to differential use of vitamin D supplementation.

1.3.1.2 PTH

PTH is coded on chromosome 11 and synthesized by the parathyroid gland and the central nervous system (16). Its synthesis is inhibited by high calcium serum levels and 1, 25 vitamin D_3. Secretion of PTH is inhibited by high calcium, 1, 25 dihydroxyvitamin D_3, glucacgon, cortisol and calcitonin. Magnesium and aluminium also inhibit PTH release.

PTH is metabolized by the liver and has half-life of less than 4 minutes and the function of the metabolized C-terminal fragments, which accumulate in the serum, is not clear. The homeostatic set point of serum calcium is increased in patients with hyperparathyroidism and reduced by oestrogen, and this may account for lower levels of PTH during pregnancy.

There are two forms of the PTH receptor. PTH1R binds both PTHrP and PTH and the receptor is found in both bone and renal cells. PTH2R binds only PTH and is found exclusively in neural tissue.

The classical actions of PTH increase ionized calcium and reduce phosphate concentrations in the blood. PTH by binding to osteocytes, leads to the release of calcium from the bone surface pool. In the longer term, PTH increases osteoclastic bone resorption by stimulating RANKL production by osteoblasts. PTH increases distal tubular resorption of calcium; decreases proximal tubular resorption of phosphate by reducing the brush border Na-P co-transporter expression; and stimulates mitochondrial 25 OHD0 1alpha OHase in the proximal tubular cells to enhance 25 to 1,25 OHD conversion, which in turn stimulates intestinal calcium absorption.

1.3.1.3 PTHrP

PTHrP is a polyhormone coded on chromosome 12; it has three principal fragments (17). The 1-9 fragment is identical to PTH and binds to PTH1R in cartilage, bone, breast, skin and kidney. The mid region, (38-94aa), binds to a different receptor and may be involved in placental calcium transfer. The final region, (107-139aa), is osteostatin, inhibiting bone resorption, stimulating

osteoblast growth as well having effects in the brain. PTHrP in the serum undergoes patient specific degradation and so requires samples to be taken in tubes with protease inhibitors present. In the adult, PTHrP mRNA is found in nearly all tissues as opposed to PTH mRNA, which is localized to bone and neural tissue.

Neither PTH nor PTHrP cross the placenta, hence circulating maternal PTHrP is not shared by the foetus. PTHrP is produced by the foetal parathyroid gland with some production by the placental syncytial trophoblasts, amnion, especially the amnion that overlies the choroid plate, umbilical cord and foetal liver. The level of PTHrP in the amnion is 40 times higher than that in foetal blood and PTHrP may have direct contact effects on skin, pulmonary and gastrointestinal cells. PTHrP from the foetal parathyroid cells is essential for the transport of calcium across the placenta and the maintenance of a calcium gradient between the foetal and maternal circulations. PTHrP is also responsible for the utero-placental vasomotor tone.

During pregnancy suppression in levels of maternal intact PTH has been demonstrated (14) and although a progressive rise in maternal PTHrP has also been measured by some, this is controversial (15). In IUGR with placental insufficiency leading to symmetrical growth failure, utero-placental vasoconstriction due to angiotensin II, is associated with an increase in placental PTHrP presumably as a compensatory mechanism. At the growth plate, PTHrP stimulates the proliferation of chondrocytes and inhibits terminal differentiation into hypertrophic chondrocytes. (20;21).

1.3.1.4 CALCITONIN

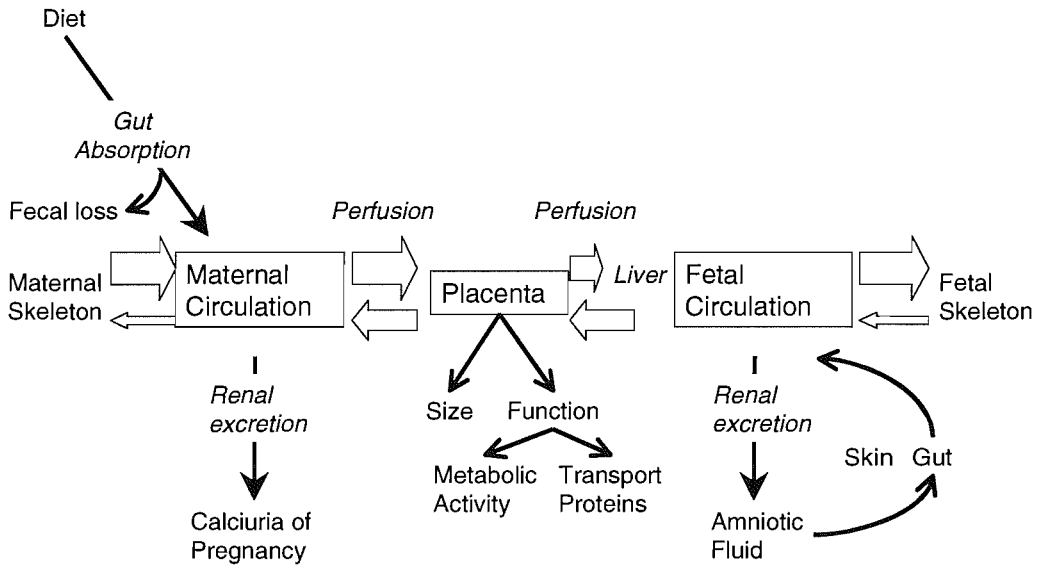
Calcitonin is a 32 amino acid peptide secreted by thyroid C cells and alternative splicing of its exons produces a 37-residue peptide in neurons, calcitonin gene related peptide. Both these cell types are derived from the neural crest. Calcitonin synthesis is stimulated by high calcium and its main action is to inhibit bone resorption by reducing the activity of osteoclasts and stimulating 1 α hydroxylase activation of vitamin D_3. In supraphysiological levels related to paracrine activity, calcitonin stimulates urinary calcium and phosphate excretion calciuria, phosphaturia, uricosuria as well as having analgesic and hypotensive effects. It also impairs glucose tolerance.

Calcitonin does not cross the placenta and during pregnancy serum levels are elevated in both the mother and the developing foetus. The physiological role of calcitonin during pregnancy is not yet known.

1.3.2 CALCIUM AND PHOSPHATE PHYSIOLOGY DURING PREGNANCY

The transfer of calcium from the mother to the growing foetus involves many key steps (Figure 4), and influencing factors include maternal placental and foetal perfusion, maternal and foetal calcium concentration and the activity of placental transport mechanisms.

FIGURE 4 MATERNO-FOETAL TRANSFER OF CALCIUM



The 30g calcium present in the neonate represents 2.5% of total maternal calcium stores and 80% is accrued in the last trimester (22). It is estimated that 170 litres of maternal plasma needs to be cleared of calcium to supply the foetus with 30g of calcium (22). As uterine perfusion in the last trimester is approximately 500ml/min, uterine blood flow is unlikely to be the limiting factor in calcium transfer. The major limitations are placental area and placental transport efficiency.

The degree to which the haemomonochorial foeto-placental unit can compensate for variations in maternal serum calcium concentrations is unclear. Mothers with hypoparathyroidism give birth to

infants with transient hyperparathyroidism; similarly mothers with hyperparathyroidism give birth to infants with transient hypoparathyroidism (23). In contrast, maternal calcium supplementation studies during pregnancy have been inconsistent (24) with a small increase in neonatal BMC only in those mothers whose diet was very low in calcium (25). In addition, vitamin D status is likely to be important since mothers with osteomalacia are more likely to give birth to infants with rickets (26).

Calcium flux across the placenta is bi-directional, with an estimated active materno-foetal flow of 40.9 μmol/min/g of placenta and a foeto-maternal flow of 39.1 μmol/min/g of placenta (22). The small difference leads to net foetal accrual of calcium. The major route of calcium flow through the placenta is transcellular (27), firstly through calcium channels (CaT1) in the apical membranes of trophoblasts (28) then through the cytoplasm by binding with the vitamin D-dependent calcium binding protein calbindin-D_{9K}. Finally the calcium is actively pumped across the baso lateral membrane by calcium ATPases (PMCA 1-4). The sodium calcium exchanger role in placental calcium transport is unclear.

The structure of the placental CaT1 calcium channel is larger than the calcium channel found in duodenal cells suggesting tissue specific alternative splicing. Regulation of the CaT1 gene is also unclear with an apparent independence to vitamin D metabolite levels in duodenal expression experiments (29). Extracellular magnesium inhibits CaT1 calcium uptake. Store operated calcium channels have been proposed as another important route for calcium entry into syncytiotrophoblasts at term however the precise molecular nature of these complexes are not yet known. PMCA 1 and 4 are expressed in almost all tissue, with PMCA 1 having the higher concentration; the other isoforms have also been found in trophoblasts. PTHrP, calmodulin with magnesium increase PMCA calcium activity.

The active transport of calcium renders the foetus relatively hypercalcaemic compared with the mother and a large amount of placental energy is expended to supply the foetus with calcium. The dependence on placental active transport to supply the foetus with calcium is evident on babies with failing placental function such as IUGR infants, who have lower total body calcium (30).

The maternal kidney does not conserve calcium during pregnancy with substantial increases in urinary calcium excretion. Maternal renal preservation of calcium is restored in the postpartum

period. The placental transfer of phosphate is less well reported but also occurs against a concentration gradient and involves active transport under the influence of both vitamin D and PTHrP.

1.4 DEVELOPMENTAL ORIGINS OF ADULT DISEASE HYPOTHESIS - PROGRAMMING

1.4.1 INTRODUCTION

The term programming describes persisting changes in structure and function due to environmental stimuli during critical periods in early development (31). 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 (32).

During early life, there are tissue-specific periods of rapid cell division called 'critical' periods (33). Tissues differ in the timing of their critical window; for example the long bones accelerate their rate of growth during the second trimester of gestation. The main adaptive response to a lack of nutrients and oxygen during this period of growth is to slow the rate of cell division, and this is amplified in tissues which are undergoing critical periods of growth. This reduction in cell division is either direct or mediated through altered concentrations of growth factors or hormones (in particular insulin, growth hormone and cortisol).

1.4.2 EPIDEMIOLOGICAL EVIDENCE FOR THE PROGRAMMING OF OSTEOPOROSIS

It has been postulated that osteoporosis in later life has its origins as a paediatric disease, with the failure of peak bone accrual (34). The programming of bone growth in response to the early environment is likely to underpin this association and there is now a large body of evidence to support the programming of osteoporosis.

1.4.2.1 PROGRAMMING OF BONE MASS

Early work on childhood growth patterns led to the development of centile charts and of the concept that an individual follows a predetermined growth trajectory or track until final height is achieved. This is supported by a Swedish cohort study, which demonstrated a strong association between height and weight at one year and adolescent height and weight (35).

Childhood skeletal status is determined, in part, by parameters at birth. Lower birth weight predicts lower femoral neck and lumbar spine BMC and BMD at eight years of age (36). Size at birth, the culmination of uterine growth, also determines peak bone mass, with a significant ($p<0.05$) association between weight at one year and BMC of the lumbar spine ($r=0.32$- 0.42) and femoral neck ($r=0.26$ - 0.40) at 21 years in women (37). Peak BMD, however, is associated with current height and childhood exercise but not birth weight. These results suggest a dual process for the development of the adult skeleton: trajectory of bone size, as measured by BMC, is set from an early age while mineralization of the skeleton, as measured by BMD, may be determined by local loading factors such as body habitus and exercise.

The relationship with growth in early life and adult BMC extends through later life. In older individuals, aged 63-73 years, weight at one year predicted BMC at the spine and hip in women and in spine BMC in men (26). The association was weaker than that found at 21 years of age, maybe due to different rates of bone loss in older age reducing the association of peak bone mass with current bone mass.

Birth weight is influenced by both the genome and the intrauterine environment. In twins only approximately 10% of the variance in birth weight is thought to be heritable (38). Furthermore recent work has demonstrated that in monozygotic twins, differences in birth weight do lead to differences in adult bone mass and density (39). These observations support the important environmental influences on both foetal growth and persisting alterations in post natal skeletal growth.

A meta-analysis of 10 observational studies from different populations around the world has confirmed the significant associations of body build in early life and skeletal status in individuals in childhood, young adulthood and the elderly (Table 1).

TABLE 1 RELATIONSHIP BETWEEN EARLY GROWTH 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)	0.20	(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. CI. Data are derived from published studies (n=10) relating weight in infancy and adult bone mass (40)

1.4.2.2 FRACTURE

Patterns of early growth also influence the risk of fragility fracture in later life. Risk of hip fracture is predicted by poor childhood growth between 7 and 15 years (hazard ratio 1.9 (95% CI 1.1-3.2); and also having a tall mother (hazard ratio 2.1 (95% CI 1.2 – 3.5) (41). Poor growth between 7 and 15 years may be due to delayed onset of puberty, a recognized risk factor for low peak bone mass. This is supported by epidemiological work examining the determinants of adiposity rebound (42), where it has demonstrated that for both male and female infants, shorter height at three years of age was associated with delayed adiposity rebound and, in the girls, delayed menarche.

A tall mother may give the genetic drive to grow, but environmental constraints on bone growth, as observed by poor birth size and childhood growth, may lead to mismatch and decreased bone strength. In combination, poor childhood growth and having a tall mother led to an increased risk of hip fracture in later life (hazard ratio 2.8 (95% CI 1.5 – 5.4). Shorter birth length was also associated with an increased hip fracture risk (hazard ratio 1.5 (95% CI 0.9-2.5).

1.4.3 PROPOSED MECHANISMS FOR PROGRAMMING OF BONE MASS

The exact mechanisms that underlie the programming of bone mass are at present unknown. Two broad hypotheses have been proposed to regulate the skeletal growth. The central hypothesis suggests there is a neural blueprint that compares expected with observed growth to influence growth rates (43). The local hypothesis, however, concentrates on effects on the growth plate in terms of stimulation and inhibition. Studies of the effects of corticosteroid inhibition on growth plate turnover and the subsequent catch up period of growth in the recovery period support a local mechanism for regulating growth (44).

The local control of bone growth is supported by the demonstration that birth weight predicts bone size rather than bone density in later life. 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. This

would change the growth trajectory of an individual throughout life. It has been proposed that the density of trabeculae within the medullary cavity does not increase during childhood; the number is established at the growth plate (45).

Alternatively, the mechanism may involve resetting endocrine responses that alter the balance between proliferation and differentiation of chondrocyte and other bone cells. 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 the vitamin D axis.

In man, the programming of the hypothalamopituitary axis has been demonstrated by studies that have confirmed persistent differences in cortisol secretion with increasing birth weight in an elderly cohort of men and women (46). The relationship between adult skeletal status and cortisol has been investigated in a series of men aged 62 to 72 years (47). In this prospective study over four years, there was a significant association between the integrated serum cortisol and levels of bone loss at the lumbar spine and femoral neck. This suggests that bone loss maybe programmed by altering the amount of cortisol secretion.

The GH/ IGF axis can also be programmed by adverse environmental influences in early life as demonstrated by animal (48) and human (49) studies; whereby either maternal undernutrition in animals or low birth weight in humans has been shown to lead to differences in IGF-1 and IGFBPs secretion or pattern of GH secretion respectively.

Similarly, animal studies have demonstrated permanent changes in leptin production by adipocytes in animal studies of undernutrition during pregnancy (50) and, in man, a relationship between low birth weight, low leptin and reduced bone mass (51).

The adult levels of 1, 25 OHD have been shown to vary by weight in early life in both post menopausal twins and singletons (211, 212). Serum 1, 25 OHD concentrations were higher in those with lower birthweight and weight at 1 year, suggesting an increased sensitivity of renal 1-alpha OHase in those who were small in early life. Increased concentrations of 1, 25 OHD were

associated with higher intestinal calcium absorption, lower aBMD and high levels of bone resorption markers.

1.4.4 SUMMARY

From the current available data, peak bone mass is an important determinant of adult bone mass and fracture risk. In addition the epidemiology of childhood fracture has identified differences in adiposity and bone mass as predictive factors. The trajectory of skeletal growth appears to be set in early life and the mechanisms influencing intrauterine foetal calcium accrual and skeletal growth include specific placental calcium transporter systems, the vitamin D and the PTH/PTHrP axis and other endocrine systems including growth hormone, glucocorticoids and leptin.

1.5 NORMAL SKELETAL GROWTH

While growth results in an increase in size, modelling is the result of environmental and genetic factors that guide growth to specific structural and physiological functions (52). Modelling factors can act in several different ways. Enabling factors either turn an activity on or off, while permissive factors are needed to allow the system to work or to be modified by other factors; rate-limiting factors set the maximum rate of growth; rate-modulating factors influence speed of growth from nil to maximum and space-guiding factors direct growth via anatomical associations. Bone growth can be affected by different types of mechanical factors including tension, due to tendons, or compression, due to loading of articular cartilage.

1.5.1 EMBRYOLOGY

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 mineralization to form mature bone. The molecular mechanisms initiating chondrogenesis have not been fully characterized but may involve cell-cell contacts by membrane bound cell adhesion molecules (53). The two types of ossification are intramembranous and endochondrial.

1.5.1.1 INTRA-MEMBRANOUS OSSIFICATION

Intramembranous ossification is the process for the development of the skull and the facial bones. The skull forms a considerable proportion of the neonatal length and up to 40% of the bone mineral content in normal neonates and with up to 80% in those with osteogenesis imperfecta (54).

Intramembranous ossification begins with a layer or membrane of mesenchymal cells which become highly vascular; the mesenchymal cells then differentiate into isolated osteoblasts that begin to secrete osteoid, which is mineralized at the end of the embryonic period to form bony spicules. The spicules become organized into lamellae; as these lamellae become concentric around blood vessels, they form Haversian systems. There is no cartilage model preceding ossification in this type of bone development.

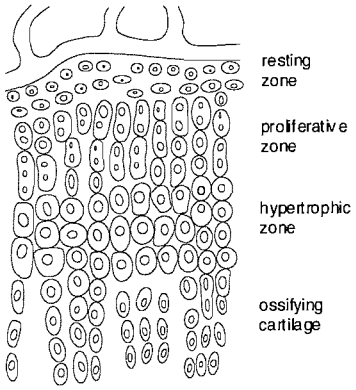
1.5.1.2 ENDOCHONDRIAL OSSIFICATION

The formation of the axial and appendicular skeleton, the main sites for fragility fracture, is by endochondral ossification. This form of ossification begins with a pre-existing cartilaginous model that undergoes vascular invasion at the diaphysis from the perichondral surface to seeding of osteoblasts. A major determinant of final bone structure is this cartilage model.

The cartilage model is formed by condensation of mesenchymal stem cells to form a primitive growth plate at five weeks gestation. It is at this time that the shape, size, position and number of skeletal elements are established. The stem cells then undergo ordered proliferation and differentiation into chondrocytes, which form a palisade of precursor to proliferative, prehypertrophic and finally hypertrophic chondrocytes (Figure 5). This expands the growth plate and forms the cartilaginous model.

The axial mesenchymal cells that go on to form the axial skeleton are derived from sclerotomes; the appendicular mesenchymal cells are derived from the lateral plate mesoderm. However, the muscles, innervation and vascular system of the appendicular skeleton are also derived from the sclerotome mesenchymal cells.

FIGURE 5 THE DEVELOPING GROWTH PLATE



Each stage of chondrocyte differentiation is characterized by modifications in the cell proliferation, morphology, matrix production, cytokine production and responsiveness (14). There is considerable expansion of the developing growth plate by both cells and the matrix, which is an important store of cytokines for later use and these cell/ matrix interactions are essential for proper differentiation to proceed.

PTHrP acts in a paracrine fashion to accelerate chondrocyte proliferation and inhibit differentiation. PTHrP release is regulated by Indian hedgehog in the developing growth plate (213). Other proliferative messengers include IGF 1, IGF 2, IGF BP 3-5, and TIMP 1-3 (55). 1, 25 dihydroxyvitamin D_3 promotes differentiation of chondrocytes in the growth plate into hypertrophic chondrocytes (56). Cbfa1 mediates mesenchymal differentiation into osteoblast progenitors as well as permitting terminal differentiation of chondrocytes (57).

Vascular invasion leads to osteoblasts, osteoclasts and haemopoietic cell aggregation in the centre of the cartilage model to form a primary ossification centre. Proteases released by osteoblasts then degrade the collagen before it is replaced by osteoid tissue. The primary ossification centre then expands longitudinally with a collar of cortical bone, secreted by perichondral osteoblasts. The surrounding matrix is then ossified by calcium hydroxyapatite crystals released from vesicles within osteoblasts (53).

1.6 NEONATAL BONE MASS.

1.6.1 MEASUREMENT OF NEONATAL BONE MASS

1.6.1.1 WHOLE BODY BMC

Whilst DXA is validated in adults, its use in younger individuals raises unique technical considerations. The smaller absolute amounts of bone mineral in neonates lead to larger percentage errors. A study of piglets using the Hologic QDR 1000, demonstrated a variation of <2.4% for whole body BMC and <1.8% for whole body BMD (58). These are higher than those reported for adults. Reassuringly, the addition of a pacifier, cotton blankets and non-metallic umbilical cord clamp had no effect on the BMC measurements (58).

Movement during the scan is common when measuring young individuals. Movement degrades the edge detection and so impairs the calculation of bone area. Movement significantly increases the DXA measurements. A study analysed DXA scans of babies, dividing them into those that moved and those that did not move during the scan; those scans with movement had increases in bone area, BMC and BMD of 4%, 13% and 9% respectively (58).

1.6.1.2 LUMBAR SPINE BMC

The measurement of the lumbar spine of neonates has several theoretical advantages over the measurement of total body bone mass. The major advantage is the reduction in scanning time needed to acquire the data, so reducing movement artefact. In addition, the vertebrae are principally composed of trabecula bone, which has greater metabolic activity and so may show more between individual variation and response to environmental influences, than cortical bone. A major theoretical concern is that the absolute amount of bone measured is small and so inaccurate edge detection may lead to large errors. However the CV for difference between paired DXA measurements at the LS were only 0.65% for BMC, 0.3% for BA and 1.8% for BMD (59).

1.6.1.3 SIZE CORRECTION

DXA measures aBMD to calculate two components of the skeleton, the total BMC and the bone area. The principal determinants of bone strength are the mineral density and the geometry of the mineral, which encompasses bone size and shape. There are many different reported methods for adjusting the DXA derived measurements to estimate volumetric or size adjusted bone mineral density. These include using areal BMD, BMAD using the method of Carter (182) and BMC adjusted for bone area using the method of Horlick (169), BMC adjusted for bone area, height and weight using the method of Prentice (170). An alternative model has been proposed to estimate bone width (bone area adjusted for height) and bone density (BMC adjusted for bone area and height) (218). Alternatively the WBBMC measurement has been compared using centiles with height, weight and lean mass measurements to determine whether the bone mass is appropriate, higher or lower than expected for the height, weight or lean mass of the infant/ child. For this reason, WBBMC, WBBMC per unit length and WBBMC per unit weight have been used in this thesis.

1.6.2 DETERMINANTS OF NEONATAL BONE MASS

1.6.2.1 GESTATIONAL AGE, GENDER AND ETHNICITY

As most foetal mineralization occurs in the final trimester, gestational age is a major determinant of total body and spine BMC and BMD, with progressive increments in bone mass with increasing gestation (59). Gender differences in bone mass are detectable during infancy (60), with males having higher whole body bone mass, whether this difference is measurable at birth is controversial with some studies finding no difference (61).

While in late childhood, Caucasians have a lower bone mass than their black peers (62); during early life the difference is small (60). Ethnic differences in LS bone mass are reduced after adjustment for both body weight and length (59). In addition, racial differences in bone mass in early life may also reflect nutritional and other lifestyle differences and not only genetic factors (63), (64).

1.6.2.2 PARENTAL BIRTH WEIGHT

Mothers who themselves were of low birth weight have lighter, shorter babies with reduced ponderal index (65) and WBBMC (66). This contrasts with low birth weight fathers who have babies, who are lighter and shorter and have reduced WBBMC but with no change in ponderal index. While this may represent genetic inheritance, such that small parents have small babies due to genetic factors, it may also represent a non genomic intergenerational effect. The mechanism for this non-genomic effect involves poor growth *in utero* of the mother, for example, retarding uterine development to an extent that it significantly limits the mother's uterus's ability in later life to supply nutrients to her growing foetus. Non genomic effects of paternal characteristics may involve differential methylation status and it is likely that these effects are due to an interaction of genetic, epigenetic and non genomic processes. The effect of maternal birth weight is not seen after adjusting for neonatal birth weight; the paternal birth weight influence on neonatal BMC is independent of neonatal birth weight.

1.6.2.3 PARENTAL ANTHROPOMETRY

Parental anthropometry is a significant predictor of final height in both girls and boys. Tall mothers have larger babies, with no difference in ponderal index (65). In addition, having a tall mother is an independent risk factor for future osteoporotic fracture (41). Maternal adiposity, as measured by triceps skinfold thickness, positively predicts neonatal bone mass; the association is present for both early and late pregnancy skinfold measurements. Tall fathers have infants that are tall and lean (65). The effect of paternal height was stronger than maternal height in predicting neonatal BMC in a multivariate model (66).

The mechanism for the association between adult anthropometry and anthropometry of their offspring could be due to genetic, epigenetic or environmental factors. Adult height is a result of both gene and environment. Using twin studies, the heritability of male height has been estimated to range from 0.87 to 0.93 while in women the heritability is lower (0.68 to 0.84) suggesting that shared environment component of the variation was more important in women. (214). The estimated heritability of adult weight and body mass index is lower ranging from 40% to 65% (215, 216).

During pregnancy, the mother gains weight independently of the foeto-placental unit. Of the approximately 10kg gained, 56% is deposition of fat mass, as measured by serial total body MRI (67). 80% is laid down subcutaneously with the rest deposited viscerally. The fat is principally laid down in the upper trunk (30%), lower trunk (44%) and thighs (19%). There is also deposition in the upper arms (4%), calves (2%) and forearms (1%).

1.6.2.4 MATERNAL NUTRITION

The transfer of nutrients from the mother's intake to foetal tissue involves many stages. Firstly, morning sickness may alter the amount of nutrients available to be absorbed by the mother. Absorption of certain nutrients, such as calcium, is influenced by endocrine factors that alter in concentration through pregnancy as described above. The maternal nutrient reserve then has to be made available to the foetus. The maternal reserve is a composite of her current nutritional intake and her pre-pregnancy size and health, which is itself a reflection of her previous nutrition.

The supply of nutrients to the foetus is also dependent on uterine vascularity and blood flow as well as the ability of the placenta to transfer nutrients to the umbilical circulation. Placental transfer of nutrients is dependent on placental size, the presence and density of specific transport proteins and hormones on both the maternal and foetal side. Finally, the uptake of specific nutrients by the developing foetal tissues is dependent on circulating foetal hormones such as insulin and IGF-1.

In an observational study of pregnant women, placental size was principally dependent on dietary patterns in early rather than late pregnancy, supporting the role of early nutrition in setting the placental growth trajectory (68). Mothers with higher carbohydrate intakes in early pregnancy and lower dairy protein intakes in later pregnancy had infants with a lower ponderal index (65).

Differences in maternal diet during pregnancy lead to measurable differences in the bone mass of their offspring in childhood. Higher maternal intakes of phosphorus, magnesium and fat during late pregnancy have been shown to predict greater childhood BMD at eight years (69). While calcium nutrient intake was not related, there was a significant relationship between maternal milk intake in late pregnancy and childhood bone mass. There appeared to be a threshold effect of maternal

calcium intake, as demonstrated by calcium supplementation studies of pregnant women, which only demonstrated a significant effect in those with low background intake.

1.6.2.5 MATERNAL EDUCATION

Maternal education is strongly related to foetal outcome with little threshold effect. In addition the number of formal years of maternal education is a predictor of osteoporosis in later years, with those with least education having a greater risk of disease (70). The mechanisms by which maternal education has such effects on her offspring are likely to involve lifestyle choices and behaviour. Higher social class is associated with less smoking, higher rates of breastfeeding and a healthier reported diet (SWS data).

1.6.2.6 MATERNAL PHYSICAL ACTIVITY DURING PREGNANCY

It is recognized that the combination of strenuous maternal physical activity during pregnancy is associated with lighter babies in women with a deficient diet (71). In studies of physical activity the measurement of physical activity is performed using different tools and some studies have not described the nature of work, using employment status or standard occupational definitions as surrogates (71). Also the dietary intake is often not assessed. In addition, women who work late into pregnancy may also differ in other class associated ways from women who stop work early. Women with high levels of physical activity both at work and at home had the highest percentage of low birth weight infants (71).

A woman's response to exercise differs during pregnancy from the non-pregnant state, owing to the biomechanical, physiological and metabolic changes of pregnancy. During pregnancy, there is an increase in resting maternal heart rate by 15 beats/ minute and in stroke volume by 10-12% (72). As the gravid uterus ascends in the abdominal cavity, the reduction in thoracic volume is compensated for by an increase in anterior-posterior diameter with an increase in tidal volume. This leads to mild respiratory alkalosis. Blood volume increases by 40%, with relative haemodilution, reducing blood viscosity. With exercise, there are further increases in heart rate, stroke volume (73) and total peripheral resistance (74). As body weight increases during pregnancy, exercise in the weight bearing position markedly increases energy consumption (75). The response of the insulin axis during

exercise also differs with an exaggerated reduction in circulating glucose with exercise (76). Such changes in the circulating nutrients together with differences in placental perfusion influence foetal growth.

The effects of maternal exercise on the foetus can be attributed to awakening of the foetus, placental transfer of catecholamines, release of foetal catecholamines, reduction in placental perfusion and an increase in maternal body temperature increasing foetal body temperature. (72). Foetal heart rate and respiratory rate increase during maternal exercise (72) (77). However, placental perfusion rates have been shown to be similar before and after brief periods of exercise. Different exercise patterns, weight bearing vs. non weight bearing, the timing during pregnancy and the mothers pre-pregnancy fitness all influence the foetal response, but are poorly described in the literature.

Starting moderate intensity exercise three to five times a week during early pregnancy has been shown to result in an increase in both birth weight and placental weight (78;79) without altering the duration of gestation (80). Women who perform regular recreational exercise have larger placental volumes in midtrimester compared with those women who do not (81).

However, higher amounts of exercise in late pregnancy are associated with lighter babies (82;83) with less percentage fat and smaller placenta (84). In addition, babies born to mothers who perform vigorous activity in late pregnancy have lower bone mass (66). As well as difference in body size and composition there may also be subtle neurobehavioural effects, with the babies of mothers who continued to exercise performing better at orientating themselves and settling after a stimuli than babies of non exercising mothers (85). However, these differences were no longer detectable at one year of age. (86).

Other studies of women who undertake high volumes of exercise, have not shown an effect of exercise reduction during pregnancy on birth weight (87-89) but this may reflect different methods for ascertaining activity.

1.6.2.7 PARENTAL SMOKING

It is well documented that mothers who smoke have lighter babies (65). The mechanism for this growth restriction in smoking mothers is at present unknown but includes reduced placental nutrient transfer due to impaired maternal haemodynamics, abnormal placental morphology or placental function, foetal hypoxia from increased carbon monoxide or a direct toxic effect on foetal growth (90). The decrease in birth weight is only partially explained by a slight decrease in gestational age. Placental weight is reduced in mothers who smoke (78), with a higher placental coefficient (ratio of placental weight to birth weight) compared to non-smoking mothers (91). The deleterious effect of smoking on birth weight, birth length and fat mass has been described in both active and passive smokers (92). Those mothers who are ex-smokers or stopped early in pregnancy do not appear to have babies with lower birth weights (93;94). Women who smoke have higher testosterone, independent of differences in body fat (95). This is likely to reflect inhibition of testosterone degradation.

Although some studies using single photon absorption densitometry have found little association between maternal smoking and BMD (96), the effect of maternal smoking during pregnancy is to reduce both the BMC and the BMD of the neonates as measured by DXA (66). The reduction in bone mass is primarily through reduced bone area. The negative effect of maternal smoking persists throughout childhood (78) and most likely demonstrates irreversible growth restriction set in early life. This is supported by the fact that maternal smoking status during childhood is not associated with reduced bone mass.

1.6.2.8 PARITY

Mothers tend to give birth to heavier babies with each successive pregnancy, with primiparous women giving birth to shorter and thinner babies (65). This is thought to reflect improved utero-placental nutrition, with enhanced uterine vascularity and placentation with each subsequent pregnancy. However, increased parity is associated with increased maternal weight in the first trimester and, the effect of parity on birth weight can be explained by including maternal weight in models.

1.7 POST NATAL GROWTH

After birth, growth can be divided into three phases: infancy, childhood, and puberty reflecting changes in the height velocity during these ages. Early work compared childhood growth to a ship following a channel; the course sailed is dependent on both the ship's characteristics and the prevailing environment (97). The principle of 'channelization' was applied to both the whole infant and for specific organ system growth. Similarly, tracking describes the tendency of an individual to maintain their ranked position in the distribution curve through time (98). Following from this concept, were observations of growth responses when an infant's environment changed to alter its channel and catch up growth.

1.7.1 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 (99). Central to this description are the concepts of a preset channel of growth and a defined insult, which is detrimental to the infant. Following removal of the insult, there is transient growth acceleration and then growth deceleration once the original growth channel has been reached.

Three patterns of catch up growth are described (97). Type 1 catch up growth has a period of rapid acceleration until the original growth channel is reached. An example of this pattern of growth is seen following successful treatment of coeliac disease in infancy/childhood. Other examples include treated hypothyroidism and anorexia nervosa. In Type 2 catch up growth, there is a delay in the timing of growth arrest, leading to an extension of the normal growth period with little or no increase in height velocity, e.g. by delaying puberty. Type 3 catch up growth is a combination of Type 1 and Type 2.

Babies who are short or light at birth may have experienced poor intrauterine growth and have a period of accelerated growth postnatally, and this may be 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. This is demonstrated by childhood growth data suggesting that in those born small there is an increased risk of childhood obesity (100).

1.7.2 POSTNATAL BONE GROWTH

In the postnatal period there is in an increase in both bone length and bone diameter. Bone length increases by either intramembranous ossification of the distal end of the phalanges and craniofacial bones, or endochondrial ossification of the remainder of the axial skeleton, through the growth plate (52). Here chondrocyte division on the metaphyseal surface of the growth plate leads to longitudinal growth. The matrix secreted by chondrocytes is ossified to form the primary spongiosa and then modelled by osteoblasts and osteoclasts to the mature secondary spongiosa. A sleeve of cartilage around the epiphysis forms the perichondral ring, which influences both the diameter and shape of the growth plate. 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.

1.7.3 DETERMINANTS OF INFANT GROWTH

It has been noted that the relation between a child's anthropometry and final height is weaker when measured at birth than at 2 years. This suggests changes in the growth rate from birth to a new 'channel' by the age of two, which is then continued to adult height (101). Evidence to support this is from the observation that the majority of infants cross centile lines during the first two years of life, with the timing of shift in growth centile different between two main groups. Those infants, who cross centiles upwards between birth and two years of age, do so from birth and tend to have taller parents. Those who shift centiles downward do so much later, starting between three and 18 months and they are likely to have shorter parents. These observations are consistent with the hypothesis that intrauterine growth has a substantial component that is independent of genetic potential as suggested by parental height while a component of postnatal growth involves resetting of growth towards a genetically predicted height.

1.7.3.1 NUTRITION

Nutrition in the early post-natal period leads to differences in height and weight during infancy. Compared with bottle fed infants, those breast-fed for the first three months of life are heavier (+0.64SD) and longer (0.5 SD) (102). The divergence in size between these two groups was completed by three months for weight and six months for length. It is known that enteral supplementation of premature infants with calcium and phosphate leads to an improvement in BMD of the lumber spine at 10 weeks of life (103). Early nutritional effects on bone mass have also been described. Supplementation of term infants with calcium and phosphate leads to greater initial gain in whole body BMC compared to those infants who are breast-fed at six months of age (104). However, breast-fed infants then have greater gains compared with the formula-fed infants such that at one year there was no difference in BMC between groups. A similar finding of an initial slowing of growth in breast fed infants with later acceleration of growth at one year has been reported in those born small for gestational age (102). These findings suggest a critical window in the postnatal period where infant nutrition leads to alterations in subsequent bone growth.

Children who were exclusively breast fed for the first three months of life had higher BMD at the spine (+0.25 SD), hip (+0.20 SD) and whole body (+0.29 SD) (105). The effect was attenuated in those born before 37 weeks gestation but persisted after adjustment for current height and weight, suggesting an effect of early feeding on both mineralization and bone size.

A major criticism of studies comparing breast to formula feeding is confounding by maternal preference. Compared with mothers who elect to bottle feed, mothers who choose to breast feed have a higher social class (social class I or II: 55.6% vs 14.8%) and maternal education level (higher education: 44.4% vs 7.4%) and are less likely to smoke (non smoking: 81.5% vs 33.3%)(102). However, even after adjustment for these known factors, the effect of breast-feeding compared with formula feeding on infant growth remains (102).

The effect of breast-feeding also appears to persists into adulthood, with those breast fed being taller in fifth decade (106). This effect was independent of parental social class in early childhood and was associated with leg not trunk length as measured by standing vs. sitting height.

Breast milk is a complex mixture of nutrients, hormones and other molecules. The protein and lactose content of milk enhances calcium absorption from the gut. Using stable isotopes in formulae containing 460 mg/L of calcium, the presence of lactose increased calcium fractional absorption from 56% to 66% (107). Fractional calcium absorption from human milk is 61% (108).

Various skeletally active mediators have also been found in breast milk. Elevated levels of osteoprotogerin (109), a potent inhibitor of osteoclasts, have been found in breast milk. PTHrP is also found in breast milk and may further influence the infants bone accrual (110). Human milk is low in phosphorus (104) this enables acidification of the faeces, limiting the growth of pathogenic organisms. In addition, as the infantile kidney cannot readily excrete surplus phosphorus, a high phosphate intake can lead to metabolic acidosis. Bottle-feeding may also reduce the bioavailability of vitamin D because of binding of this fat soluble vitamin to the plastic bottle (111). The effect of mineral and vitamin D supplementation in infancy leading to permanent changes bone in growth have also been reported. In contrast the premature infant appears to have a lower demand for vitamin D. Vitamin D supplementation in this group does not enhance accrual of bone mass (112). However, there may be extra skeletal benefits in terms of reduced respiratory complications and requirements for mechanical ventilation in higher dose vitamin D supplementation (960 vs 200 IU/kg/day till three months of age).

1.8 CHILDHOOD SKELETAL GROWTH AND PEAK BONE MASS

The importance of childhood bone growth and peak bone mass for bone strength during later life was initially suggested by cross-sectional observations that the variance of bone mass does not widen with age. This led to the hypothesis that bone mass tracks throughout life and that an individual at a high centile of the population distribution at age 30 years is likely to remain at that end at age 70 years. Recent longitudinal studies have described the same tracking across the pubertal growth spurt (113).

Body size, height and weight during adolescence predicted peak bone mass at the proximal femur and lumbar spine in both girls and boys (12). Adiposity during adolescence, as measured by BMI, also predicted peak bone mass at these sites.

Genetic factors are important in determining adult height, bone size, age of onset of puberty, sex and race, all of which influence peak bone mass. Environmental factors are also important and can act pre- or post-natally. Postnatal factors include diet, exercise, smoking and drug therapy such as glucocorticoids.

1.8.1 GESTATIONAL AGE

The importance of gestational age for skeletal growth is evident in those born prematurely, who have persisting reductions in childhood bone mass, even after adjusting for current size and age (114). While premature infants may regain normal height, BMC measurements are reduced suggesting a delay or deficit in skeletal mineralization compared to growth of the skeletal envelope in premature infants. Such differences in bone mass may be site specific. LS measurements are no different in premature infants and with no difference by one year (115) or in childhood (116).

1.8.2 FRACTURE

Fractures are common in childhood and are attributed to trauma rather than reduced bone strength. However, in a four-year prospective study of fracture in girls, previous fracture, total body BMD and body weight were independent predictors for future fracture (117); these factors match those known to predict adult osteoporotic fracture (Table 2).

TABLE 2 RISK FACTORS FOR FUTURE FRACTURE IN CHILDREN

	Hazard ratio	95% CI
Previous Fracture	3.3	1.4- 7.6
Total body BMD	1.92 per SD reduction	1.3 – 2.8
Body weight	1.49 per SD reduction	1.1 – 2.1

Adapted (117)

The predictive effect of previous fracture may reflect either reduced bone strength or increased fall-related behaviour. A prospective study has, however, demonstrated no difference in physical activity levels in girls who sustained a further fracture compared with girls who did not (117). While the study confirmed the predictive capability of total body BMD and previous fracture, higher not lower body weight was associated with increased risk of fracture, proposing either falling with greater force or reduced physical activity in those who are obese as mechanisms. This has been confirmed by another case control study (217). There therefore maybe more than one phenotype associated with higher risk of fracture in childhood. No difference in pubertal development or age at menarche has been demonstrated in those who fracture, arguing against delayed pubertal maturation as a contributor to the lower BMC in girls who fractured.

1.8.3 PUBERTY

Puberty is the period during which the characteristic gender differences in bone mass observed in adults becomes fully expressed, with marked acceleration in mineralization. While in pre-pubertal children, height is a strong predictor of bone mass (118); this close relationship vanishes during pubertal maturation and BMD values are poorly correlated with height, the pattern observed in adults.

The most important difference to emerge during pubertal maturation is the greater increase in bone size, with greater periosteal apposition in males as compared with females (119). These gender differences in size contrast with similar values for volumetric bone mineral density between sexes. Studies based on histomorphometry and QCT indicate no difference in volumetric trabecula density at the end of the period of pubertal maturation.

The greatest increase in bone mass occurs between 12 and 15 years in girls and 14 to 17 years in boys, with achievement of peak bone mass between 25 and 35 years (120); by the end of puberty 85-90% of peak bone mass is achieved (121). The greater demands for calcium during this time are met by an increase in intestinal calcium absorption at the onset of puberty (122).

The timing of puberty is an important determinant of peak bone mass (123). The earlier puberty occurs, the earlier chondral division ceases, leading to ossification of the growth plate, and the shorter the individual. Hence, girls, who have an earlier onset of puberty, are shorter than those with a later puberty. However, the timing of the onset of puberty is not the only factor determining bone mass accrual; variation in maturation rate and time to menarche also influences bone growth. For example, girls who mature slowly have lower bone mass (98).

1.8.4 GENETIC DETERMINANTS OF PEAK BONE MASS

That bone mass has a genetic component is demonstrated by a positive family history of fracture being a risk factor for low bone mass (124). Studies of the heritability of bone mass have used twin studies, offspring of osteoporotic individuals and offspring of non-osteoporotic individuals. However the magnitude of genetic effects on bone mass may be overestimated due to similarities in environmental influences between parents and offspring (125), or through indirect influences on bone mass by lean mass (126).

From twin studies, it is estimated that between 0.42 – 0.98 of the variability in peak bone mass may be hereditable (127). In singletons, 46-62% of the variance in BMD of sons and daughters was attributable to heredity (128). The daughters of osteoporotic women have low bone mass (126), and the association is strongest while the mothers are still pre-menopausal (129). The age at which these genetic factors operate is not known; the relationship between a mother's and daughter's bone mass is detectable in the pre-pubertal period and tracks through puberty (113). This relationship was significant at the proximal femur and lumbar spine, and for BMC, BA and BMD. However, while significant relationships exist between the bone mass of a mother and her daughter, there is no such association between grandmothers and their grand daughters (130); this suggests the importance of environmental determinants of bone mass or varied bone loss; however the rate of bone loss is more similar in monozygotic than dizygotic twins (120).

Candidate genes for regulation of bone mass include those coding calcitropic hormones, bone matrix components, growth factors and adhesion molecules. Using linkage analysis, initially unrelated metabolic systems also seem to influence bone mass (131). Currently, genetic polymorphisms have been found to make only a modest contribution to bone mass in populations.

Differences in VDR polymorphisms account for 1-2 % of bone mass variation (132). Interactions between VDR and ER polymorphisms may contribute more to bone mass. Other candidate molecules include IL-6, a regulator of osteoclasts and TGFβ, which influences osteoblast osteoclast interactions.

To date only a small proportion of the variance in adult bone mass has been attributed to specific genetic polymorphisms. This may be because low BMD and fracture have their own specific genetic risk factors. Using twin data, while there was an important genetic contribution to the risk of wrist fractures, this did not appear to be mediated via low BMD (133). This once again highlights that BMD measurement is a surrogate and not an end point in studies of osteoporosis.

1.8.5 ENVIRONMENTAL DETERMINANTS OF PEAK BONE MASS

The environmental influences on peak bone mass can be ranked in order of effect size (Table 3

TABLE 3 ENVIRONMENTAL INFLUENCES ON PEAK BONE MASS

Major Effect	Moderate Effect	Minor Effect
Medication (corticosteroids)	Exercise	Lactation
Co morbidity (rheumatoid arthritis)	Calcium intake	Parity
	Other nutrients	Oral contraceptives
	Smoking	Caffeine
	Excess alcohol	

Modified (120)

1.8.5.1 NUTRITION

During childhood, differences in calcium intake influence have been shown to influence bone mass accrual through adolescence and achievement of peak bone mass. However, the tools available to measure dietary intake during childhood and adolescence vary by study and these may account for the differing findings of the effect of diet on bone mass.

During puberty, there is a significant increase in intestinal calcium absorption (122); this suggests that during childhood growth, the same amount of dietary calcium has varying bioavailability adding further complexity to assessment of calcium intake. Studies of dietary calcium intake are either observational or involve trials of different foods or supplements.

In ten year olds, using a seven-day food diary, protein intake was positively associated with whole body bone area while negative associations were observed with phosphorus and sodium (134). In addition current calcium intake was a significant predictor of size adjusted whole body bone BMC suggesting an effect on bone mineralization. However, the estimated daily calcium intake was high in this Danish cohort and this may limit its generalizability to other populations (boys: mean 1.2g Ca/day [SD 0.4g]; girls: mean 1.1g Ca/day [SD 0.3g]). This does support a role for calcium intake in bone mineralization in not only calcium deficient children but also in those with the higher ranges of calcium intake.

Observational studies of diet during adolescence have identified dietary vitamin D but not calcium as positively predicting bone mass (12). The effect of dietary vitamin D was only significant in females and also lumbar spine during adolescence and at the proximal hip in early adulthood. Furthermore the principle determinant of 25 (OH), vitamin D_3 status is photo conversion and dietary sources are only important during periods of low ultraviolet exposure. Seasonal variation of vitamin D status, have also been shown to influence BMC. During winter, reductions in skin exposure to ultraviolet light may lead to insufficiency of vitamin D levels (25 hydroxyvitamin D_3 <40 nmol/l) in those adolescents with borderline low vitamin D status. However these seasonal effects have not been shown to reduce bone growth as measured at the radius, compared with those with higher vitamin D levels (135).

Calcium supplementation does increase BMC in adolescents; however this appears to be due to accelerating maturation with and earlier age of menarche in the supplemented group (136). The method of supplementation is also likely to be important; supplementation with calcium as a mineral produces transient increases in BMC, while dairy supplementation may led to a sustained increase in BMC (137).

As well as the method of supplementation, the baseline calcium intake is also important, with the increase in BMC from calcium supplementation greater in those with low calcium intakes as expected. The consumption of an extra 714mg/d of calcium for one year in those with very low intake (<330mg/day) led to an increase in BMC at the mid-radius at the end of supplementation (138) and this was sustained for at least a year after the children returned to a low calcium diet (139). In contrast, there was no difference in either linear growth or timing of puberty in the supplemented group. The children were supplemented at age 10.3 years.

Supplementation with dairy products increases nutrient intake of calcium, phosphorus, vitamin D as well as energy and fat. Sustained effects on bone mass in some dairy supplementation studies may be due to the higher fat intake precipitating an earlier menarche. The calcium: phosphate ratio may also be important as dietary phosphate, found in carbonated drinks, is known to bind to calcium in the gut to produce a non-absorbable salt. Other factors include acidity, caffeine, sugar or salt content. However, the primary deleterious effect of carbonated beverages on calcium intake is likely to be due to milk displacement (140).

Another 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 (141). This has lead to investigations of the predictors of childhood calcium intake and it is of interest to note that the mother's calcium intake during the third trimester of pregnancy does weakly predict the calcium intake of her child ($r=0.17$) (105), suggesting a non genomic familial component to certain aspects of diet.

1.8.5.2 EXERCISE

There is large variability in exercise patterns amongst adolescents and this leads to variation in bone mineral accrual (142). Studies have classified exercise by amount of exercise, type of exercise in observational or interventional study designs.

The pattern of exercise is likely to be important with impact sports involving loading of the skeleton leading to greater gains in BMD than non-impact exercise, such as swimming (143). Weight-bearing

exercise may have a site-specific effect on bone growth; activities involving impact such as running and jumping have the biggest effect at the femoral neck compared with the lumbar spine (143). However, intervention studies of jumping have shown effects at both these sites (128), where the increase in BMC was through size at the proximal femur and density at the lumbar spine.

Activities that load the skeleton by muscular contraction alone, such as swimming, do not seem to augment bone growth. Whether gender influences the relationship between activity and bone mass is not clear. While some studies show no difference (143), others demonstrate a strong relationship between activity and bone mass in boys (12); this may reflect gender differences in the type and intensity of physical activity.

1.9 CHANGES IN MATERNAL BONE MASS DURING PREGNANCY

During pregnancy, there is a large supply of calcium (33g) to the developing foetus; 80% of this in the third trimester (22) and the maternal skeleton undergoes changes to meet these demands.

1.9.1 BONE MASS

The changes in bone histology during pregnancy have been described in both early and late pregnancy (144). In early pregnancy, bone volume decreases with an increase in resorption cavities. In late pregnancy, bone volume recovers with an increase in osteoid and seam width and mineralization. This is mirrored by a reduction in QUS measurements during early and late pregnancy (126;144). All studies have demonstrated a loss of trabecula bone (15;18); the effect on cortical bone is not clear with some suggesting gain (18) and others loss (19) during pregnancy.

Calcaneal QUS measurements continue to fall in the postpartum period (145). Nulliparity and adolescent age of the mother were predictors of higher loss rates. Smoking and physical activity status did not influence loss rates. However, the effect of breast-feeding was not examined.

1.9.2 BONE MARKERS

There are significant changes in maternal bone markers during pregnancy. There is a progressive increase in bone resorption markers throughout pregnancy (146) with bone formation markers only rising in late pregnancy (15;18). The change in bone markers and bone histology suggests in initial period of resorption in the early pregnancy followed by increased bone formation. Although the change in maternal bone markers may be due to changes in the developing foetal skeleton, using isomers specific to foetal tissue, the foetal contribution to maternal resorption is less than 10% (18).

1.9.3 MECHANISMS

The endocrine mechanism underlying dissociated bone resorption in early pregnancy with suppression of formation despite the hyper-oestrogenic milieu of pregnancy is not fully characterized. In addition to the classic hormones of calcium homeostasis, other pregnancy related hormones, such as placental lactogen may also have a role in determining maternal skeletal status during pregnancy.

Maternal calcitonin levels are elevated during pregnancy and may act to protect the maternal skeleton from some of the catabolic effects of pregnancy. PTHrP may stimulate renal 1α hydroxylation of vitamin D thereby maximizing absorption of calcium from the maternal gut. However measurement of maternal PTHrP has not demonstrated significant changes during pregnancy (19). The high levels of 1, 25 dihydroxyvitamin D also dampen classic PTH secretion from the parathyroid glands. Recovery of bone mass is associated with resumption of menses, with further bone loss during postpartum amenorrhea (147).

1.10 OUTSTANDING AREAS OF RESEARCH

While maternal lifestyle and anthropometric characteristics during pregnancy have been identified to predict neonatal bone mass it is not known if the maternal characteristics in the pre-pregnant period also influence foetal growth. For example, it is recognized that there is only a small effect of maternal diet during pregnancy on foetal growth suggesting the pre-pregnancy nutrient status is

more important. Also, lifestyle characteristics such as smoking and physical activity change during pregnancy and it is not known whether the pre-pregnant lifestyle status has persisting effects on foetal growth. There is little data to describe whether these maternal determinants of foetal growth have persisting effects on post-natal growth. In addition, while there is data to support a reduction in maternal bone quality during pregnancy, the determinants for this have not been fully characterized and the relationship to foetal mineralization has not been described.

2 OBJECTIVES

Aims and Objectives of investigation:

To test the hypothesis that maternal birth weight, pre-conceptional body mass index, maternal smoking status, maternal fat stores during pregnancy, and maternal energy and protein intake during pregnancy are determinants of neonatal bone mineral content.

To examine the extent to which maternal calcaneal bone mineral in pregnancy predicts neonatal bone mass.

To examine the extent to which pre-conceptional body build and maternal nutrition influence maternal bone mineral changes through pregnancy.

To examine the extent to which maternal predictors of neonatal bone mass lead to differences in bone mass at nine years.

3 MATERNAL PREDICTORS OF NEONATAL BONE MASS

3.1 SUBJECTS AND METHODS

3.1.1 INTRODUCTION

The Southampton Women's Survey is a prospective cohort study assessing lifestyle and body composition in 12,500 non-pregnant women aged 20-34 years registered with a general practitioner (GP) in the city of Southampton (148). All women eligible for the survey were sent an information letter and telephoned for an appointment for initial interview at the women's own homes. Only women, whom the GP considered unsuitable because of physical, psychiatric or other problems, were not approached. Those women not on the telephone were contacted by letter or in person.

A research nurse administered the questionnaire at the initial interview at the woman's home. The questionnaire included socio-demographic characteristics, smoking habit, alcohol consumption and the level of physical activity (Table 4). Physical activity was measured using two questions: reported walking speed (fairly slow, slow, normal, fairly brisk, fast) and reported activity (both leisure and job related) graded as vigorous (sufficient to cause breathlessness and exhaustion), moderate (sufficient to cause exhaustion) and gentle (sufficient to cause tiredness). Milk intake was recorded as a measure of calcium intake. In addition the following measurements were made of the woman: height using a stadiometer, weight using calibrated electronic scales, skin fold thickness using Harpenden callipers at four sites (triceps, biceps, sub-scapular and superior iliac) and circumference (mid-upper arm, waist, hip, thigh and calf).

Those women who subsequently became pregnant were invited to attend the Princess Anne Maternity Hospital, Southampton, at early (11 weeks) and late (34 weeks) gestation for measurement of foetal growth and reassessment of lifestyle and body composition characteristics (**Appendix II, Appendix III**). All the pregnant women were divided according to GP into bone and pulmonary research arms and at 34 weeks those in the bone research arm were given an information sheet about bone health (**Appendix VI**).

TABLE 4 MATERNAL CHARACTERISTICS RECORDED DURING SURVEY

Demographics	Reproductive	Lifestyle	Anthropometry
Age	Parity	Smoking status	Height
Employment	Age of Menarche	Alcohol use	Weight
Social Class	Cycle regularity	Walking speed	BMI
Education	Use of oral contraceptive	Vigorous activity	Skinfold thickness (Triceps, Biceps, Sub-scapular, Supra-iliac)
Qualification		Diet including milk intake and food supplements	Circumferences (Mid upper arm, waist, hip, thigh calf)

Legend: Employment was recorded full time (>30 hours per week), part time (<30 hours per week) or none. Educational qualifications were recorded as the highest qualification achieved (none, CSE, GCSE D-G, GCSE A-C, A'level, HND and degree).

At birth, samples of the cord blood and placenta were taken and frozen at –70°C. Neonatal birth circumstances and detailed anthropometry were recorded within 48 hours of birth (**Appendix VII**). The neonatal measurements at birth were: birth weight; circumferences of the head, mid upper arm, chest and abdomen; skinfold thickness at triceps, sub-scapular and mid-thigh; crown heel and crown rump length. All measurements were performed by research nurses following a protocol and with regular assessments of inter-observer variation.

After delivery of their child, the parents who had been given the leaflet at 34 weeks were asked if they were agreeable to their baby having a DXA scan. Infants were excluded from the DXA study if born prior to 37 weeks' gestation, had major congenital deformities, were born of multiple gestations or could not return for a DXA scan within two weeks after birth.

All participants were given written information at every phase of this study, and asked to give written informed consent for the measurement of both their heel bone mass and the bone mass of their infant. The local Southampton and South West Hampshire Research Ethics Committee approved the study and all women gave written informed consent.

3.1.2 STATISTICAL METHODS

All data was double entered and analyses were performed using Stata intercooled vs 7. Data checking was performed using distribution plots and two-way scatter plots. Those variables with skewed distributions were log transformed. Univariate analysis of parental determinants of neonatal body composition was performed and the significant univariate predictors were used to generate a multiple linear regression models. Power calculations were based on the likely association of maternal skinfold thickness and maternal smoking on neonatal whole body bone mineral content. The models for pre-, early and late predictors were performed using the maximum number of individuals at each time point; repeating the analysis restricted to those with complete data did not alter the models.

3.1.3 NEONATAL DXA MEASUREMENTS

The neonates were all scanned on the same Lunar DPX-L instrument (**Appendix VIII**). The paediatric small scan mode was selected using scan widths of 280mm for total body and 90mm for the lumbar spine. To reduce artefact from scanning air, two rice bags were placed each side of the baby during the lumbar spine scan. The babies were fed with milk if required and, if necessary, a pacifier was used. They were swaddled in a cotton towel and placed unrestrained in the supine position on an incontinence sheet on the DXA table. After the baby had settled, the scan was commenced. If the baby moved during the scan, the scan was repeated once.

3.1.3.1 IMAGE ANALYSIS

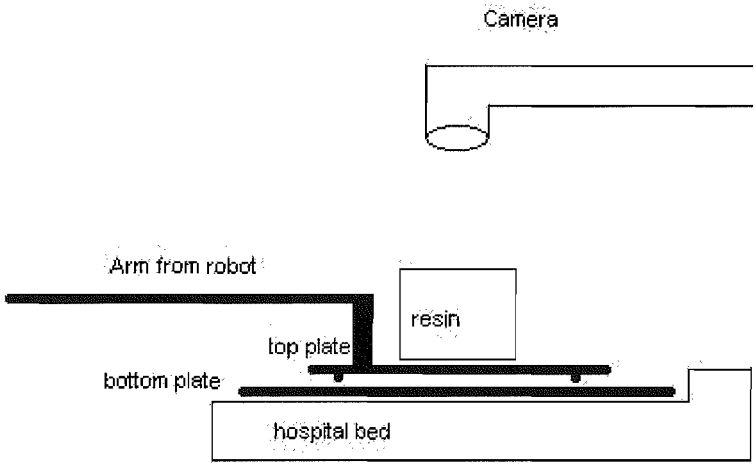
Using the manual analysis option, exclusion regions of interest were placed around areas of the scan image from extraneous material, such as the rice bags. Next the regional markers were manually adjusted on the whole body DXA image following manufacturers guidelines (Lunar reference 3.61), to delineate the head, neck, dorsal spine, lumbar spine, pelvis, ribs, arm and leg regions. The rib markers were however placed wider than recommended as the neonatal spines were often not

straight. Each whole body image was graded by site of movement (yes/no) at varies sites (head, torso, arms, legs) by the observer to generate movement scores for each image both by site and number of sites. Once all the whole body spine images had been adjusted, the same two operators together reviewed each image again; the images were further adjusted until both operators agreed.

3.1.3.2 DXA MOVEMENT ARTIFACT STUDY

Movement artefact was assessed using two methods. The overall effect of movement on DXA imaging was assessed with the aid of a gantry robot (Figure 6). The settings of the DXA instrument were set to total body scan type and paediatric small scan mode with a scan width of 300mm and a scan length of 250mm, reflecting the settings used to scan the neonates.

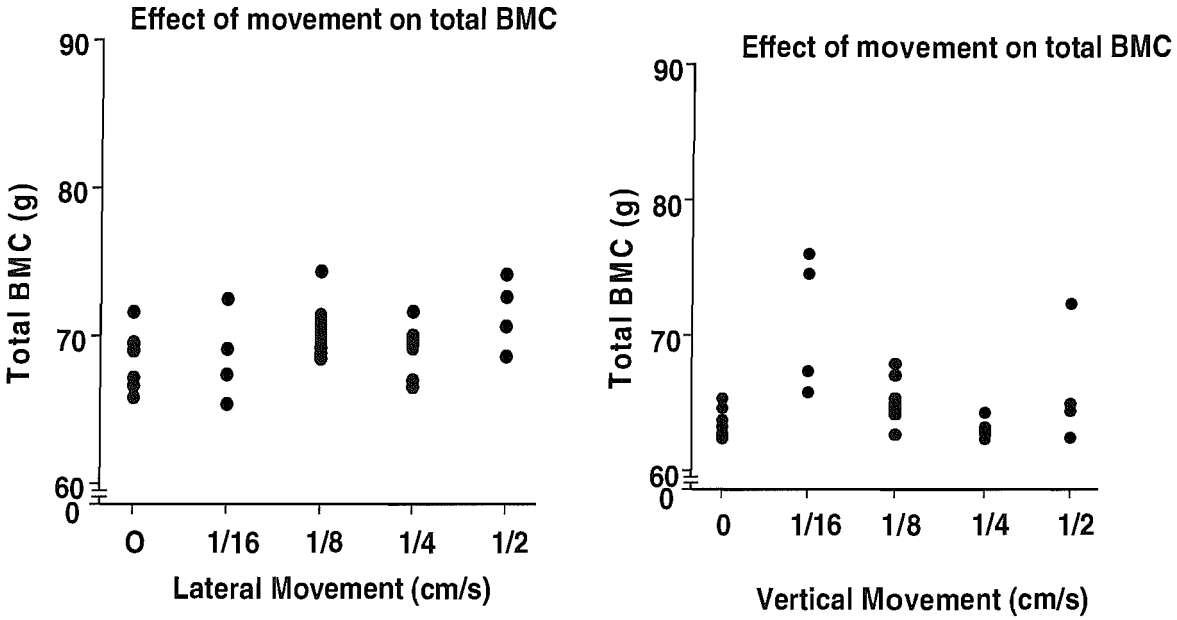
FIGURE 6 PHANTOM MOVEMENT STUDY USING A GANTRY ROBOT



A Hologic adult lumbar spine phantom was placed on a wooden platform mounted on steel bearings on the DXA table. This was mounted on another wooden board using bearings to reduce friction. The platform was connected to a gantry robot and the phantom was scanned at 4 four speeds (stationary, 0.0625 cm/s, 0.125 cm/s, 0.25 cm/s and 0.5 cm/s) with either lateral or vertical movement. In each axis the phantom was displaced by 2 cm and moved as a sine wave function. The onset movement of the robotic arm was set to coincide with start of movement of the DXA instrument's scanning arm.

The different types of movement were performed in a random order generated using the statistical package Stata v7 in blocks of 10. All the images were then analyzed to include the entire scan area and the total BMC, BA and BMD were recorded. The total BMC for each of the movements of the robotic arm at the different movement settings is shown in Figure 7

FIGURE 7 EFFECT OF MOVEMENT ON BMC MEASUREMENT OF HOLOGIC SPINE PHANTOM



3.1.3.3 NEONATAL MOVEMENT SCORE

The second method for assessing the effect of movement used the observed movement scores. As described above, each whole body scan was graded by site of movement (head, torso, arms, and legs) to generate movement scores for each image both by site and number of sites. The effect of these scores on DXA measurements was then investigated.

There was little difference in BMC measurements according to site of the movement. However, with increasing cumulative movement score there is a reduction in the values of the absolute estimates of BMC, lean mass and fat mass (Table 5). Different methods were then used to minimize this by adjusting for the measured length or weight. The weight used here is that derived from the

sum of the DXA measurements. From the table, it is evident that adjusting the DXA measurement to the mean measured DXA weight reduces the variability of the movement. The other methods of size adjustment using length increased the movement variability. For this reason movement score was added to the final regression models as a categorical variable.

TABLE 5 MEAN NEONATAL DXA Z-SCORE VALUES[1] BY CUMULATIVE MOVEMENT SCORE.

Movement score	0 N=73	1 N=117	2 N=100	3 N=41	4 N=32	P value[2]	R^2
Neonatal DXA							
BMC	0.19	0.10	0.03	-0.16	-0.34	0.01	2.0%
Bone area	0.19	-0.00	0.03	-0.13	-0.34	0.01	2.0%
BMD	0.12	0.05	-0.01	-0.2	-0.18	0.16	1.1%
BMC/kg	0.18	-0.02	0.08	-0.21	-0.31	0.02	2.3%
adjBMC/kg^3	0.12	-0.04	0.12	-0.23	-0.21	0.11	1.8%
BMC/cm	0.21	0.01	0.03	-0.18	-0.37	0.006	2.5%
adjBMC/cm^3	0.26	0.01	0.00	-0.13	-0.39	0.004	2.6%
Lean	0.11	-0.02	-0.01	0.03	-0.21	0.13	0.7%
Lean/kg	-0.17	-0.12	0.13	0.12	0.29	0.03	2.4%
adjLEAN/kg^3	-0.07	-0.15	0.12	0.16	0.13	0.35	1.6%
Lean/cm	0.14	-0.03	-0.02	0.06	-0.22	0.09	0.9%
adjLEAN/cm^3	0.12	-0.3	-0.05	0.18	-0.2	0.14	1.1%
Fat	0.18	0.09	-0.1	-0.6	-0.35	0.01	2.3%
Fat/kg	0.18	0.11	-0.11	-0.07	-0.35	0.01	2.5%
adjFAT/kg^3	0.06	0.15	-0.13	-0.15	-0.11	0.4	1.6%
Fat/cm	0.21	0.09	-0.11	-0.08	-0.36	0.008	2.7%
adjFAT/cm^3	0.18	0.13	-0.17	-0.06	-0.28	0.03	2.7%

[1]Mean z scores (internally derived) for each component of body composition both measured and derived are shown by cumulative movement score.

[2]P-values contrast values in movement score 0 vs 4 in a categorical linear bivariate model.

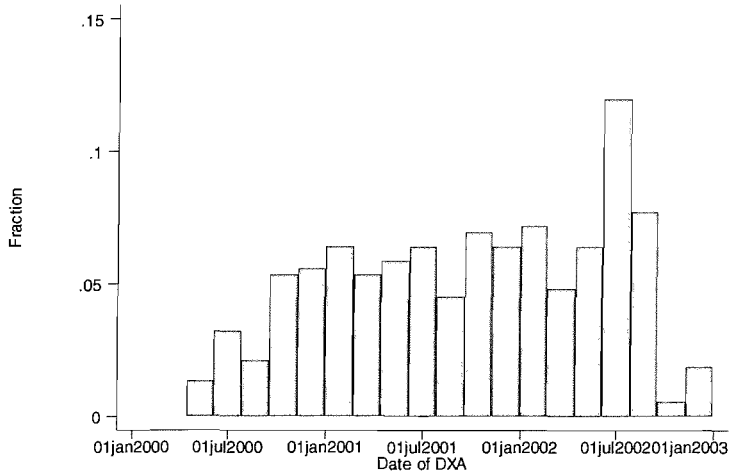
[3]DXA measure adjusted for either length or weight in bivariate regression model

3.2 RESULTS

3.2.1 DESCRIPTION OF THE NEONATES

198 male and 165 female neonates completed a whole body DXA scan and the timing of recruitment is shown in Figure 8 .

FIGURE 8 RECRUITMENT OF BABIES FOR DXA SCAN.



The gestational age of the neonates is shown in Figure 9 and the baseline characteristics are shown in Table 6 Neither gestational age or birth weight were significantly different between male and female neonates. Despite the lack of difference in overall birth weight, there were significant differences in body composition by gender. Male infants were slightly longer than females and had a greater head circumference. However, female infants had greater fat mass as measured by skinfold thickness (triceps, sub-scapular and thigh) and by DXA (Table 7). There was, however, no significant difference in mid-upper arm, chest or abdominal circumference by gender.

FIGURE 9 GESTATIONAL AGE AT BIRTH OF THE NEONATES IN THE DXA COMPONENT OF STUDY

TABLE 6 ANTHROPOMETRIC CHARACTERISTICS OF 363 NEONATES WHO HAD WHOLE BODY DXA ASSESSMENT WITHIN 16 DAYS AFTER BIRTH

Characteristic	Boys n=198	Girls n=165	P- value
	Mean (SD)	Mean (SD)	
Gestation age (weeks)	39.9 (1.5)	40.0 (1.6)	0.42
Birth weight (g)	3510 (521)	3460 (549)	0.46
Length			
Crown Rump (cm)	33.8 (1.7)	33.4 (1.8)	0.05
Crown Heel (cm)	50.2 (2.2)	49.7 (2.3)	0.07
Circumference			
Head (cm)	35.3 (1.4)	34.6 (1.3)	<0.001
Mid upper arm (cm)	11.4 (1.0)	11.5 (1.0)	0.33
Chest (cm)	33.4 (1.8)	33.4 (1.9)	0.97
Abdominal (cm)	31.5 (2.2)	31.7 (2.2)	0.40
Skin fold thickness			
Triceps (mm)	4.7 (0.9)	4.9 (1.0)	0.01
Sub-scapular (mm)	4.9 (1.0)	5.1 (1.0)	0.02
Thigh (mm)	6.4 (1.4)	6.9 (1.6)	<0.001

Legend: Unadjusted mean (SD) values shown for each of the body compartments measured by DXA.

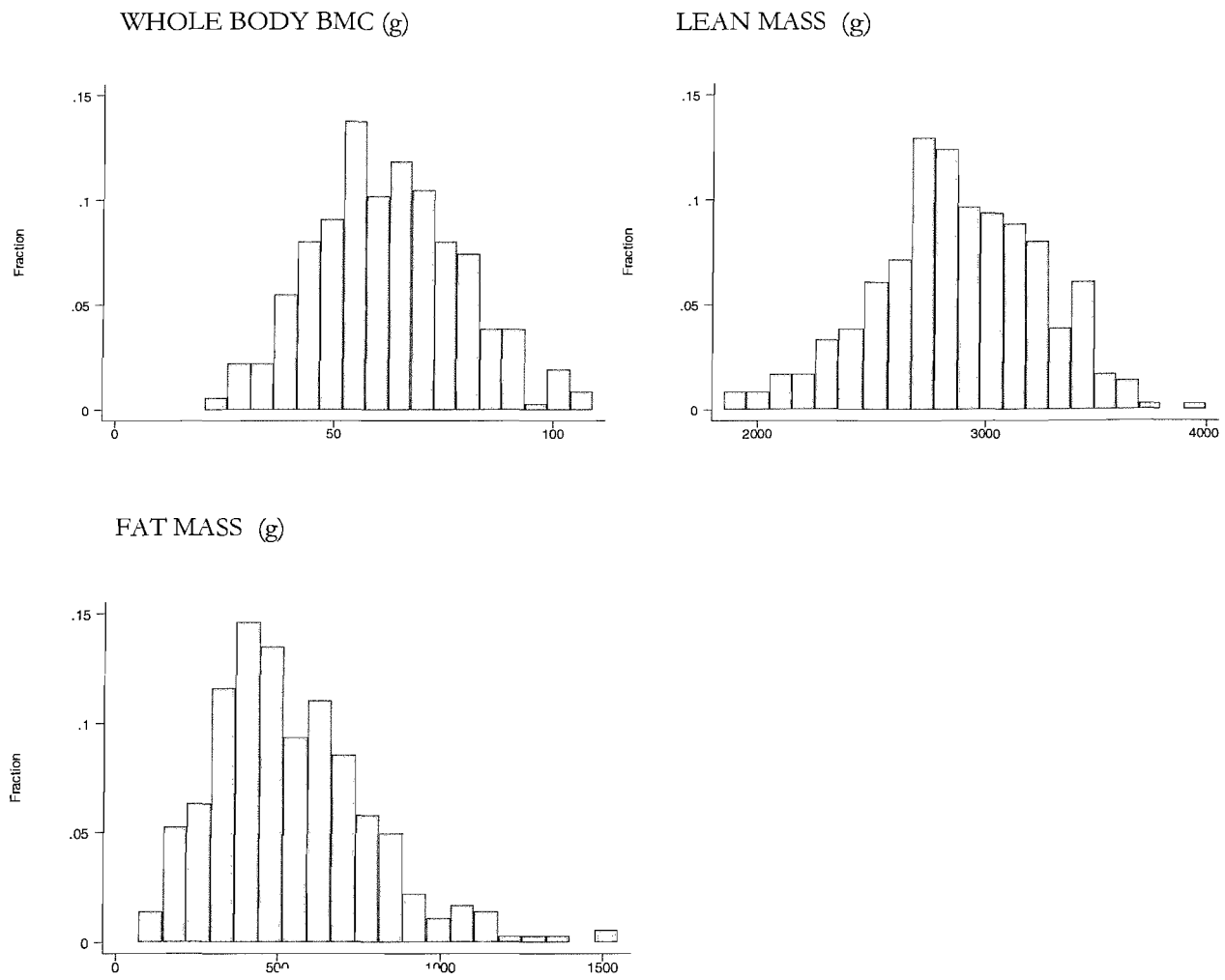
TABLE 7 BODY COMPOSITION AS MEASURED BY DXA IN 363 NEONATES WHO HAD WHOLE BODY DXA ASSESSMENT WITHIN 16 DAYS AFTER BIRTH.

Characteristic	Boys n=198	Girls n=165	P- value
	Mean (SD)	Mean (SD)	
Whole body composition			
WBBMC (g)	63.4 (17.4)	60.7 (16.0)	0.19
Bone Area (cm^2)	118.0 (28.3)	115.0 (27.4)	0.28
BMD (g/cm^2)	0.533 (0.028)	0.526 (0.027)	0.008
Lean mass (g)	2930 (363)	2820 (362)	0.006
Fat mass (g)[1]	518 (238.2)	572 (240.9)	0.01
Proportionate composition[2]			
BMC %	1.79% (0.3)	1.74% (0.3)	0.13
Lean %	84.0% (5.0)	82.2% (4.7)	<0.001
Fat %	14.2% (4.8)	16.1% (4.6)	<0.001

Legend: Mean (SD) shown for each body compartment (unadjusted) as measured by DXA.
[1]Geometric mean and SD
[2]Proportionate body composition derived using total weight as measured by DXA as denominator

The body composition measurements using DXA are shown in Table 7 and Figure 10 . The bone mineral compartment accounted for 1.77% of the total weight; while boys had a higher relative fraction of total body composition attributable to bone mineral than girls, this did not reach statistical significance. However at birth boys had a significantly higher proportion of lean mass and lower fat mass than girls.

FIGURE 10 DISTRIBUTION OF NEONATAL BODY COMPOSITION AS MEASURED BY DXA IN 363 NONATES



As shown in Table 8 there was a significant correlation between the absolute measurements of each body compartment, which were attenuated to a small degree by adjustment for gestational age.

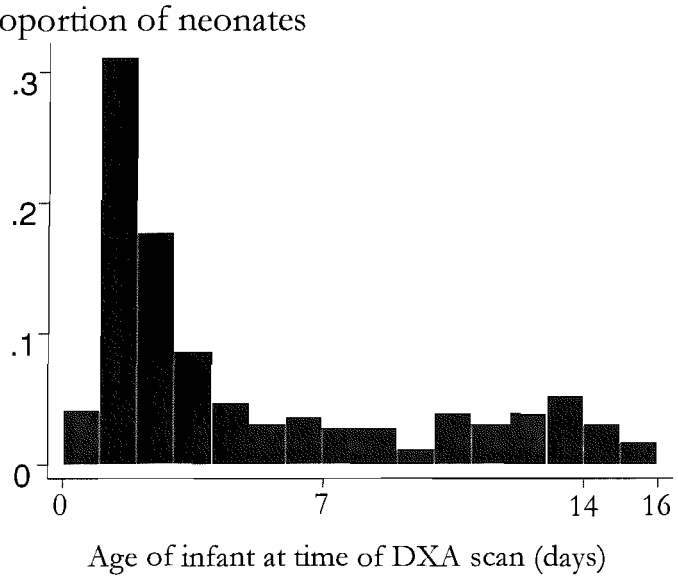
TABLE 8 RELATIONSHIP BETWEEN EACH NEONATAL BODY COMPARTMENT AS MEASURED BY DXA

R(p)	WBBMC		Lean Mass	
	unadjusted	adjusted	unadjusted	adjusted
Lean mass (unadjusted)	0.73 (<0.001)			
Lean mass (adjusted)		0.64 (<0.001)		
Fat mass[1] (unadjusted)	0.74 (<0.001)		0.64 (<0.001)	
Fat mass[1] (adjusted)		0.68 (<0.001)		0.55 (<0.001)

Legend: Pearson correlation coefficients between each body compartment unadjusted and adjusted for gestational age.
[1]Log transformed

The age of the neonates at the time of the DXA scan is shown in Figure 11 and 52.8% of the neonates were DXA scanned within 48 hours.

FIGURE 11 AGE OF NEONATES AT TIME OF DXA SCAN



As can be seen in Figure 12 in the first few postnatal days, there was a reduction in body weight as measured by DXA compared with birth weight with an increase in weight occurring about day 7. Of the body compartments, only lean mass was significantly associated with gestational age (Figure 13).

However, the effect size was small and as age at time of scan was not considered to bias the relationship between maternal factors and neonatal body composition, age of scan was not added to the models.

FIGURE 12 DIFFERENCE IN BIRTH WEIGHT AND WEIGHT DERIVED FROM DXA BY AGE OF NEONATE AT THE TIME OF SCAN

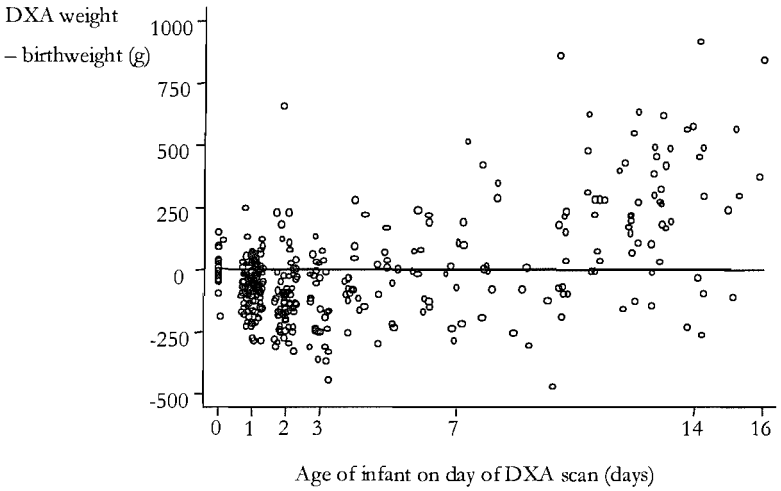
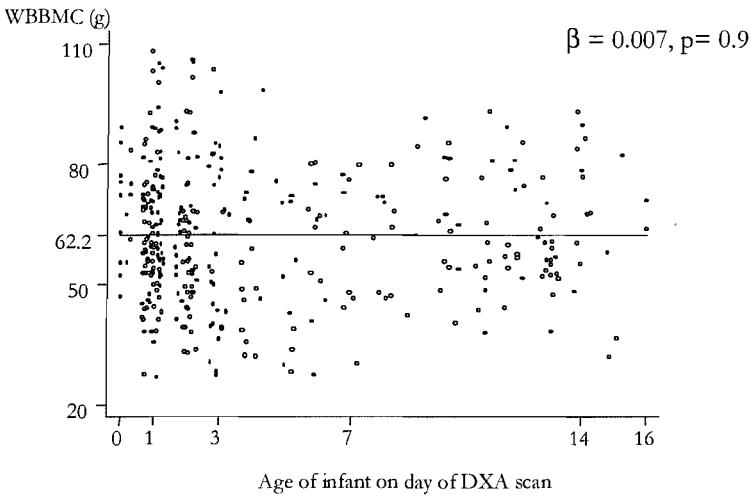
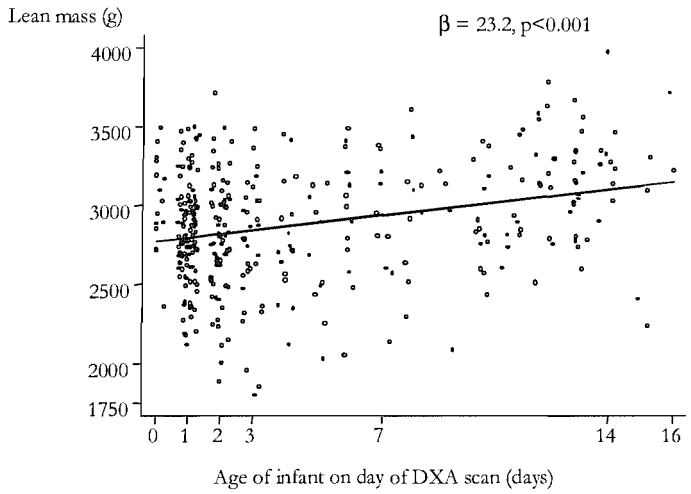


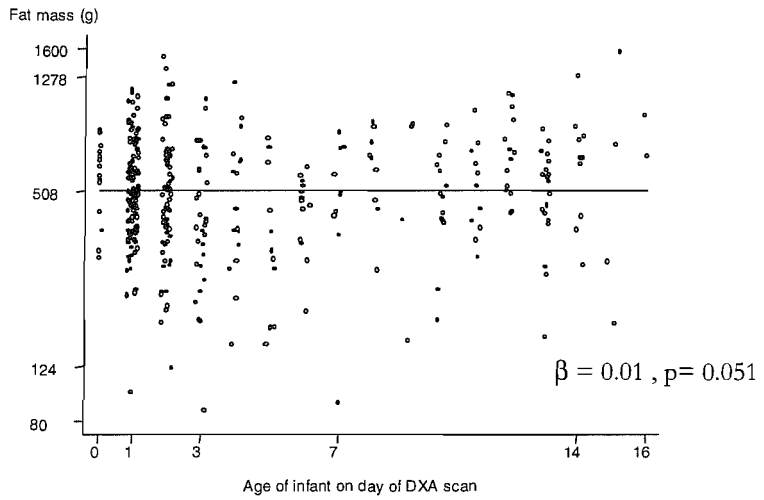
FIGURE 13 NEONATAL BODY COMPOSITION BY AGE AT TIME OF SCAN



Legend: Line shows mean WBBMC of all neonates



Legend: Line shows regression line for whole body lean mass and age on day of DXA scan



Legend: Line shows mean fat mass of all neonates

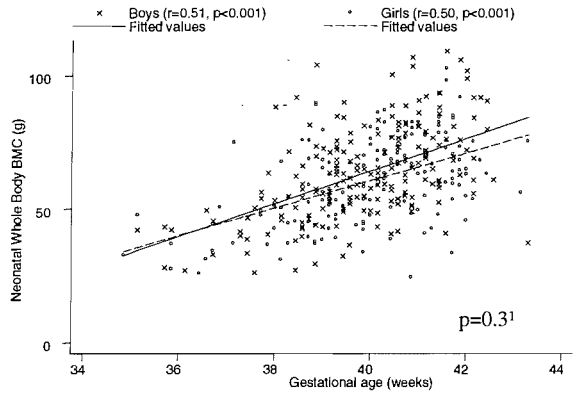
The mean gestational age was 39.9 weeks (SD 1.5) with no statistical difference by gender (Table 6). Birth size increased with increasing gestational age and the increase in birth size was evident in all body compartments. There was a disproportionate greater increase in fat mass and bone mass compared with lean mass. In females, there appeared to be greater proportionate fat accrual than lean mass compared to males (p=0.03).

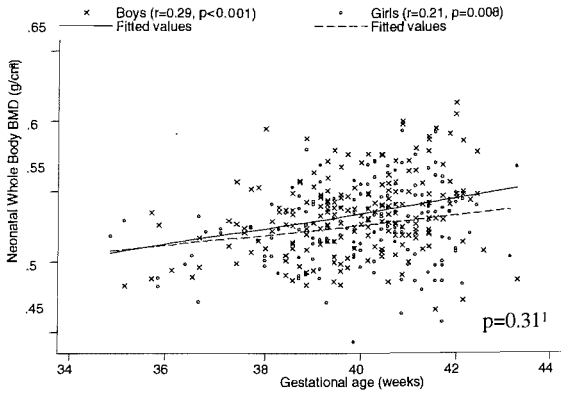
Neonatal whole body BMC and aBMD were significantly correlated with both crown heel length, birth weight and gestational age (Figure 14). However there were no significant differences by gender in the relationship between the measures of neonatal size and gestational age and DXA

derived estimation of whole body BMC. There was a statistically significant ($p=0.008$) difference in the slopes of whole body BMC against bone area in boys ($\beta=0.60$) compared with girls ($\beta=0.57$) (Figure 15) but as the coefficients were similar, subsequent analyses were performed on both sexes combined.

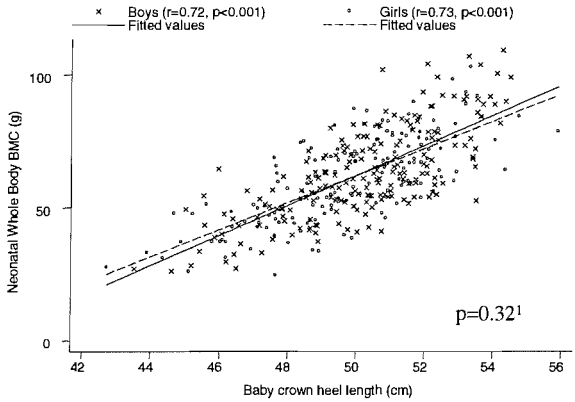
FIGURE 14 RELATIONSHIP BETWEEN NEONATAL WHOLE BODY BMC AND ABMD WITH NEONATAL GESTATIONAL AGE (A), LENGTH (B) AND WEIGHT (C)

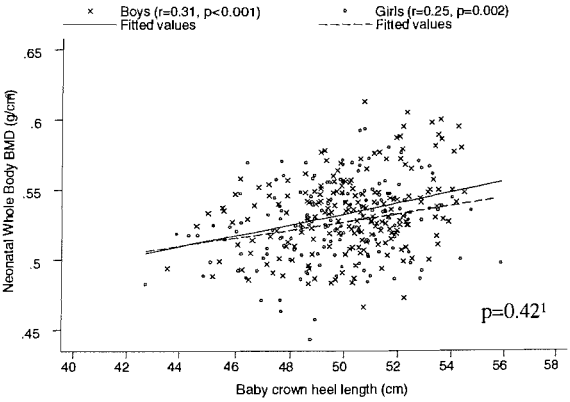
(A)



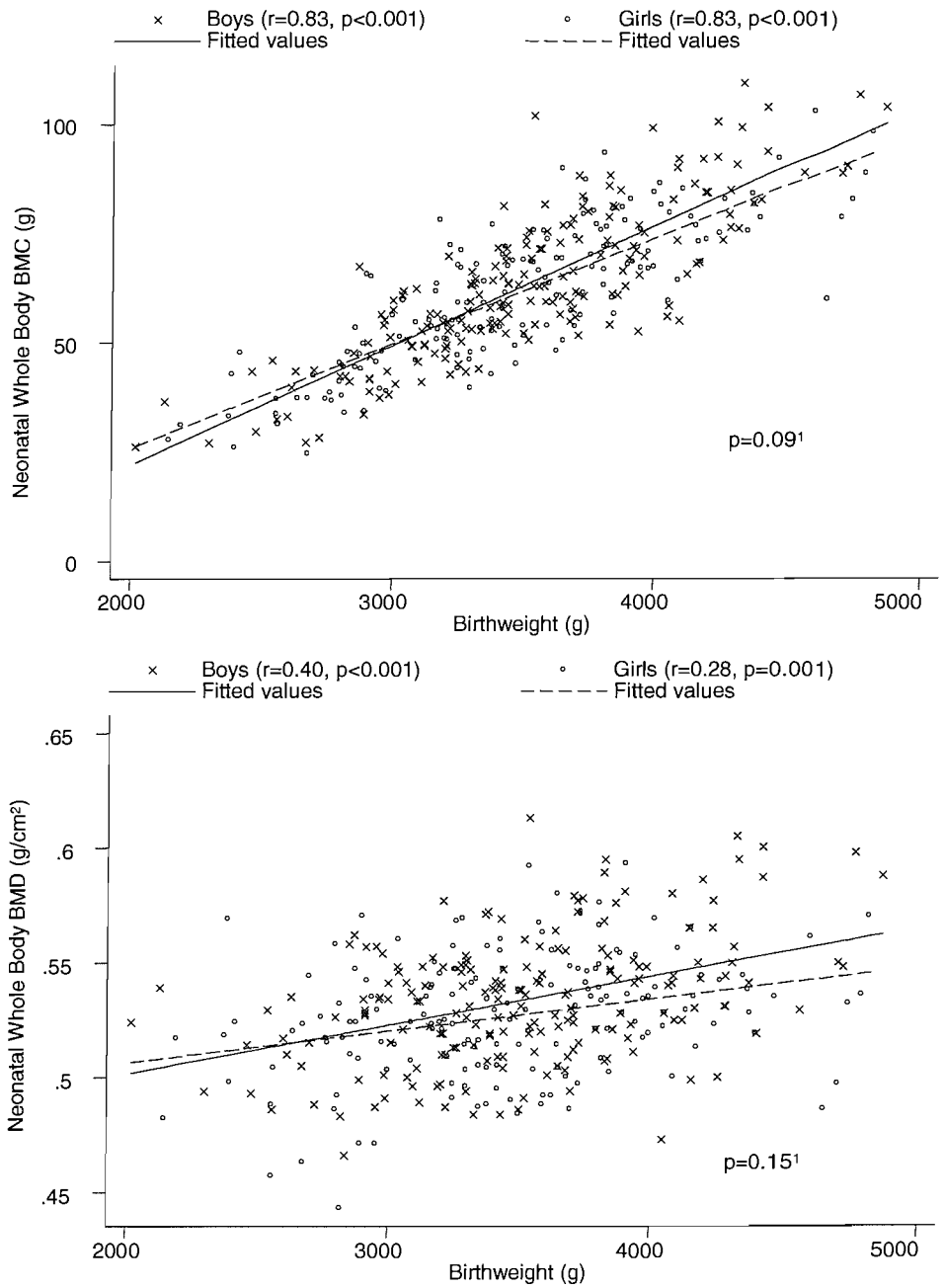


(B)



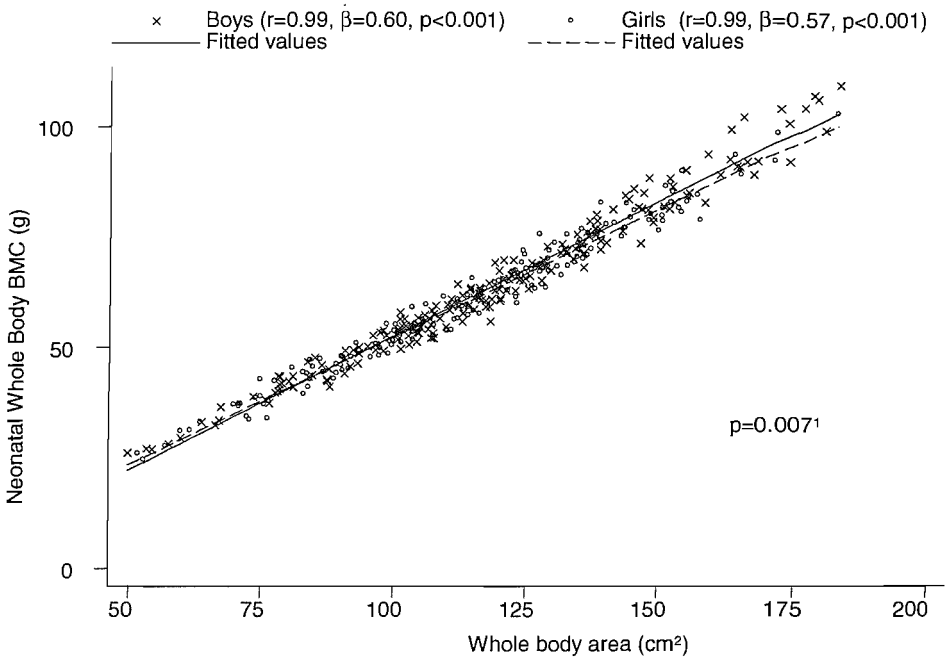


(c)



Legend: Scatter plot illustrates relationship between gestational age, birth length and birth weight with whole body BMC and aBMD in boys and girls separately.
Pearson's correlation, r, with significance p for each gender.
[1]Significance value, p, shown for the difference in β by gender as derived using students T test.

FIGURE 15 RELATIONSHIP BETWEEN WHOLE BODY BMC AND AREA



Legend: Scatter plot illustrates relationship between whole body BMC and bone area boys and girls separately. Pearson's correlation, r, with significance p and β for slope shown for each gender.

[1]Significance value, p, shown for the difference in β by gender as derived using students T test.

3.2.3 DESCRIPTION OF MATERNAL COHORT

From the 363 completed neonatal DXA images, 346 had complete pre-pregnancy measurements, 279 early pregnancy measurements and 336 late pregnancy measurements.

The descriptive characteristics of the study cohort are shown in Table 9 . We had limited data on non-responders; there was no significant difference in age, height or weight in pre-pregnancy comparing those in the bone component and those in the rest of the survey.

TABLE 9 SOCIO-DEMOGRAPHIC, LIFESTYLE AND BODY COMPOSITION CHARACTERISTICS OF MOTHERS WHOSE OFFSPRING UNDERWENT NEONATAL DXA SCAN AS RECORDED PRE-PREGNANCY, EARLY PREGNANCY AND LATE PREGNANCY

Maternal characteristic	Pre-pregnancy n=346	Early pregnancy n=279	Late pregnancy n=336
	Mean (SD)	Mean (SD)	Mean (SD)
Age (years)	28.5(3.8)	29.8 (3.7)	30.0 (3.8)
Qualification[3]:			
<2	9.8%	-	-
-4	54.2%	-	-
-6	36.0%	-	-
Employed (%):			
Not working (%)	20.8%	19.6%	47.6%
Part time (%)	29.2%	31.9%	22.6%
Full time (%)	50.0%	48.5%	29.8%
Parity (%):			
0	47.8	-	-
1	37.7	-	-
>1	14.5	-	-
Birth weight (kg)	3.2 (0.55)	-	-

Smoking (%):			
Never	55.5%	-	-
Ex smoker	16.5%	24%[2]	-
Current	28.0%	14.0%	14.0%
Alcohol (units/wk):			
0	8.4%	24.8%	28.3%
-3	27.0%	60.8%	61.0%
-14	47.7%	11.5%	10.4%
>14	16.9%	2.9%	0.3%
Vigorous activity (%)	61.2%	40.3%	27.7%
Walking speed[4]:			
1-2	7.5%	12.6%	64.6%
3	38.7%	50.7%	28.9%
4	48.8%	32.0%	6.6%
5	4.9%	4.7%	0.0%
Milk (%) (pints per day):			
0	4.3%	26.2%	10.5%
-0.25	21.3%	3.7%	16.0%
-0.5	35.0%	25.2%	22.6%
-1.0	35.0%	36.0%	37.5%
>1.0	5.5%	9.0%	13.5%
% Food supplements	46.8%	96.8%	48.2%

Mean values (and standard deviation [SD]) are shown. [1]Median values (and inter-quartile range [IQR]) [2]smoked at LMP. [3]Qualifications: 1-none, 2-CSE/GCSE D-F, 3-Olevel/GCSE A-C, 4-Alevel/City and Guilds, 5- HND/RGN, 6 Degree/NVQ5. [4]Walking speed:1- very slow; 2-stroll at easy pace, 3-normal, 4-fairly brisk, 5-fast.

TABLE 10 MATERNAL ANTHROPOMETRY PRE-, EARLY AND LATE PREGNANCY IN THOSE MOTHERS WHOSE OFFSPRING UNDERWENT NEONATAL DXA SCANNING.

Maternal characteristic	Pre-pregnancy n=346	Early pregnancy n=279	Late pregnancy n=336
	Mean (SD)	Mean (SD)	Mean (SD)
Height (m)	1.63 (0.06)	-	-
Leg length (cm)	98.2 (4.8)	-	-
Head circumference (cm)	-	55.0 (1.5)	-
Weight (kg)[1]	64.5 (58.0, 73.4)	66.5 (59.2, 75.3)	77.6 (69.9, 87.8)
BMI (kg/m^2)[1]	24.0 (22.1, 27.6)	24.6 (22.4, 28.5)	28.9 (26.5, 32.6)
Skin fold thickness:			
Triceps (mm)[1]	19.1 (15.3-24.4)	19.1 (15.0, 25.0)	20.6 (16.2, 25.9.)
Biceps (mm)[1]	9.9 (6.6, 14.8)	9.5 (7.0, 13.8)	11.2 (7.6, 14.8)
Sub-scapular (mm)[1]	16.2 (11.8, 24.9)	16.8 (11.9, 25.9)	20.6 (15.5, 30.0)
Supra-iliac (mm)[1]	19.7 (13.7, 28.0)	21.3 (15.4, 29.0)	26.9 (20.7, 34.3)
Circumference:			
Mid upper arm (cm)[1]	28.2 (26.2-31.3)	28.6 (26.3, 31.7)	29.3 (27.4, 32.3)
Waist (cm)[1]	77.5 (72.5, 85.4)	81.8 (76.2, 90.7)	-
Hip (cm)[1]	101.3 (96.5, 108.4)	102.7 (98.0, 109.5)	-
Thigh (cm)[1]	54.3 (50.5, 58.8)	54.8 (51.4, 59.5)	57.2 (54.0, 62.0)
Calf (cm)[1]	36.0 (34.3, 38.4)	36.2 (34.5, 39.0)	38.0 (36.0, 40.6)
Heel Width (cm)			
Inter-malleolar	-	6.3 (0.4)	6.4 (0.4)
Soft tissue	-	5.5 (0.5)	5.7 (0.5)

Legend: Mean values (SD) are shown.
[1]Median values (and inter-quartile range [IQR])

Of the skinfold thickness measurements, triceps measurements had the lowest CV (2.8%) compared with measurements at the biceps (CV 4.9%), sub-scapular (CV 3.4%) and supra-iliac (CV 4.4%) sites. Durnin and Womersly have suggested using the sum of skin folds to estimate total body fat mass. These methods are not validated in during pregnancy and it became clear

that the much larger absolute differences in the truncal skin thicknesses compared to triceps and biceps skin fold thickness would dominate the estimates of fat mass. For this reason triceps skinfold thickness was the measure of adiposity used in the models of neonatal bone mass.

The median duration from the initial pre-pregnancy interview to the early pregnancy interview was 13.7 months (range 9 to 187 weeks). During this interval, there was a significant increase in the body size of the women (Table 11 . The observed increase in maternal weight, BMI and waist circumference was significantly associated with the interval between pre-pregnancy and early pregnancy interview, suggesting an increase in adiposity with age. Weight increased by 15g per week ($p=0.02$); BMI increased by 0.006 kg/m^2 per wk ($p=0.02$) and the circumference around the waist increased by 0.3 mm/wk ($p<0.001$).

As with other maternal measurements, the skinfold measurements in pre-pregnancy and early pregnancy were highly collinear (triceps $\beta=0.94$, $p<0.001$, $R^2=72\%$). However there was no significant relationship between the interval between pre-pregnancy and early pregnancy with any skinfold thickness [triceps ($p=0.14$)] or circumferences [mid upper arm ($p=0.14$); calf ($p=0.77$); thigh ($p=0.98$)]. This suggests that these measures of adiposity are relatively stable before pregnancy. As expected maternal body size increased through pregnancy (Table 11).

TABLE 11 INCREMENT IN MATERNAL ANTHROPOMETRY BETWEEN PRE-, EARLY AND LATE PREGNANCY MEASUREMENTS

	Pre to early pregnancy		Early to late pregnancy	
Maternal measurement	%Increment	p	%Increment	p
Weight (kg)	2.0% (-1.7, 5.5)	<0.001	16.8% (12, 21))	<0.001
BMI (kg/m^2)	2.0% (-1.7, 5.5)	<0.001	16.8% (12, 21)	<0.001
Skin fold thickness:				
Triceps (mm)	2.5% (-12, 15)	0.05	9.2% (-4, 19)	<0.001
Biceps (mm)	0.8%(-19, 15)	0.70	18.2% (-5, 36)	<0.001
Sub-scapular (mm)	4.9% (-14, 32)	<0.001	27.9% (4.2, 46))	<0.001
Supra-iliac (mm)	13.6% (-12, 19)	<0.001	39.1% (1.6, 63)	<0.001
Circumference:				
Mid upper arm (cm)	0.9% (-2.8, 4.6)	0.008	3.0% (0, 6.2)	<0.001
Waist (cm)	5.0% (0.4, 8.8)	<0.001	-	-
Hip (cm)	1.1% (-1.5, 2.6)	<0.001	-	-
Thigh (cm)	1.2% (-2.5, 5.1)	<0.001	4.7% (0.9, 8.2))	<0.001
Calf (cm)	0.5% (-1.7, 2.6)	0.04	4.4% (2.2, 6.5)	<0.001
Heel width				
Inter-malleolar	-	-	2.1% (-1.5, 4.7)	<0.001
Soft Tissue	-	-	4.6% (0, 9.5))	<0.001

Legend: Mean (IQR) are shown. P-values contrasts measurement at different stages of pregnancy (pre-pregnancy vs. early pregnancy; early pregnancy vs. late pregnancy).

3.2.3.1 MATERNAL SMOKING

Before pregnancy 28.0% of women reported smoking regularly, 16.5% of woman was ex-smokers and 55.5% had never smoked. Approximately half of those smoking before pregnancy had stopped by 11 weeks of pregnancy; most of them stopping after becoming pregnancy (Table 12). 20% of those smoking in early pregnancy stopped by 34 weeks. There was a reduction in the median number of cigarettes smoked per day by smokers during pregnancy from 10 in pre-pregnancy to 7 in early pregnancy and 8 in late pregnancy. Maternal social class was available in a subset of the sample and, as expected, maternal smoking was more prevalent in the lower social classes, with the inter class difference becoming more distinct through pregnancy (Table 13). While there was no significant difference in maternal height, weight or BMI by smoking status, women who smoked had smaller triceps skinfold thickness. This was statistically significant at pre-pregnancy (-0.23 SD, p=0.05) and late pregnancy (-0.3 SD, p=0.05) but not at early pregnancy (-0.25SD, p=0.13). Ex-smokers had similar triceps skinfold thickness to non-smokers.

TABLE 12 FREQUENCY OF MATERNAL SMOKING THROUGH PREGNANCY

Smoking Status		n	EARLY		LATE	
			NO	YES	NO	YES
PREPREGNANCY	NO	192	100%	0%	100%	0%
	EX	37	97.8%	2.2%	100%	0%
	YES	97	46.0%	54.0%	52.4%	47.6%
EARLY	NO	240			99.1%	0.9%
	YES	39			20%	80%

TABLE 13 MATERNAL SMOKING BY MATERNAL SOCIAL CLASS

Maternal	Pre-pregnancy		Early pregnancy		Late pregnancy	
Social Class:	n	Smoking %	n	Smoking %	n	Smoking %
I. Professional	19	16.7%	12	0%	19	0%
II. Management	123	58.5%	105	4.8%	118	7.6%
IIIn. Skilled non manual	104	65.3%	81	21.0%	94	18%
IIIm. Skilled manual	20	60.0%	14	21.4%	18	22%
IV. Partly skilled	24	93.3%	18	50.0%	22	36%
V. Unskilled	5	50.0%	2	0%	4	0%
Total	295		232		275	
P		0.03		<0.001		0.002

3.2.3.2 MATERNAL ALCOHOL CONSUMPTION DURING PREGNANCY

Approximately 17% of women reported drinking more than 14 units of alcohol per week before pregnancy. One quarter of women who drank before pregnancy stopped when they became pregnant and of the remainder 60% of women continued to consume small amounts of alcohol (< 3 units/week). There was a significant positive association between increasing alcohol intake and current smoking both in early (p=0.004) and late (p=0.001) pregnancy but not pre-pregnancy (p=0.37). One woman consistently consumed more than 50 units a week before and during pregnancy and subsequent analyses of alcohol intake were performed with and without this participant as a sensitivity analysis.

3.2.3.3 MATERNAL EMPLOYMENT STATUS AND PARITY

50% of women reported working more than 30 hours per week at the pre-pregnancy interview, with 29% working on a part-time basis. During pregnancy, there was little change in employment status at 11 weeks but by 34 weeks the number of women not working had risen from 20% to 48% with 30% still continuing full time employment.

48.7% of the women were nulliparous at the pre-pregnancy assessment and maternal parity was strongly associated with maternal employment, such that nulliparous women were more likely (86% vs. 16%) to be in full time employment compared with those with one or more children (Table 15). By 34 weeks, while 79% of women with more than one child had stopped working, only 38% of those with no children had stopped working. Full time employment increased with educational status and this relationship was present through pregnancy (Table 14).

TABLE 14 FULL TIME EMPLOYMENT STATUS BY EDUCATIONAL LEVEL

	Pre-pregnancy			Early pregnancy	Late pregnancy
Educational level Equivalent	N	% Full time	n	% Full time	% Full time
Nil	8	0%	1	0%	0%
CSE	26	27%	17	29%	12%
O'level	98	48%	66	53%	32%
A'level	89	49%	66	48%	29%
HND	34	56%	28	61%	32%
Degree	77	61%	60	60%	43%

TABLE 15 MATERNAL EMPLOYMENT BY PARITY

Employment	Parity		
	0	1	>1
Pre pregnancy			
N	165	130	50
None	5.5%	26.2%	58%
Part time	9.1%	55.4%	26%
Full time	85.5%	18.5%	16%
Early pregnancy			
N	133	88	28
None	4.5%	21.6%	60.7%
Part time	10.5%	61.3%	28.6%
Full time	85.0%	17.1%	10.7%
Late pregnancy			
N	133	88	28
None	37.6%	48.9%	78.6%
Part time	9.0%	38.6%	17.9%
Full time	53.4%	12.5%	3.6%

3.2.3.4 MATERNAL PHYSICAL ACTIVITY

Maternal physical activity was measured in two ways: walking speed and frequency of strenuous, moderate and gentle exertion. The relationship between these two measures of physical activity through pregnancy is shown in Table 16 . There was little difference in reported strenuous activity across the different walking speeds by late pregnancy.

TABLE 16 THE RELATIONSHIP BETWEEN MATERNAL WALKING SPEED AND STRENUOUS PHYSICAL ACTIVITY THROUGH PREGNANCY.

Stage Pregnancy		Pre-		Early		Late
Walking speed	n	Strenuous (%)	n	Strenuous (%)	n	Strenuous (%)
Very slow	2	0%	1	0%	52	23%
Stroll	24	58%	34	15%	165	30%
Normal	132	55%	141	44%	97	24%
Fairly brisk	168	66%	90	41%	22	36%
Fast	17	71%	13	62%	0	-
P						0.87

p relates to K-Wallis test of walking speed by strenuous activity in three groups.

3.2.4 MATERNAL PREDICTORS OF NEONATAL BODY COMPOSITION

The significant parental univariate predictors of neonatal WBBMC in pre-pregnancy, early and late pregnancy will now be described.

3.2.4.1 PRE-PREGNANCY

TABLE 17 PRE-PREGNANCY MATERNAL PREDICTORS OF NEONATAL BONE MASS

Maternal characteristic		WBBMC (g)	Lean Mass (g)	Fat Mass (g)	Birth weight (g)	Length (cm)
	n	Mean	Mean	Mean	Mean	Mean
Height (m)						
<1.60	106	59.3	2771	465	3355	49.4
-1.66	131	62.2	2901	488	3474	50.0
>1.66	108	64.2	2947	538	3601	50.5
r, p		0.12, 0.03	0.21, <0.001	0.15, 0.007	0.21, <0.001	0.28, <0.001
Weight (kg)						
<60	112	58.4	2848	458	3362	49.5
-69	111	61.1	2845	485	3440	49.7
>69	120	66.4	2935	550	3624	50.6
r, p		0.25, <0.001	0.16, 0.003	0.24, <0.001	0.30, <0.001	0.27, <0.001
BMI (kg/m^2)						
<22.6	113	59.4	2877	479	3421	49.8
-26	113	59.3	2823	452	3378	49.6
>26	117	67.2	2930	565	3630	50.4
r, p		0.23, <0.001	0.09, 0.11	0.20, <0.001	0.23, <0.001	0.18. <0.001
MUAC (cm)						
<27	114	59.0	2866	461	3369	49.7
-30	124	60.6	2838	474	3435	49.7
>30	106	66.8	2934	564	3647	50.5
r, p		0.26, <0.001	0.12, 0.02	0.23, <0.001	0.28, <0.001	0.20, <0.001
Triceps skinfold (mm)						
<16	107	59.5	2865	458	3386	49.7
-22	119	61.0	2836	495	3450	49.7

>22	118	65.2	2929	533	3592	50.4
r, p		0.15, 0.004	0.07, 0.17	0.16, 0.003	0.20, <0.001	0.18, 0.001
Birth weight (kg)						
<3.0	74	59.4	2803	449	3337	49.4
-3.4	84	60.8	2881	491	3448	49.7
>3.4	86	66.8	2943	552	3621	50.5
r, p		0.17, 0.007	0.16, 0.01	0.20, 0.002	0.25, <0.001	0.20, 0.002
Parity						
0	165	59.5	2779	446	3365	49.6
1	130	64.1	2955	535	3561	50.3
>1	50	64.0	2988	569	3618	50.2
P[1]		0.01	<0.001	<0.001	<0.001	0.01
Smoking						
Never	192	62.3	2886	498	3509	50.1
Ex	57	61.2	2878	498	3497	50.4
Yes	97	61.6	2853	478	3398	49.6
P[1]		0.85	0.73	0.61	0.15	0.05
Walking Speed						
Slow	26	63.5	2862	495	3500	50.2
Normal	134	62.6	2902	512	3516	50.0
Fairly Brisk	186	61.2	2858	484	3444	49.9
r, p		-0.05, 0.32	-0.04, 0.47	-0.06, 0.31	-0.04, 0.42	-0.03, 0.63
Employment						
No		65.1	2979	555	3604	50.1
Part-time		63.7	2957	531	3557	50.4
Full-time		59.5	2785	454	3375	49.7
P		<0.001	<0.001	<0.001	<0.001	0.009

Legend: Mean values are shown with Pearson r and p value. [1] ANOVA

TABLE 18 PRE-PREGNANCY MATERNAL ANTHROPOMETRIC AND LIFESTYLE DETERMINANTS OF PROPORTIONATE BODY COMPOSITION.

Maternal characteristic		Proportionate WBBMC	Proportionate Lean Mass	Proportionate Fat Mass
	n			
Height (m)				
<1.60	106	1.76%	83.7%	14.6%
-1.66	131	1.76%	83.7%	14.6%
>1.66	108	1.77%	82.4%	15.8%
R, p		-0.01, 0.84	-0.10, 0.08	0.10, 0.07
Weight (kg)				
<60	112	1.70%	84.1%	14.2%
-69	111	1.76%	83.4%	14.8%
>69	120	1.83%	82.3%	15.9%
R, p		0.17, 0.002	-0.22, <0.001	0.21, <0.001
BMI (kg/m^2)				
<22.6	113	1.71%	83.7%	14.5%
-26	113	1.73%	84.1%	14.2%
>26	117	1.85%	81.9%	16.2%
R, p		0.19, <0.001	-0.20, <0.001	0.19, <0.001
MUAC (cm)				
<27	114	1.71%	84.1%	14.2%
-30	124	1.76%	83.6%	14.6%
>30	106	1.84%	82.0%	16.2%
R, p		0.21, <0.001	-0.22, <0.001	0.21, <0.001
Triceps skinfold (mm)				
<16	107	1.72%	84.2%	14.1%
-22	119	1.76%	83.1%	15.2%
>22	116	1.81%	82.7%	15.5%
R, p		0.11, 0.05	-0.15, 0.004	0.15, 0.005
Birth weight (kg)				
<3.0	74	1.75%	84.1%	14.1%
-3.4	82	1.74%	83.5%	14.8%
>3.4	85	1.84%	82.3%	15.9%
R, p		0.08, 0.23	-0.17, 0.006	0.17, 0.006

Parity				
0	165	1.76%	84.1%	14.2%
1	130	1.77%	82.7%	15.5%
>1	50	1.74%	82.3%	15.9%
P		0.82	0.009	0.007
Smoking				
Never	192	1.76%	83.2%	15.0%
Ex	57	1.75%	82.9%	15.3%
Yes	97	1.77%	83.6%	14.6%
P		0.87	0.64	0.62
Walking Speed				
Slow	26	1.80%	83.3%	14.9%
Normal	134	1.76%	83.0%	15.2%
Fairly Brisk	186	1.76%	83.4%	14.8%
r, p		-0.01, 0.74	0.03, 0.55	-0.03, 0.55
Employment				
No	72	1.78%	82.5%	15.7%
Part time	100	1.76%	82.8%	15.5%
Full time	171	1.76%	83.9%	14.3%
P		0.85	0.03	0.03

Legend: Mean values are shown with Pearson r and p value. [1] ANOVA

As expected, maternal anthropometry had significant effects on neonatal body composition. Increasing maternal height was associated with an increase in all measures of neonatal body size (weight, length, WBBMC, lean and fat mass). However this increase in neonatal size was due to a disproportionate increase in fat mass as compared with lean mass with little effect on the proportion of WBBMC. Like maternal height, maternal pre-pregnancy weight predicted neonatal birth weight, length, WBBMC, lean and fat mass. In contrast, however, both fat and bone mass were disproportionately increased with a lower proportion of lean mass.

The birth weight of the mother was also positively related to birth size, but in contrast to measures of the maternal height and adiposity, there was a positive effect on both proportional WBBMC and lean mass, and a non-significant negative effect on proportionate fat mass. Change in maternal anthropometry from the pre-pregnancy, early and late pregnancy assessments did not predict neonatal absolute or percentage body composition in univariate models.

Of the other maternal factors recorded in pre-pregnancy, parity, but not maternal age, was significantly associated with increased birth size. Parity increased proportionate fat and reduced proportionate lean but did not change in proportionate BMC. Women who did not work had a similar pattern on increased neonatal size with a greater proportionate fat and lower proportionate lean but no change in proportionate BMC. Those mothers who did not work were more likely to be multiparous ($p<0.001$) and hence working status during pregnancy seemed to be behaving as a surrogate for parity.

3.2.4.2 EARLY PREGNANCY

As with the pre-pregnancy maternal characteristics, measures of maternal adiposity (maternal weight, BMI, MUAC and triceps skin fold thickness) in early pregnancy were significantly associated with increased birth size, proportionate fat and WBBMC and lower proportionate lean mass (Table 19). With the exception that neonatal lean mass was not significantly predicted by BMI or triceps skinfold thickness and proportionate BMC was not predicted by triceps skinfold thickness.

Smoking during early pregnancy was associated with significantly shorter babies with a non-significant trend to lower BMC, lean and fat. There was no difference in proportionate body composition. Employment during early pregnancy had a weaker effect than pre-pregnancy employment and non-working mothers had bigger babies without significant differences in the proportionate body composition. While there was no association between walking speed as recorded in pre-pregnancy and neonatal composition, mothers reporting faster walking speed in early pregnancy had a non-significant increase in lean and reduction in fat but no effect on birth weight, length or BMC. In addition, faster walking speed was significantly associated with increased proportionate lean and reduced proportionate fat.

TABLE 19 EARLY PREGNANCY MATERNAL ANTHROPOMETRIC AND LIFESTYLE DETERMINANTS OF NEONATAL BODY COMPOSITION

Maternal Characteristic		WBBMC (g)	Lean Mass (g)	Fat Mass (g)	Birth weight (g)	Length (cm)
	N	Mean	Mean	Mean	Mean	Mean
Weight (kg)						
<61	88	58.2	2872	456	3408	49.6
-72	93	65.3	2883	523	3535	50.1
>72	97	64.5	2905	537	3609	50.6
r, p		0.23, <0.001	0.15, 0.01	0.26, <0.001	0.32, <0.001	0.32, <0.001
BMI (kg/m^2)						
<23	81	60.5	2918	486	3481	50.0
-27	93	61.1	2810	468	3408	49.7
>27	90	65.9	2927	554	3640	50.6
r, p		0.21, <0.001	0.07, 0.24	0.22, <0.001	0.25, <0.001	0.19, 0.002
MUAC (cm)						
<27	91	59.6	2887	479	3441	49.8
-30	91	62.1	2835	471	3438	49.7
>30	94	66.5	2939	569	3683	50.8
r, p		0.23, <0.001	0.10, 0.09	0.24, <0.001	0.28, <0.001	0.24, <0.001
Triceps skinfold (mm)						
<16.4	91	62.2	2922	477	3482	50.1
-22	94	61.3	2826	484	3449	49.6
>22	94	64.8	2916	559	3632	50.6
r, p		0.15, 0.02	0.04, 0.45	0.19, 0.002	0.20, 0.001	0.20, <0.001
Smoking						
No	240	62.9	2896	512	3541	50.2
Yes	39	62.0	2834	466	3390	49.5
P		0.71	0.25	0.18	0.06	0.03
Walking Speed						
Slow	35	60.9	2809	556	3501	49.9
Normal	141	63.2	2883	504	3528	50.0
Fairly Brisk	103	62.8	2921	491	3519	50.3
r, p		0.02, 0.73	0.09, 0.13	-0.09, 0.14	0.0, 0.9	0.07, 0.22
Employment						
No	66	64.6	2957	551	3607	50.1
Part time	107	64.7	2965	537	3579	50.4
Full time	163	58.9	2786	452	3372	49.7
P		<0.001	<0.001	<0.001	<0.001	0.005

Legend: Mean values are shown with Pearson r and p value. [1] ANOVA

TABLE 20 EARLY PREGNANCY MATERNAL ANTHROPOMETRIC AND LIFESTYLE DETERMINANTS OF PROPORTIONATE NEONATAL BODY COMPOSITION

Maternal characteristic	n	Proportionate WBBMC	Proportionate Lean Mass	Proportionate Fat Mass
Weight (kg)				
<61	88	1.69%	84.3%	14.0%
-72	93	1.85%	82.6%	15.5%
>72	97	1.80%	82.3%	15.9%
R, p		0.13, 0.04	-0.27, <0.001	0.26, <0.001
BMI (kg/m^2)				
<23	81	1.72%	83.7%	14.6%
-27	93	1.79%	83.8%	14.5%
>27	90	1.82%	82.1%	16.1%
R, p		0.15, 0.01	-0.24, <0.001	0.23, <0.001
MUAC (cm)				
<27	91	1.71%	83.8%	14.5%
-30	91	1.80%	83.6%	14.6%
>30	94	1.83%	81.8%	16.4%
R, p		0.17, 0.005	-0.25, <0.001	0.24, <0.001
Triceps skinfold (mm)				
<16.4	91	1.77%	83.8%	14.4%
-22	94	1.77%	83.5%	14.8%
>22	94	1.80%	81.9%	16.3%
R, p		0.10, 0.11	-0.21, <0.001	0.20, 0.005
Smoking				
No	237	1.77%	82.9%	15.3%
Yes	39	1.81%	83.8%	14.4%
P		0.43	0.26	0.23
Walking Speed				
Slow	35	1.74%	81.5%	16.8%
Normal	138	1.79%	83.0%	15.2%
Fairly Brisk	103	1.77%	83.7%	14.6%
P		0.02, 0.73	0.14, 0.02	-0.14, 0.02
Employment				
No	160	1.76%	83.1%	15.1%
Part time	75	1.76%	82.9%	15.4%
Full time	98	1.77%	83.7%	14.5%
P		0.94	0.41	0.38

Legend: Mean values are shown with Pearson r and p value. [1] ANOVA

3.2.4.2.1 LATE PREGNANCY

TABLE 21 LATE PREGNANCY MATERNAL ANTHROPOMETRIC AND LIFESTYLE DETERMINANTS OF NEONATAL BODY COMPOSITION

Maternal characteristic		WBBMC (g)	Lean Mass (g)	Fat Mass (g)	Birth weight (g)	Length (cm)
	n	Mean	Mean	Mean		
Weight (kg)						
<72	103	56.5	2810	428	3302	49.3
-83	110	62.7	2882	504	3470	49.9
>83	115	66.0	2925	560	3656	50.6
r, p		0.31, <0.001	0.20, <0.001	0.26, <0.001	0.38, <0.001	0.33, <0.001
BMI (kg/m^2)						
<27.5	84	59.2	2892	454	3416	49.9
-31	74	62.3	2838	496	3455	49.6
>31	86	65.0	2892	548	3626	50.6
r, p		0.26, <0.001	0.10, 0.10	0.19, 0.002	0.31, <0.001	0.20, 0.001
MUAC (cm)						
<28	106	57.9	2856	459	3377	49.7
-31.5	126	61.7	2855	480	3439	49.7
>31.5	95	66.2	2918	564	3656	50.6
r, p		0.26, <0.001	0.12, 0.03	0.19, <0.001	0.30, <0.001	0.24, <0.001
Triceps skinfold (mm)						
<17.2	109	58.1	2834	449	3353	49.5
-23.4	106	62.9	2893	493	3501	50.0
>23.4	112	64.3	2893	550	3590	52.0
r, p		0.20, <0.001	0.08, 0.12	0.20, <0.001	0.23, <0.001	0.23, <0.001
Smoking						
No	289	62.7	2895	511	3528	50.2
Yes	47	57.2	2759	421	3218	48.9
P		0.01	0.007	0.003	<0.001	<0.001
Walking Speed						
Slow	215	63.1	2895	505	3508	50.1
Normal	96	60.0	2839	493	3450	49.7
Fairly Brisk	22	58.7	2857	441	3416	50.1
P		-0.13, 0.02	-0.08, 0.13	-0.08, 0.12	-0.9, 0.10	-0.07. 0.16
Employed						
No	157	62.4	2894	501	3518	50.0
Part time	76	62.8	2918	528	3518	50.3

Full time	100	60.4	2815	468	3409	49.7
P		0.43	0.07	0.15	0.13	0.14

Legend: Mean values are shown with Pearson r and p value. [1] ANOVA

TABLE 22 LATE PREGNANCY MATERNAL ANTHROPOMETRIC AND LIFESTYLE DETERMINANTS OF PROPORTIONATE NEONATAL BODY COMPOSITION

Maternal characteristic		Proportionate WBBMC	Proportionate Lean Mass	Proportionate Fat Mass
	n			
Weight (kg)				
<72	103	1.69%	84.8%	13.5%
-83	110	1.78%	83.2%	15.0%
>83	115	1.81%	81.9%	16.3%
r, p		0.19, <0.001	-0.31, <0.001	0.30, <0.001
BMI (kg/m^2)				
<27.5	84	1.71%	84.5%	13.8%
-31	74	1.79%	83.3%	15.0%
>31	86	1.82%	81.9%	16.2%
r, p		0.19,0.003	-0.29, <0.001	0.28, <0.001
MUAC (cm)				
<28	106	1.69%	84.2%	14.1%
-31.5	126	1.77%	83.5%	14.7%
>31.5	95	1.83%	81.8%	16.3%
r, p		0.20, <0.001	-0.28, <0.001	0.28, <0.001
Triceps skinfold (mm)				
<17.2	109	1.71%	84.4%	13.9%
-23.4	106	1.79%	83.5%	14.8%
>23.4	112	1.79%	82.0%	16.2%
r, p		0.14, 0.009	-0.24, <0.001	0.23, <0.001
Smoking				
No	289	1.77%	83.0%	15.3%
Yes	47	1.73%	84.9%	13.3%
P		0.34	0.008	0.008

Walking Speed				
Slow	215	1.78%	83.1%	15.2%
Normal	96	1.73%	83.4%	14.9%
Fairly Brisk	22	1.71%	84.5%	13.8%
r, p		-0.09, 0.09	0.10, 0.07	-0.09, 0.09
Employed	100			
No	157	1.76%	83.1%	15.1%
Part time	76	1.76%	82.9%	15.4%
Full time	100	17.7%	83.7%	14.5%
P		0.94	0.41	0.38

Legend: Mean values are shown with Pearson r and p value. [1] ANOVA

As with the earlier stages of pregnancy, there were similar relationships between late pregnancy measures of maternal adiposity and increasing birth size, proportionate fat and WBBMC with a lower proportionate lean mass.

Mothers smoking in late pregnancy had shorter, lighter, smaller babies with significantly reduced BMC, lean, fat and greater reduction in proportionate fat. There was now a significant reduction in WBBMC in those who reported faster walking speed in late pregnancy. In contrast to associations described in pre and early pregnancy, employment status in late pregnancy had little effect on neonatal size.

3.2.4.2.2 MATERNAL ALCOHOL CONSUMPTION

Maternal alcohol consumption before or during pregnancy was not significantly associated with neonatal WBBMC, lean mass, birth weight or length after excluding the woman with a high alcohol intake. While the number of units consumed before pregnancy was negatively associated with neonatal fat mass (Spearman r=-0.12, p=0.01); there was no significant relationship with maternal consumption during pregnancy and neonatal fat mass.

3.2.4.2.3 MATERNAL SKINFOLD THICKNESS BEFORE AND CHANGE DURING PREGNANCY

There was a high correlation between each of the skin fold measurement sites during pre-, early and late pregnancy. There was a suggestion that there was a higher correlation between the arm thickness measurements and between the truncal thickness measurements than between the arm and truncal measurements.

TABLE 23 RELATIONSHIP BETWEEN SKIN FOLD THICKNESS MEASUREMENTS PRE-, EARLY AND DURING LATE PREGNANCY

n=265	Triceps	Biceps	Supra-scapular
Pre pregnancy			
Biceps	0.80		
Sub-scapular	0.78	0.77	
Supra – iliac	0.75	0.74	0.8
Early Pregnancy			
Biceps	0.83		
Sub-scapular	0.81	0.81	
Supra – iliac	0.76	0.72	0.82
Late pregnancy			
Biceps	0.74		
Sub-scapular	0.77	0.71	
Supra – iliac	0.69	0.57	0.75

Legend: Pearson correlations, with significance $p<0.0001$ shown for log transformed skin fold thickness measurements

We measured maternal skin fold thicknesses at various sites. However, triceps skin fold thickness had the strongest and most robust association with neonatal bone mass. While this may in part be due to greater precision with triceps measurements, the findings would suggest that the triceps skin fold thickness is different to measurements of truncal fat using subscapular and supra-iliac skin folds and a better predictor of neonatal bone mass.

While absolute maternal weight and MUAC gain during early to late pregnancy significantly predicted neonatal WBBMC, there was not significant relationship between change in the other measures of estimated maternal adiposity during pregnancy and neonatal WBBMC. These findings indicate the importance of pre-pregnancy fat stores over the change in fat stores during pregnancy.

TABLE 24 RELATIONSHIP BETWEEN MATERNAL LATE PREGNANCY SKIN FOLD THICKNESS MEASUREMENTS AND NEONATAL WBBMC

Maternal measure N=306	WBBMC (g) per SD late pregnancy measurement	WBBMC (g) per SD change in measurement during pregnancy
Weight	4.2 (<0.001)	3.2 (<0.001)
Triceps	2.8 (<0.001)	1.2 (0.2)
Biceps	1.6 (0.04)	0.4 (0.7)
Subscapular	1.8 (0.02)	0.9 (0.3)
Suprailiac	1.1 (0.2)	-0.4 (0.7)
MUAC	3.7 (p<0.001)	2.0 (0.02)

Legend: Regression coefficient with significance shown for neonatal WBBMC (g) per Z score late pregnancy maternal measurements and per Z score change between early and late pregnancy.

TABLE 25 MATERNAL PREDICTORS OF NEONATAL BONE MASS

Maternal Characteristic	Pre - pregnancy	Early pregnancy	Late pregnancy
Age	x		
Height	+		
Weight	+	+	+
Birthweight	+		
BMI	+	+	+
MUAC	+	+	+
Triceps skinfold	+	+	+
Parity	+		
Smoking	x	x	-
Employment full time	-	-	x
Faster Walking speed	x	x	-

Legend: Direction of association with neonatal bone mass: + = positive; - = negative; x = no significant association

3.2.5 PATERNAL PREDICTORS OF NEONATAL BONE MASS: PRE-, EARLY AND LATE PREGNANCY.

The fathers' characteristics are shown in Table 26 Compared with the mothers, the fathers were 2.5 years older (SD 4.5; p = 0.42); 16 cm taller (SD 7; p<0.001); and 15kg heavier (p<0.001). Taller fathers had bigger babies with more bone, fat and lean mass (Table 27). Both maternal and paternal height had similar relationships with proportionate neonatal composition with bigger babies having proportionately greater bone and fat mass with less lean mass (Table 28).

While there was a strong effect of maternal weight, there is no effect of paternal weight on neonatal size or proportional body composition. While maternal birth weight had significant effects on neonatal size, the father's birth weight only weakly predicted BMC and not any other measure of neonatal size or proportion.

TABLE 26 PATERNAL ANTHROPOMETRIC AND LIFESTYLE CHARACTERISTICS

Partner's Characteristic		N
Age (years)	32.4 (5.4)	242
Height (m)	1.79 (0.07)	257
Weight (kg)[1]	79.4 (73.0, 89.2)	135
BMI (kg/m^2)[1]	25.1 (23.6, 27.8)	135
Birth weight (kg)	3.4 (0.7)	137
Working (y/ n)	96.8%	278
None	3.2%	9
Part time	1.4%	4
Full time	95.4%	266

[1]Median values (and inter-quartile range [IQR])

TABLE 27 PATERNAL DETERMINANTS OF NEONATAL BONE MASS

Partner's Characteristic	n	WBBMC (g)	Lean mass (g)	Fat mass (g)	Birth weight (g)	Length (cm)
Height (m)						
<1.75	65	62.0	2838	479	3461	49.8
-1.82	94	61.4	2849	510	3507	49.8
>1.82	98	65.3	2972	525	3591	50.7
r, p		0.13, 0.03	0.19, 0.003	0.12, 0.06	0.15, 0.02	0.23, <0.001
Weight (kg)						
<75	42	62.1	2859	504	3565	50.3
-85	40	62.6	2855	481	3493	50.0
>85	53	64.2	2919	523	3524	50.3
r, p		0.02, 0.84	0.11, 0.21	0.03, 0.77	-0.02, 0.84	0.0, 0.9
BMI (kg/m^2)						
<24.0	48	64.2	2908	499	3585	50.4
-26.0	42	62.3	2862	491	3520	50.2
>26.0	45	62.5	2871	523	3474	50.1
r, p		-0.08, 0.38	-0.03, 0.76	-0.01, 0.88	-0.11,0.21	-0.11, 0.2
Birth weight (kg)						
<3.2	47	63.6	2913	520	3555	50.1
-3.7	40	60.8	2865	517	3533	50.4
>3.7	50	67.8	2959	565	3657	50.7
r, p		0.15, 0.08	0.07, 0.45	0.07, 0.41	0.08, 0.38	0.09, 0.3
Full time job						
No	13	55.4	2906	421	3437	49.2
Yes	266	63.1	2888	510	3525	50.2
P		0.05	0.83	0.10	0.51	0.07

TABLE 28 PATERNAL DETERMINANTS OF PROPORTIONATE NEONATAL BONE MASS

Partner's Characteristic	n	Proportionate WBBMC (g)	Proportionate Lean mass (g)	Proportionate Fat mass (g)
Height (m)				
<1.75	65	1.79%	83.5%	14.7%
-1.82	94	1.76%	82.9%	15.3%
>1.82	98	1.80%	82.9%	15.3%
r, p		0.05, 0.4	-0.07, 0.23	0.07, 0.25
Weight (kg)				
<75	42	1.78%	83.0%	15.3%
-85	40	1.79%	83.4%	14.8%
>85	53	1.80%	82.9%	15.3%
r, p		-0.03, 0.71	0.01, 0.87	-0.01, 0.89
BMI (kg/m^2)				
<24.0	48	1.81%	83.3%	14.9%
-26.0	42	1.78%	83.2%	15.0%
>26.0	45	1.77%	82.7%	15.5%
r, p		-0.07, 0.44	0.02, 0.78	-0.02, 0.81
Birth weight (kg)				
<3.2	47	1.79%	82.9%	15.3%
-3.7	40	1.74%	82.6%	15.7%
>3.7	50	1.84%	82.0%	16.2%
r, p		0.13, 0.15	-0.05, 0.55	0.05, 0.60
Full time Working (yn)				
No	13	1.60%	85.5%	12.9%
Yes	266	1.79%	82.9%	15.3%
P		0.02	0.05	0.07

Both maternal and paternal height predicted neonatal WBBMC (R^2=1.5, p=0.05 and R^2= 2.1%, p=0.02) and birth length (R^2=9.0, p<0.001 and R^2= 5.5%, p<0.001) respectively (Table 29). While there was a weak positive correlation between parental height (r=0.18, p=0.02) there was no association between parental BMIs (r=-0.02, p=0.84). When both parental heights were entered into a simultaneous regression model, although maternal height was no longer statistically

significant (p=0.1) there was little change in the beta scores for predicting WBBMC and both remained significant predictors of neonatal length.

TABLE 29 RELATIONSHIP BETWEEN MATERNAL AND PATERNAL HEIGHT WITH NEONATAL BODY COMPOSITION IN 243 SUBJECTS NEONATES WHERE PARENTAL HEIGHTS WERE RECORDED

Parental Height		Maternal height (m)	Paternal height (m)
WBBMC (g)	Unadjusted	27.8, 0.05	27.1, 0.02
	Adjusted	23.7, 0.1	24.4, 0.04
Bone Area (cm^2)	Unadjusted	52.1, 0.03	48.4, 0.01
	Adjusted	44.9, 0.06	43.2, 0.03
Lean mass (g)	Unadjusted	1180, <0.001	765, 0.006
	Adjusted	1070, 0.001	640, 0.02
Fat mass (g)[1]	Unadjusted	0.80, 0.06	0.73, 0.04
	Adjusted	0.69, 0.10	0.65, 0.06
Birth weight (g)	Unadjusted	1450, 0.002	904, 0.02
	Adjusted	1330, 0.004	745, 0.05
Birth length (cm)	Unadjusted	8.8, <0.001	5.7, <0.001
	Adjusted	8.0, <0.001	4.8, 0.001

Legend: β and significance p shown for parental height predicting gestational adjusted neonatal whole body composition unadjusted and adjusted for the height of the partner in a linear regression model.
[1]Logged outcome

3.2.6 PREDICTORS OF SIZE ADJUSTED NEONATAL WBBMC

Neonatal WBBMC is a measure of bone area and vBMD and correlated with both length and birth weight. To provide an estimate of vBMD, neonatal WBBMC was adjusted for bone area and also height in linear regression models.

3.2.6.1 PARENTAL PREDICTORS OF AREAL BMD

Derived whole body aBMD was not associated with parental height, birth weight or age. Of the maternal factors there was a weak positive relationship between the amount of milk consumed during early pregnancy, and MUAC with aBMD (R^2 =1.6; p=0.02). In late pregnancy, faster walking speed was negatively associated with aBMD (β= -0.01, p=0.028).

3.2.6.1.1 PARENTAL PREDICTORS OF NEONATAL WBBMC/ HEIGHT

Neonatal WBBMC was then size adjusted by linear methods using birth length. Of the pre-pregnancy maternal characteristics, all measures of maternal anthropometry except skinfold thickness at the biceps, subscapular and superior iliac crest, were significantly ($p<0.05$) associated with length-adjusted WBBMC. In addition, maternal birth weight (R^2 = +2.1%, p=0.02), parity (R^2 = +1.2%, p=0.05), fast walking speed (β=0.5, p=0.03) and receipt of benefits (R^2 = +1.3%, p=0.03) were positively associated with length adjusted WBBMC. There were negative correlations with supplement use (R^2 = -1.2%, p=0.04) and current working status (R^2 = –2.3%, 0.02).

During early pregnancy, only maternal head circumference (R^2= 2.2%; p=0.02); weight (R^2= 4.4; $p <0.001$); BMI (R^2= 3.3) and maternal circumference, but not skinfold measurements at various sites, were associated with neonatal WBBMC adjusted for birth length. The only significant lifestyle predictor was maternal working status (R^2=3.4%, 0.003).

During late pregnancy, maternal age had a negative association with neonatal WBBMC adjusted for birth length (β–0.01, p=0.01). All measures of maternal size, including heel width but excluding the skinfold thickness, were significantly ($p<0.05$) predictive of neonatal WBBMC adjusted for length.

3.2.7 INDEPENDENT PREDICTORS OF NEONATAL WBBMC AFTER ADJUSTMENT FOR GESTATION AGE AT BIRTH.

The independent predictors of neonatal WBBMC are shown in Table 30 Maternal, but not paternal, height significantly predicted neonatal WBBMC. Adipose Mothers had babies with greater bone mass independently of maternal height. Maternal fat mass was measured pre-pregnancy and during early and late pregnancy. At every time point, maternal fat mass significantly predicted neonatal WBBMC. However, this was greater in measurements in late (R^2=4.8%) compared to early (R^2=2.1%) or pre-pregnancy (R^2=2.3%). However, the increment in fat mass during pregnancy, did not predict neonatal bone mass.

TABLE 30 INDEPENDENT PREDICTORS OF NEONATAL WBBMC AFTER ADJUSTMENT FOR GESTATION AGE AT BIRTH.

Maternal characteristic	β, p
Height (m)	25.4, 0.04
Triceps (mm)[1,2]	6.7, 0.004
Smoking [1]	-4.3, 0.05
Parity	3.8, 0.01
R^2	***8.0%, p<0.001***

Legend: Maternal predictors for neonatal whole body BMC adjusted for gestational age.
[1]Late pregnancy
[2]Log Transformed

Maternal smoking during late pregnancy, but not early and pre-pregnancy, significantly reduced WBBMC by 8.8%. Although smokers had smaller triceps skinfold (-0.3 SD, p=0.05), both had independent effects on neonatal WBBMC and, after adjustment for other maternal characteristics and gestational age, the effect on WBBMC was reduced to 6.6%. Parity had a persisting effect on neonatal bone mass, independent of maternal size and age. There was a reduction in neonatal WBBMC in those mothers who continued to walk faster (β =-6.3, p=0.08), comparing fast with slow walking in late pregnancy, however this was not significant in a multivariate model.

After adding movement score to the model, maternal smoking was slightly weakened (β=-3.4 , p=0.07, however we were unable to detect an effect of maternal smoking status on neonatal movement score and the model was otherwise unchanged.

3.3 DISCUSSION

The results of this study support the role of maternal lifestyle and anthropometric factors in determining intra uterine bone mineral accrual. Of the previously identified independent predictors of neonatal bone mass (66), we were able to replicate the independent influence of maternal fat stores, smoking and birth weight with a weaker effect of exercise in late pregnancy on neonatal bone mass.

At birth, boys were longer with greater lean mass and aBMD than girls. The gender differences in body composition were also observed with derived proportionate body composition, with boys having a higher percentage of lean mass but a lower percentage of fat mass than girls. For girls, there appears to be an accrual of fat mass at the detriment of lean mass. In both genders however, increased birth weight was associated with greater accrual of fat mass than lean mass, and in neonates bone mass appeared to track with fat mass with a negative relationship with proportionate lean mass. As expected with increasing gestational age, birth weight and birth length there was an increase in neonatal WBBMC, which was attenuated after adjustment for bone area to calculate aBMD.

At each time point, pre-pregnancy, early and late pregnancy, maternal fat mass predicted neonatal WBBMC and proportionate WBBMC. The closest correlation was between measurements of maternal adiposity in late pregnancy. The increment in maternal adiposity from pre to late pregnancy did not however predict neonatal body composition, suggesting that the association between maternal fat mass and the body composition is not related to pregnancy-associated changes in maternal fat mass but is a more general reflection of maternal fat stores in the pre pregnant state. This emphasizes the importance of pre-pregnant maternal nutrition.

Both maternal and paternal height predicted neonatal bone mass, and although paternal height was no longer significant in the multivariate model after adjustment for maternal fat mass and smoking, the β coefficient was not greatly reduced. While maternal adiposity was a robust independent predictor of neonatal WBBMC, paternal adiposity, as measured by BMI, did not predict neonatal body composition. This suggests that effects of height on neonatal growth are

shared between the parents but it is maternal, and not paternal, adiposity that has additional influences on intra uterine growth and it may be the progressive increasing adiposity of women of child bearing age that is accounting for, in part, the increase in height over the last century.

As previously reported (78), we have demonstrated a deleterious effect of smoking on foetal growth. 28% of women reported smoking before pregnancy, and 14% of all mothers continued to smoke during pregnancy. This reduction in smoking frequency occurred after the LMP date for the pregnancy suggesting that mothers stopped smoking when they knew they were pregnant as opposed to when they were trying to conceive. No mother took up smoking during pregnancy, emphasizing the importance of primary prevention of smoking in women. The effect of maternal smoking on neonatal WBBMC was significant in mothers who continued to smoke in late pregnancy, a time of greatest mineral accrual. However, there were significant deleterious effects on other aspects of neonatal growth such as lean mass and birth length apparent by smoking status pre pregnancy and during early pregnancy as well. This suggests smoking had effects on foetal growth during early pregnancy, possibly before the mother is aware she is pregnant. An important determinant of smoking in late pregnancy is an under appreciation of the mother of the deleterious effects of smoking on her child (149). Furthermore, intensive individualized smoking cessation therapies for pregnant women have been demonstrated to increase smoking cessation and it has been suggested that during pregnancy, smokers are more sensitive to smoking cessation interventions. However, it may be necessary to emphasize the importance of smoking cessation before conception to parents wishing to have children, to reduce smoking related effects on foetal growth.

We have also demonstrated that multiparity is associated with significant increases in each of bone, lean and fat mass. This was independent of increased maternal adiposity. The accrual of fat mass was greater than the increment in lean mass in multiparous compared to primiparous mothers. However, the effect of smoking in late pregnancy on neonatal WBBMC was similar in primiparous and multiparous women, suggesting that parity does not protect the growing foetus from other maternal factors.

As in the previous study (66), walking speed in late pregnancy predicted lower WBBMC and aBMD, suggesting an effect of late pregnancy exercise on foetal mineralization in contrast to bone size. However the relationship between maternal physical activity and neonatal bone mass

was no longer statistically significant in multivariate models including other maternal characteristics. There was no observable relationship between maternal strenuous activity and neonatal bone mass. Further studies of maternal lifestyle may have to utilize more accurate measures of maternal physical activity than reported walking speed or strenuous activity.

In comparison to WBBMC, there were few robust predictors of aBMD. Maternal MUAC in early, but not late, pregnancy was positively associated with aBMD. However no other measure of maternal adiposity was predictive of aBMD. When neonatal WBBMC was adjusted for birth length, another method of size correction; there was a positive relationship between maternal adiposity at pre-pregnancy, early and late pregnancy.

In summary, we have demonstrated that maternal height, adiposity, smoking status in late pregnancy and parity are independent determinants of neonatal WBBMC in a series of healthy term pregnancies.

4 CHANGES IN MATERNAL CALCANEAL QUANTITATIVE ULTRASOUND DURING PREGNANCY AND ITS DETERMINANTS

4.1 ABSTRACT

Introduction:

During pregnancy, mineralization of the foetal skeleton and obligate urinary calcium losses require adaptations of maternal calcium homeostasis, including increased intestinal calcium absorption and bone resorption. However the determinants of maternal bone resorption during pregnancy in healthy adult mothers has not been previously described.

Methods:

We therefore conducted a population based longitudinal study of 307 term pregnancies using an established cohort of women living in the Southampton area. During early and late pregnancy bone quality was measured at the left calcaneus using a Hologic Sahara Quantitative Ultrasound device.

Results:

There was a significant decline in both SOS and BUA during pregnancy. Those women pregnant for the first time, with low milk intakes and reduced fat mass had the greatest reduction in heel bone measurements. Furthermore, there was a seasonal effect such that mothers whose pregnancy included the winter season had the greatest losses in calcaneal QUS measurements.

Conclusions:

The seasonal effect on maternal calcaneal QUS loss during pregnancy may suggest a role for vitamin D supplementation during winter in pregnant women, especially those with low milk intakes and pregnant for the first time.

4.2 INTRODUCTION

During pregnancy, mineralization of the foetal skeleton and preparation for lactation necessitate maternal adaptations to meet the increase in calcium demands (150). The developing foetus requires in total 21g (range 13-33g) of calcium and 80% of the transfer of mineral occurs in the third trimester (144). The maintenance of a normal plasma ionized calcium concentration with expansion of the plasma volume and higher urinary calcium losses, secondary to increases in glomerular filtration rate, place further demands on maternal calcium homeostasis. Maternal adaptations include altered bone turnover, renal calcium transport and intestinal calcium absorption. The changes in bone turnover lead to a net reduction in bone mass as measured by both dual energy X-ray absorptiometry (151) and quantitative ultrasound (QUS) (152).

However, there is a marked inter individual variation in change of bone mass during pregnancy, with some mothers gaining bone mineral as measured by QUS. We therefore assessed the determinants of maternal bone change during pregnancy in a cohort of healthy women assessed before and during pregnancy and in a sub-sample compared the observed calcaneal bone change during pregnancy with the bone mass of the offspring.

4.3 SUBJECTS AND METHODS

This sample was drawn from the Southampton Women's Survey (148) as previously described. In addition to the questionnaire and anthropometric measurements performed, at each visit the mother's bone mineral was measured by quantitative ultrasound (QUS) of the left foot using a calcaneal ultrasound device instrument (Sahara, Hologic Inc. CA). The QUS instrument measures speed of sound (SOS), bone ultrasound attenuation (BUA) and calcaneal width. The instrument was calibrated daily using its own phantom. In a repeatability study of healthy non-pregnant women the coefficients of variation were calculated for SOS (0.8%) and BUA (3.0%). Calcaneal scans with a chi^2 of greater than 50 were excluded as per manufacturer's guidelines. In a sub-sample, the whole body BMC of the offspring, as measured by Lunar DPX-L, were available for comparison.

The local research ethics committee approved the study and all women gave written informed consent.

4.3.1 STATISTICAL ANALYSIS

A sample size of 400 gave 80% power to detect a 0.3 SD difference in SOS at p=0.05 significance. The data were analyzed using STATA v7.0. The dynamic measurement range of SOS and BUA differ markedly, hence change in SOS and BUA during pregnancy was expressed as a Z score using the SD of the measurements at 11 weeks. Change in SOS and BUA measurements during pregnancy were found to be associated with changes in heel width, hence both SOS and BUA were adjusted to mean heel width during early and late pregnancy as appropriate.

The effect of season during pregnancy was investigated using the following; spring: March-May; summer: June-August; autumn: September-November; winter: December-February.

Univariate analysis of determinants of baseline QUS was performed; significant univariate predictors were used to generate a multiple linear model of determinants of both baseline and change in QUS during pregnancy.

4.4 RESULTS

Between April 1998 and April 2001, 8700 women completed the baseline interview. Among those who subsequently became pregnant between October 1999 and January 2002, 340 had calcaneal QUS measurements performed during early (11 weeks) and late (34 weeks) pregnancy. Of these, 307 calcaneal measurements were suitable for analysis. While there was no difference in age, those excluded had higher SOS and BUA measurements both at early and late pregnancy ($p<0.0001$) suggesting a systematic overestimation of QUS measurement in those excluded. Those excluded also had wider heels at early (median 38.6 [36.3, 41.6] vs valid 37.4 [34.5, 39.9] cm, $p=0.01$) but not late (median 40.5 [37.7, 43.2] vs valid 37.1 [37.9, 43.0] cm $p=0.77$) pregnancy

The anthropometric and lifestyle characteristics of the 307 mothers are shown in Table 31 The mean age of the mothers was 29.5 years and the median interval between pre-pregnancy interview and early pregnancy assessment was 1.1 yrs (IQR 0.6 to 1.8 yrs). The mean interval between early (11 weeks) and late (34 weeks) pregnancy calcaneal measurements was 22.8 weeks (SD 0.7 weeks).

For 44% of women this was their first term pregnancy. As expected, the mothers gained weight (mean increment 10.6 kg [SD 4.0]) during pregnancy as well as both mid upper arm circumference and triceps skinfold thickness. As the pregnancy progressed, reported vigorous physical activity diminished. 24% of the women reported smoking before pregnancy and 57% of them continued to smoke during pregnancy. In line with current nutritional advice for pregnant women, highest supplement use was observed during early pregnancy (94%), with use rates returning to pre-pregnancy levels by 34 weeks. Up to 30% of women drank less than ¼ pint of milk per day either before or during pregnancy.

TABLE 31 ANTHROPOMETRIC AND LIFESTYLE CHARACTERISTICS OF THE 307 MOTHERS WITH SINGLETON PREGNANCIES WHO HAD CALCANEAL QUS MEASUREMENTS THROUGH PREGNANCY.

Maternal characteristic	Early Pregnancy (11 weeks)	Late Pregnancy (34 weeks)
	Mean (SD)	
Age (yrs)	29.5 (3.9)	-
Height (m)	1.63 (0.064)	-
Birth weight (kg)	3.220 (0.58)	-
Parity		
0	44.3%	-
1	39.3%	-
>1	16.4%	-
	Median (IQR)	
Weight (kg)	66.7 (59.1, 47.9)	77.3 (70.0, 85.7)
Triceps skinfold thickness (mm)	19.0 (15.4, 23.1)	20.2 (16.2, 24.9)
Mid upper arm circumference (cm)	28.6 (26.4, 31.3)	29.2 (27.1, 31.6)
Smoking (%)	12.7%	13.0%
Vigorous activity[1] (%)	38.4%	27.3%
Nutritional Supplements (%)	94.2%	50.9%
Milk intake (pints/day):		
< 0.25	30.9%	23.1%
- 0.5	30.6%	31.9%
- 1.0	32.9%	34.2%
> 1.0	6.5%	10.8%

Legend: SD = standard deviation, IQR = inter-quartile range
[1]Sufficient to cause subjective breathlessness and rapid heart beat

During pregnancy, there was a significant ($p<0.001$) decline in calcaneal SOS and BUA (Table 32).

TABLE 32 CALCANEAL QUS DURING EARLY AND LATE PREGNANCY IN 307 SINGLETON PREGNANCY WOMEN.

Calcaneal QUS measurement	Early pregnancy	Late pregnancy	Z score change	p-value
Speed of sound (m/s)	1548 (25.8)	1540 (22.2)	-0.30 (0.59)	<0.001
Bone Ultrasound Attenuation (dB/Hz)	72.3 (11.1)	69.4 (10.5)	-0.26 (0.60)	<0.001
Calcaneal width (mm)	37.1 (4.4)	40.5 (4.2)	+0.77 (1.2)	<0.001

Legend:Values are mean (SD)
P-values are for the difference between early and late pregnancy.

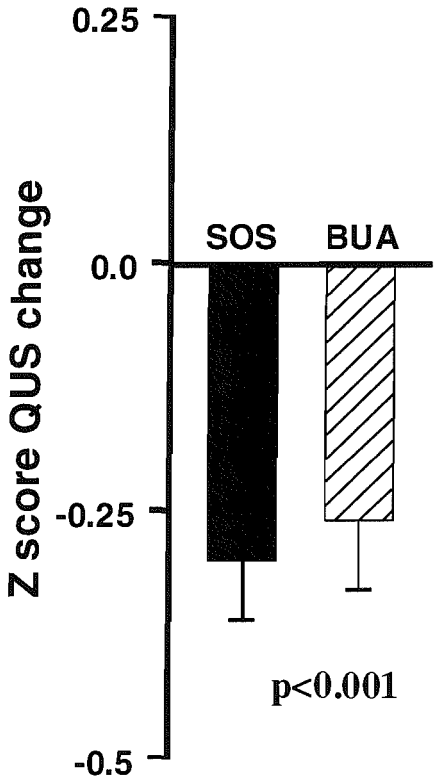
Calcaneal width increased during this time and, while there was no significant relationship between baseline either SOS or BUA and calcaneal width, the change in calcaneal width was significantly ($p<0.01$) positively correlated with change in SOS and negatively with BUA measurements (Table 33). However, the observed reductions in both calcaneal SOS and BUA during pregnancy persisted after adjustment for change in calcaneal width. Furthermore, mothers who had less than a 0.25 SD change in calcaneal width during pregnancy, had reductions in both SOS (-0.32SD) and BUA (-0.32) of a similar magnitude as the remainder of the cohort.

TABLE 33 RELATIONSHIP BETWEEN CHANGES IN MEASUREMENTS OF CALCANEAL SOS, BUA AND WIDTH DURING PREGNANCY IN 307 MOTHERS.

Change in calcaneal QUS during pregnancy.	BUA Z score	Width Z score
SOS (Z score)	0.26(<0.001)	0.38 (<0.001)
BUA (Z score)	-	-0.17 (0.002)

Legend: Pearson's correlation coefficient with significance shown. Contrasts relationships of calcaneal measurements between early (11 weeks) and late (34 weeks) gestation.

FIGURE 16 REDUCTION IN CALCANEAL SOS AND BUA FROM EARLY TO LATE PREGNANCY IN 307 MOTHERS.



Legend: Mean (95% CI) Z-score change for calcaneal SOS and BUA between early (11 weeks gestation) and late (34 weeks gestation) after adjustment for differences in calcaneal width.

4.4.1 MATERNAL PREDICTORS OF CHANGE IN CALCANEAL QUS

A positive correlation was observed between maternal age (20.4 to 37.1 years) and calcaneal QUS measurements at both early (SOS: r=0.19, p=0.001; BUA: r=0.15, p=0.01) and late (SOS: r=0.22, p<0.001; BUA: r=0.18, p=0.002) pregnancy. This relationship was independent of calcaneal width and maternal parity. Maternal age however, did not predict calcaneal QUS change during pregnancy. Age of menarche was also not correlated with any measure of calcaneal QUS.

Higher maternal parity was associated with lower early pregnancy maternal SOS (r= -0.14, p=0.01) and an attenuated reduction in calcaneal SOS during pregnancy (p=0.01) (Figure 17).

Maternal educational level was also positively correlated with calcaneal SOS at early (r=0.16, p=0.005) and late (r=0.15, p=0.01) pregnancy but not SOS change during pregnancy. The effects of maternal educational level on calcaneal SOS were independent of maternal age and parity.

There was a significant (p<0.01) positive relationship between calcaneal width and each of maternal height, weight and mid-upper arm circumference (MUAC). Maternal weight, however, did not predict calcaneal SOS at baseline, change during pregnancy or the change in calcaneal width during pregnancy. Maternal height also did not predict change in calcaneal QUS during pregnancy. However, maternal adiposity, as measured by late pregnancy MUAC, was positively associated with calcaneal BUA at early (r=0.12, p=0.04), late (r=0.23, p<0.001) and change during pregnancy (r=0.16, p=0.005); such that mothers with greater fat stores had an attenuated reduction in BUA during pregnancy (Figure 17). This was independent of changes in calcaneal width. Maternal adiposity was not significantly associated with calcaneal SOS. Increases in maternal adiposity during pregnancy did not predict change in calcaneal BUA. Reported maternal birth weight was also not associated with QUS measurements during pregnancy.

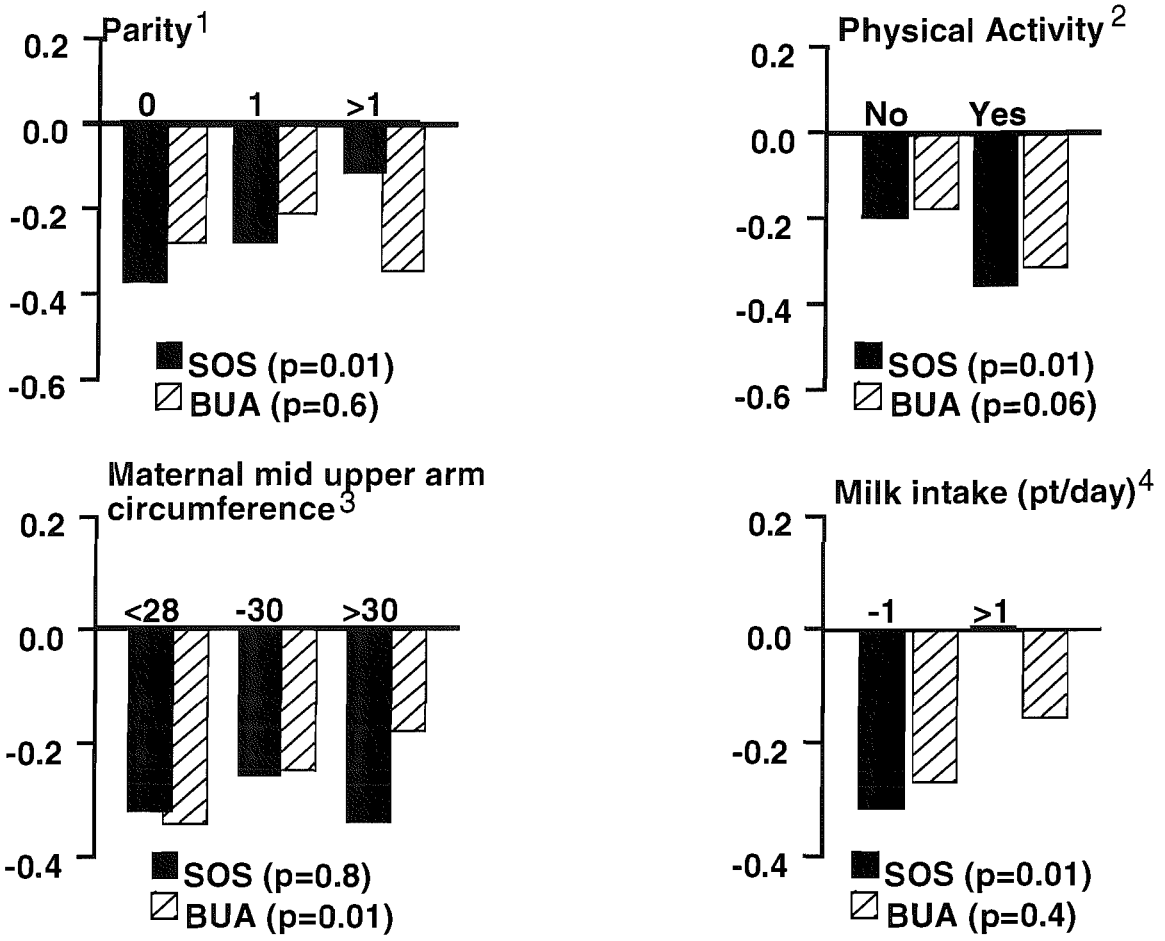
Those mothers reporting physical activity sufficient to cause subjective breathlessness and a rapid heart at early pregnancy had higher early pregnancy SOS (+0.3SD, p=0.01) and higher BUA (+0.2SD, p=0.07) measurements. Reported vigorous activity in early pregnancy was also associated with a greater reduction in SOS (-0.16 SD, p=0.01) and BUA (-0.13 SD, p=0.06). Change in reported vigorous activity during pregnancy did not significantly (p>0.3) influence

QUS changes at the heel. Maternal working status (full-time, part-time or none) was also not associated with calcaneal QUS measurements during pregnancy.

Maternal smoking status before pregnancy or during pregnancy was not associated with either baseline or change in calcaneal QUS measurements. Women who smoked during early pregnancy did have a greater increment in heel width (0.43 SD, $p=0.03$) during pregnancy, independent of weight gain during pregnancy.

Maternal milk intake was not associated with calcaneal BUA during pregnancy. However, those mothers drinking more than one pint of milk per day before pregnancy tended to preserve calcaneal SOS during pregnancy (+0.32SD, $p=0.01$) (Figure 17). Maternal nutritional supplement use during early pregnancy was significantly correlated with maternal education ($p<0.001$). While there was no association between supplement use and calcaneal SOS, those mothers who continued to use supplements into late pregnancy did have higher late pregnancy BUA measurements ($p=0.01$) but not to change in BUA during pregnancy.

FIGURE 17 MATERNAL DETERMINANTS OF CHANGE IN CALCANEAL SOS AND BUA DURING PREGNANCY IN 307 MOTHERS.



Legend: The figure shows mean values for Z score change in SOS and BUA during pregnancy after adjustment for change in calcaneal width. Significance values for change in SOS and BUA shown separately.

[1]Parity refers to the number of term births before the index pregnancy. Spearman correlation significance.

[2]Vigorous physical activity is sufficient to cause subjective breathlessness and rapid heart beat in early pregnancy. T-test significance.

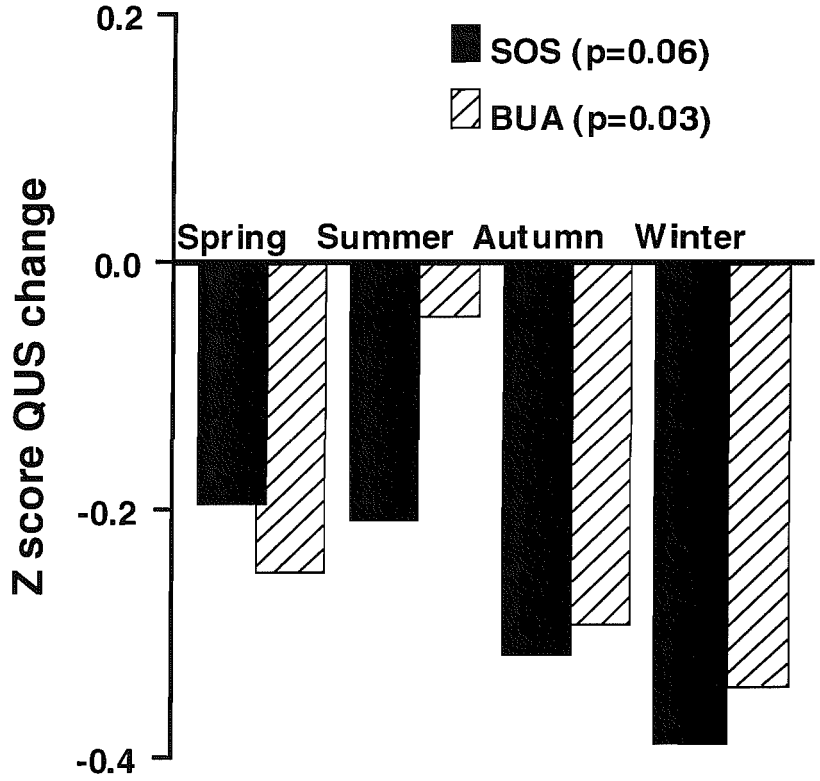
[3]As measured in late pregnancy. Pearson correlation significance.

[4]As recorded as pints per day during early pregnancy. T-Test significance.

4.4.1.1 SEASONAL EFFECTS ON CALCANEAL QUS

Calcaneal width, was 5mm (1.2 SD, $p<0.001$) greater for summer measurements compared to those performed in winter. While the season during late pregnancy did not influence the change in SOS ($p=0.38$) or BUA ($p=0.10$) measurements, there was a notable association between season during early pregnancy and the subsequent change in both SOS ($p=0.06$) and BUA ($p=0.03$). Such that early pregnancies during spring and summer had blunted reductions SOS and BUA, while those in autumn and winter had greater reductions in SOS and BUA (Figure 18). These effects persisted after adjustment for changes in calcaneal width.

FIGURE 18 RELATIONSHIP BETWEEN SEASON AT TIME OF EARLY PREGNANCY AND SUBSEQUENT CHANGE IN CALCANEAL SOS AND BUA DURING PREGNANCY IN 307 MOTHERS.



Legend: The figure shows mean values for Z score change in SOS and BUA during pregnancy, adjusted for change in calcaneal width, by season at time of early pregnancy scan (Spring – March, April, May; Summer – June, July, August; Autumn- September, October, November; Winter – December, January, February).
Significance values for change in SOS and BUA, from analysis of variance, are shown separately.

There was a weaker non significant relationship with season at time of the second QUS measurement in later pregnancy.

Using multiple linear regression modelling, the mutually independent predictors of early pregnancy SOS were age ($p<0.001$); vigorous activity ($p=0.03$) and lower parity ($p=0.01$) (Table 34). Calcaneal BUA during early pregnancy was determined by age ($p=0.01$) with a weaker effect of maternal fat stores ($p=0.06$). The change in both SOS and BUA were influenced by season of the time of the early pregnancy visit. Change in calcaneal SOS during pregnancy was also independently predicted by parity and milk intake (> 1 pint/day) before pregnancy. As maternal fat stores varied by season, it did not remain as an independent predictor of calcaneal BUA change during pregnancy once seasonality was added to the model.

TABLE 34 INDEPENDENT DETERMINANTS OF CALCANEAL QUS DURING PREGNANCY IN 307 MOTHERS.

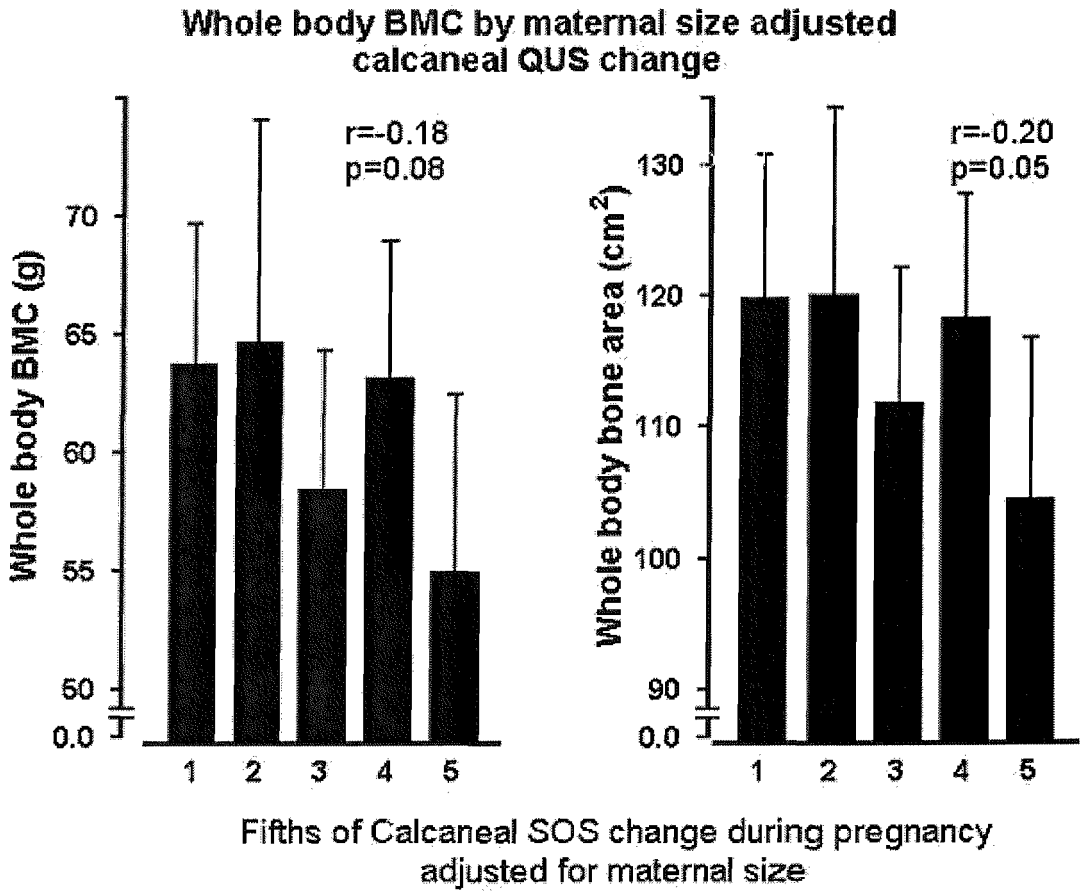
Calcaneal QUS	Determinant	β	95% CI	p- value
Early pregnancy				
SOS	Maternal age (yrs)	1.4	0.6 to 2.1	<0.001
	Parity (per child)	-4.5	-8.0 to -0.96	0.01
	Vigorous activity (y/n)	6.8	0.7 to 12.9	0.03
BUA	Maternal age (yrs)	0.4	0.08 to 0.72	0.01
	MUAC (cm)[1]	9.6	-0.24 to 19.6	0.06
Change in SOS[2]	Parity (per child)	0.12	0.05 to 0.19	0.001
	Milk intake (>1 pint)	0.31	0.07 to 0.55	0.01
	Season[3] (summer)	0.2	-0.01 to 0.4	0.067
Change in BUA[2]	Season[3] (summer)	0.2	0.1 to 0.5	0.004

Legend: The regression coefficients β (95% CI), with significance p, are shown.
[1] Log transformed.
[2] Change from early to late pregnancy adjusted for change in calcaneal width.
[3] Compares summer to winter

4.4.2 MATERNAL CALCANEAL QUS DURING PREGNANCY AND NEONATAL BONE MASS

There was no significant ($p>0.05$) relationship between adjusted baseline maternal QUS measurements during pregnancy and whole body bone mineral content (BMC) or body composition of her offspring. However, after adjustment for maternal size, there was a significant relationship between increment in SOS and both whole body bone area ($r= -0.21$, $p=0.05$) and birth length ($r= -0.28$, $p=0.006$) and weaker relationships with whole body BMC ($r= -0.19$, $p=0.07$) and birth weight ($r= -0.17$, $p=0.12$) (Figure 19), such that mothers with greater reductions in calcaneal SOS during pregnancy had babies with greater bone area and longer babies. This relationship was not weakened by adjustment for changes in maternal heel width, parity, milk intake or season at early pregnancy.

FIGURE 19 RELATIONSHIP BETWEEN MATERNAL SIZE ADJUSTED CALCANEAL QUS CHANGE DURING PREGNANCY AND NEONATAL BONE MASS IN 106 PREGNANCIES



Legend: Mean (95% CI) shown by equal fifths of change in maternal SOS during pregnancy, such that those in the lowest fifth had a greatest reduction and those in the highest fifth had the greatest increase in SOS during pregnancy

4.5 DISCUSSION

We have examined the determinants of change in calcaneal QUS during pregnancy in 307 healthy pregnancies. Our data demonstrate that there is considerable reduction in maternal calcaneal SOS and BUA during pregnancy and that maternal adiposity, parity, milk intake and physical activity influence the magnitude of the reduction in calcaneal QUS. Furthermore, the season during early pregnancy influences the reduction in QUS measurements during pregnancy.

To meet the increase in calcium demand during pregnancy there are a number of maternal physiological adaptations including mobilization of calcium from the maternal skeleton to that of the foetus during pregnancy (153). Bone histomorphometric studies of women during early pregnancy and at term have demonstrated changes in bone structure evident as early as eight weeks gestation (144). In early pregnancy, bone volume decreases with an increase in resorption cavities, while in late pregnancy bone volume recovers with an increase in osteoid and seam width and postulated mineralization rate.

The biphasic response during pregnancy is mirrored by corresponding changes in bone resorption and formation markers; with a progressive increase in bone resorption markers throughout pregnancy (146) and the markers of bone formation only rising in late pregnancy (18;19). Although the change in maternal bone markers may be due to changes in the developing foetal skeleton, using isomers specific to foetal tissue, the foetal contribution is less than 10% (18).

The reduction in maternal bone mass during pregnancy has also been demonstrated using DXA. In a study of women wishing to become pregnant for the first time, there was a 2.1% reduction in lumbar spine and a 3.8% reduction in distal radial BMD between pre-pregnancy and post delivery (151). Similar reductions in BMD at trabecula sites have been reported in another longitudinal study using whole body DXA measurements (18).

Previous work has identified that there is a progressive decline in SOS and BUA measurements with the greatest loss in the last trimester, the time of greatest foetal demand for mineral (146;152;154). The magnitude of the decline in QUS measurements in our study is in accord with that of Sowers et al (155), who demonstrated a 3.6% decline in BUA between 16 weeks pregnancy and 6 weeks postpartum. However they were unable to demonstrate a significant decline in SOS and no mention is made of differences in, and adjustment for, ankle oedema during pregnancy.

Changes in heel width accounted for a proportion of the observed change in QUS during pregnancy. Total heel width comprises bone volume, marrow volume and extra osseous soft tissue. In a study of patients with dependent pitting oedema, of an average 6.3 mm, increased oedema was associated with a reduction in both SOS and BUA measurements (156). While in this longitudinal study BUA was reduced with increased ankle oedema, SOS measurements were slightly but significantly increased. The cause for this is not as yet apparent, and it is possible that the relationship between increased heel width and calcaneal QUS during pregnancy is not solely due to soft tissue oedema.

We have shown that increased maternal adiposity rather than weight gain during pregnancy is associated with higher calcaneal BUA measurements and an attenuated loss during pregnancy. This is in agreement with a cross sectional study of children and young adults (157), suggesting that increased loading of the calcaneus increases BUA measurements. Surprisingly, maternal smoking status during pregnancy had no significant effect on maternal QUS measurements.

A previous cross sectional study has also demonstrated increased bone loss in nulliparous compared with parous young women and adolescents (155), and this is in accord with our observations. While the average decline in BUA during pregnancy was similar (3.6% vs. 4.0% in this study), the effect of parity was restricted to change in BUA and did not affect SOS.

We have also demonstrated that maternal milk intake, a marker of calcium intake, before and not during pregnancy predicted her skeletal response to pregnancy. The lack of effect of milk intake during pregnancy suggests that the maternal diet in the pre-pregnancy period determine the maternal skeletal response to pregnancy, although the mechanism for this is not known.

Season during early but not late pregnancy assessment influenced the subsequent change in maternal calcaneal SOS and BUA during pregnancy. This is likely to be due to seasonal variation in vitamin D status influencing subsequent maternal bone loss rates during pregnancy. There is large inter individual variation in sunlight exposure and in the absence of serum maternal D concentrations at the different stages of pregnancy, the observed seasonal influence on pregnancy related QUS change are likely to be underestimated in this study. Other possible mechanism include seasonal variation in physical activity and diet.

The endocrine mechanism underlying dissociated bone resorption in early pregnancy is not fully characterized. In addition to the increase in weight, pregnancy is a high oestrogen state which should, through inhibition of osteoclast recruitment and activity, maintain bone mass. Higher maternal calcitonin levels during pregnancy also protect the maternal skeleton from increased bone resorption (158). During early pregnancy, maternal serum PTH levels are suppressed (19) and while there is an increase in 1,25 $(OH)_2$ vitamin D from placental 1α OHase activity, this is matched by an increase in vitamin D binding protein during pregnancy and free 1,25 $(OH)_2$ vitamin D concentration only rises in late pregnancy. However, there is now evidence to support activity of bound vitamin D (159).

The seasonal effect on maternal bone quality during pregnancy suggest that the maternal skeleton at this time is still sensitive to changes in vitamin D status. High levels of 1, 25 dihydroxyvitamin D would inhibit classic PTH secretion from the parathyroid glands and increase absorption of calcium from the maternal gut.

The placenta also produces PTHrP, with a PTH like N-terminal end able to stimulate bone resorption (160). PTHrP stimulates renal 1α hydroxylation of vitamin D and may be responsible for the increase in maternal 1, 25 $(OH)_2$ vitamin D concentration during pregnancy. However, changes in maternal PTHrP concentration have not been consistently demonstrated (19).

Other candidate hormones include βhCG, which has been associated with osteolytic tumours (161) and IGF-1, whose concentration in the maternal serum rises in pregnancy preceding the rise in bone formation markers (19) and was negatively associated with changes in maternal BMD during pregnancy (18). Serum prolactin, secreted by maternal pituitary and uterine decidua, rises

during pregnancy and the inhibition of prolactin secretion during pregnancy is associated with reduced bone turnover (162). Furthermore, leptin, a marker of adiposity, inhibits prolactin production and this may explain the protective effect of maternal adiposity on calcaneal QUS changes that we have demonstrated (163). Recovery of bone mass is associated with resumption of menses, with further bone loss during postpartum amenorrhea (147).

We have also demonstrated that the greater the reduction in SOS the longer the neonate and the greater the whole body BMC and bone area. This trend was apparent in unadjusted models and became statistically significant after adjusting for maternal size, another independent predictor of neonatal WBBMC. Adding change in SOS during pregnancy to maternal height and body mass index increased the explained variance of the model for neonatal WBBMC from 10% to 13%. The question arises whether the relationship between maternal and foetal skeletal status is driven by maternal supply or by foetal demand.

A study of foetal reduction suggests that neonatal growth maybe the principal drive. When a triplet pregnancy was electively reduced to twins in assisted conception pregnancies, levels of maternal markers of bone turnover were related to foetal number (164). After foetal reduction from 3 to 2, the serum concentration of ICTP, a marker of bone resorption, was reduced to that expected in a twin pregnancy. There was however little change in levels of bone formation. This is a relatively large change in foetal mineral demand and does not preclude a component of maternal constraint in determining foetal growth in singleton pregnancies.

There were several limitations to the calcaneal QUS component of the study. We were unable to measure calcaneal SOS and BUA before pregnancy and have used the measurements recorded at 11 weeks as baseline. There is histological evidence for increased bone resorption even before this point of pregnancy(144). It is therefore likely that a pre-pregnancy measurement of QUS would have demonstrated even greater changes in maternal QUS during the whole of pregnancy. While there were significant reductions in both SOS and BUA during pregnancy these were less than the least significant change for each measure of calcaneal QUS. DXA has a lower reproducibility error than QUS measurements, however the perceived radiation hazard precludes its use during pregnancy. The greater reproducibility error in QUS measurements may account for the small proportion in the variance of QUS accounted for by the final independent models. Also, while we have demonstrated that those mothers consuming less than one pint of milk a day

had lower rates of QUS loss at the heel, without data on other dietary sources of calcium or vitamin D, we are unable to estimate an adequate calcium intake needed to maintain maternal bone mass during pregnancy. Season was used as a surrogate for 25 OH vitamin D status, this clearly does not take into account the large inter-individual differences in sunlight exposure in terms of clothing and time spent outdoors. In addition more subtle meteorological variations in sunlight exposure due to cloud were also not taken into account. In the absence of maternal and neonatal serum values, it is still speculative to suggest that the season effect on calcaneal QUS is due to vitamin D insufficiency.

In summary, maternal calcaneal BUA and SOS measurements fall during pregnancy indicating a loss of bone mass. These changes are augmented in women who are pregnant for the first time, consuming less than one pint of milk per day before but not during pregnancy and those who were pregnant during autumn or winter for the first stage of the pregnancy. Seasonal variation in vitamin D status may therefore influence the maternal bone response to pregnancy and also foetal growth.

5 MATERNAL PREDICTORS OF CHILDHOOD BONE MASS

ABSTRACT

Introduction:

Evidence is accumulating that the risk of osteoporotic fracture in later life may be determined, in part, by environmental influences during intrauterine and early postnatal life. We have previously demonstrated that maternal lifestyle and body build during pregnancy influence intra uterine bone mineral accrual of her offspring. However, it is not known whether maternal factors during pregnancy have persisting effects on skeletal growth during childhood.

Methods: The study sample of children was drawn from population-based study of maternal nutrition and foetal growth. The mothers were characterized for lifestyle factors and body build through pregnancy and, in a follow up study, we now relate maternal lifestyle and anthropometry during pregnancy and neonatal characteristics with the childhood bone mass and body composition at nine years of age.

Results: There were significant positive associations between the child's birth weight and each of whole body BMC ($r=0.31$, $p<0.001$); whole body lean mass ($r=0.44$, $p<0.001$); but not whole body fat mass ($r=0.12$, $p=0.12$), after adjustment for gestational age at birth. Reduced maternal height, lower maternal pre-pregnancy weight, reduced maternal fat stores during late pregnancy, a history of maternal smoking during pregnancy and lower maternal social class were associated with reduced whole body BMC of the child at nine years. Of the umbilical vein measurements of calcium homeostasis, lower calcium concentration predicted lower bone mass at nine years.

Conclusions: Birth size predicts childhood bone mass, even after adjustment of gestational age, confirming the sensitivity of post-natal bone mass accrual to perturbations during intra-uterine growth. Both maternal height and cord serum calcium independently predicted the bone mass of the child at nine years suggesting that the capacity of the placenta to maintain a positive calcium gradient is critical for ensuring an optimum trajectory of post-natal skeletal growth.

5.1 INTRODUCTION

There is a growing body of evidence supporting the hypothesis that the risk of osteoporosis in later life is increased by adverse environmental stimuli acting during early development. Epidemiological studies have shown that weight at birth and in infancy predicts peak bone mass (37) and bone mass in later life (165-168). Furthermore, poor growth during childhood is associated with an approximate doubling of hip fracture risk six decades later (41).

These findings have led to the evaluation of maternal nutritional and lifestyle factors during pregnancy that influence neonatal bone mass. We have previously reported that mothers who smoked, had lower fat stores, or reported vigorous physical activity in late pregnancy had offspring with lower whole body bone mass (66). However, it is not clear whether these maternal factors have persisting effects on skeletal growth after birth.

We have therefore tested the hypothesis that maternal lifestyle and body build during pregnancy have persisting effects on childhood bone growth using a population-based British cohort of healthy children and explored potential mechanisms for associations found.

5.2 SUBJECTS AND METHODS

5.2.1 INTRODUCTION AND INITIAL COHORT ASSESSMENT

The sample population were recruited from children born to 596 Caucasian women who had participated in a study of maternal nutrition and foetal growth at the Princess Anne Maternity Hospital, Southampton UK between 1991 and 1992 (68). The women were aged over 16 years and registered before 17 weeks gestation at the antenatal clinic. The women completed a lifestyle questionnaire (**Appendix X**) during early (median 15.3 weeks) and late (median 32.7 weeks) pregnancy. The women were asked about previous obstetric history, current smoking habits, pre-pregnancy weight and were requested to contact their parents in order to ascertain their own birth weight. Social class was derived from the woman's current or last occupation. A food frequency questionnaire was administered to assess consumption of 100 foods or food groups in the three months preceding the visit. At each visit the women had the following measurements recorded: height using a stadiometer, weight using calibrated electronic scales and mid upper arm circumference.

All the pregnancies were singleton and gestational age was calculated from the date of the last menstrual period and confirmed by ultrasound measurements of foetal size at the initial visit.

Following delivery, two trained fieldworkers recorded neonatal anthropometrical measures (birth weight, head, abdominal and mid upper arm circumference, total length and crown rump length). After clamping of the umbilical cord and before placental delivery, umbilical venous blood samples were taken. The placental weight was measured after removing any obvious clots, cutting the umbilical cord flush with its insertion into the placenta and stripping both the foetal and maternal membranes.

The cord blood samples were stored at –70°C degrees and serum calcium, albumin, phosphate and alkaline phosphate were measured using a Beckman CX-7 analyzer (Department of Clinical Chemistry, Southampton, UK).

At nine months, the infants were visited again at home for repeat anthropometry by measurement of weight, head, abdomen and mid upper arm circumference and crown heel and crown rump length. The type of infant feeding used (exclusive breast, exclusive bottle, combined and the time bottle feeding was introduced) was asked about, as well as maternal smoking status at the time of the visit.

5.2.2 FOLLOW-UP RECRUITMENT AND CURRENT ASSESSMENT

The children from this cohort still resident in Hampshire were then invited to attend a further assessment of cardiac, neurological and bone status at nine years. Using an interviewer-administered questionnaire, the lifestyle characteristics of both the mother and child, were recorded (**Appendix XIII**). The children had their height measured using a stadiometer and weight using calibrated electronic scales. In addition, the children had whole body BMC and body composition measurements performed using a Lunar DPX-L instrument using specific paediatric software (v 4.7c, Lunar Corporation.)

At the time of the scan, the children's height and weight were also recorded. The instrument was calibrated every day and all scans were performed with the children wearing light clothing.
The study was approved by the local research ethical committee and both mother and child gave informed consent.

5.2.2.1 IMAGE ANALYSIS

Using the manual analysis option, exclusion regions of interest were placed around areas of the scan image from extraneous material. After this, the dividers on the whole body DXA images were adjusted, according to manufacturers guidelines, to delineate the head, neck, dorsal spine, lumbar spine, pelvis, ribs, arm and leg regions. Once all the images had been adjusted, the same two operators together reviewed each scan and the dividers were further adjusted until both operators agreed. There was no significant movement artefact on the scanned images and so movement scores were not required.

5.2.2.2 DATA ANALYSIS

The data were analysed using STATA v7.0. Body weight, skinfold thickness, body mass index and fat mass, as measured by DXA, were positively skewed and were log transformed to approximate normality for subsequent analyses. We performed univariate analysis and then multivariate linear regression methods to generate the final independent model.

Despite the narrow age-range of the sample studied (1.2 years), whole body bone area, BMC and BMD were significantly ($p<0.05$) associated with the age at time of DXA scan. In addition, gestational age at the time of birth was weakly predictive of childhood WBBMC (19g per extra week of gestation, $p<0.0001$); where appropriate, results were adjusted to a gestational age of 40 weeks and to the mean age of the children at the time of scan. Measurements at infancy were adjusted using linear regression to an age of nine months.

Whole body BMC is a measure of both size and mineral density and is strongly correlated with height and weight. In order to adjust for size to give an estimate of mineral density, whole body BMC was analysed unadjusted and after partial correction for the following: height, weight, height with height-adjusted weight, total bone area; BMC corrected for bone area [BMCa], using the method of Holick et al.(169); and BMC corrected for bone area, weight and height [BMCp] using the method of Prentice et al (170). The method of Horlick uses computations of BMC and bone area while the method of Prentice used adjusts BMC for the current height and weight of the child using height and the residual of weight on height. Proportionate whole body BMC, lean mass and fat mass were calculated using total body weight as measured by DXA as the denominator.

The amount of calcium in cord blood was adjusted for albumin concentration using:
Corrected calcium = calcium (mmol/L) + 0.01x(38-albumin (g./L)).

The determinants of sex-adjusted whole body BMC, BA and BMD were calculated using univariate analysis of infant and parental factors. Significant determinants were then used to build a multiple linear model of determinants for nine-year whole body BMC. In a similar way, determinants of corrected cord calcium were derived.

5.3 RESULTS

Firstly the response rate and the characteristics of the children will be described followed by the parental predictors and finally the conditional modelling of both childhood and parental determinants.

5.3.1 RESPONSE RATE AND OUTLIER DETECTION

Of the 596 infants in the original cohort, 461 were still resident in the area and were invited to attend. 226 mothers responded and 216 agreed to participate with the DXA component of the follow-up survey. Women who took part in the follow-up study were older at the time of the first pregnancy assessment (27.0 years vs. 25.9 years [p=0.009]) and were less likely to have smoked in late pregnancy (17.0% vs. 25.8% [p=0.04]) than those who were not followed up (Table 35). However, there was no significant difference in maternal social class, birth weight and body build. In addition, the children followed up in this study were of similar birth size had similar umbilical vein measurements but were of shorter gestation (median gestation 277 days vs. 280 days [p=0.02]) than the infants in the remainder of the initial cohort.

TABLE 35 DIFFERENCES IN MATERNAL AND NEONATAL CHARACTERISTICS BETWEEN NON RESPONDERS AND RESPONDERS IN THE FOLLOW UP STUDY

Characteristic	Non Responders N=394	Responders N=226	p[5]
Maternal			
Age (yrs)	25.9 (4.9)	27.0 (4.9)	0.008
Menarche (yrs)	13.0 (1.52)	13.2 (1.4)	0.22
Prepregnant weight[1] (kg)	60 (54, 67)	59 (53, 65)	0.21
Height (cm)	1.63 (6.3)	1.63 (0.07)	0.31
Prepregnant BMI[1]	22.2 (20.4, 24.6)	21.9 (20.3, 24.3)	0.29
MUAC[1] (late pregnancy)	26.8 (25, 29.5)	26.5 (25, 28.6)	0.38
Birth weight (kg)	3.32 (0.55)	3.28 (0.52)	0.394
Social class (%)			0.47
i	12.1	9.6	
Ii	22.0	19.6	
Iiin	9.1	12.3	
Iiim	35.7	36.8	
Iv	14.6	15.5	
V	6.6	6.4	
Education[2] (%)			0.007
None	12.0	6.2	
CSE's	21.3	15.0	
O-level	32.2	36.7	
A-level	20.2	27.4	
HND	6.9	4.4	
Degree	7.5	10.2	
Smoking (early pregnancy)	28.1	21.2	0.061
Smoking (late pregnancy)	25.2	17.8	0.040
Smoking (infancy)	31.8	24.3	0.055

Alcohol (early pregnancy)	56.2	58.9	0.53
Alcohol (late pregnancy)	51.2	55.2	0.35
Vigorous activity (early pregnancy)	17.5	17.8	0.93
Vigorous activity (late pregnancy)	18.4	22.4	0.23
Infant			
Birth weight (kg)	3.4 (0.58)	3.4 (0.62)	0.11
Gestational age	282 (274-289)	280 (271-287)	0.02
Placental weight (kg)	0.53 (0.12)	0.53 (0.13))	0.97
Head circumference	35.1 (1.4))	35.0 (1.4)	0.17
Abdominal circumference	33.6 (1.9)	33.4 (2.3)	0.3
Crown rump length (cm)	33.2 (1.55)	33.2 (1.7)	0.99
Umbilical vein			
Corrected calcium[3]	2.75 (0.14)	2.75 (0.14)	0.91
Infant feeding[4]			0.86
Breast only	29	30	
Bottle only	31	29	
Both bottle and breast	40	41	

Legend: Mean and (standard deviations) or percentages shown were appropriate. For skewed data[1] median (IQR) are shown

[2] Highest educational level at time of initial assessment

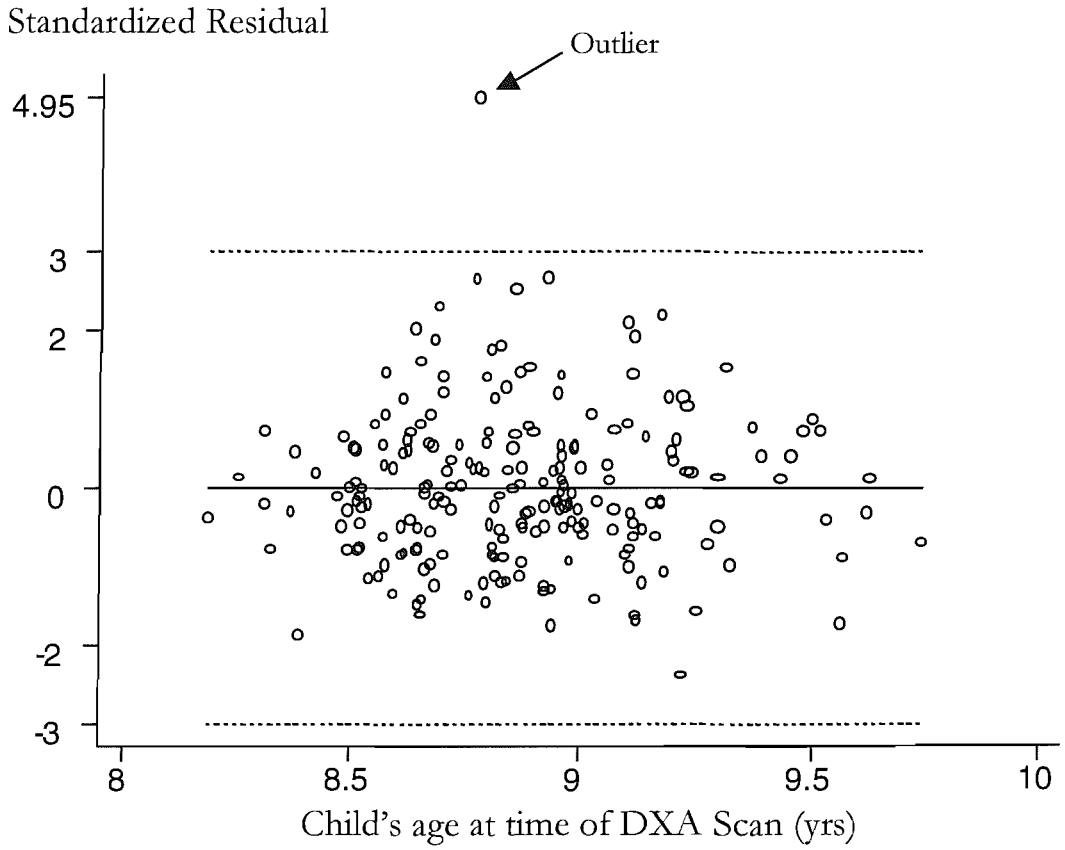
[3] Umbilical venous calcium concentration corrected for concentration of umbilical venous albumin

[4] Infant feeding pattern up to 90 days postnatally.

[5] P values contrast responders with non responders

From an initial analysis of the DXA measurements, a potential outlier was identified. This individual had a standardized residual whole body BMC of +4.94 SD (Figure 20 . In a bi-variate model of child's whole body BMC and age, his leverage score (h_i = 0.047) was more than twice the score of the whole group (h = 0.0043), indicating a substantial effect of this individual's results on the model of the group. On further checking, this individual was found to have an African father. The study inclusion criteria only excluded non-Caucasian mothers but not fathers. No other African fathers were identified in the sample. Therefore, for both statistical and biological reasons, this subject was excluded from further analysis, reducing the total number of children to 215.

FIGURE 20 AGE AND WHOLE BODY BMC IN 216 CHILDREN AGED 9YR



Legend: Standardized residual of whole body BMC by child's age at time of DXA scan. Dotted lines represent +/- 3SD limits.

5.3.2 CHILDHOOD CHARACTERISTICS

The anthropometric and birth characteristics of the children are shown in Table 36 The mean age of both boys and girls was 8.7 years. There were significant gender differences in anthropometry in the children. Boys were significantly (p=0.006) taller, had greater whole body BMC (boys 1.2kg, girls 1.1kg) and lean mass measurements (boys 22.4 kg, girls 20.2 kg) than girls. However, the boys had a lower fat mass (boys 4.7kg, girls 7.0kg) than the girls; resulting in no significant difference in body weight by gender. The boys' higher whole body BMC (WBBMC) and bone area (BA) was attributable to their greater height; however, the difference in BMD was still significant after adjustment for current height.

TABLE 36 CHILDHOOD AND BIRTH ANTHROPOMETRIC CHARACTERISTICS OF 215 CHILDREN STUDIED AT AGE NINE YEARS

Characteristic	Male n=114	Female n=101	p-value[3]
CHILDHOOD			
Age (yrs)	8.7 (0.24)	8.7 (0.21)	0.3
Height (m)	1.32 (0.06)	1.29 (0.06)	0.003
Weight (kg)[1]	28.0 (25.7-31.6)	28.2 (25.1-31.6)	0.58
Whole body BMC (kg)	1.2 (0.18)	1.1 (0.16)	0.002
Proportionate BMC[2] (%)	4.0% (0.4)	3.8% (0.4)	<0.001
Lean mass (kg)	22.5 (2.9)	20.2 (2.4)	<0.001
Proportionate lean mass[2] (%)	77.3% (7.3)	70.9% (6.5)	<0.001
Fat mass (kg) [1]	4.7 (3.6-6.9)	7.0 (5.2, 9.3)	0.001[4]
Proportionate fat mass[2] (%)[1]	17.4% (13.5-21.1)	24.8% (19.6-30.3)	<0.001[4]
BIRTH			
Gestational age (weeks)[1]	39.9 (38.6- 40.9)	40.1 (38.9- 41)	0.23
Birth weight (kg)	3.37 (0.44)	3.25 (0.65)	0.05
Placental weight (kg)	0.52 (0.12)	0.53 (0.22)	0.61
Crown heel length (cm)	50.2 (1.9)	49.3 (1.9)	<0.001

Legend: Mean values (standard deviation) are shown. [1]Median values (inter-quartile range) for variables not normally distributed. [2]Proportionate BMC, fat and lean mass were derived using the total weight derived from the DXA measurements as the denominator. [3]P-values contrast male and female data using T-test or [4]Mann Whitney U test.

TABLE 37 RELATIONSHIPS BETWEEN CHILDHOOD WHOLE BODY COMPOSITION AS MEASURED BY DXA

	Height	Weight[1]	BMC	Bone area	Lean
Height					
Weight[1]	0.74				
BMC	0.75	0.80			
Bone Area	0.83	0.83	0.95		
Lean	0.79	0.76	0.83	0.87	
Fat[1]	0.38	0.80	0.51	0.50	0.29

Legend: Pearson correlation coefficient shown for whole body composition, $p<0.001$.
[1]Log transformed.

As can be seen in Table 37 there is considerable correlation between the different measurements of the children's anthropometry. The associations between DXA derived fat mass and the child's height and lean mass are the weakest. As bone mass makes up only 4% of total body composition, a high inverse relationship between proportionate lean and fat mass was expected (**Error! Reference source not found.**).

Whole body BMC was significantly, negatively related to both weight and fat mass, suggesting that heavier children had a smaller proportion of body mass attributed to the bone compartment; there was no relationship between height and proportionate whole body BMC.

5.3.3 HEAD VS. SUBTOTAL BMC

Head BMC represented 31% of whole body BMC. While there were significant ($p<0.001$) differences in the absolute head BMC in boys (357g (SD 41g)) vs. girls (337g (SD 35g)), there was no difference in the proportion of head BMC from whole body BMC by gender (boys: 31.1%g, girls 31.5%g, $p=0.2$). Within the narrow age range of the group, there was a decline in the proportion of head BMC to whole body BMC with increasing age (-0.6 SD per year, $p=0.01$).

Proportionate whole body BMC was highly correlated with proportionate subtotal BMC ($r=0.92$, $p<0.001$). There was no significant difference in the determinants in the final multivariate model for whole body BMC or subtotal BMC.

5.3.4 NEONATAL AND CHILDHOOD PREDICTORS OF WHOLE BODY BMC AND BODY COMPOSITION AT NINE YEARS

For every additional week of gestation, whole body BMC at nine years increased by 0.019 kg ($p<0.001$). When gestational age and birth weight were included in a bi-variate model, birth weight remained as the significant predictor of bone mass at nine years. After adjustment for gestational age, the child's birth weight was also a significant predictor of whole body lean mass but not fat mass of the child at nine years (Figure 21 . Other measures of neonatal and placental size were also predictive of nine-year whole body BMC (Table 38).

The type of feeding during infancy (exclusive breast, bottle or combined) did not appear to affect whole body BMC at nine years. However, in those children who drank milk at nine years, childhood milk intake at nine years was significantly ($p=0.04$) correlated with whole body BMC. Current physical activity, as measured by number of days playing sport or days walking for more than 15 minutes, did not influence whole body BMC.

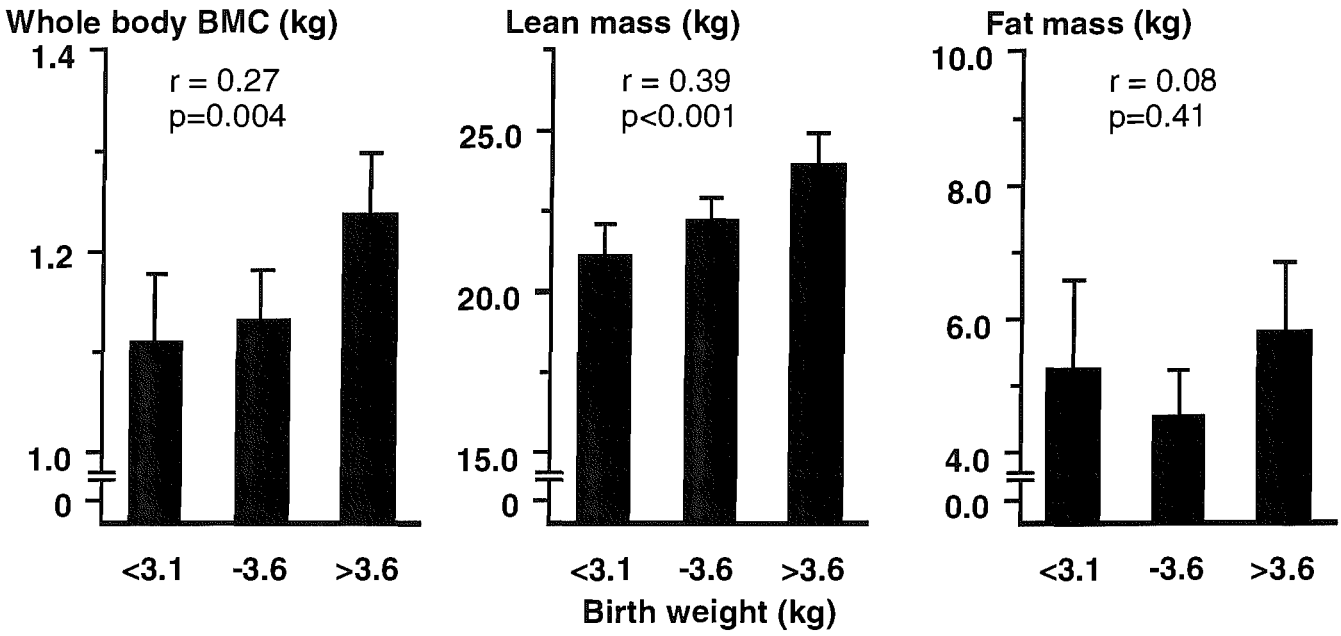
TABLE 38 RELATIONSHIP BETWEEN MEASUREMENTS AT BIRTH AND CHILDHOOD WHOLE BODY BONE MINERAL CONTENT (WBBMC) AT NINE YEARS

Birth measurement	All children N=215	Boys N=114	Girls N=101
	WBBMC (kg)	WBBMC (kg)	WBBMC (kg)
Birth weight (kg)			
<3.1	1.07	1.11	1.04
-3.6	1.10	1.13	1.07
>3.6	1.23	1.24	1.20
r (p)	0.33(<0.001)	0.31 (0.001)	0.36 (<0.001)
Placental weight (g)			
<480	1.10	1.13	1.08
-570	1.10	1.13	1.06
>570	1.18	1.24	1.11
r (p)	0.21 (0.003)	0.30 (0.001)	0.12 (0.24)
Birth length (cm)			
<49	1.06	1.10	1.03
-51	1.12	1.14	1.10
>51	1.21	1.23	1.17
r (p)	0.38 (<0.001)	0.30 (0.001)	0.40 (<0.001)
Mid-upper arm circumference (cm)			
<11	1.08	1.11	1.05
-12	1.12	1.15	1.08
>12	1.19	1.23	1.14
r (p)	0.23 (<0.001)	0.19 (0.05)	0.26 (0.009)
Pre-pregnant BMI (kg/m^2)			
<21.0	1.089	1.125	1.049
-23.0	1.141	1.182	1.072
>23.0	1.125	1.152	1.103
r (p)	0.10 (0.2)	0.12 (0.2)	0.10 (0.3)

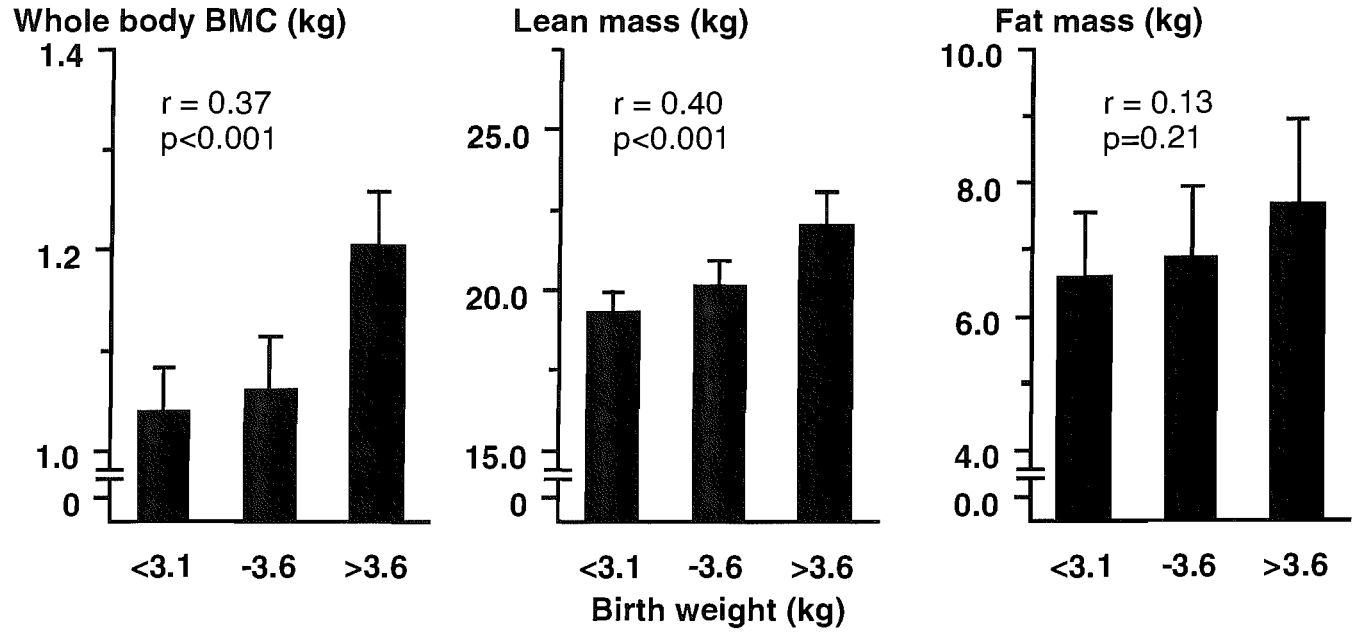
Legend: Mean values for whole body BMC at nine years are shown for each third of distribution of birth weight, placental weight, birth length and neonatal upper arm circumference adjusted for gestation. After adjustment of whole body BMC for current age of the child, the partial correlation coefficient, r, with significance, (p) are shown.

FIGURE 21 RELATIONSHP BETWEEN BIRTH WEIGHT AND CHILDHOODWHOLE BODY BMC

Boys



Girls



Legend: Mean values for whole body BMC at nine years are shown for each third of distribution of birth weight, After adjustment of whole body BMC for current age of the child, the partial correlation coefficients, r, with significance, (p) are shown.

5.3.5 PARENTAL BASELINE CHARACTERISTICS AND PREDICTORS OF CHILDREN'S BONE MASS AND BODY COMPOSITION AT NINE YEARS

The anthropometric and lifestyle characteristics of the 215 mothers, at the time of the index pregnancy, are shown in Table 39 . At the time of birth of their children, the women in the study had a mean age of 27 years (SD 4.9yrs), 55% were primiparous and 21% reported smoking at some time during the pregnancy.

TABLE 39 ANTHROPOMETRIC AND LIFESTYLE CHARACTERISTICS AT THE TIME OF THE INITIAL ASSESSMENT OF MOTHERS WHOSE CHILDREN UNDERWENT BODY COMPOSITION ASSESSMENT BY DXA

Maternal characteristic	
(n=215)	*Mean value (SD)*
Age (years)	27 (4.9)
Height (m)	1.63 (0.07)
	Median (IQR)
Pre-pregnant weight (kg)	59 (53- 65)
Pre-pregnant BMI (kg/m^2)	21.9 (20.3-24.3)
Mid upper arm circumference (cm) [during late pregnancy]	26.5 (25.0- 28.6)
Primiparous	55%
Smoking during pregnancy (%)	21%
Vigorous activity (%)[1] (> twice per week during late pregnancy)	23%

Legend: SD = standard deviation, IQR = inter-quartile range
[1]Sufficient to cause subjective breathlessness and a fast pulse.

5.3.5.1 PARENTAL ANTHROPOMETRY AND CHILDHOOD BONE MASS AND BODY COMPOSITION

Maternal height, pre-pregnancy weight and late pregnancy MUAC measurements (Figure 22), but not estimated maternal pre-pregnant BMI, were significantly ($p<0.001$) associated with childhood whole body BMC and bone area at nine years (Table 40). Similar relationships were observed for predicting whole body bone area.

Paternal height had a similar relationship with childhood body composition as maternal height. Paternal height (cm) was positively associated with childhood whole body BMC (R^2= 8%, $p< 0.001$), bone area (R^2= 10%, $p<0.001$), height (R^2=18%, $p< 0.001$), weight (R^2= 4%, $p<0.01$) but not proportionate whole body bone mass or other estimates of volumetric bone mineral density.

Of the predictors of childhood height; maternal height (R^2=20%, $p<0.001$), maternal birth weight (R^2= 3%, $p=0.02$) and pre-pregnant weight (R^2=2%, $p=0.32$), but not pre-pregnant BMI or late pregnancy MUAC, were the significant determinants (Table 40 . Paternal height also significantly predicted the child's height. All recorded measures of maternal body size were positively correlated with the current weight of her child at nine years of age: maternal height (R^2=10%, $p<0.001$), pre-pregnant weight (R^2=11%, $p<0.001$), pre-pregnant BMI (R^2=3%, $p<0.01$) MUAC in late pregnancy (R^2=10%, $p<0.001$)) and birth weight ((R^2= 3%, $p=0.02$). Paternal height did not predict childhood weight.

Maternal pre-pregnant weight (R^2=4%, $p<0.01$), BMI (R^2=3%, $p<0.01$) and late pregnancy MUAC (R^2=5%, $p<0.001$), but not height ($p=0.7$), were negatively correlated with proportionate whole body BMC (Table 41). There was a significant positive relationship between measures of maternal adiposity and childhood proportionate fat mass and a negative relationship with proportionate lean mass. Paternal measurements did not predict the proportionate body composition of the child.

TABLE 40 RELATIONSHIP BETWEEN PARENTAL ANTHROPOMETRY AND THE WHOLE BODY BMC AND BODY BUILD OF THE CHILD AT AGE OF NINE YEARS

Parental Characteristic		WBBMC (kg)	Bone area (cm^2)	Lean Mass (kg)	Fat Mass (kg)	Weight (kg)	Height (m)
MATERNAL	N						
Height (m)							
<1.6	64	1.08	1230	20.3	5.85	27.6	1.28
-1.65	66	1.08	1230	21.0	5.06	27.3	1.29
>1.65	81	1.18	1340	22.5	6.38	30.6	1.34
r (p)		0.33	0.40	0.38	0.11 (0.08)	0.31	0.45
		(<0.001)	(<0.001)	(<0.001)		(<0.001)	(<0.001)
Pre-pregnancy weight (kg)							
<55	66	1.07	1230	20.5	5.11	27.0	1.30
-62	73	1.11	1270	21.5	5.48	28.4	1.30
>62	75	1.18	1320	22.0	7.06	30.7	1.32
r (p)		0.26	0.27	0.23	0.30	0.33	0.15 (0.03)
		(<0.001)	(<0.001)	(<0.001)	(<0.001)	(<0.001)	
Pre-pregnancy BMI (kg/m^2)							
<21	70	1.09	1250	20.9	4.99	27.3	1.31
-23	67	1.14	1300	22.0	5.97	29.6	1.32
>23	73	1.13	1270	21.2	6.43	29.0	1.29
r(p)		0.10 (0.2)	0.09 (0.2)	0.05 (0.5)	0.25 (<0.001)	0.18 (0.009)	-0.08 (0.3)
MUAC (cm) (late pregnancy)							
<25.5	68	1.06	1230	20.8	4.88	27.0	1.30
-28	73	1.16	1300	21.7	6.00	29.3	1.31
>28	71	1.15	1300	21.8	6.73	29.9	1.31
r (p)		0.25	0.24	0.17 (0.01)	0.37	0.33	0.1 (0.1)
		(<0.001)	(<0.001)		(<0.001)	(<0.001)	

Birthweight		WBBMC	Bone area	Lean Mass	Fat Mass	Weight	Height
(kg)		(kg)	(cm^2)	(kg)	(kg)	(kg)	(m)
<3.0	59	1.08	1250	21.1	5.21	27.8	1.30
-3.6	83	1.13	1270	21.1	6.06	28.7	1.30
>3.6	56	1.16	1320	22.3	6.31	30.2	1.33
r(p)		0.19 (0.009)	0.17 (0.02)	0.19 (0.007)	0.11 (0.1))	0.17 (0.02)	0.16 (0.02)
PATERNAL							
Height (m)							
<1.72	45	1.07	1220	20.4	5.65	27.7	1.28
-1.8	84	1.12	1280	21.5	5.79	28.7	1.30
>1.8	79	1.16	1320	22.0	5.96	29.6	1.33
r(p)		0.29	0.32	0.27	0.06 (0.4)	0.19 (0.006)	0.42
		(<0.001)	(<0.001)	(<0.001)			(<0.001)
Birthweight							
(kg)							
<3.15	56	1.08	1250	21.0	5.13	27.7	1.30
-3.6	45	1.12	1280	21.5	6.05	28.9	1.32
>3.6	74	1.15	1300	21.8	5.84	29.1	1.31
r(p)		0.16 (0.03)	0.15 (0.05)	0.13 (0.09)	0.06 (0.4)	0.1 (0.2)	0.13 (0.1)

Legend: Mean values for whole body BMC at nine years are shown for each third of distribution of each maternal characteristic. The partial correlation coefficient, r, with significance, (p), between each parental characteristic and WBBMC adjusted for the current age of the child are shown.

TABLE 41 RELATIONSHIP BETWEEN PARENTAL ANTHROPOMETRY AND PROPORTIONATE WHOLE BODY COMPOSITION OF THE CHILD AT AGE OF NINE YEARS

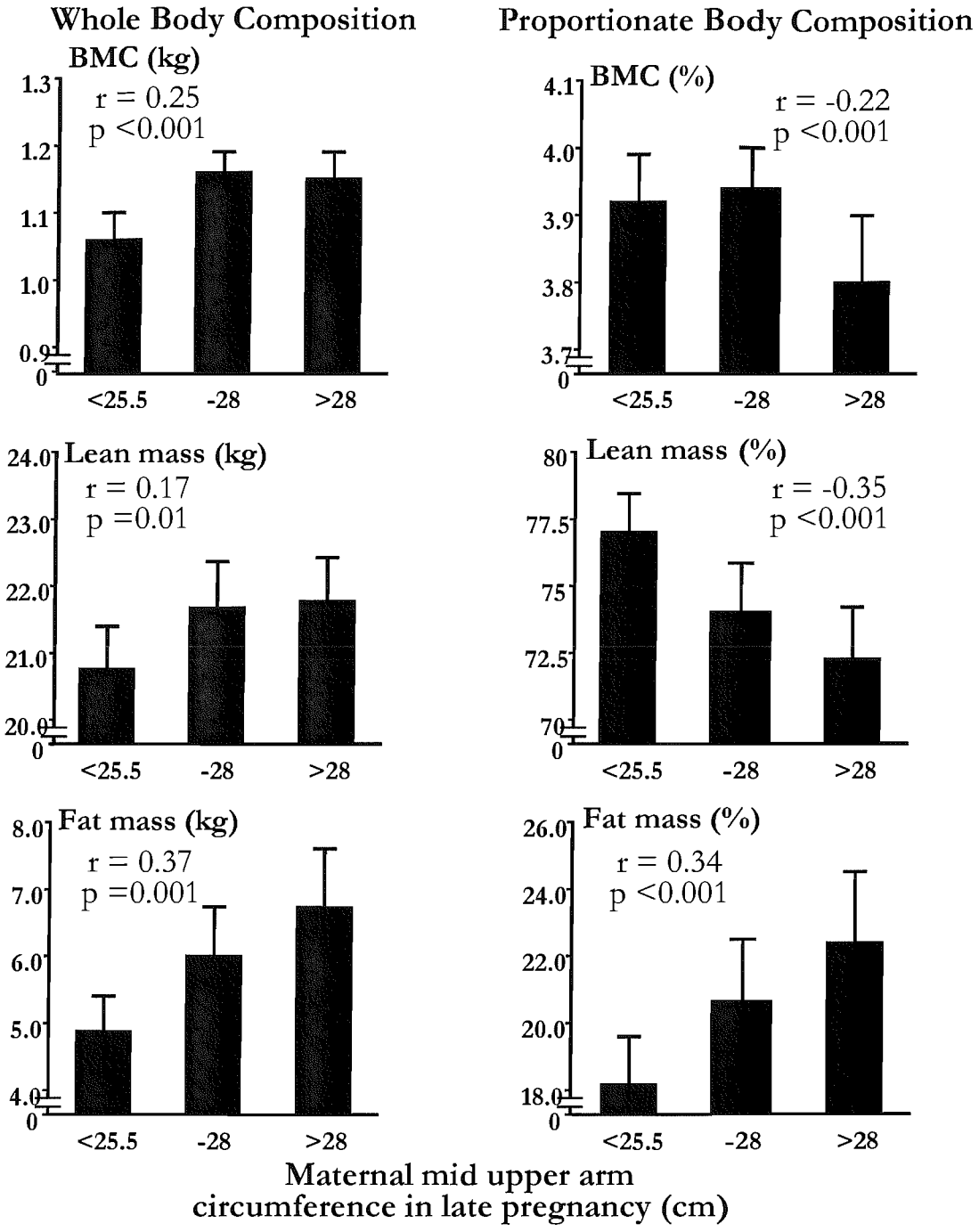
Parental Characteristic		BMC% (kg)	Lean mass% (cm^2)	Fat mass% (kg)
MATERNAL	n			
Height (m)				
<1.6	64	3.91%	73.6%	21.2%
-1.65	66	3.91%	76.4%	18.6%
>1.65	81	3.86%	73.7%	21.0%
R (p)		-0.02 (0.7)	-0.02 (0.8)	0.01 (0.9)
Pre pregnancy weight (kg)				
<55	66	3.96%	76.0%	19.0%
-62	73	3.89%	75.4%	19.4%
>62	75	3.81%	71.7%	23.0%
R (p)		-0.2 (0.003)	-0.26 (<0.001)	0.25 (<0.001)
Pre-pregnancy BMI(kg/m^2)				
<21	70	3.96%	76.6%	18.5%
-23	67	3.82%	74.4%	20.2%
>23	73	3.85%	72.7%	22.1%
r(p)		-0.18 (0.008)	-0.24 (<0.001)	0.24 (<0.001)
MUAC (cm) (late pregnancy)				
<25.5	68	3.92%	77.0%	18.2%
-28	73	3.94%	74.0%	20.6%
>28	71	3.80%	72.3%	22.3%
r (p)		-0.22 (0.001)	-0.35 (<0.001)	0.34 (<0.001)
Birthweight (kg)		BMC% (kg)	Lean mass% (cm^2)	Fat mass% (kg)
<3.0	59	3.89%	75.8%	19.0%
-3.6	83	3.91%	73.7%	21.1%
>3.6	56	3.84%	73.9%	20.8%
r(p)		-0.02 (0.8)	-0.04 (0.6)	0.05 (0.4)

PATERNAL				
Height (m)				
<1.72	45	3.83%	74.0%	20.5%
-1.8	84	3.89%	74.6%	20.3%
>1.8	79	3.92%	74.6%	20.2%
r(p)		0.13 (0.07)	0.03 (0.7)	-0.02 (0.8)
Birthweight (kg)				
<3.15	56	3.90%	76.0%	18.7%
-3.6	45	3.84%	74.0%	20.7%
>3.6	74	3.95%	74.8%	20.2%
r(p)		0.05 (0.5)	-0.02 (0.8)	0.03 (0.7)

Legend: Mean values for whole body BMC at nine years are shown for each third of distribution of each maternal characteristic. The partial correlation coefficient, r, with significance, (p), between each parental characteristic and WBBMC adjusted for the current age of the child are shown.

Of the derived estimates of whole body volumetric bone mineral density (vBMD), a weak negative relationship between whole body BMC adjusted for bone area (BMCa) and maternal height was observed (R^2=1.3%, $p<0.05$). No maternal predictors of whole body BMC adjusted using the Prentice method (170) (BMCp) could be demonstrated.

FIGURE 22 MATERNAL MUAC IN LATE PREGNANCY AND CHILDHOOD BODY COMPOSITION



Legend: Mean values for whole body BMC at nine years are shown for each third of distribution of birth weight, After adjustment of whole body BMC for current age of the child, the partial correlation coefficients, r, with significance, (p) are shown.

5.3.5.2 PARENTAL SMOKING

Maternal smoking status was recorded at the LMP before index pregnancy, early pregnancy, late pregnancy, infancy and at time of the child's DXA scan at nine years; changes in maternal smoking status are shown in Table 42 .

TABLE 42 CHANGING MATERNAL SMOKING STATUS

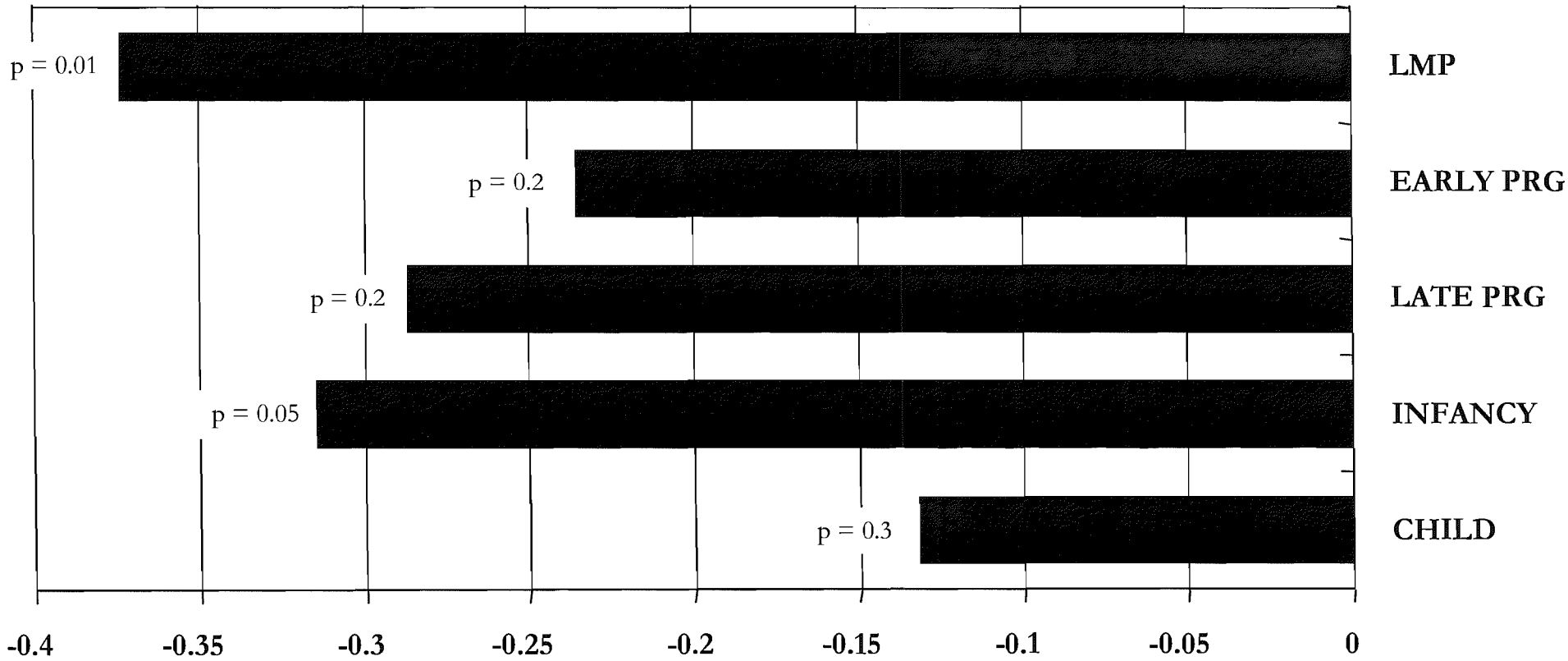
			Early	Late	Infancy	Childhood
		n	Yes	Yes	Yes	Yes
LMP	No	146	0%	0%	1%	20%
	Yes	69	64%	53%	72%	96%
Early	No	171		1%	8%	31%
	Yes	44		77%	86%	98%
Late	No	176			10%	35%
	Yes	36			97%	97%
Infancy	No	163				28%
	Yes	52				98%
Childhood	No	118				
	Yes	96				

Legend: Percentage Maternal smoking at different time points of study shown: LMP – at LMP of index pregnancy; EARLY – early pregnancy; LATE – late pregnancy; INFANCY- time of infant interview; CHILDHOOD – time childhood assessment of her child.

Reported maternal smoking was associated with lower whole body BMC measurements in their children (Figure 23). This reduction was significant in children whose mothers reported smoking at their LMP (-64g, p=0.01) and during infancy (-53g, p=0.05). Although mothers who smoked were significantly younger than non-smoking mums by 1.75 years ($p<0.001$) in pre-pregnancy, adding maternal age to the model did not weaken the effect of smoking.

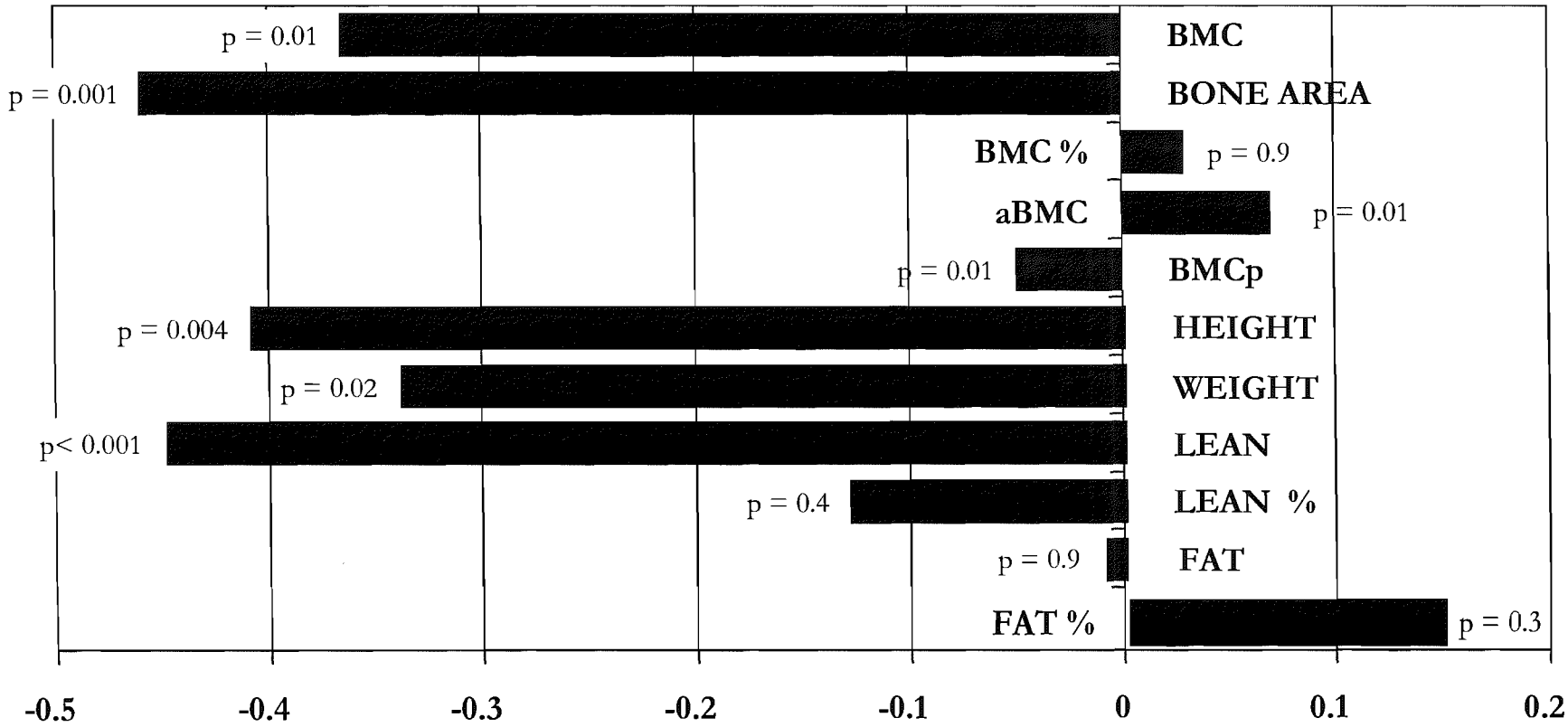
Smoking at the LMP of the index pregnancy was associated with a 0.18kg lower birth weight and adjusting for birth weight weakened the relationship between smoking at LMP and childhood WBBMC (unadjusted β= -0.064kg, p=0.01; adjusted β=-0.041kg, p=0.08).

FIGURE 23 MATERNAL SMOKING STATUS AT VARIOUS TIMEPOINTS AND REDUCTION IN CHILDHOOD BONE MASS



Standardized change (SD) in childhood whole body BMC in mothers who smoked compared to non smokers at the same time point of censure

FIGURE 24 MATERNAL SMOKING STATUS AT LMP AND BODY COMPOSITION OF HER CHILD



Standardized change (SD) in childhood whole body BMC in mothers who smoked compared to non smokers at the same time point of censure

As well as lower childhood BMC, maternal smoking was associated with lower whole body bone area and height but not size adjusted WBBMC suggesting an effect on size rather than mineral density. The current fat mass of the child was not associated with reported maternal smoking at LMP ($p=0.9$).

Maternal smoking status was not associated with proportionate childhood whole body BMC at nine years; however maternal smoking during pregnancy was associated with higher BMCa ($p<0.05$). Paternal smoking status was available only at the time of the childhood assessments and was not associated with the bone mass of the child at age nine years.

5.3.5.3 SOCIAL CLASS, EDUCATIONAL LEVEL AND PARITY

Lower social class was negatively correlated with childhood size. Compared to those in the highest social class, those born to mothers in the lowest social class at the time of the index pregnancy had a 6.6% lower childhood whole body BMC ($R^2=5\%$, $p<0.01$), 4.9% lower bone area ($R^2=5\%$, $p<0.01$), 1.7% lower height ($R^2=4\%$, $p<0.01$) and 4.0% lower weight ($R^2=2\%$, $p<0.05$) at nine years. Adding maternal smoking status weakened the effect of social class on nine-year body composition. Social class difference also exist in other lifestyle factors such as diet, physical activity; in addition phenotypic differences in height exist between the social classes which may also act to influence the bone mass of the offspring.

Mothers educational level was measured both at the time of pregnancy and at the time of her child's DXA scan at age nine years. The mother's educational level at the time the child was nine years, but not at index pregnancy, was weakly positively associated with childhood size. Compared to those in the highest educational level, those in the lowest level had a 2% lower childhood whole body bone area ($R^2=2\%$, $p<0.05$) and 2.6% lower height ($R^2=3\%$, $p<0.01$). Paternal educational level at the time of childhood assessment was not associated with any measure of childhood body composition.

Maternal parity, as recorded at the time of the index pregnancy, was weakly negatively associated with the current height of her child ($R^2=2\%$, $p<0.05$) but not any other measure of childhood body composition.

5.3.5.4 PARENTAL BIRTH WEIGHT

Maternal birth weight was significantly correlated with the whole body BMC (kg) (R^2=3%, p=0.009), bone area (R^2=3%, p=0.02), height (R^2=3%, p=0.02) and weight (R^2=3%, p=0.02) of her child at nine years of age (Table 40 , Table 41 . There was no significant influence of maternal birth weight on proportionate whole body BMC or estimates of volumetric bone mineral density, either BMCa or BMCp. Of the measures of childhood anthropometry, paternal birth weight was only significantly associated with whole body BMC (R^2=3%, p=0.03) and bone area (R^2=2%, p=0.05), however only 175 paternal birth weights were available, limiting the statistical power.

5.3.5.5 OTHER MATERNAL CHARACTERISTICS

A weak positive association was observed between late pregnancy maternal alcohol intake (y/n) and whole body BMC and bone area of her child at nine years. No significant persistent effect was seen of maternal alcohol intake (units/week) during pregnancy on whole body BMC or other anthropometric measurements of her child at nine years of age.

There was no significant relationship between maternal dietary protein, total energy, calcium or vitamin D intake in early or late pregnancy and the whole body BMC of her child at nine years old.

The mean age of menarche of the mothers was 13.1 years (SD 1.4; range 9-17). While age of menarche was positively correlated with maternal height (r=0.25, p<0.001), it was not predictive of childhood WBBMC.

5.3.6 INDEPENDENT MATERNAL PREDICTORS OF CHILDHOOD BONE MASS

In a multivariate linear model, the independent predictors of childhood whole body BMC were: maternal height, paternal height, maternal adiposity (pre-pregnant weight or late pregnancy MUAC) and maternal smoking at LMP (Table 43 . Maternal smoking status weakened the association between social class and childhood bone mass. The association between parental birthweight and childhood bone mass was weakened by including measures of parental height in the model. The independent predictors of childhood whole body bone area were similar to those for whole body BMC.

TABLE 43 INDEPENDENT PARENTAL PREDICTORS OF CHILDHOOD WBBMC

Maternal characteristics	
	β, p
Height (m)	8.3, <0.001
MUAC (mm)[1,2]	234, 0.02
Smoking [3]	-54.6, 0.02
R^2	*16.5%*

Legend: Maternal predictors for childhood whole body BMC adjusted for gestational age.
[1]Late pregnancy mid upper arm circumference
[2]Log Transformed
[3]time of last menstrual period

Smoking status during late pregnancy and maternal height were significant independent predictors of BMCa, however most of the variation in whole body BMC was accounted for by whole body bone area (R^2=90.2%), and adding maternal height and smoking status only marginally improved the model (R^2=90.3%). BMCp accounted for 91% of the variation in childhood whole body BMC and none of the recorded maternal characteristics were independently associated with BMCp.

Maternal smoking status at LMP, maternal height and paternal height, were the only independent predictors of the height of the child at nine years. Maternal height took account of the

associations between childhood height and maternal birth weight, pre-pregnant weight and parity. Smoking at LMP was a more robust predictor of childhood height than smoking status recorded at late pregnancy. Smoking at LMP together with maternal height accounted for the effect of social class on childhood height.

Childhood weight was predicted by parental heights and late pregnancy maternal MUAC. However, paternal measures of adiposity were not recorded in this part of the study and we are therefore unable to comment on the influence of paternal adiposity. Maternal MUAC measurements weakened the association between maternal smoking and children's weight.

Measures of maternal adiposity (late pregnancy MUAC, pre-pregnant weight or pre-pregnancy BMI) were the principal determinants of proportionate childhood body composition. These measures of maternal size were negatively related to proportionate BMC and lean mass but positively associated with measures of proportionate whole body fat mass. Maternal smoking status at late pregnancy was also independently negatively associated with proportionate lean mass and positively with fat mass proportions.

5.3.7 UMBILICAL VEIN CALCIUM CONCENTRATION

Of the 215 children, 156 (89 boys) had cord serum markers of calcium homeostasis measured at birth. These included calcium, albumin, phosphate and total alkaline phosphatase (Table 44).

TABLE 44 UMBILICAL CORD CONSTITUENTS OF CALCIUM HOMEOSTASIS

Umbilical Vein Concentration	Boys n=89	Girls n=67	p value
Calcium (mmol/l)	2.70 (0.01)	2.71 (0.02)	0.7
Albumin (g/l)	32.8 (3.1)	34.3 (3.2)	0.005
Corrected calcium[3]	2.75 (0.13)	2.75 (0.14)	0.8
Phosphate (mmol/l)	1.80 (0.26)	1.79 (0.23)	0.6
Alkaline phosphate (iu/l)	335 (110)	376 (12)	0.03

Legend: Mean (SD) shown. P-values contrast levels in boys and girls.

Both umbilical cord calcium (β= 0.03 per SD, R^2 5.8%, p=0.002) and albumin concentrations (β= 0.014 per SD, R^2 3.6%, p=0.02) were significantly predictive of whole body BMC (kg) at age nine years. After adjustment for gestational age, cord albumin concentrations no longer predicted childhood BMC (Table 45). No significant association was observed between childhood WBBMC and umbilical venous concentrations of phosphate, alkaline phosphatase or creatinine.

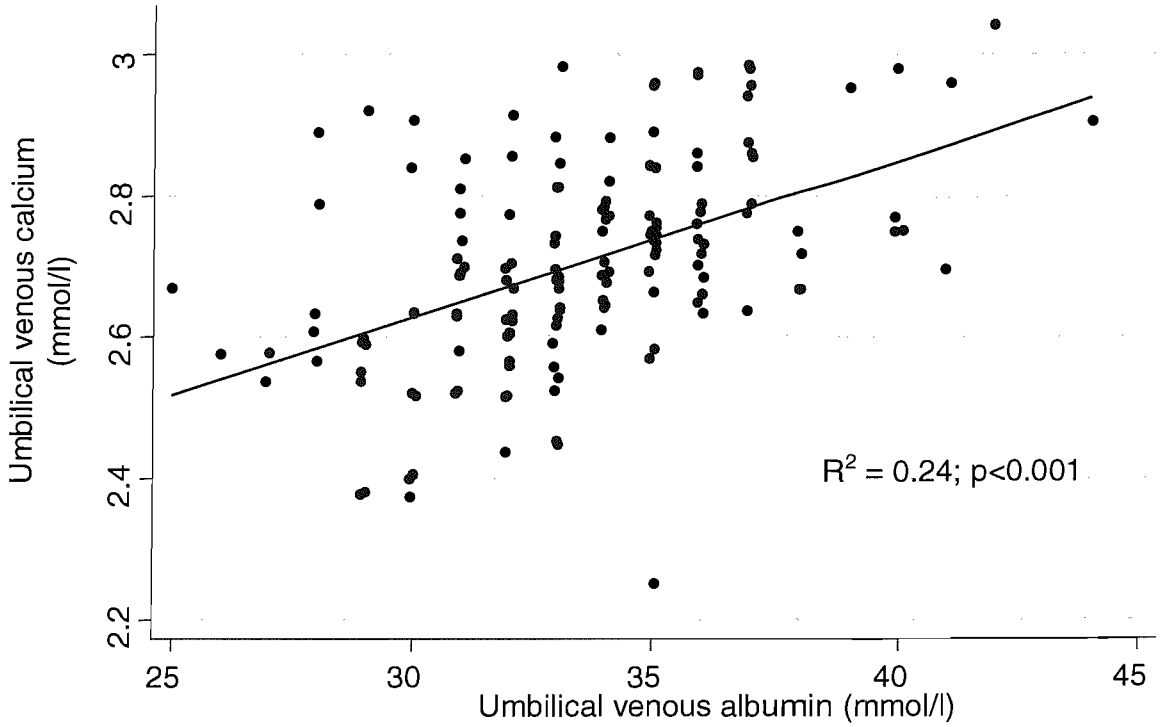
TABLE 45 RELATIONSHIP BETWEEN UMBILICAL VEIN CONSTITUENTS OF CALCIUM HOMEOSTASIS AND CHILDHOOD WHOLE BODY BONE MASS.

Umbilical vein blood	Combined [β, R^2 (p)]	Boys n= 89 [β, R^2 (p)]	Girls n= 67 [β, R^2 (p)]
Calcium (mmol/l)	30, 2.8%, (0.04)	21, 1.2% (0.3)	41, 7.5% (0.03)
Albumin (mmol/l)	15, 0.7%, (0.3)	44, 4.6% (0.03)	5.5, 0.1% (0.77)
Phosphate (mmol/l)	1.5, 0.0%, (0.9)	-19, 1.2% (0.3)	30.5 3.2% (0.1)
Alkaline phosphatase (iu/l)	-3.8, 0.0%, (0.9)	36, 3.3% (0.09)	-32, 4.4% (0.09)

Legend: The β per SD and percentage variance in WBBMC (R^2) accounted for by each umbilical vein constituent is shown with significance level (p). WBBMC was adjusted for current age and the umbilical vein constituents were adjusted for gestational age at birth.

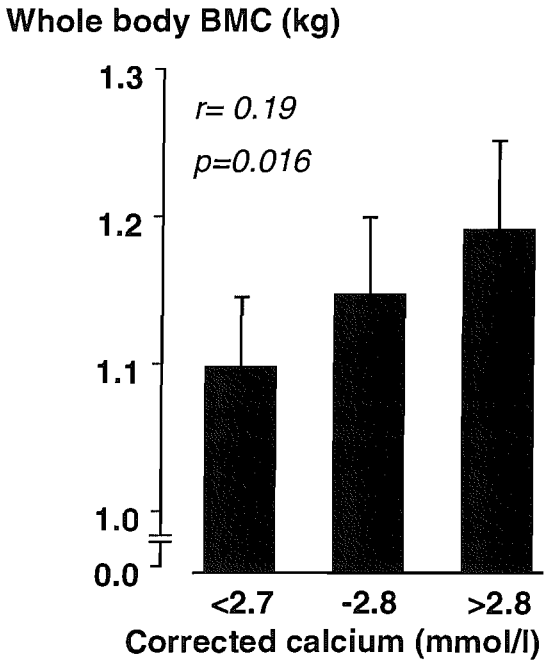
As venous concentrations of both albumin and calcium were highly correlated (Figure 25 , albumin corrected venous calcium concentrations (coca) were derived using linear regression (coca = 0.89x cord calcium + 0.34).

FIGURE 25 RELATIONSHIP BETWEEN UMBILICAL CORD CALCIUM AND ALBUMIN CONCENTRATIONS



Derived albumin corrected calcium concentration in the umbilical vein (coca) remained as the significant predictor of childhood WBBMC (Figure 26). This relationship was significant in girls (girls: R^2= 9.3%, p=0.01; boys: R^2= 0.4%, p=0.5).

FIGURE 26 RELATIONSHIP BETWEEN ALBUMIN ADJUSTED CORD SERUM CALCIUM CONCENTRATIONS AND WHOLE BODY BONE MINERAL CONTENT IN CHILDREN AGED NINE YEARS.



Legend: Mean (95% CI) are shown for whole body BMC at nine years of age by thirds of umbilical vein calcium concentration adjusted for albumin concentration. Pearson correlation coefficient r (p) and significance level, after adjustment for gestational age and current age, is shown.

Umbilical venous calcium was also positively associated with both birth weight and birth length (Table 46). This relationship was significant in girls and remained significant after adjustment for gestational age; however, there was no significant difference in the slopes of the regression lines between the genders.

TABLE 46 RELATIONSHIP BETWEEN UMBILICAL VENOUS CALCIUM CONCENTRATION AND BIRTHSIZE BY GENDER

Neonatal size	Boys	Girls
	n=89	n=67
	[β, R^2 (p)]	[β, R^2 (p)]
Birth weight (g)	48.1, 1.1% (0.3)	173, 16% (0.001)
Birth length (cm)	0.05, 0.0% (0.8)	0.62, 14.7% (0.002)

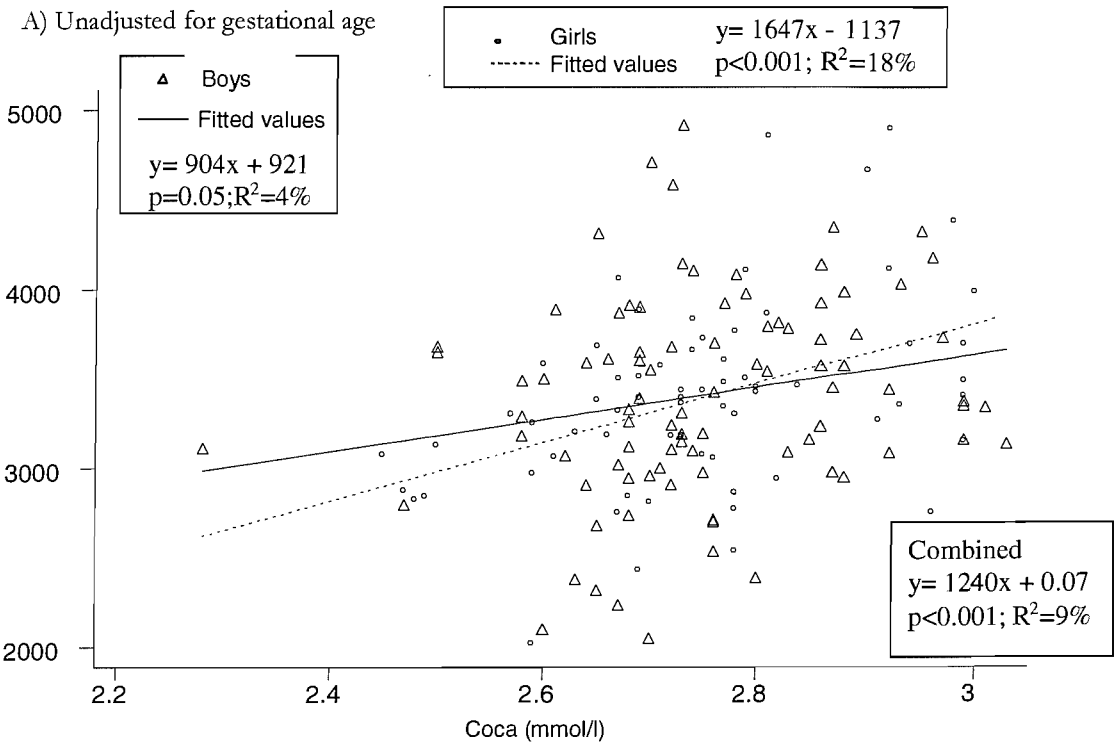
Legend: The β per SD and percentage variation, with significance level (p), in birth size accounted for by albumin adjusted cord calcium concentration adjusted for gestational age.

As birth size also predicted childhood size, we tested whether the relationship between coca and childhood WBBMC was independent of birth weight. Approximately, 31% of the association between coca and childhood WBBMC was independent of birth weight and 54% independent of birth length.

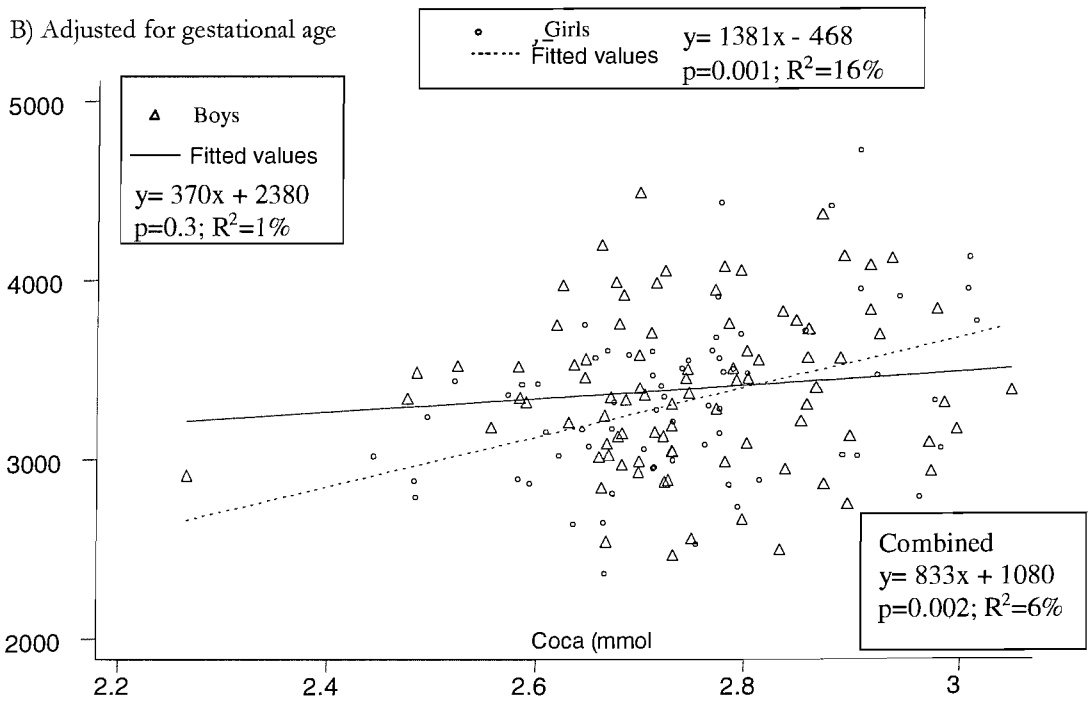
FIGURE 27 RELATIONSHIP BETWEEN UMBILICAL VENOUS CALCIUM CORRECTED FOR ALBUMIN LEVELS AND BIRTH SIZE BY GENDER

BIRTH WEIGHT (g)

A) Unadjusted for gestational age

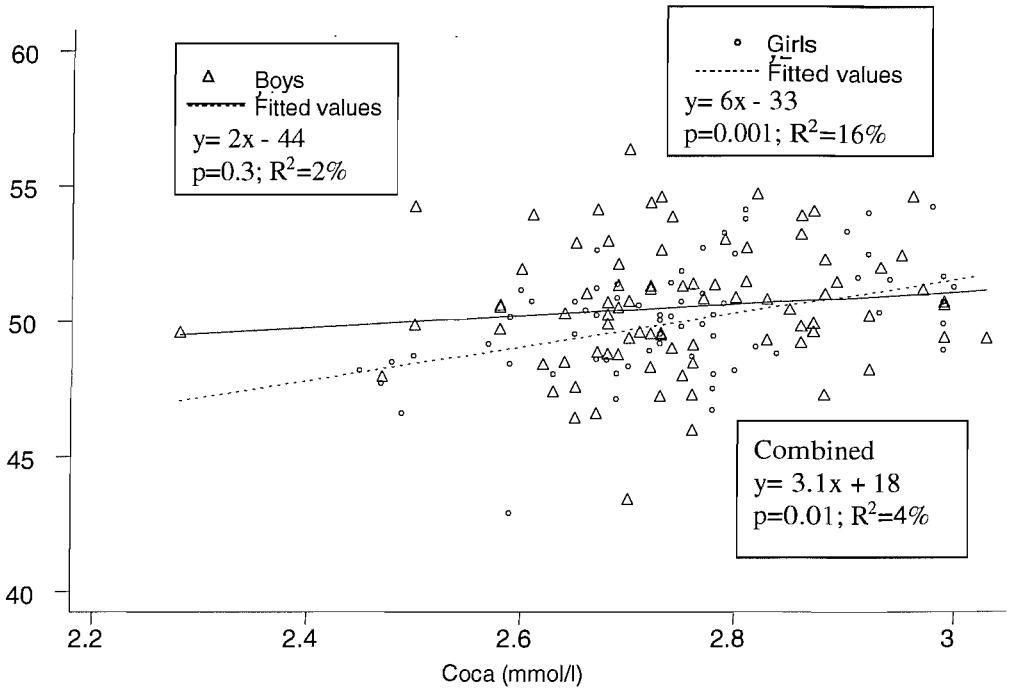


B) Adjusted for gestational age

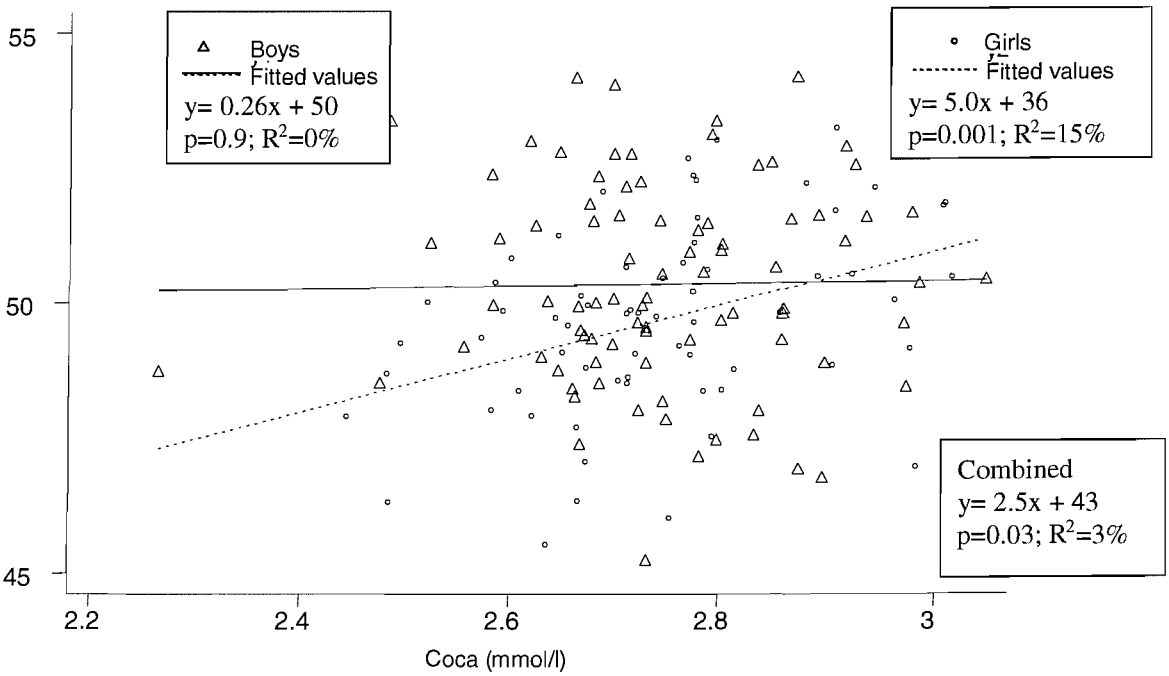


CROWN HEEL LENGTH (cm)

A) Unadjusted for gestational age



B) Adjusted for gestational age



Legend: Umbilical venous calcium concentration adjusted for albumin and birth weight and length. Regression equations with significance and %variance shown for each gender and combined.

Umbilical venous calcium concentration also predicted other measures of childhood size (Table 47).

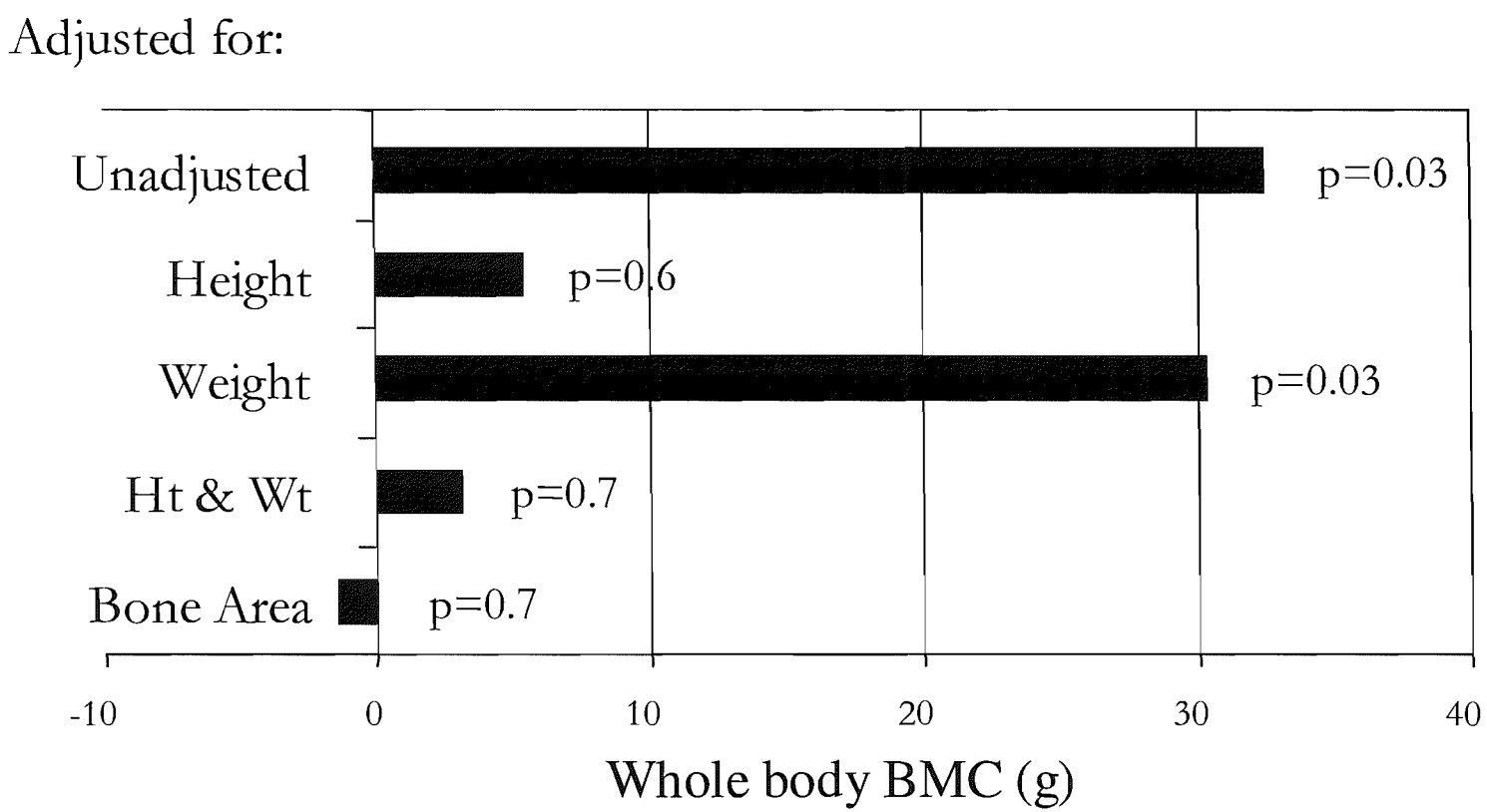
TABLE 47 RELATIONSHIP BETWEEN CHILDHOOD SIZE AND UMBILICAL VENOUS CALCIUM CONCENTRATION

Childhood Size	Boys n=89 [β, R^2 (p)]	Girls n=67 [β, R^2 (p)]
Height (m)	0.008, 0.0% (0.9)	2.3, 15.5% (0.001)
Weight (kg)[1]	0.008, 0.0% (0.7)	0.05, 8.4% (0.02)
Bone area	7.4, 0.0% (0.65)	42.0 11.1% (0.006)

Legend: The β per SD change in umbilical cord calcium concentration adjusted for gestational age albumin shown with variance R^2 and significance level (p)are shown.
[1]Log transformed

As WBBMC measurements were also highly correlated with other measures of body size such as height and weight, the relationship between coca and WBBMC was adjusted for differences in childhood height and weight (Figure 28). As shown by the figure, the relationship between coca and childhood WBBMC is principally accounted for by measures of childhood height, suggesting the umbilical venous calcium predicts linear bone growth rather than volumetric bone mineral density.

FIGURE 28 RELATIONSHIP BETWEEN ALBUMIN CORRECTED CORD CALCIUM CONCENTRATION AND CHILDHOOD WHOLE BODY BMC AFTER ADJUSTMENT FOR CHILDHOOD HEIGHT, WEIGHT AND BONE AREA.



Legend: Beta coefficients for one SD increment in umbilical vein calcium on childhood WBBMC unadjusted and after adjustment for current childhood height, weight, height & weight and bone area.

Using multiple linear regression modelling, umbilical venous calcium did not appear to influence the relationship of the parental determinants with childhood WBBMC (Table 48). There was a small non-significant attenuation of the coefficients for maternal fat mass (-15%; p=0.9) and smoking (-11%; p=0.9).

TABLE 48 INDEPENDENT PREDICTORS OF CHILDHOOD WHOLE BODY BONE MASS IN CHILDREN WITH AND WITHOUT CORD CALCIUM RESULTS AVAILABLE

	All children n=215	Children with cord measurements unadjusted n=156	Children with cord measurements adjusted for calcium (n=156)
Maternal Height	7.2 (<0.001)	8.4 (<0.001)	8.1 (<0.001)
Paternal Height	5.2 (<0.001)	5.2 (0.003)	4.9 (0.006)
Maternal fat mass[1]	29.9 (0.007)	23.4 (0.1)	19.8 (0.2)
Maternal smoking[2]	- 47.8 (0.04)	-42.6 (0.1)	-37.7 (0.2)
R^2	22.1% (20.5%)	21.4% (19.3%)	23.4% (20.8%)

Legend: β coefficients with significance p shown for the independent predictors of whole body BMC.
[1]as measured by logged mid upper arm circumference
[2]at time of last menstrual period
[3]Adjusted for umbilical venous albumin concentration
R^2 and R^2 adjusted for variance in predictors in parenthesis, are also shown

5.3.8 CONDITIONAL BIRTH AND INFANT DETERMINANTS OF WHOLE BODY BMC IN CHILDHOOD

5.3.8.1 INTRODUCTION AND METHODS

It was not clear if parental characteristics were influencing childhood bone mass by changing prenatal growth or having independent effects on post natal growth. The measurement of infant length and weight at nine months enabled modelling the effects of parental factors on childhood bone mass independent of effects on intrauterine growth. In the conditional models, growth was divided into three phases: intrauterine, infancy (from birth to nine month assessment) and childhood (from infant to childhood assessment). Infant height and weight were adjusted for birth length and weight respectively using the residual regression method. For example, infant length gain was derived from the residual of infant length regressed on birth length. Using a similar method, size at time of the childhood assessment was adjusted for infant size.

Increasing gestational age at birth had significant ($p<0.001$) negative influences on conditional estimates of infancy weight and height gain. However, there were no effects of gestational age on conditional childhood weight and height gain, hence only the models of intrauterine and infant growth were adjusted for gestational age at time of birth.

5.3.8.2 RESULTS

From the conditional models of childhood growth, the effect of birth length on childhood WBBMC were no longer significant after length in infancy or height at nine years were taken into account (Table 49). Birth weight; however, appeared to have persisting influences on childhood BMC even after adjustment for subsequent weight gain. Similarly there was a persisting effect of infant weight, but not length, on childhood bone mass.

TABLE 49 RELATIONSHIP BETWEEN CHILDHOOD WHOLE BODY BONE MASS AND CONDITIONAL INFANT/BIRTH SIZE

	Childhood whole body BMC z-score		
	Unadjusted	Adjusted for infant size	Adjusted for child hood size
Birth weight	0.35 (<0.001)	0.12 (0.09)	0.09 (0.02)
Infant weight	0.51 (<0.001)	-	0.18 (<0.001)
Birth length	0.38 (<0.001)	0.07 (0.41)	0.0 (0.99)
Infant length	0.51 (<0.001)	-	0.0 (0.97)

Legend: The SD change (p value) in childhood WBBMC per SD change in predictor, both unadjusted and adjusted for subsequent growth are shown.

5.3.8.3 DETERMINANTS OF CONDITIONAL HEIGHT GAIN

Maternal height, maternal smoking status at late pregnancy and parity were the mutually independent predictors of crown-rump-heel length at birth (Figure 29). Paternal height also independently predicted infant length; however in the multivariate model of infant length, paternal birth weight and not paternal height remained as the significant independent determinant. Maternal pre-pregnancy weight and MUAC in late pregnancy were no longer associated with birth length when maternal height was entered in the model. Smoking status in late pregnancy weakened the association of mother's social class on birth length. Despite the significant relationship between maternal height and birth length, there was no relationship between paternal height and the birth length of the child (p=0.3).

Parity was not a determinant of conditional infant linear growth. Both maternal and paternal height independently predicted conditional linear growth from birth to age nine months and from nine months to nine years. There was a positive association between conditional infant height gain and maternal age at menarche (p=0.006), but this was no longer significant when maternal height was introduced into the model as maternal height and age of menarche were significantly correlated (r= 0.19, p=0.005).

Maternal parity and maternal smoking status during infancy were negatively associated with childhood height gain. The positive univariate association between breast-feeding and childhood height gain was no longer apparent when maternal smoking status was added to the model.

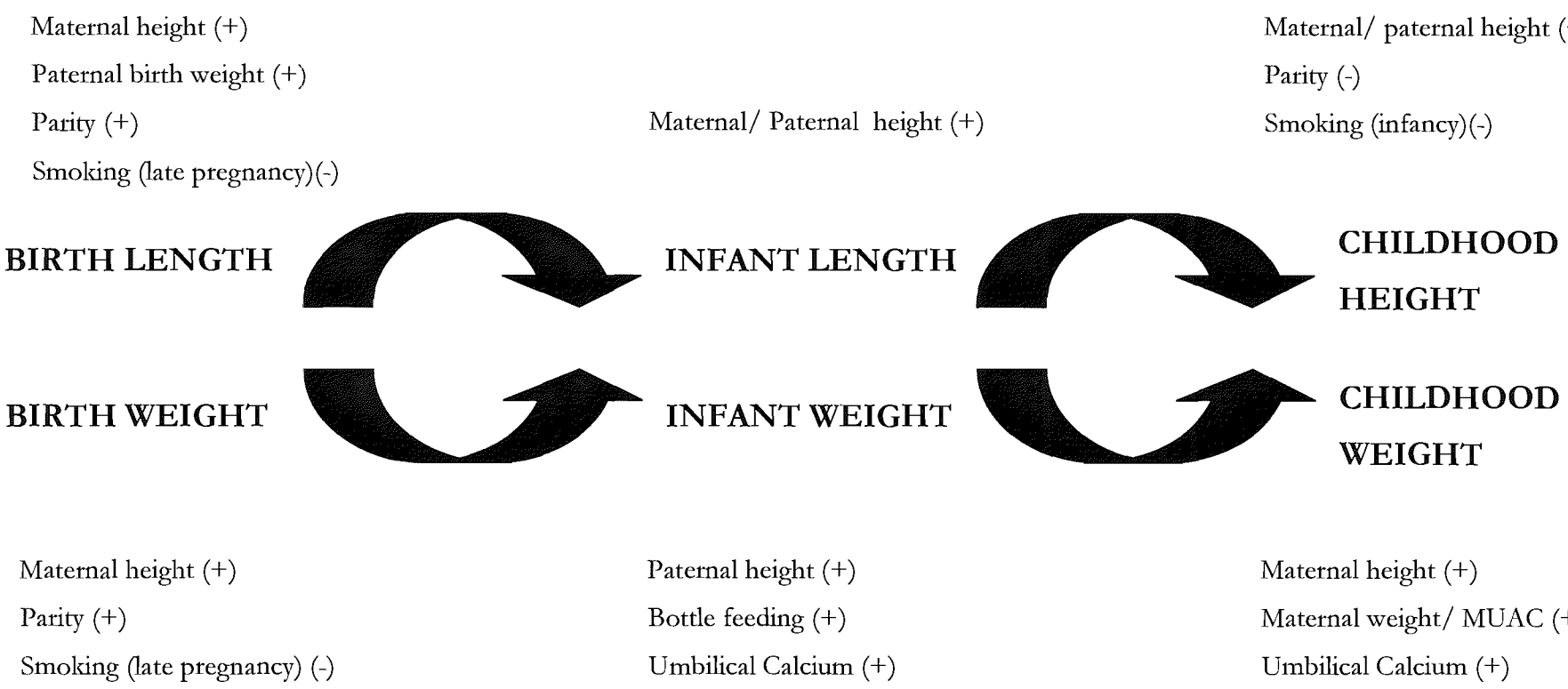
5.3.8.3.1 CONDITIONAL WEIGHT GAIN

Maternal height, parity and smoking were the principal independent determinants of birth weight (Figure 29). Maternal height accounted for the relationship between maternal pre-pregnant weight, MUAC measurements and maternal birthweight on the child's birth weight. Smoking status removed the effect of social class on birth weight.

Paternal, but not maternal height, independently predicted conditional infant weight gain. Other significant independent predictors were bottle-feeding and age of menarche. Maternal parity was not associated with infant weight gain. In addition, maternal height was no longer significantly associated with conditional infant weight gain once menarchal age or breast-feeding status was added to the model. The concentration of unadjusted calcium in the umbilical cord was weakly associated with conditional infant weight gain ($p=0.027$). However this was no longer significant after adjustment for the concentration of albumin.

While maternal height had a positive influence on conditional childhood weight gain from nine months to age nine years, the effect of paternal height was no longer statistically significant ($p=0.08$). Other significant ($p<0.05$) predictors of conditional childhood weight gain were maternal pre-pregnant weight and MUAC measurements during late pregnancy and albumin corrected umbilical venous calcium concentrations. The effect of maternal smoking during the LMP of the index pregnancy on childhood conditional weight gain was no longer significant when maternal adiposity was added to the model.

FIGURE 29 SUMMARY INDPENDENT PREDICTORS OF CONDITIONAL GROWTH DURING CHILDHOOD.



Legend: Independent determinants shown with direction of association in parenthesis.

5.3.9 RELATIONSHIP BETWEEN MATERNAL ANTHROPOMETRY AND NEONATAL AND CHILDHOOD BODY COMPOSITION

We now wished to compare and contrast the relationship between maternal height and adiposity with both neonatal and childhood bone mass. As the only measurement of maternal adiposity in late pregnancy in the children's cohort was MUAC, which encompasses not only lean but also fat and bone size, maternal adiposity in late pregnancy was estimated by deriving MUAC adjusted for maternal height in a linear model. The body composition of the offspring were compared unadjusted and after adjusting for length/height and weight, to give an estimate of size corrected bone mass.

5.3.9.1 MATERNAL HEIGHT

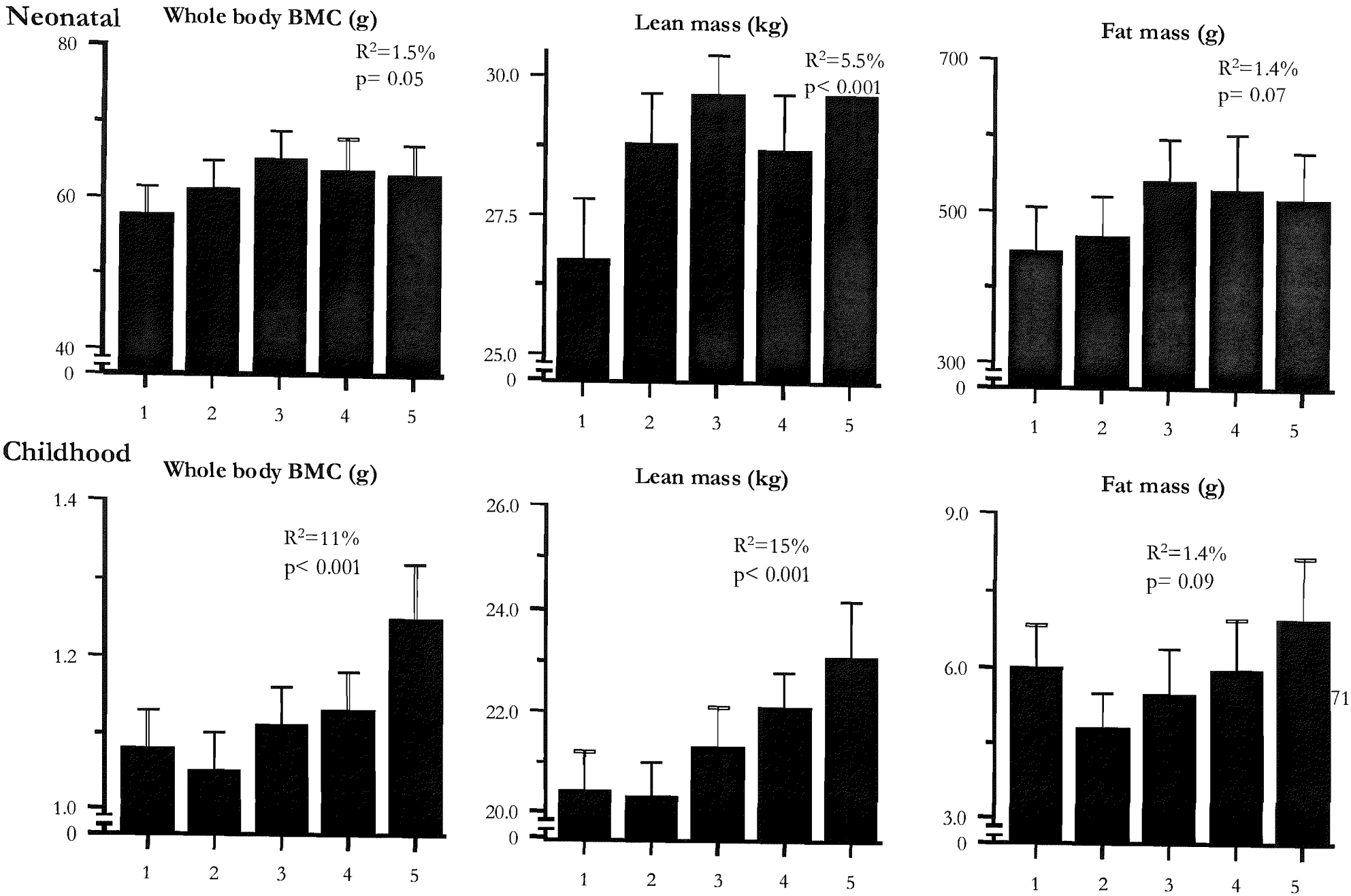
Maternal height predicted both neonatal and childhood bone and lean but not fat mass. The associations were strongest for lean mass and for childhood body composition. The associations with neonatal bone and lean mass were no longer significant after adjusting for length, while the childhood associations while attenuated remained statistically significant. There was no significant relationship between maternal height and weight-adjusted body composition of her offspring at any site.

5.3.9.2 MATERNAL ADIPOSITY

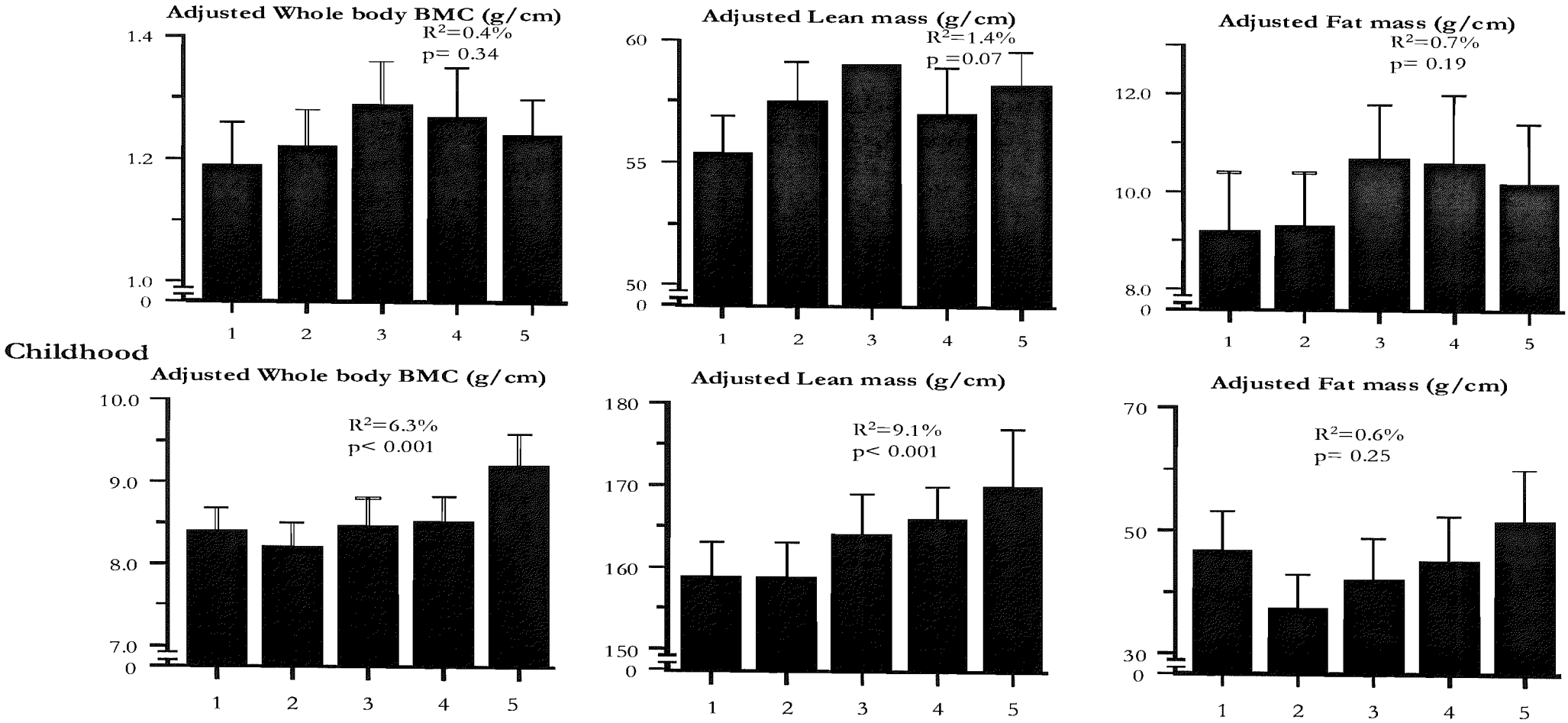
Higher maternal adiposity in later pregnancy was predicted greater neonatal and childhood bone and fat mass with no effect on lean mass both in childhood and neonatal life. The effect on fat mass appeared stronger in childhood for fat mass, with little difference in bone mass during either neonatal life or childhood. These relationships persisted after adjusting the offspring's

body composition for height and weight. However, after adjusting for lean mass for weight, a significant negative association was sign with maternal late pregnancy adjusted MUAC.

FIGURE 30 NEONATAL AND CHILDHOOD BODY COMPOSITION BY EQUAL FIFTHS OF MATERNAL HEIGHT



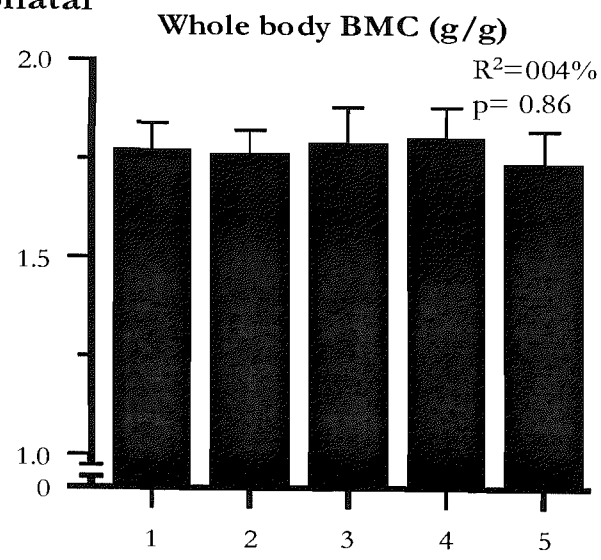
Neonatal and childhood body composition per unit length by equal fifths of maternal height

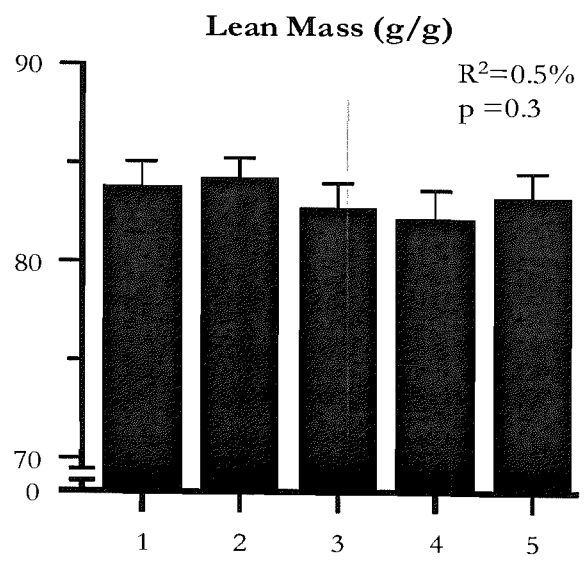


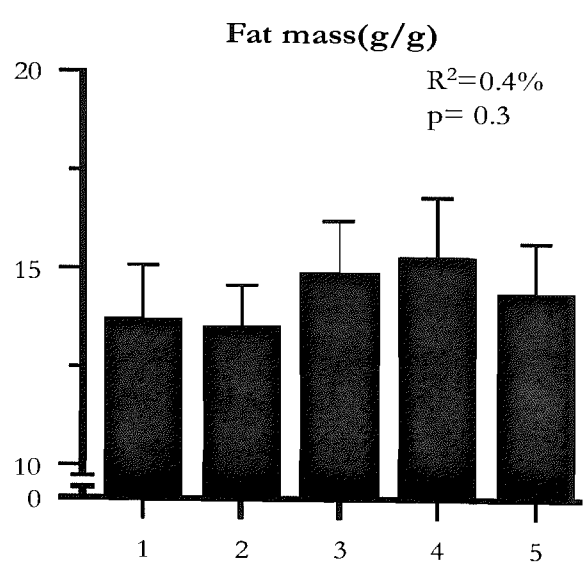
Legend: Mean (95% CI) shown for offspring unadjusted and adjusted body composition by equal fifths of maternal height. R^2 (p) shown after adjustment for gestational age or current childhood age.

Neonatal and childhood body composition per unit weight by equal fifths of maternal height

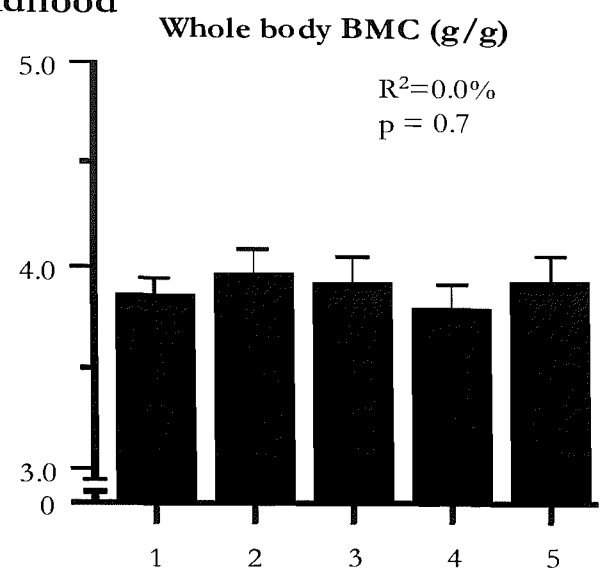
Neonatal

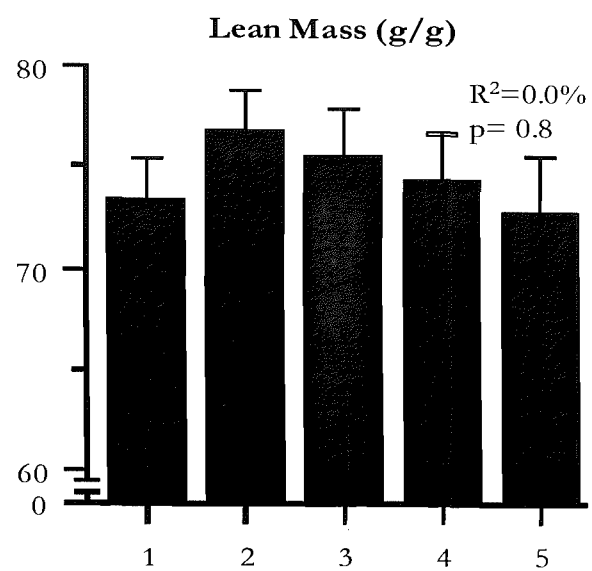






Childhood





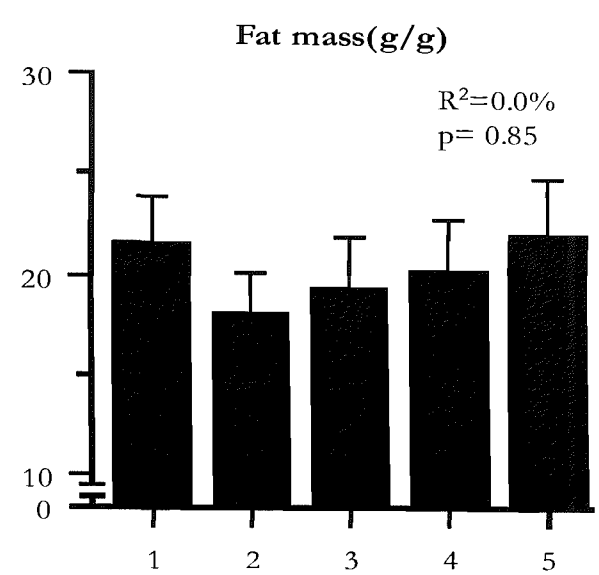
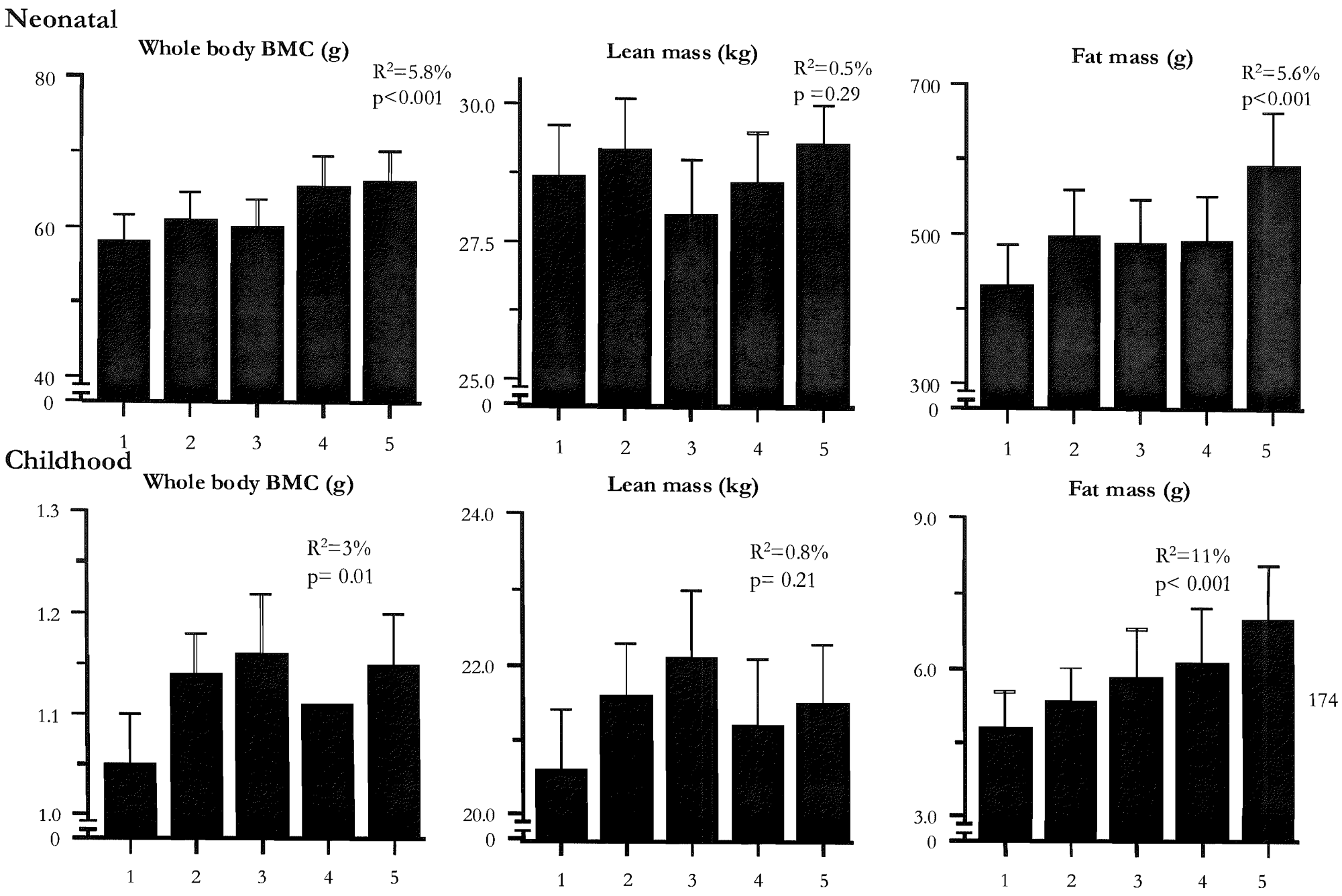
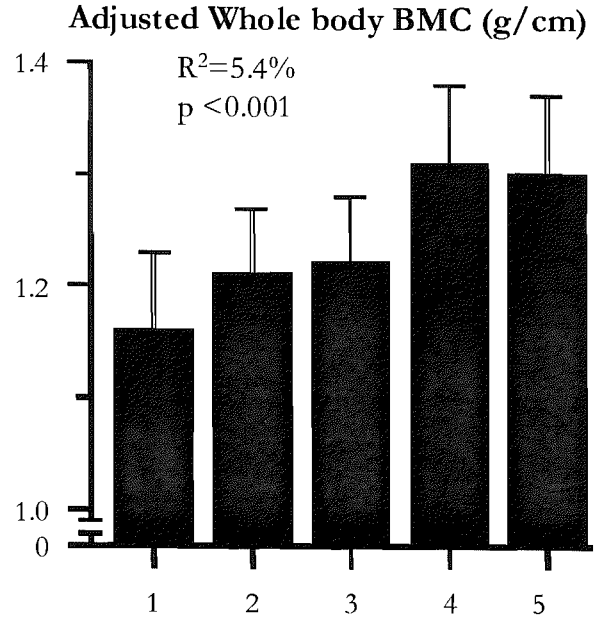


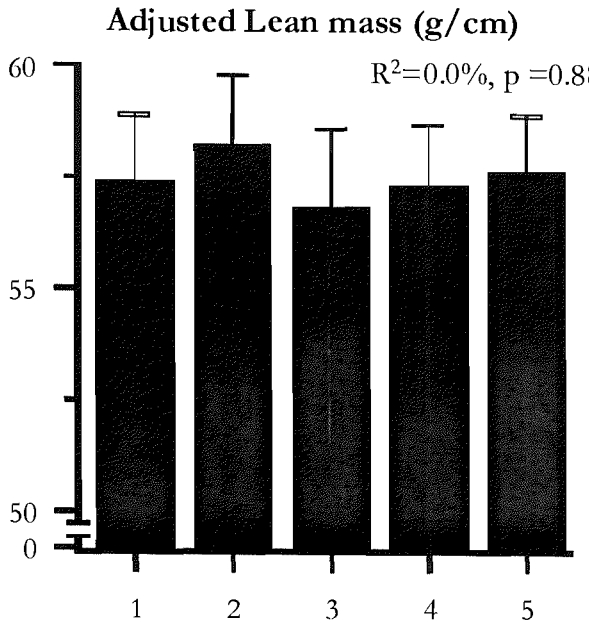
FIGURE 31 NEONATAL AND CHILDHOOD BODY COMPOSITION BY EQUAL FIFTHS OF MATERNAL LATE PREGNANCY MUAC ADJUSTED FOR HEIGHT

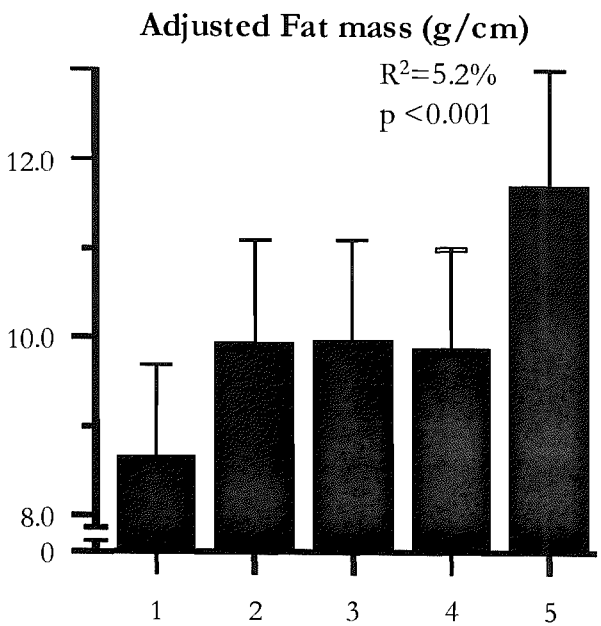


Neonatal and childhood body composition per unit length by equal fifths of maternal late pregnancy MAUC adjusted for height

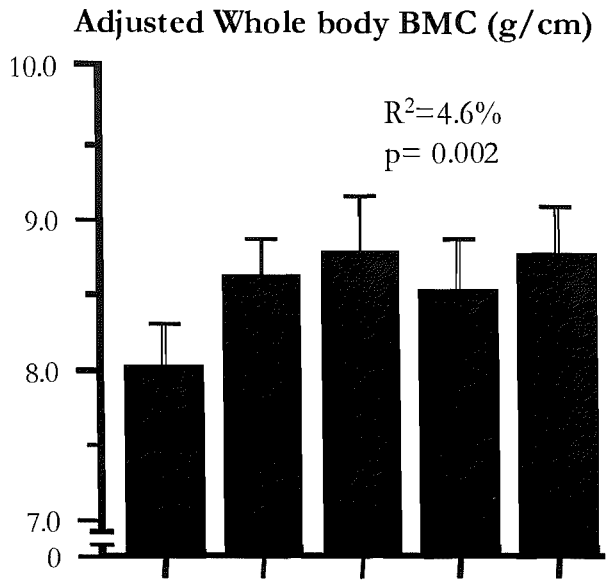
Neonatal

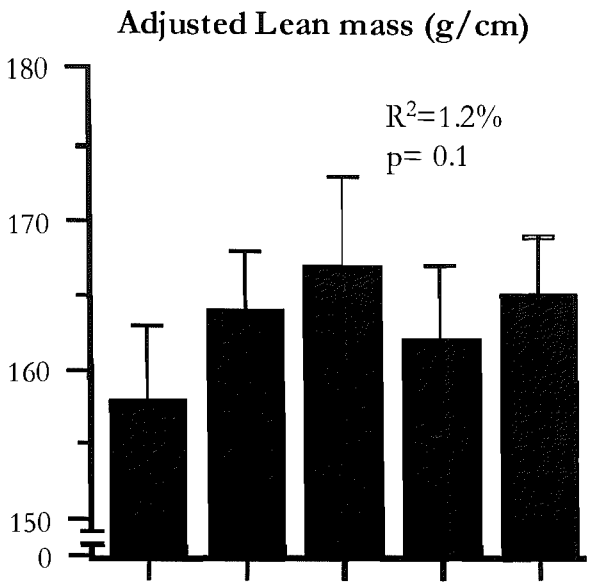


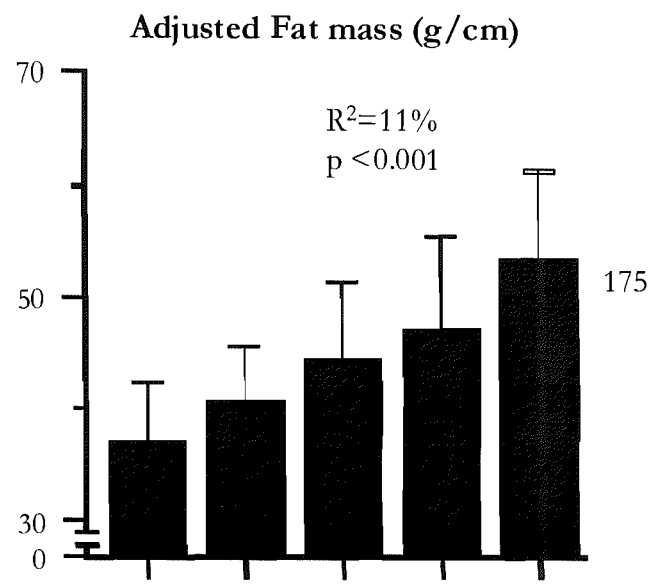




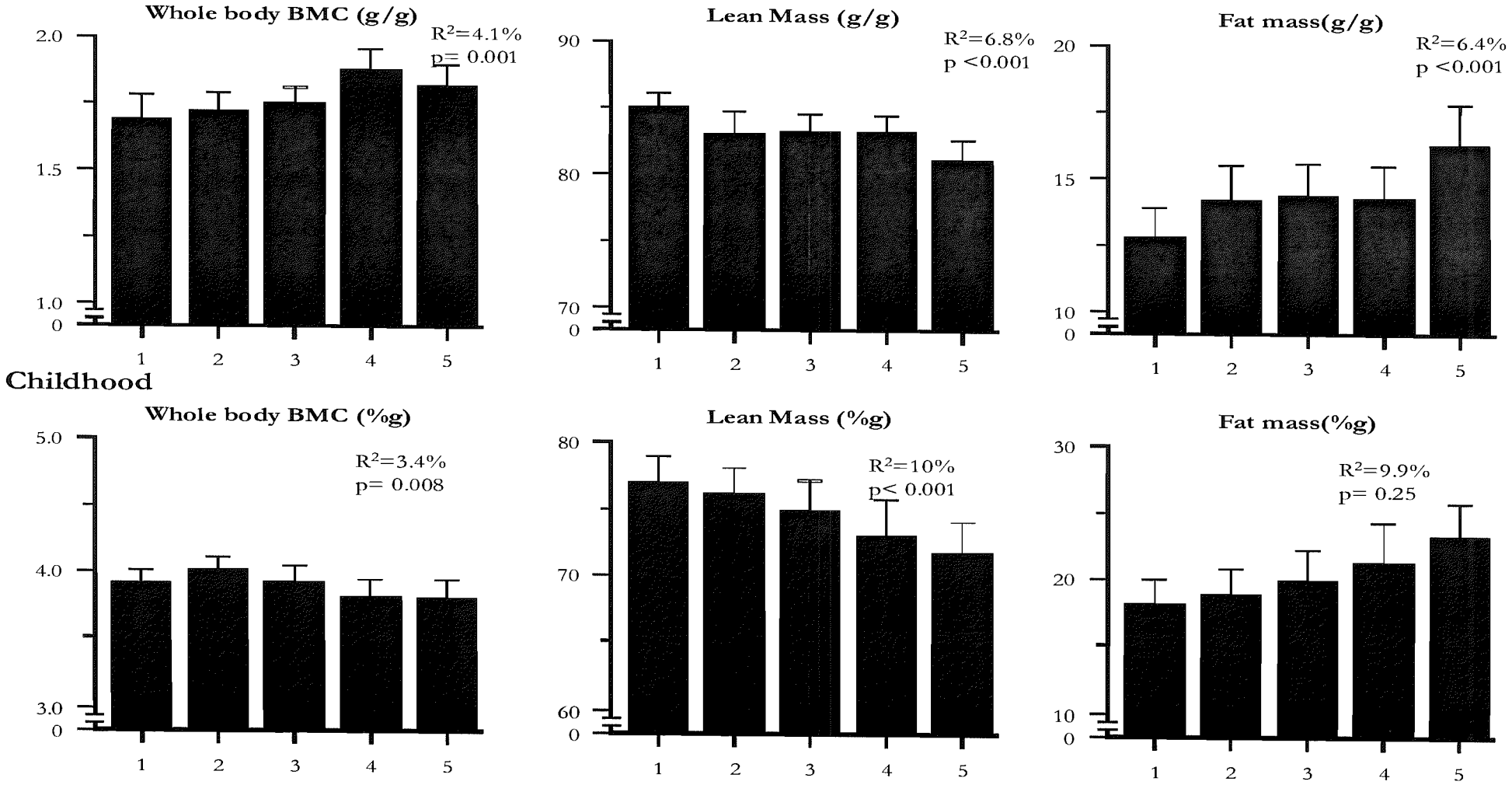
Childhood







Neonatal and childhood body composition per unit weight by equal fifths of maternal late pregnancy MAUC adjusted for height



Legend: Mean (95% CI) shown for offspring body composition by equal fifths of maternal MUAC adjusted for height. R^2 (p) shown after adjustment for gestational age or current childhood age.

5.4 DISCUSSION

The results of this study support a role for the prenatal maternal environment in determining the trajectory of bone mineral accrual postnatally, during childhood. Thus, the positive relationship between birth weight and whole body BMC that we have previously reported (66) was found to persist to age nine years; it also remained after adjustment for gestational age. In like manner, the previously demonstrated determinants of neonatal bone mass such as maternal fat stores and smoking during pregnancy, retained their influence on bone mass in later childhood. Finally, the study demonstrated that umbilical venous ionised calcium concentration was strongly correlated with whole body BMC some nine years later.

We, and others, have previously shown that weight at birth and, more strongly, weight at one year predict bone mass in later life (37). These relationships are independent of known genetic and adult environmental determinants of bone mass (39). Postnatal feeding patterns have been linked with infant weight and bone mass in childhood (132), but none of the follow-up studies of infants born at term have found significant associations between the type of feeding and bone mass in later adult life. Mathematical analyses of growth after birth suggest the transition between foetal and childhood phases occurs at around age one year, and that infant growth rates are strongly influenced by the trajectory of intrauterine growth (171). These observations suggest that influences which determine the foetal phase of growth may have longer term implications for the risk of osteoporosis. Our study provides direct evidence that intrauterine growth, as reflected in anthropometric dimensions at birth, is significantly correlated with bone mineral accrual at age nine years.

The mechanisms underlying the long-term effect of the intrauterine environment are not known, but include the foetal programming of endocrine systems which influence skeletal metabolism, and the persisting effects of altered skeletal growth and development in utero. There is a growing body of evidence suggesting that these effects are mediated through epigenetic mechanisms, such as the methylation status of imprinted genes that regulate foetal and placental growth, as well as specific placental transport systems (172). There are two broad mechanisms for epigenetic

inheritance: an extrinsic or intrinsic environmental insult or series of insults altering the epigenetic profile of the gamete at the time of gametogenesis. For example in girls, during oocytogenesis during her intrauterine development, a low vitamin D level (extrinsic) may alter the epigenetic profile of her oocytes which would then lead to altered phenotype expression of her offspring. If the epigenetic profile of the gametes could be altered in the post gametogenesis phase by either altering stochastic methylation or by more targeted remethylation, then the critical period for environmental insults would be longer. In an intrinsic model, the epigenetic phenotype of the parent would be for example the behaviour of parental grooming of the offspring, which would alter the epigenetic status of the offspring such that the offspring repeating the determining environmental phenotype, grooming, for their offspring in subsequent generations. Animal models are also consistent with the programming of skeletal growth; maternal undernutrition during pregnancy reduces bone size and alters the trabecular architecture of metaphyseal bone in the offspring (173). Furthermore, the offspring of undernourished pregnant rats have abnormal growth plate architecture (174) and reduced sensitivity of mesenchymal osteoblast precursor cells to growth promoters such as 1,25 $(OH)_2$ vitamin D and IGF-1 (175).

We observed that the concentration of calcium in umbilical venous blood predicted post natal bone mineral accrual, suggesting a role for intra uterine foetal calcium metabolism. The foetus accumulates approximately 30g of calcium from the mother in utero, and 80 per cent of this transfer occurs in the last trimester of pregnancy (176). The maternal capacity to supply the foetus with calcium is dependent on many factors including maternal calcium intake and vitamin D status; intestinal calcium absorption; maternal bone turnover; maternal renal function; and the capacity for placental calcium transfer (150). Of these, placental calcium transport is the critical final step in determining foetal supply; previous studies have documented an association between poor placental function and reduced total body calcium in utero (30).

In animal models, transfer of calcium through the placenta is bi-directional, with an estimated active transport of around 40 μmol/min/g of placenta, and a similar rate of passive transport (177). The active component of transport involves uptake by placental trophoblasts through specific calcium transporter channels; within the trophoblasts, calcium is bound to calbindin D_{9k} before being pumped into the foetal circulation by both Ca/ATPase and Na/Ca exchanges (178).

The mechanism by which hormones such as 1, 25 $(OH)_2$ vitamin D and parathyroid hormone related peptide (PTHrP) regulate placental calcium transfer remains uncertain.

There are several weaknesses in our study. First, a minority of the original cohort was traced. We showed, however, that those participating in the study did not differ from non-responders in regard to maternal body build or lifestyle; furthermore, it is difficult to see how differences in response rate would have spuriously revealed an association between umbilical venous calcium concentration and childhood bone mass. Second, the lower numbers of subjects with umbilical venous calcium measurements limited the statistical power of our study to investigate the extent to which this measure might mediate the effects of the maternal environment. Third, mid upper arm circumference was used as a measure of maternal fat stores. While this measure includes upper arm musculature, it remains highly correlated with measures of peripheral adiposity such as triceps skinfold thickness in previous Southampton studies ($r=0.79$, $p<0.001$).

There are no widely accepted methods available for the correction of circulating total calcium concentrations for protein binding in childhood (179). We therefore used a method analogous to that used in adults, which depended upon adjustment for umbilical venous albumin concentration. Finally, our study relied upon DXA for measurement of bone mass. While validated in adults, its use in children raises unique technical considerations. The smaller absolute amounts of bone mineral lead to greater percentage precision errors. A study in piglets demonstrated coefficients of variation up to 2.4% for whole body BMC and 1.8% for BMD (180); these are greater than those reported in adults. Furthermore, the variability between the proportion of intra-osseous marrow fat and that present within lean tissue may lead to an inaccuracy in the estimation of BMC by as much as 20% (181). Again, it is difficult to see how this would have led to a spurious relationship between umbilical venous calcium and whole body BMC. We corrected bone mineral measurements for bone size using three separate mathematical algorithms (169;169;170;170;182). Performing these adjustments significantly weakened the relationship between umbilical cord serum calcium concentration and bone mass at age nine years, suggesting that the determinants of bone size differ from those of volumetric bone mineral density.

In summary, our study confirms the association between birth weight and bone mass measured by DXA some nine years later. The adverse effects of maternal smoking and poor maternal fat stores on bone mass in the offspring appear to be maintained well into later childhood. In addition that umbilical venous serum calcium concentration predicts childhood bone mass suggests that the ability of the placenta to maintain an optimum calcium supply to the growing foetus represents a critical determinant of the childhood trajectory of bone mineral accrual.

6 SUMMARY DISCUSSION CONTRASTING DETERMINANTS OF NEONATAL AND CHILDHOOD BONE MASS

6.1 PRINCIPAL FINDINGS

1. Maternal height, smoking during pregnancy and birthweight independently predicted neonatal bone mass.

2. Pre – pregnancy maternal adiposity and not the increment in adiposity during pregnancy predicted neonatal bone mass

3. Paternal height but not paternal adiposity predicted neonatal bone mass

4. Maternal height and adiposity before and during pregnancy predicted childhood bone mass.

5. Maternal smoking status at the time of the last menstrual period and during infancy, but not during pregnancy and childhood, was associated with a reduction in childhood bone mass.

6. Weight at birth predicted later childhood bone and lean but not fat mass.

6.2 MATERNAL PREDICTORS OF NEONATAL AND CHILDHOOD BONE MASS

6.2.1 MATERNAL HEIGHT

Maternal height in both cohorts predicted neonatal and childhood whole body bone mass (Figure 30 Maternal height significantly predicted both neonatal and childhood whole body bone and lean mass with a weaker effect on fat mass.

Although a lower R^2 is expected in the neonatal compared with the childhood models because of poorer scan acquisition quality as discussed in section 6.4, there does appear to be stronger effect of maternal height on body composition during childhood than at birth. This suggests that there is a greater influence of maternal height on postnatal growth rather than prenatal growth of the offspring.

In childhood but not neonates, the relationship between maternal height and body composition remained significant after adjusting for height, such that the children of taller mothers had greater bone and lean mass per height at aged 9 years. This suggests that maternal height has effects on post-natal bone mineral and lean mass accrual in addition to those on linear growth. Adjusting neonatal and childhood body compositions for weight removed the association between maternal height and the offspring's bone and lean mass, demonstrating that there is little association between maternal height and proportionate body composition by weight of her child.

6.2.2 MATERNAL ADIPOSITY

While maternal height predicted the WBBMC and lean mass of her offspring, maternal adiposity independent of maternal height, was positively associated with both neonatal and childhood WBBMC and fat mass but not lean mass (Figure 30 and Figure 31). The relationship was stronger for fat mass than WBBMC and while adjusting for neonatal length or weight attenuated the association between neonatal body composition and maternal height, the associations with maternal adiposity were little changed after the above adjustments and remained statistically

significant. A fatter mother had a larger baby, though there was an apparent disproportion, with greater accrual of fat mass at the expense of lean mass. After adjusting for unit weight of the offspring, increasing maternal adiposity was positively associated with fat mass and negatively associated with lean mass.

While absolute maternal weight and MUAC gain during early to late pregnancy significantly predicted neonatal WBBMC, there was not significant relationship between change in the other measures of estimated maternal adiposity during pregnancy and neonatal WBBMC. These findings indicate the importance of pre-pregnancy fat stores over the change in fat stores during pregnancy.

The relationships between maternal adiposity and the proportion of BMC by body weight were different at birth compared with those at nine years. Fatter mothers had bigger babies with a greater proportion of fat and bone in comparison to lean per weight. However, by nine years, the offspring of the fatter mothers had lower proportion of bone per weight, emphasizing that the weight gain from fat mass accrual is proportionately greater than either lean or bone mineral accrual by childhood.

As higher maternal adiposity was associated with greater bone mass at birth and childhood, avoiding being underweight as a mother is the critical factor for enhancing bone growth of her child. However, advice regarding the optimum upper limit of adiposity in regards to bone mass is less clear. The negative relationship between maternal adiposity and lean mass of the offspring is of concern. It would suggest that advice to mothers is to maintain a measure of adiposity within the normal range and while thinness leads to poor bone mass, increased fatness may lead to higher bone mass and fat mass at the expense of lean mass in the offspring which may have implications for other aspects of health, especially taking into account the current epidemic of childhood obesity.

On the one hand we have demonstrated that birth size is a poor predictor of fat mass in childhood and yet by adulthood, maternal, but not paternal, fat mass, is a strong independent predictor of the offspring's bone mass. In addition, maternal but not paternal adiposity is a significant predictor of the child's adiposity at birth and childhood. This suggests that regulation

of fat mass accrual during childhood and adulthood is critical for determining the bone mineral accrual and height of the next generation.

In early pregnancy, the SWS mothers were significantly ($p<0.001$) older (mean age 29.8yrs (SD 3.73]) than the PAH women (mean age 27.0 [SD 4.8]). Although the neonatal (SWS) and childhood (PAH) cohorts were less than a decade apart, there were striking differences in maternal and neonatal anthropometric measurements. There was a no significant difference in maternal height between the two cohorts (1.631 vs. 1.627 cm, $p=0.34$), however there was a marked increase in pre-pregnancy weight (59 vs. 64.5 kg) in the SWS and PAH cohorts respectively. This remained significant even after adjusting for height or age ($p<0.001$). There was also a difference in BMI (SWS: 25.0 vs. PAH: 22.4 kg/m2 respectively [$p<0.001$]). It may be argued that the PAH pre-pregnancy weights are recalled weights and therefore subject to recall bias and an underestimation of pre-pregnancy weight. However, maternal adiposity during late pregnancy, as measured by MUAC, was also higher in the SWS cohort to the extent that the quintiles for the PAH cohort were within the first three quintiles of the SWS cohort.

The differences in maternal body size were echoed by differences in neonatal size. Even after adjusting for sex and gestational age, SWS babies were 396g heavier ($p=<0.001$) and 1.1cm longer ($p<0.001$) than the PAH children born a decade earlier. These observations suggest that within the relatively short time span of a decade, increments in maternal adiposity lead on to increments in neonatal size. Hence, further work investigating the genetic and environmental factors influencing fat mass accrual during childhood and adolescence, especially in girls is required to shed light on the epidemiology of height and peak bone mass.

The mechanism whereby maternal adiposity influences the bone mass of her offspring during pregnancy is not known. Possible mechanisms include genetic or environmental factors or a combination of the two. The lack of effect of paternal adiposity strongly suggests an environmental effect which could be either general or via specific mediators. Maternal triceps skin fold thickness maybe a measure of the maternal diet and lifestyle and so a marker of nutrient reserve in the pre-pregnant mother. In this way, it would predict the general availability of calorific, protein or fat capacity or more specific nutrients such as the fat soluble vitamins including vitamin A, D, K and E for the foetus. While the role of vitamin D in skeletal status has

been well described the other vitamins may also influence bone health. The vitamin A receptor forms a heterodimer with the vitamin D receptor and vitamin K is required for the carboxylation of the osteocalcin. The mechanism may also involve messengers such as leptin or adiponectin secreted by the adipocytes. Recent work has also highlighted the action of lipoprotein receptor 5 in determining bone mass. Other adipocyte functions include insulin sensitivity and also immunomodulation, both of which can affect foetal growth. The target effect of maternal adiposity may involve altering placental transport of calcium and other nutrients, or may influence the linear growth of the foetus or the ability of the foetal skeleton to mineralize. Of all the measures of skinfold thickness, triceps rather than truncal measurements had the greatest predictive value; the mechanism for peripheral rather than truncal measures of fat maternal influencing neonatal bone mass is at present unknown as are the determinants of regional differences in fat deposition.

6.2.3 MATERNAL SMOKING

For over four decades maternal smoking during pregnancy has been associated with reductions in both birth weight and length (183), as replicated in this study. The deleterious effects of smoking are related to the number of cigarettes and persist even after adjustment for confounders such as alcohol consumption. In this thesis, the effect of smoking on neonatal bone mass was most marked in those women who were still smoking at 34 weeks and appeared to reduce bone and fat mass more than lean mass. A dose response effect of number of cigarettes on neonatal body composition was apparent in pre-pregnancy and early pregnancy. However at nine years smoking history at the time of the last menstrual period was associated with the greatest reduction in childhood bone mass. We were unable to demonstrate an independent effect of passive smoking in the home on bone accrual of the child at birth or at nine years.

There are three principal mechanisms for the effect of maternal smoking on bone accrual during intra-uterine and childhood. Firstly, maternal smoking during pregnancy is associated with many socio-demographic factors such as lower maternal educational level and employment status and so smoking could be a surrogate for another environmental factor. However, it is known that the constituents of cigarette smoke have direct inhibitory effects on osteoblasts (184). Another mechanism by which smoking may influence bone growth is affecting gross motor activity. It is known that prenatal exposure to tobacco increases the risk of the offspring for cognitive deficits, attention deficit/hyperactivity disorder, conduct disorder, criminality, smoking and alcohol abuse. Nicotine readily crosses the placenta and as the foetal brain contains nicotinic receptors, mothers who smoke during pregnancy may alter the development of their child's central nervous system and programme both child and adult behaviour (185). If this led to a perturbed neural development effecting developmental milestones and physical activity in later childhood, this would in part help explain the longer term deficits in bone growth of children born to mothers who smoked.

The under reporting of smoking habit during pregnancy may have introduced a negative bias to these results (186). Smokers may vary the amount of cigarettes smoked and also the different brands may expose the foetus to different amounts of tar, nicotine and carbon monoxide (187).

Serum cotinine has been used as a biological marker of smoke exposure. In studies of pregnant women, the 1-2% of mothers reported non-smoking whilst they had elevated cotinine levels. In contrast 4-5% of mothers who reported occasional smoking had levels of cotinine below the smoking threshold, suggesting that during pregnancy occasional smokers were over reporting the amount they smoked.

Community based education using both information pack and personalized mailings of dietary and lifestyle interventions during pregnancy have been reviewed (188). These may be supplemented with individual counselling in a clinic or home setting; however only small differences in knowledge and attitude were found with no difference in dietary behaviour and further research is needed for the development of health promotion interventions.

6.2.4 MATERNAL PHYSICAL ACTIVITY

In contrast to findings from other studies (66;84;189;190) we were unable to demonstrate a robust influence of maternal physical activity during pregnancy on neonatal or childhood bone mass accrual. Any effects were adjusted for by differences in maternal fat mass and smoking status. While this does not discount an effect of physical activity on neonatal bone growth, it does suggest that some of the effects are mediated through changes in maternal body composition and lifestyle associations, such as smoking or parity. The question of whether increased physical activity in pregnancy, such as through occupation, influences intra uterine growth is important and future studies and analysis would require more detailed description of physical activity during pregnancy than those in this study.

6.2.5 MATERNAL ALCOHOL INTAKE

While a substantial proportion of women continued to drink alcohol through pregnancy, only small reductions in neonatal size and body composition were evident with no dose effect. However, only approximately 50% of the alcohol sold is measured by self-reported questionnaires with under reporting more likely in those who drink wine, higher amounts of alcohol and those with irregular drinking patterns (191). Gender, signs of addiction or drinking alone vs. socially were not related to under reporting. Alcohol has little direct toxic effect on

mature osteoblasts but may affect responsiveness of osteoblasts to circulating mitogens (192). However, as stated above, we were unable to detect an independent effect of alcohol consumption during pregnancy on intrauterine growth.

6.2.6 MATERNAL PARITY, SOCIAL CLASS AND EDUCATION

The positive association between maternal parity and birth weight of the offspring is well recognised in studies from a number of international studies (193). The association appears to be mediated through both increased maternal adiposity and an independent effect of increased intra uterine vascularity. Parity, however, did not protect the foetus from the deleterious effects of maternal thinness or smoking, suggesting that targeting only primiparous mothers for lifestyle interventions would be insufficient.

6.3 NEONATAL CHARACTERISTICS AND CHILDHOOD BODY COMPOSITION/ CONDITIONAL MODELLING

6.3.1 BIRTH SIZE

The relationship between birth weight and postnatal BMC and BMD has been demonstrated in both children (36) and in adults (165). Furthermore, infant size has been shown to predict peak bone mass. This study confirms the significant relationship between birth size and pre-pubertal bone size and lean mass but not fat mass. The weaker, non-significant relationship between birth size and post natal fat mass is in accord with previous work in children and adults using bioimpedence (194) and DXA (165). There are evolutionary theoretical advantages for this. In a poor-quality environment, maternal investment in the foetus is altered to favour fat deposition to ensure survival in the weaning period. In a good-quality environment, to maximize the reproductive success of her offspring, the mother expends more resources by increasing foetal lean mass.

It is also now well recognized that foetal growth does not reach full genetic potential and there is constraint of foetal growth. This is due to the competing interests of maternal constraint to

produce a foetus and retain the ability to reproduce again while for the father interest is to produce the largest offspring possible (195). These opposing interests are thought to be mediated by selective methylation of foetal growth factors including IGF-2 (196). However, in this study paternal height had an equal magnitude of effect on neonatal length as maternal height.

6.3.2 INFANT AND CHILDHOOD MILK INTAKE

By nine years of age, we were unable to detect any influence of pattern of infant feeding for the first three months of life on childhood body composition. However, those exclusively bottle-fed did appear to have accelerated weight gain during infancy. This is well recognized and compared to formula fed infants, breast-fed term infants grow slower during the first few months of life and then have an accelerated growth such that by the end of one year there is no overall measurable difference (197). However, altering the tempo of growth during the first year of life may lead to differences in bone strength that are not detectable by aBMD including bone geometry. Whilst there is observational evidence to suggest that breast feed infants go on to be taller adults, this observation is open to significant confounding (198); we were unable to demonstrate an effect of breast-feeding on childhood height. .

6.3.3 CHILDHOOD PHYSICAL ACTIVITY

Using participation in sports and number of days walking more than 30 minutes as a marker for physical activity, we were unable to demonstrate a positive relationship between physical activity and BMC at nine years. In adolescents measured serially through childhood, a positive relationship between physical activity, measured using a five point scale, and BMC, however, has been demonstrated (199;200). However, whether this is an independent effect of loading is not known. Increased physical activity requires an increased calorie intake and this together with other lifestyle factors needs to be taken into account. The frequency, type and duration of physical activity and its relation to bone mineral accrual is not clearly understood; physical activity effects on skeletal status may be site specific (201) and may also be developmentally specific, such that age of first walking (202) vs. exercise during pubertal growth may have different effects.

6.4 LIMITATIONS

6.4.1 DXA DERIVED BODY COMPOSITION ESTIMATIONS

Measurements of whole body BMC, lean mass and fat mass are in accord with other published reference data on neonatal body composition as assessed using DXA (203). While direct chemical analysis of cadavers is the most accurate means of assessing body composition (204), DXA measures whole body regional skeletal size and body composition using a three-compartment model. However, DXA instruments are designed principally for use with adult body size and with the smaller body sizes there are extra limitations reducing accuracy with greater error with smaller masses (203). While software modifications have lowered the bone-detection thresholds, the precision and overall accuracy may be compromised (204), with underestimation of bone mass and overestimation of fat mass. Work from Bolotin et al. (205) has called into question accuracy of dual beam technologies in measuring bone due to variation in the marrow fat and haemopoietic composition.

6.4.2 MOVEMENT ARTIFACT AND AGE AT SCANNING

The effect of movement has been studied using both a robot phantom and also movement scores for each scan image. Using the robot phantom the precision was lower with greater size and frequency of movement. This was supported using regional dichotomous movement scores with a 13.6% reduction in WBBMC in those with gross movement compared with those with no movement. However, there was no significant parental predictor of movement score and higher movement score was an independent predictor of lower bone mass in neonates. In the children, despite the 12 minute image acquisition time, there was little or no movement visible on the DXA scan. The poorer scan images in the neonates compared with the children is likely to account for the lower predicted variance from the models in the neonates.

Delay in the scanning of the neonates was associated with a significant reduction in lean mass but this was small in comparison to the overall variance in body composition measurements. Weight loss of up to 10% is common in the first two weeks of life and is due to dehydration. This would be expected to affect measurement of lean mass rather than fat mass or bone mass as we have

found. The above differences may have lead to misclassification errors in the neonates and children bone mass scores.

6.4.3 ESTIMATION OF VBMD VS. BONE SIZE AND SUB REGIONAL ANALYSIS

DXA measures two components of the skeleton, the total BMC and the bone area. From these two measurements one is able to drive estimates of skeletal size and more importantly mineral density. There is no gold standard for estimating volumetric or size adjusted bone mineral density and methods include areal BMD, BMAD and BMC adjusted for bone area using the method of Horlick (169), BMC adjusted for bone area, height and weight using the method of Prentice (170), BMC per unit length, BMC per unit weight. The ideal size adjustment should fulfil two criteria. Firstly it should have a high validity. Validity itself exists on two planes. Firstly, the estimate should reflect either actual areal bone mineral density or volumetric density. As the neonatal skeleton is under mineralized compared with the adult skeleton, edge detection may become compromised affecting estimates of bone area, and hence aBMD may have lower accuracy. However, at this age it may well be that the size of the bone envelope is more important than the degree of its mineralization for future growth. Secondly, it should predict bone strength and risk of fracture to a similar degree that adult measures of aBMD predict fragility fracture risk. Longer term follow up of these children with recording of incident fracture, which is common in childhood, will allow testing of these skeletal measures and their association with future fracture risk.

The second criterion is precision. The estimate of bone volume should be relatively insensitive to uncontrolled factors such as movement artefact, gestation age or postnatal age. The relationship between the different size adjusted bone estimates and the movement scores has been tested. In addition, the effect of movement from the gantry-robot study on bone measurements has been performed. While in adults, subject is in the anatomical position, this is clearly the case only in a minority of neonatal images. It is relatively common for bones to overlap, for example the arms across the chest, or legs crossing each other. This would falsely elevate the aBMD and lower the whole body bone area but have little effect on whole body BMC measurements. For this reason WBBMC was the principal bone outcome used in the analyses of this study.

Previous investigators have not found a relationship between childhood age and whole body BMC, and attributed this to the large variance in whole body BMC explained by the skull, which does not increase linearly with age (54). Different parts of the skeleton mature at different rates and this is true for the axial and appendicular skeleton (206), and it is well recognized that skull size increases rapidly during infancy and slows during childhood. However, in this larger study we were able to demonstrate an age-related increase in whole body BMC even within a narrow age limit.

6.4.4 DIETARY EVALUATIONS

We were unable to demonstrate an association between childhood diet and bone mass using milk intake as a surrogate for calcium intake. There is no gold standard for the measurement of dietary calcium intake in children. In most Western countries, more than two-thirds of dietary calcium intake is through milk and diary intake (207), and in the childhood component of this study, we were only able to record milk intake with no measure of other sources of calcium or of total energy intake to allow nutrient densities to be estimated. In adults, under reporting of energy intake is a major source of inaccuracy when calculating nutrient intake. For this reason, derived calcium density has been has been used. However, in children there is a strong positive relationship between calcium and energy intake and calcium density has not been shown to a better predictor of BMC compared with using unadjusted calcium intake (208). This would in validate the use of unadjusted calcium intake as done in this study.

6.5 FUTURE WORK

6.5.1 DETERMINANTS OF TRABECULAR BONE MASS USING LUMBAR SPINE DATA

Whole body BMC is a measure of mainly cortical bone, and while the proximal femur is an important site for fragility fracture, trabecular bone is critical for vertebral body strength and is regarded as the most metabolically active component of the bony skeleton. It will be of considerable interest to see whether the predictors of whole body BMC match those of lumbar spine BMC. In addition, using the models of Carter et al. (182) to derive BMAD, predictors of estimated volumetric BMD can be compared to begin to investigate differential determinants of bone size, density and both.

6.5.2 MEASUREMENT OF BIOLOGICAL NUTRIENTS IN MATERNAL AND UMBILICAL BLOOD: ROLE OF VITAMIN D/ PTH AXIS WITH UMBILICAL VENOUS CALCIUM

The identification of umbilical venous calcium concentration as a predictor of childhood bone mass has informed a new direction of research for the mechanisms for the programming of skeletal growth. Of the determinants of cord calcium, maternal vitamin D status and the PTH/ PTHrP paracrine system are likely to play critical roles and future studies measuring these components of the calcium axis would shed light on the underlying mechanisms of the associations shown.

6.5.3 DETERMINATION OF EPIGENETIC CHARACTERISTICS OF FOETAL AND PLACENTAL TISSUE

Further investigation of the mechanisms involved in skeletal programming is likely to focus on variable epigenetic methylation status of key placental transport systems. Placental size in late pregnancy is a good predictor of neonatal size (209) and in animal studies, reducing placental size,

reduces foetal size. However, placental growth occurs early in pregnancy pre-empting foetal demand and so the factors influencing placental development in the first and second trimester have persisting effects on foetal growth throughout gestation. One important mechanism involves the variable inactivation of local growth factors, such as IGF-2, and placental transport proteins and study of the relationship between these and maternal environmental characteristics will add considerably to our understanding.

6.5.4 DETERMINATION OF AGE OF WALKING, CHILDHOOD FRACTURES, PUBERTAL ONSET AND PEAK BONE MASS

Loading has significant effects on bone growth and trabecula orientation. There is also evidence to suggest that the age of walking is responsible for some of the variation in female pelvic shapes attained in adulthood (202). It will therefore be of interest to explore the relationship between maturation of the nervous system to permit full weight bearing and the still growing and plastic skeletal system and whether the infants weight at the time of walking influences the shape of the proximal femur.

This study has demonstrated the maternal effects on intrauterine growth and persistence to age nine. Incident fracture rates during childhood are high (210) and it will be of interest to ascertain whether any of the parental determinants of the offspring's bone mass also predict fracture risk.

The key question is whether programmed effects on skeletal growth effect fragility fracture in late adulthood, when they have a significant morbidity, mortality and health care cost. It is likely that such mechanism would modulate osteoporotic fracture by altering peak bone mass and it will be critical to evaluate these pre-natal parental factors in relation to the offspring's bone mass at the time of skeletal maturation.

Whilst adolescence is a key period of bone accrual, with as much bone laid down during the adolescent years as is lost in the remainder of life (199), little is known as to whether factors during pregnancy influence onset of puberty. Childhood adiposity and leptin concentration is

critical to maturation of the growth hormone axis to permit the onset of puberty. In the children, it would appear that adiposity was the only component of the child's whole body composition that was independent of birth size. This suggests that the maternal environment or genetic factors act on postnatal fat accrual independently of intrauterine growth or that there are effects on the growing foetus in ways that can not be detected using the anthropometric measurements used at birth in this study. Alternatively, it is the postnatal environment that determines fat accrual. As the cohorts mature, these hypotheses can be tested.

6.5.5 CHARACTERIZATION OF PATERNAL SKELETAL STATUS

Only crude measures of paternal body composition such as height and weight were available. A more detailed assessment of paternal body composition using DXA of the whole body, lumbar spine and femoral neck, together with calcaneal QUS and a brief lifestyle questionnaire would permit comparison of each parental attributes effect on the growth of their offspring to allow better identification of the low bone mass infant.

6.6 CONCLUSION

In summary, our study confirms the association between birth weight and bone mass measured by DXA some nine years later. The adverse effects of maternal smoking and poor maternal fat stores on bone mass in the offspring appears to be maintained well into later childhood; the observation that these influences may be mediated by umbilical venous serum calcium concentration suggests that the ability of the placenta to maintain an optimum calcium supply to the growing foetus represents a key determinant of the childhood trajectory of bone mineral accrual.

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8 LIST OF APPENDICES

FOR APPENDICES: I-XIV, see separate document

APPENDICES

MATERNAL NUTRITION, MATERNAL BODY COMPOSITION DURING PREGNANCY AND NEONATAL BONE MASS

Dr Muhammad Kassim Javaid

2005

Doctoral Thesis

MRC Epidemiology Resource Centre,
University of Southampton, Southampton, UK

APPENDICES

CONTENTS

APPENDIX I : SWS MATERNAL PRE- PREGNANCY QUESTIONNAIRE

SWS serial number

SOUTHAMPTON Women's SURVEY

QUESTIONNAIRE

Name: _____

Address: _____

_____

Postcode: | | | | | | | |

Phone No: | | | | | | | | | | | |

Interviewer: | | | Date of interview: d d | | m m | | y y | |

If the woman wants to have a cup of tea/coffee with you and has not eaten or drunk anything in the past hour, do the mouthwash sample first but remember to obtain the woman's ***consent****. If not, go to section 1.*

Mouthwash sample provided (0 = No, 1 = Yes) | |

Time of mouthwash sample
(24 hr clock) | | | | |

1: OCCUPATION

I would like to start by talking about any paid work that you do.

1.1 Were you in paid employment or self-employed in the week ending last Sunday? | |

0. No, *go to 1.3*

1. Yes, *go to 1.2*

1.2 Were you working full time or part time?

0. Full time (more than 30 hours) *go to 1.6b*
1. Part time (30 hours or fewer) *go to 1.3*

1.3 Are you going to college full time?

0.No *if working part-time go to 1.6a*
if not working go to 1.5
1.Yes

1.4 *If yes,* what are you studying? _____

If working part time go to 1.7

If not working go to section 2

1.5 *If not working or studying* were you

Unemployed ? (1)

Permanently unable to work because

of long term sickness or disability ? (2)

looking after home or family? (3)

other ? (specify)_____ (4)

1.6a *If **not** working or working part-time*, what was your last **full-time** job ?

If only ever part-time ask for last part time job.

Then if currrently working part time go to 1.7, otherwise go to section 2.

Job Position _____

Self-employed/manager/foreman/employee

Industry _____

1.6b *If working full-time*, what is your job ? *(Then go to section 2)*

Probe industry & self-employed/manager/foreman/employee

Job Position

Self-employed/manager/foreman/employee

Industry _____

1.7 *If working part-time now,* what is your current job?

Job Position _____

Self-employed/manager/foreman/employee

Industry _____

1.8 *If working part time,* how many hours per week do you work?

☐☐ ☐☐ MINS

2: ACTIVITY AND EXERCISE

Now I'm going to ask you about your activity and exercise patterns over the last three months.

We would like you to divide up a "typical" day into three types of activity. These are:

(1) sleeping or lying, (2) sitting, (3) standing or walking.

2.1 Over a typical 24 hour day how many hours do you generally spend sleeping or lying with your feet up? ☐☐ hrs ☐☐ mins

(*ask time usually go to bed & wake up, including any at work!*)

This would indicate xx hours sitting or on your feet.

2.2 Of those hours how many on a typical day do you spend sitting down? (*e.g. includes sitting at work, mealtimes, driving, reading, watching TV*)

☐☐ hrs ☐☐ mins

2.3 This would mean that you spend about xx hours a day on your feet. Does this sound about right?

☐☐ hrs ☐☐ mins

2.4 Out of these xx hours spent on your feet, about how much of the time are you **actively on the move** (rather than standing fairly still)?

*

1.	Very little	10%
2.	Some	30%
3.	About half	50%
4.	Most	70%
5.	Almost all	90%

☐

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

*

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

☐ ☐

FFQ categories 1-7 >x1

AND **ON AVERAGE** ABOUT HOW LONG D☐

each period of activity last? ☐ hrs ☐☐ mins

b) **moderate exercise** which normally leaves you exhausted but not breathless, e.g. brisk walking, dancing, easy swimming or cycling, badminton, sailing.

FFQ CATEGOR☐-7 ☐☐

AND **ON AVERAGE** ABOUT HOW LONG D☐

each period of activity last? ☐ hrs ☐☐ mins

c) **gentle exercise** which normally leaves you tired but not exhausted, e.g. walking, heavy housework (including washing windows and polishing), gardening, DIY, golf.

FFQ categories 1-7 ☐ >x1 ☐☐

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

2.6 On a typical day, how many hours do you generally spend watching television? *

1. More than 5 hours
2. 4-5 hours
3. 3-4 hours
4. 2-3 hours
5. 1-2 hours
6. Less than one hour
7. None

2.7 Which of the following best describes your walking speed? *

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

3: DIETARY QUESTIONS

3.1 Now I am going to ask you about the foods you eat. To do this I have a list of foods and I would like you to tell me how often you have eaten each food during the past 3 months. The list may include foods you never eat or you may find foods which you eat a lot are missing. These can be added on at the end. *(Define the 3 month period)*

FOOD CODE	FOOD DESCRIPTION	FREQUENCY EATEN							
		Never	Once every 2-3 Months	Once a Month	Once a Fortnight	1-2 Times per Week	3-6 Times per Week	Once a day	More than once a day
1	WHITE BREAD	1	2	3	4	5	6	7	☐
	WHEN YOU EAT BREAD/TOAST/SANDWICHES, HOW MANY SLICES/ROLLS DO YOU EACH AT A TYPICAL MEAL? ☐☐ ☐ *ROLLS (COUNT AS 2 SLICES)* . *FRENCH BREAD (2″ COUNTS AS 1 SLICE)*								
2	BROWN AND WHOLEMEAL BREAD/ROLLS	1	2	3	4	5	6	7	☐
	How many slices/rolls do you eat at a typical meal? *Rolls (count as 2 slices)* ☐☐ . ☐								

3	Crackers and cheese biscuits	1	2	3	4	5	6	7	☐
4	Wholemeal and rye crackers	1	2	3	4	5	6	7	☐
5	'Bran' breakfast cereals	1	2	3	4	5	6	7	☐

	FOOD DESCRIPTION	FREQUENCY EATEN							
FOOD CODE		Never	Once every 2-3 Months	Once a Month	Once a Fortnight	1-2 Times per Week	3-6 Times per Week	Once a day	More than once a day
6	Other breakfast cereals	1	2	3	4	5	6	7	☐
7	Added bran to foods	1	2	3	4	5	6	7	☐
8	CAKES AND GATEAUX	1	2	3	4	5	6	7	☐

9	Buns	1	2	3	4	5	6	7	
10	Pastries	1	2	3	4	5	6	7	
11	Biscuits – chocolate, digestive and ginger	1	2	3	4	5	6	7	
12	Other biscuits	1	2	3	4	5	6	7	
13	Fruit puddings	1	2	3	4	5	6	7	
14	Milk based puddings and sauces	1	2	3	4	5	6	7	

	FOOD DESCRIPTION	FREQUENCY EATEN							
FOOD CODE		Never	Once every 2-3 Months	Once a Month	Once a Fortnight	1-2 Times per Week	3-6 Times per Week	Once a day	More than once a day
15	Other puddings	1	2	3	4	5	6	7	☐
16	Yogurt and fruit fools	1	2	3	4	5	6	7	☐
17	POTATOES - BOILED AND JACKET	1	2	3	4	5	6	7	☐
	WHEN YOU EAT THESE HOW MANY POTATOES DO YOU EAT AT A TYPICAL MEAL? ☐☐ ☐ *Large baking (count as 3)/new (count as 0.5)*								
18	Roast potatoes and chips	1	2	3	4	5	6	7	☐

	When you eat these how many potatoes do you eat at a typical meal? ☐☐.☐								
19	Yorkshire puddings and savoury pancakes	1	2	3	4	5	6	7	☐
20	Brown and white rice	1	2	3	4	5	6	7	☐
21	Pasta and dumplings	1	2	3	4	5	6	7	☐

	FOOD DESCRIPTION	FREQUENCY EATEN							
FOOD CODE		Never	Once every 2-3 Months	Once a Month	Once a Fortnight	1-2 Times per Week	3-6 Times per Week	Once a day	More than once a day
22	Tinned vegetables	1	2	3	4	5	6	7	☐
23	Peas and green beans	1	2	3	4	5	6	7	☐
24	Carrots	1	2	3	4	5	6	7	☐
25	Parsnips, swede and turnip	1	2	3	4	5	6	7	☐
26	Sweetcorn and mixed veg	1	2	3	4	5	6	7	☐

27	Beans and pulses	1	2	3	4	5	6	7	☐
28	Tomatoes	1	2	3	4	5	6	7	☐
29	Spinach	1	2	3	4	5	6	7	☐
30	BROCCOLI, BRUSSELS SPROUTS AND SPRING GREENS	1	2	3	4	5	6	7	☐

	FOOD DESCRIPTION	FREQUENCY EATEN							
FOOD CODE		Never	Once every 2-3 Months	Once a Month	Once a Fortnight	1-2 Times per Week	3-6 Times per Week	Once a day	More than once a day
31	Cabbage and cauliflower	1	2	3	4	5	6	7	☐
32	Peppers and watercress	1	2	3	4	5	6	7	☐
33	Onion	1	2	3	4	5	6	7	☐
34	Green salad	1	2	3	4	5	6	7	☐
35	Side salads in dressing	1	2	3	4	5	6	7	☐

36	Courgettes, marrow and leeks	1	2	3	4	5	6	7	☐
37	Mushrooms	1	2	3	4	5	6	7	☐
38	Vegetable dishes	1	2	3	4	5	6	7	☐
39	Vegetarian foods	1	2	3	4	5	6	7	☐

	FOOD DESCRIPTION	FREQUENCY EATEN							
FOOD CODE		Never	Once every 2-3 Months	Once a Month	Once a Fortnight	1-2 Times per Week	3-6 Times per Week	Once a day	More than once a day
40	Tinned fruit not including grapefruit, prunes, figs or blackcurrants	1	2	3	4	5	6	7	☐
41	Cooked fruit not including blackcurrants	1	2	3	4	5	6	7	☐
42	Dried fruit	1	2	3	4	5	6	7	☐
43	Fresh apples and pears	1	2	3	4	5	6	7	☐
44	Fresh oranges and orange juice	1	2	3	4	5	6	7	☐☐

45	Grapefruit and grapefruit juice	1	2	3	4	5	6	7	
46	Blackcurrants, ribena and hi-juice blackcurrant drinks	1	2	3	4	5	6	7	
47	Other fruit juices (not squashes)	1	2	3	4	5	6	7	
48	DIET COKE AND PEPSI NOT INCLUDING CAFFEINE FREE	1	2	3	4	5	6	7	

	FOOD DESCRIPTION	FREQUENCY EATEN							
FOOD CODE		Never	Once every 2-3 Months	Once a Month	Once a Fortnight	1-2 Times per Week	3-6 Times per Week	Once a day	More than once a day
49	Coke and Pepsi	1	2	3	4	5	6	7	☐☐
50	Soft drinks not including diet drinks (low calorie or low sugar)	1	2	3	4	5	6	7	☐☐
51	Bananas	1	2	3	4	5	6	7	☐
52	Fresh peaches, plums, cherries and grapes	1	2	3	4	5	6	7	☐
53	Strawberries and raspberries	1	2	3	4	5	6	7	☐

54	Fresh pineapple, melon, kiwi fruit and other tropical fruits	1	2	3	4	5	6	7	☐
55	Nuts	1	2	3	4	5	6	7	☐
56	Bacon and gammon	1	2	3	4	5	6	7	☐
57	Pork	1	2	3	4	5	6	7	☐
58	Chicken and turkey	1	2	3	4	5	6	7	☐

	FOOD DESCRIPTION	FREQUENCY EATEN							
FOOD CODE		Never	Once every 2-3 Months	Once a Month	Once a Fortnight	1-2 Times per Week	3-6 Times per Week	Once a day	More than once a day
59	Lamb	1	2	3	4	5	6	7	☐
60	Beef	1	2	3	4	5	6	7	☐
61	Minced meat dishes	1	2	3	4	5	6	7	☐
62	Meat pies	1	2	3	4	5	6	7	☐
63	Liver and kidney	1	2	3	4	5	6	7	☐
64	Paté and liver sausage	1	2	3	4	5	6	7	☐

65	Faggots and black pudding	1	2	3	4	5	6	7	☐
66	Sausages	1	2	3	4	5	6	7	☐
67	Ham and luncheon meat	1	2	3	4	5	6	7	☐
68	White fish	1	2	3	4	5	6	7	☐

	FOOD DESCRIPTION	FREQUENCY EATEN							
FOOD CODE		Never	Once every 2-3 Months	Once a Month	Once a Fortnight	1-2 Times per Week	3-6 Times per Week	Once a day	More than once a day
69	Fish fingers and fish dishes	1	2	3	4	5	6	7	☐

70	Oily fish	1	2	3	4	5	6	7	☐
71	Shellfish	1	2	3	4	5	6	7	☐
72	Boiled and poached eggs	1	2	3	4	5	6	7	☐
73	Omelette and fried eggs	1	2	3	4	5	6	7	☐
74	Cottage Cheese	1	2	3	4	5	6	7	☐
75	Cheese	1	2	3	4	5	6	7	☐
76	PIZZA, QUICHES AND CHEESE FLANS	1	2	3	4	5	6	7	☐

77	Soup	1	2	3	4	5	6	7	
78	Mayonnaise and salad cream	1	2	3	4	5	6	7	

	FOOD DESCRIPTION	FREQUENCY EATEN							
FOOD CODE		Never	Once every 2-3 Months	Once a Month	Once a Fortnight	1-2 Times per Week	3-6 Times per Week	Once a day	More than once a day
79	Pickles, chutney, tomato ketchup and brown sauce	1	2	3	4	5	6	7	
80	Chocolate	1	2	3	4	5	6	7	
81	Other sweets	1	2	3	4	5	6	7	

82	Ice cream and chocolate desserts	1	2	3	4	5	6	7	☐
83	Cream	1	2	3	4	5	6	7	☐
84	Crisps and savoury snacks	1	2	3	4	5	6	7	☐☐
85	Sweet spreads	1	2	3	4	5	6	7	☐
86A	Gravy granules and powders	1	2	3	4	5	6	7	☐
86B	Stock cubes and Marmite	1	2	3	4	5	6	7	☐

	FOOD DESCRIPTION	FREQUENCY EATEN							
FOOD CODE		Never	Once every 2-3 Months	Once a Month	Once a Fortnight	1-2 Times per Week	3-6 Times per Week	Once a day	More than once a day
87	Drinking chocolate and milk shakes not including McDonald style milkshakes	1	2	3	4	5	6	7	☐
88	Decaffeinated coffee and tea	1	2	3	4	5	6	7	☐☐
89	Tea	1	2	3	4	5	6	7	☐☐
90	Coffee	1	2	3	4	5	6	7	☐☐
93	Spreading fat (1) F ☐☐☐☐☐ _____	1	2	3	4	5	6	7	☐

94	Spreading fat (2) _____ F	1	2	3	4	5	6	7	☐
95	Spreading fat (3) _____ F	1	2	3	4	5	6	7	☐
96	Frying fat or oil (1) _____ F	1	2	3	4	5	6	7	☐
97	Frying fat or oil (2) _____ F	1	2	3	4	5	6	7	☐
98	Frying fat or oil (3) _____ F	1	2	3	4	5	6	7	☐

	FOOD DESCRIPTION	FREQUENCY EATEN							
FOOD CODE		Never	Once every 2-3 Months	Once a Month	Once a Fortnight	1-2 Times per Week	3-6 Times per Week	Once a day	More than once a day
99	Other vegetable oil (1) e.g. salad dressings, _____ marinades F	1	2	3	4	5	6	7	☐

100	Other vegetable oil (2) e.g. salad dressings, _____ marinades	F	1	2	3	4	5	6	7	☐

3.2 Are there food or drinks which you have eaten or drunk **once a week or more** which are not on the list?

0. No/1. Yes ☐

IF YES

NAME OF FOOD/DRINK	1-2 times per week	3-6 times per week	Once a day	More than once a day
				☐
				☐
				☐
				☐
				☐

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

3.3 Which types of milk have you used regularly in drinks and added to breakfast cereals over the last 3 months?

1. Whole pasteurised
2. Semi-skimmed pasteurised
3. Skimmed pasteurised
4. Whole UHT
5. Semi-skimmed UHT
6. Skimmed UHT
7. Other

Milk 1 ☐ Other (specify)_____

Milk 2 ☐ Other (specify)_____

Milk 3 ☐ Other (specify)_____

3.4 On average over the last 3 months how much

* of each milk have you consumed per day?

Milk 1 ☐.☐☐ pints

Milk 2 ☐.☐☐ pints

Milk 3 ☐.☐☐ pints

3.5 Do you add sugar to breakfast cereals, tea & coffee, puddings etc.? ☐

0. No *go to 3.7*
1. Yes

3.6 Approximately how many teaspoons of sugar do you add each day? ☐☐

3.7 When you eat meat, how much of the fat do you usually cut off (including chicken skin)? ☐

1. all 100%
2. most 60%
3. some 30%
4. none 0%
9. not applicable

4: FOOD SUPPLEMENTS

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

0. No 1. Yes

If yes, please state which:

(for number per day, record number of tablets/capsules/teaspoons per day, as appropriate)

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

5: GENERAL DIET QUESTIONS

5.1 Are the **past three months** typical of the way you generally eat? ☐

0. No
1. Yes
2. Reasonably

5.2 Still thinking about your normal pattern of eating - in a typical **week** how often do you:

*	NEVE	< once/ week	1-2 times	3-6 times	everyday
eat breakfast					
eat lunch					
eat an evening meal					
go out in the evening not necessarily to eat but also to socialise					

5.3 Just thinking about the **past week** how many servings did you eat of:

vegetables and vegetable-containing dishes (excluding potatoes)?	
fruit and pure fruit juices?	
meat and fish and their dishes?	

6: DIETING

6.1 Which of the following describes you best?

* 1. I have **NEVER** been on a diet to lose weight ☐

2. I have **ONLY ONCE** been on a diet to lose weight
3. I **USED TO** diet **REGULARLY** to lose weight but **DON'T ANYMORE**
4. I go on a diet to lose weight **EVERY NOW AND AGAIN**
5. I am **USUALLY** on a diet to lose weight

If 2, 4 or 5 ask 6.2 otherwise go to section 7

6.2 Are you currently trying to lose weight by dieting? ☐

0. No

1. Yes

7: ALCOHOL CONSUMPTION

I'd like to ask you a few questions about your drinking and smoking habits.

7.1 Do you ever drink alcohol? 0. No *go to section 8* ☐

1. Yes

During the past three months:

7.2 a) How often have you drunk

Shandy or Low Alcohol Beer/Lager/Cider? FFQ 1-7 ☐ >x1 ☐☐

(don't include alcohol **free** lager etc)

b) When you drank these how many pints did you normally have? ☐ . ☐☐

(if range given code mid-point)

7.3 a) How often have you drunk

Beer/Stout/Lager/Cider/Alcopops? FFQ 1-7 ☐ >x1 ☐☐

b) When you drank these how many pints did you normally have? ☐ . ☐☐

(if range given code mid-point)

7.4 a) How often have you drunk

Low alcohol wine? FFQ 1-7 ☐ >x1 ☐☐

b) When you drank this how many glasses did you normally have? ☐.☐☐

(if range given code mid-point)

7.5 a) How often have you drunk

Wine/Sherry/Martini/Cinzano? FFQ 1-7 ☐ > x1 ☐☐

b) When you drank these how many glasses did you normally have? ☐.☐☐

(if range given code mid-point)

7.6 a) How often have you drunk

Spirits/Liqueurs? FFQ 1-7 ☐ > x1 ☐☐

b) when you drank these how many measures did you normally have? ☐.☐☐

(IF RANGE GIVEN CODE MID-POINT)

8: SMOKING

8.1 Have you ever smoked regularly (at least once a day for a year or more) ? ☐

0. No *go to section 9*
1. Yes

8.2 How old were you when you first smoked regularly ? ☐☐

8.3 Are you currently smoking ?

0. No *go to section 9*

1. Yes *go to 8.4*

8.4 How many per day? *Record maximum stated*

9: FAMILY BACKGROUND

Now I'd like to ask some questions about your family.

Tell the woman that she may find some of these questions difficult or impossible to answer. Explain that you would like to leave a form for her to complete where possible by asking her parents for the details. Answers that she can give us now (even approximately) are useful but if she can supplement them later that would be extremely helpful.

Starting with your **FATHER**:

9.1 Is your father still alive?
0.No, 1.Yes, 7. Adopted, 8. Don't talk about him, 9. Don't know

9.2 What was his full-time job when you were born?
or if unemployed or part time, last full time job before that time.

Probe industry & self-employed/manager/foreman/employee.

If full time student give subject.

Job Position

Self-employed/manager/foreman/employee

Industry

9.3 Approximately what is/was his height?

In feet and inches? ft ins

. *OR In centimetres* cms

9.4 Approximately what is/was his current/latest weight?

In stones and pounds? ☐☐ st ☐☐ l bs

OR In kilograms? ☐☐.☐ kg

9.5 WHAT WAS HIS BIRTH WEIGHT?

In pounds and ounces? ☐☐ lbs ☐☐ . ☐ oz

OR In grams? ☐☐☐☐ grams

Now your **MOTHER**:

9.6 Is your mother still alive? ☐
0. No, 1.Yes, 7. Adopted, 8. Don't talk about her, 9. Don't know

9.7 and what was her **full** name when you were born? _____

_____

9.8 What is/was her date of birth?

d d	m m	y y
☐☐	☐☐	☐☐

9.9 Where was she born?

If in UK: Town/Village _____

County _____

If abroad: Country _____

9.10 WHAT IS/WAS HER HEIGHT?

In feet and inches ☐ ft☐☐.☐ ins

OR In centimetres? ☐☐☐.☐ cm

9.11 WHAT DID SHE WEIGH BEFORE YOU WERE CONCEIVED?

In stones and pounds? ☐☐ st☐☐ lbs

OR In kilograms? ☐☐☐.☐ kg

9.12 WHAT WAS HER BIRTH WEIGHT?

In pounds and ounces? ☐☐ lbs☐☐ .oz

OR In grams? ☐☐☐☐ g rams

Returning to **YOURSELF**:

9.13 What is your date of birth? d d m m y y

9.14 What was your birth weight?

In pounds and ounces? ☐☐ lbs ☐☐ oz

Or In grams? ☐☐☐☐ grams

9.15 Where were you born?

If in UK: Town/Village_____

County _____

***If abroad*: Country** _____

9.16 Were you born at home or in hospital ?

1. Home ☐
2. Hospital - specify _____

9.17 Were you part of a multiple birth (twin, triplet etc.)?

0. No ☐
1. Yes

9.18 Were you born early, late or when you were expected?

1. Early ☐
2. When expected *go to 9.20*
3. Late
9. Don't know

9.19 How early/ late were you? ☐☐ weeks ☐ days

1. Certain
2. Not certain or mid point of a range ☐

9.20 How many children did your mother have before you were born (including stillbirths)? ☐☐

9.21 Do you have any sisters aged 20 or over? ☐

0.No, 1.Yes

10: EDUCATION

I would like to ask you briefly about your education.

10.1 How old were you when you left full-time education ? ☐☐

(don't round up; enter current age if still studying)

yrs

(count a year or less out as continuous education)

10.2 Have you passed any exams or do you have any formal qualifications ?

1. None
2. CSE/ School cert/ GCSE grade D or lower/ NVQ1/ Foundation GNVQ
3. O levels/ Matric/ GCSE grade A,B,C/ RSA secretarial/ NVQ2/ Intermediate GNVQ ☐
4. A levels/ City & Guilds/ EN(G)/ ONC/ NNEB/ BTech (day release)/ NVQ3/ Advanced GNVQ/ OND / HNC
5. HND/ RGN/ Teaching Cert/ NVQ4
6. Degree/ NVQ5
7. Other (specify)

11: ETHNIC GROUP

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

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

12: MARITAL STATUS

12.1 What is your marital status?

1. Single (never married)
2. Married (living with husband)
3. Separated
4. Divorced
5. Widowed

13: HOUSING

13.1 WHAT TYPE OF ACCOMMODATION DO YOU LIVE IN?

1. Detached house/bungalow
2. Semi-detached house/bungalow
3. End terraced house
4. Terraced house
5. Purpose built flat/maisonette
6. Converted flat/maisonette
7. Dwelling with business premises
8. Bedsitter in multiple occupation
9. Bedsitter other
10. Hostel
11. Hall of residence
12. Other student accommodation
13. Other (specify)_____

☐☐

13.2 On what floor is the main part of living accommodation?
(If more than one code the lowest)

1. Basement
2. Ground floor/street level
3. 1st floor
4. 2nd floor
5. 3rd floor
6. 4th to 9th floor
7. 10th to 19th floor
8. 20th floor or higher

☐

13.3 Do you own your own home, or are you buying it on a mortgage, or do you rent it in some way?

*

1. Owns outright or buying with mortgage
2. Rent from private landlord
3. Rent from council or housing association
4. Other rented accommodation (hostel, hall of residence, B& B)
5. Lives with parents
6. Other (specify)_____

☐

13.4 Here is a list of some problems that people often have with their homes. Please tell me if you think that each one is a big problem, a small problem or not a problem for you and your family? *(Tick appropriate boxes)*

*	Big problem	Small problem	Not a problem
CONDENSATION			
Rising or penetrating damp			
Difficulty in keeping home warm			
Leaking roof			
Rot in window frames, timbers or floorboards			
Not enough space			

14: HOUSEHOLD COMPOSITION AND CHILDREN

14.1. Does anyone else live in the house with you? ☐
0 = No *go to 14.2*
1 = Yes

For each person living in the household (apart from the woman herself) complete one line.
A household is defined as a group of people who share a living room or eat together for at least one meal a day. People living in hostels or halls of residence are classed as living alone.
For ***all children*** *(see younger generation list) record* ***date of birth*** *(or age if d.o.b. is not available).*
For the ***woman's own children*** *give the child's* ***birthweight.***
For all adults, record whether they currently smoke at least once a day. 0=No, 1=Yes
Days per week is for anyone who is only in the household part-time. *Record the average number of days per week that person lives in the household.*

KEY:

Own Generation

H	=	*Husband*
C	=	*Cohabitee*
S	=	*Sibling (brother/sister)*
AS	=	*Adopted sibling*
SIL	=	*Sibling-in-law (sister/brother-in-law)*
SS	=	*Stepsibling*
FS	=	*Foster sibling*

Older Generation

P	=	*Parent*
FP	=	*Foster parent*
SP	=	*Step parent*
PIL	=	*Parent-in-law*
GP	=	*Grandparent*

Younger Generation

OC	=	*Own child (son/daughter)*
SC	=	*Step child*
AC	=	*Adopted child*
FC	=	*Foster child*
CIL	=	*Child-in-law (son/daughter–in-law)*
CC	=	*Cohabitee's child*
GC	=	*Grandchild*
SB	=	*Still born child*

Other

OR	=	*Other relative*
ON	=	*Other non-relative*

Person number	Relationship to woman	Sex M	Sex F	Date of birth Day	Date of birth Mth	Date of birth Yr	Age (yrs)	BIRTHWEIGHT lb	BIRTHWEIGHT oz	BIRTHWEIGHT grams	Smoker	Days per week
1												
2												
3												
4												
5												
6												
7												
8												
9												
10												
11												
12												
13												
14												
15												
16												
17												
18												

14.2. HOW MANY CHILDREN HAVE YOU HAD, INCLUDING ANY STILLBIRTHS? ☐

(ANY NOT INCLUDED ABOVE ADD TO THE TABLE WITH 0 DAYS/WEEK)

14.4 *If the woman has a child under the age of two years:* Are you breastfeeding your (youngest) child? *(Any amount of breastfeeding counts as yes)* ☐

0. No 1. Yes

15: PARTNER'S OCCUPATION

If there is a husband or partner living in the house (if not go to 16):

15.1 Was your husband/partner in paid employment or self-employed in the week ending last Sunday?

0. No *go to 15.3*
1. Yes *go to to 15.2*

15.2 Was he working full time or part time?

0. Full time (more than 30 hours) *go to 15.6b*
1. Part time (30 hours or fewer) *go to 15.3*

15.3 Was he going to college full time?

0.No *if working part-time go to 15.6a*
if not working go to 15.5
1.Yes

15.4 *If yes,* what is he studying? _____

If working part time go to 15.7

If not working go to section 16.

15.5 *If not working or studying* was he

Unemployed ? (1)

Permanently unable to work because of long term sickness or disability ? (2)

looking after home or family? (3)

other ? (specify)_____ (4)

15.6a *If **not** working or working part-time*, what was his last **full-time** job?

If only ever part-time give last part time job.

Then if currently working part time go to 15.7, otherwise go to section 16

Job Position _____

Self-employed/manager/foreman/employee

Industry _____

15.6b *If working full-time,* what is his job ? *(Then go to section 16)*

Probe industry & self-employed/manager/foreman/employee

Job Position

Self-employed/manager/foreman/employee

Industry _____

15.7 *If working part-time now,* what is his current job?

Job Position _____

Self-employed/manager/foreman/employee

Industry _____

15.8 *If working part time,* how many hours per week does he work?

☐☐ hrs ☐☐ mins

16: CHILDCARE ARRANGEMENTS

16.1 *If the woman works (part-time or full-time) and has children at home under the age of twelve years: (if not go to section 17)*

Which of the following best describes the way you arrange for your children aged 12 or under to be looked after while you are at work?
Tick up to three boxes.

*	1st mention	2nd mention	3rd mention
1. I work only while they are at school			
2. They look after themselves until I get home			
3. I work from home			
4. My husband/partner looks after them			
5. A nanny or mother's help looks after them at home			
6. They go to a work-place nursery			
7. They go to a day nursery			
8. They go to a child minder			
9. A relative looks after them			
10. A friend or neighbour looks after them			
11. Other (specify)			

17: BENEFITS

17.1 Are you (or your husband/partner) receiving any of the following benefits?

* (Income support/job seekers allowance/family credit/housing benefit)

0 = No *go to section 18* ☐

1 = Yes

17.2 How long have you been receiving them?

(0=No, 1=<1 year, 2=1-2 years, 3=2+years, 9=Don't know)

(a) Income support ☐

(b) Job seekers allowance ☐

(c) Family credit ☐

(d) Housing benefit ☐

*IF NOT DONE BEFORE, GET **CONSENT** HERE*

18: BODY MEASUREMENTS

18.1 Pulse (30sec) ☐☐☐

(Double the value to give pulse for 1 minute)

18.2 Which hand do you write with ? ☐

1. Right
2. Left
3. Completely ambidextrous

18.3 Weight ☐☐☐ . ☐ kg

18.4 Height ☐☐☐ . ☐ cm

MARK AND MEASURE UP THE NON-DOMINANT ARM AND SIDE OF THE BODY

(measure the left if completely ambidextrous)

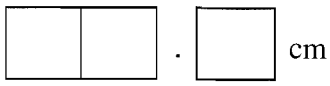
18.5 Leg length ☐☐☐ . ☐ cm

18.6 Waist circumference ☐☐☐ . ☐ cm

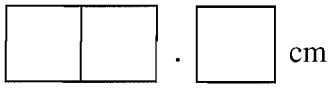
18.7 Hip circumference ☐☐☐ . ☐ cm

18.8 Mid-thigh circumference ☐☐ . ☐ cm

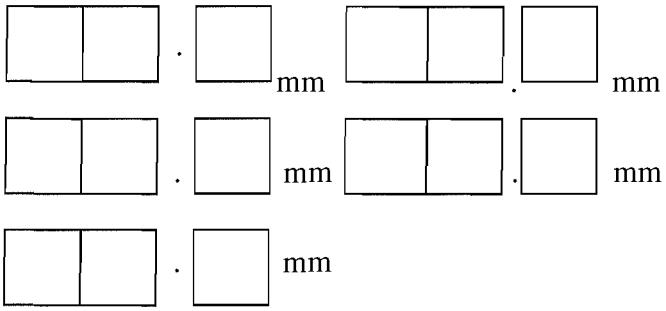
18.9 Calf circumference



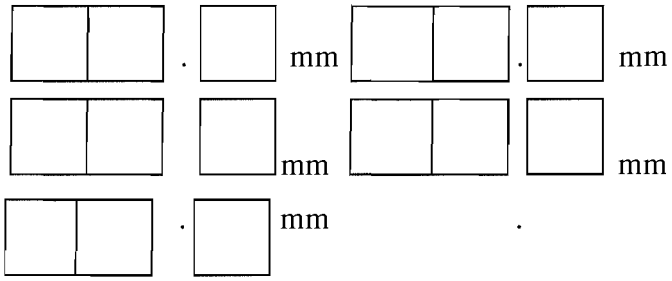
18.10 Mid-upper arm circumference
(non-dominant side)



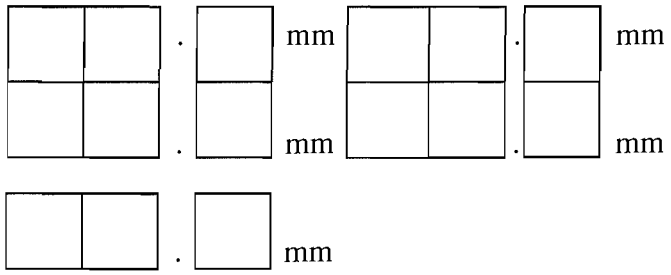
18.11 Triceps skinfold
(non-dominant side)

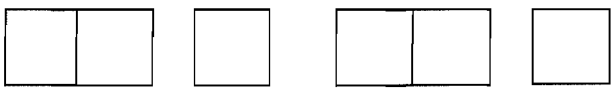


18.12 Biceps skinfold
(non-dominant side)



18.13 Subscapular skinfold
(non-dominant side)





18.14 Upper suprailiac skinfold . mm . mm

(non-dominant side) ☐☐.☐ mm ☐☐.☐ mm

☐☐.☐ mm

18.15 Skinfold calipers used ☐☐☐

18.16 Time (24 hr clock) ☐☐☐☐

19: MOUTHWASH SAMPLE

If the mouthwash sample was obtained at the beginning, go to section 20

19.1 Mouthwash sample provided

(0=No, 1=Yes) ☐

19.2 Time of mouthwash sample (24 hr clock) ☐☐☐☐

20: GENERAL HEALTH

20.1 How is your health in general? Would you say it was:

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

20.2 Do you have any long-standing illness, disability or infirmity? By long standing, I mean anything that has troubled you over a period of time or that is likely to affect you over a period of time.

0. No *go to 20.4*
1. Yes ☐

20.3 What is the illness/disability/infirmity?_____

(Do not record headaches, indigestion, aches and pains. We are interested in major problems such as diabetes , multiple sclerosis, rheumatoid arthritis, muscular dystrophy – anything which might affect growth or body composition)

20.4 To what extent do you feel that the stress or pressure you have experienced in your life has affected your health?

* 1. None
2. Slightly
3. Moderately ☐
4. Quite a lot
5. Extremely

20.5 In general, how much stress or pressure have you experienced in your daily living in the last 4 weeks?

* 1. None
2. Just a little ☐
3. A good bit
4. Quite a lot
5. A great deal

21: MENSTRUAL CYCLE AND PREGNANCIES

21.1 What was the date of the first day of your last menstrual period?

d d m m y y

☐☐ ☐☐ ☐☐

21.2 How long is your usual cycle between the start of one period and the start of the next period? ☐☐ days

(Don't know 99)

21.3 Is your usual cycle regular, or has it varied by more than 5 days between periods in the last 6 months? ☐

1: Regular

2: Varied by more than 5 days

21.4 How old were you when you had your first period ? ☐☐.☐ yrs

(Don't know 99.9)

21.5 Within the last 3 months have you taken the oral contraceptive pill or had the Depot injection or other hormonal treatment? ☐

0. No *go to 21.8*

1. Yes

21.6 Which? Specify (most recent if several) _____

21.7 Are you currently taking this? ☐

0. No

1. Yes

21.8 Do you anticipate trying for a baby within the next 12 months? ☐

0. No

1. YES

That is the end of the questionnaire but we would be grateful for your help with some extra items.

Use the explanations in fieldworker notes for the following items but please mark the results below:

Have you left a birth details form?

0. No ☐

1. Yes

Have you left a food diary?

0. No ☐

1. Yes

Is there agreement to a blood sample?

(Remember to mark the woman's record card as well)

0. No ☐

1. Yes

*Has **consent** been obtained for the GP to notify us if the woman becomes pregnant?*

0. No ☐

1. Yes

Is the woman willing to be approached for other studies related to the SWS?

0. No ☐

1. Yes

Don't forget to leave a fridge magnet, pregnancy reply card, two prepaid envelopes (one large and one small), and, if the woman is interested, an information leaflet.

THANK YOU VERY MUCH FOR ALL YOUR HELP. THE INFORMATION YOU HAVE GIVEN US IS VERY IMPORTANT FOR IMPROVING THE HEALTH OF WOMEN. THE MORE WOMEN WHO TAKE PART, THE MORE VALUABLE ALL THE DATA BECOME SO WE WOULD BE VERY GRATEFUL IF YOU WOULD ENCOURAGE YOUR FRIENDS TO TAKE PART.

MANY THANKS AGAIN

Local Research Ethics
Committee No 276/97

APPENDIX II : SWS MATERNAL EARLY PREGNANCY QUESTIONNAIRE

SWS Serial Number

EARLY PREGNANCY
QUESTIONNAIRE

Name: (Forename, Surname) _____

Address: _____

_____

Postcode: | | | | | | | |

Date of Birth: d d m m y y | | | | | | |

Interviewer: | | Date of interview: d d m m y y | | | | | |

We would like to send details of your ultrasound scan report to your GP to assist in your care during pregnancy. Are you happy for us to do this?

0. No
1. Yes | |

If yes: *May I just confirm your GP's name and address:*

GP's name: _____

Surgery Address: _____

1: ACTIVITY AND EXERCISE

Can I firstly ask you about your activity and exercise patterns over the last three months? As before, we would like you to divide up a "typical" day into three types of activity. These are:

(1) sleeping or lying, (2) sitting, (3) standing or walking.

1.1 Over a typical 24 hour day how many hours have you generally spent sleeping or lying with your feet up? ☐☐ hrs ☐☐ mins

(ask time usually go to bed & wake up, including any at work!)

This would indicate xx hours sitting or on your feet.

1.2 Of those hours how many on a typical day have you spent sitting down? *(e.g. includes sitting at work, mealtimes, driving, reading, watching TV)*

☐☐ hrs ☐☐ mins

1.3 This would mean that you have spent about xx hours a day on your feet. Does this sound about right?

☐☐ hrs ☐☐ mins

1.4 Out of these xx hours spent on your feet, about how much of the time were you **actively on the move** (rather than standing fairly still)?

* ☐

1.	Very little	10%
2.	Some	30%
3.	About half	50%
4.	Most	70%
5.	Almost all	90%

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

a) **strenuous exercise** which made your heart beat rapidly **AND** left you breathless e.g. jogging, vigorous swimming or cycling, aerobics.

FFQ categories 1-7 ☐ >x1 ☐

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

b) **moderate exercise** which left you exhausted but not breathless, e.g. brisk walking, dancing, easy swimming or cycling, badminton, sailing.

FFQ categories 1-7 ☐ >x1 ☐☐

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

c) **gentle exercise** which left you tired but not exhausted, e.g.walking, heavy housework (including washing windows and polishing), gardening, DIY, golf.

FFQ categories 1-7 ☐ >x1 ☐☐

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

1.6 * Which of the following best describes your walking speed at present? ☐

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

2: DIETARY QUESTIONS

2.1 Now I am going to ask you about the foods you have eaten over the past 3 months. To do this I have a list of foods and I would like you to tell me how often you have eaten each food. As before the list may include foods you <u>never</u> ate or you may find foods which you eat a lot are missing. These can be added on at the end. *(Define the 3 month period)*

	FOOD DESCRIPTION	FREQUENCY EATEN							
FOOD CODE		Never	Once every 2-3 Months	Once a Month	Once a Fortnight	1-2 Times per Week	3-6 Times per Week	Once a day	More than once a day
1	White Bread	1	2	3	4	5	6	7	☐
	When you ate bread/toast/sandwiches, how many slices/rolls did you eat at a typical meal? ☐☐.☐ *Rolls (count as 2 slices)* *French bread (2" counts as 1 slice)*								
2	Brown and wholemeal bread/rolls	1	2	3	4	5	6	7	☐
	How many slices/rolls did you eat at a typical meal? ☐☐.☐ *Rolls (count as 2 slices)*								

3	Crackers and cheese biscuits	1	2	3	4	5	6	7	☐
4	Wholemeal and rye crackers	1	2	3	4	5	6	7	☐
5	'Bran' breakfast cereals	1	2	3	4	5	6	7	☐

	FOOD DESCRIPTION	FREQUENCY EATEN							
FOOD CODE		Never	Once every 2-3 Months	Once a Month	Once a Fortnight	1-2 Times per Week	3-6 Times per Week	Once a day	More than once a day
6	Other breakfast cereals	1	2	3	4	5	6	7	☐
7	Added bran to foods	1	2	3	4	5	6	7	☐
8	Cakes and gateaux	1	2	3	4	5	6	7	☐

9	Buns	1	2	3	4	5	6	7	
10	Pastries	1	2	3	4	5	6	7	
11	Biscuits-chocolate, digestive and ginger	1	2	3	4	5	6	7	
12	Other biscuits	1	2	3	4	5	6	7	
13	Fruit puddings	1	2	3	4	5	6	7	
14	Milk based puddings and sauces	1	2	3	4	5	6	7	

FOOD CODE	FOOD DESCRIPTION	FREQUENCY EATEN							
		Never	Once every 2-3 Months	Once a Month	Once a Fortnight	1-2 Times per Week	3-6 Times per Week	Once a day	More than once a day
15	Other puddings	1	2	3	4	5	6	7	☐
16	Yogurt and fruit fools	1	2	3	4	5	6	7	☐
17	Potatoes – boiled and jacket	1	2	3	4	5	6	7	☐
	When you ate these how many potatoes did you eat at a typical meal? ☐☐ ☐ *Large baking (count as 3)/ new (count as 0.5)*								
18	Roast potatoes and chips	1	2	3	4	5	6	7	☐
	When you ate these how many potatoes did you eat at a typical meal? ☐☐ ☐								

19	Yorkshire puddings and savoury pancakes	1	2	3	4	5	6	7	☐
20	Brown and white rice	1	2	3	4	5	6	7	☐
21	Pasta and dumplings	1	2	3	4	5	6	7	☐

	FOOD DESCRIPTION	FREQUENCY EATEN							
FOOD CODE		Never	Once every 2-3 Months	Once a Month	Once a Fortnight	1-2 Times per Week	3-6 Times per Week	Once a day	More than once a day
22	Tinned vegetables	1	2	3	4	5	6	7	☐
23	Peas and green beans	1	2	3	4	5	6	7	☐
24	Carrots	1	2	3	4	5	6	7	☐
25	Parsnips, swede and turnip	1	2	3	4	5	6	7	☐

26	Sweetcorn and mixed veg	1	2	3	4	5	6	7	☐
27	Beans and pulses	1	2	3	4	5	6	7	☐
28	Tomatoes	1	2	3	4	5	6	7	☐
29	Spinach	1	2	3	4	5	6	7	☐
30	Broccoli, Brussels sprouts and spring greens	1	2	3	4	5	6	7	☐

	FOOD DESCRIPTION	FREQUENCY EATEN							
FOOD CODE		Never	Once every 2-3 Months	Once a Month	Once a Fortnight	1-2 Times per Week	3-6 Times per Week	Once a day	More than once a day
31	Cabbage and cauliflower	1	2	3	4	5	6	7	☐
32	Peppers and watercress	1	2	3	4	5	6	7	☐
33	Onion	1	2	3	4	5	6	7	☐
34	Green salad	1	2	3	4	5	6	7	☐
35	Side salads in dressing	1	2	3	4	5	6	7	☐
36	Courgettes, marrow and leeks	1	2	3	4	5	6	7	☐
37	Mushrooms	1	2	3	4	5	6	7	☐

38	Vegetable dishes	1	2	3	4	5	6	7	☐
39	Vegetarian foods	1	2	3	4	5	6	7	☐

	FOOD DESCRIPTION	FREQUENCY EATEN							
FOOD CODE		Never	Once every 2-3 Months	Once a Month	Once a Fortnight	1-2 Times per Week	3-6 Times per Week	Once a day	More than once a day
40	Tinned fruit not including grapefruit, prunes, figs or blackcurrants	1	2	3	4	5	6	7	☐
41	Cooked fruit not including blackcurrants	1	2	3	4	5	6	7	☐
42	Dried fruit	1	2	3	4	5	6	7	☐
43	Fresh apples and pears	1	2	3	4	5	6	7	☐
44	Fresh oranges and orange juice	1	2	3	4	5	6	7	☐☐

45	Grapefruit and grapefruit juice	1	2	3	4	5	6	7	
46	Blackcurrants, ribena and hi-juice blackcurrant drinks	1	2	3	4	5	6	7	
47	Other fruit juices (not squashes)	1	2	3	4	5	6	7	
48	Diet Coke and Pepsi not including caffeine free	1	2	3	4	5	6	7	

	FOOD DESCRIPTION	FREQUENCY EATEN							
FOOD CODE		Never	Once every 2-3 Months	Once a Month	Once a Fortnight	1-2 Times per Week	3-6 Times per Week	Once a day	More than once a day
49	Coke and Pepsi	1	2	3	4	5	6	7	
50	Soft drinks not including diet drinks (low calorie or low sugar)	1	2	3	4	5	6	7	
51	Bananas	1	2	3	4	5	6	7	

52	Fresh peaches, plums, cherries and grapes	1	2	3	4	5	6	7	☐
53	Strawberries and raspberries	1	2	3	4	5	6	7	☐
54	Fresh pineapple, melon, kiwi and other tropical fruits	1	2	3	4	5	6	7	☐
55	Nuts	1	2	3	4	5	6	7	☐
56	Bacon and gammon	1	2	3	4	5	6	7	☐
57	Pork	1	2	3	4	5	6	7	☐
58	Chicken and turkey	1	2	3	4	5	6	7	☐

	FOOD DESCRIPTION	FREQUENCY EATEN							
FOOD CODE		Never	Once every 2-3 Months	Once a Month	Once a Fortnight	1-2 Times per Week	3-6 Times per Week	Once a day	More than once a day
59	Lamb	1	2	3	4	5	6	7	☐
60	Beef	1	2	3	4	5	6	7	☐
61	Minced meat dishes	1	2	3	4	5	6	7	☐
62	Meat Pies	1	2	3	4	5	6	7	☐
63	Liver and kidney	1	2	3	4	5	6	7	☐
64	Paté and liver sausage	1	2	3	4	5	6	7	☐
65	Faggots and black pudding	1	2	3	4	5	6	7	☐

66	Sausages	1	2	3	4	5	6	7	☐
67	Ham and luncheon meat	1	2	3	4	5	6	7	☐
68	White fish	1	2	3	4	5	6	7	☐
	FOOD DESCRIPTION	FREQUENCY EATEN							
FOOD CODE		Never	Once every 2-3 Months	Once a Month	Once a Fortnight	1-2 Times per Week	3-6 Times per Week	Once a day	More than once a day
69	Fish fingers and fish dishes	1	2	3	4	5	6	7	☐
70	Oily fish	1	2	3	4	5	6	7	☐
71	Shellfish	1	2	3	4	5	6	7	☐
72	Boiled and poached eggs	1	2	3	4	5	6	7	☐

73	Omelette and fried eggs	1	2	3	4	5	6	7	
74	Cottage Cheese	1	2	3	4	5	6	7	
75	Cheese	1	2	3	4	5	6	7	
76	Pizza, quiches and cheese flans	1	2	3	4	5	6	7	
77	Soup	1	2	3	4	5	6	7	
78	Mayonnaise and salad cream	1	2	3	4	5	6	7	

FOOD CODE	FOOD DESCRIPTION	FREQUENCY EATEN							
		Never	Once every 2-3 Months	Once a Month	Once a Fortnight	1-2 Times per Week	3-6 Times per Week	Once a day	More than once a day
79	Pickles, chutney, tomato ketchup and brown sauce	1	2	3	4	5	6	7	☐
80	Chocolate	1	2	3	4	5	6	7	☐☐
81	Other sweets	1	2	3	4	5	6	7	☐☐
82	Ice cream and chocolate desserts	1	2	3	4	5	6	7	☐
83	Cream	1	2	3	4	5	6	7	☐
84	Crisps and savoury snacks	1	2	3	4	5	6	7	☐☐
85	Sweet spreads	1	2	3	4	5	6	7	☐

86A	Gravy granules and powders	1	2	3	4	5	6	7	
86B	Stock cubes and Marmite	1	2	3	4	5	6	7	

	FOOD DESCRIPTION	FREQUENCY EATEN							
FOOD CODE		Never	Once every 2-3 Months	Once a Month	Once a Fortnight	1-2 Times per Week	3-6 Times per Week	Once a day	More than once a day
87	Drinking chocolate and milk shakes not including McDonald style milkshakes	1	2	3	4	5	6	7	
88	Decaffeinated coffee and tea	1	2	3	4	5	6	7	
89	Tea	1	2	3	4	5	6	7	
90	Coffee	1	2	3	4	5	6	7	
93	Spreading fat (1) _____ F	1	2	3	4	5	6	7	
	F								

94	Spreading fat (2) _____	1	2	3	4	5	6	7	☐
95	Spreading fat (3) _____	1	2	3	4	5	6	7	☐
96	Frying fat or oil (1) _____	1	2	3	4	5	6	7	☐
97	Frying fat or oil (2) _____	1	2	3	4	5	6	7	☐
98	Frying fat or oil (3) _____	1	2	3	4	5	6	7	☐

FOOD CODE	FOOD DESCRIPTION	FREQUENCY EATEN							
		Never	Once every 2-3 Months	Once a Month	Once a Fortnight	1-2 Times per Week	3-6 Times per Week	Once a day	More than once a day
99	Other vegetable oil (1) e.g. salad dressings, marinades _____ F ☐☐☐☐ ☐ ☐	1	2	3	4	5	6	7	☐
100	Other vegetable oil (2) F ☐☐☐☐	1	2	3	4	5	6	7	☐

	e.g. salad dressings, _____ marinades								

2.2 Are there food or drinks which you have eaten or drunk **once a week or more** which are not on the list? Include breakfast bars such as Nutrigrain and Kellogg's

0.No/1. Yes ☐

IF YES

NAME OF FOOD/DRINK	1-2 times per week	3-6 times per week	Once a day	More than once a day
☐☐☐☐☐				☐
☐☐☐☐☐				☐
☐☐☐☐☐				☐
☐☐☐☐☐				☐
☐☐☐☐☐				☐

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

2.3: Which types of milk have you used regularly in drinks and added to breakfast cereals over the last 3 months?

0. None
1. Whole pasteurised
2. Semi-skimmed pasteurised
3. Skimmed pasteurised
4. Whole UHT
5. Semi-skimmed UHT
6. Skimmed UHT
7. Other

Milk 1 ☐ Other (specify)_____

Milk 2 ☐ Other (specify)_____

Milk 3 ☐ Other (specify)_____

2.4 On average over the last 3 months how much

* of each milk have you consumed per day?

Milk 1 ☐.☐☐ pints

Milk 2 ☐ ☐☐ pints

Milk 3 ☐ ☐☐ pints

2.5 Have you added sugar to breakfast cereals, tea & coffee, puddings etc.?

0. No *go to 2.7* ☐
2. Yes

2.6 Approximately how many teaspoons of sugar have you added each day?

☐☐

2.7 When you eat meat, how much of the fat have you usually cut off (including chicken skin)?

1. all 100%
2. most 60%
3. some 30%
4. none 0%
4. not applicable

☐

2.8 Just thinking about the **past week** how many servings did you eat of:

Vegetables and vegetable-containing dishes (excluding potatoes)?	
fruit and pure fruit juices?	
meat and fish and their dishes?	

3: FOOD SUPPLEMENTS & DIETARY CHANGES

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

0. No 1. Yes

If yes, please state which:

(for number per day, record number of tablets/ capsules/ teaspoons per day, as appropriate)

Supplement	Number per day	How many days in the last 90?	Did you start taking this: 1: Less than 1 month ago 2: 1-2 months ago 3: More than 2 months ago
☐☐☐☐			
☐☐☐☐			
☐☐☐☐			
☐☐☐☐			
☐☐☐☐			

3.2 We have asked you about your diet on 2 occasions. In the time between our **first visit**

*

in .....(month) .....(year) and your **last menstrual period** in

.....(month) .....(year) were there **major** changes in any of the following?

0: No ☐

1: Yes

If no go to Section 4.

3.3 (a) How often you were eating meat and meat dishes?

1: more

2: same ☐

3: less

4: stopped completely

(b) How often you were eating fruit and vegetables?

1: more

2: same ☐

3: less

(c) The amount of milk and other dairy products you were consuming

1: more

2: same ☐

3: less

(d) The amount of alcoholic drinks you were consuming.

1: more

2: same ☐

3: less

4: stopped completely

APPETITE AND NAUSEA DURING PREGNANCY

4.1: Have you experienced any nausea or sickness since becoming pregnant? ☐

0. No
1. Yes

If yes, has this been:

1. Mild (nausea only)
2. Moderate (sometimes sick) ☐
3. Severe (regularly sick, can't retain meals)

4.2 **Since you became pregnant,** are you eating:

1. More
2. The same ☐
3. Less <u>in amount</u>

4.3 If **more,** is this

* 1. Because you feel more hungry
2. To prevent you feeling sick ☐
3. Because you feel it is best for the baby
(9. Not sure/other reason)

If **less**, is this

* 1. Because you feel less hungry
2. Because of nausea/sickness ☐
3. Don't want to put on too much weight
(9. Not sure/other reason)

5: ALCOHOL CONSUMPTION

I'd like to ask you a few questions about your drinking and smoking habits.

5.1 Do you ever drink alcohol? ☐

0. No *go to section 6*
1. Yes

5.2 **During the past three months:**

a) How often have you drunk

Shandy or Low Alcohol Beer/Lager/Cider? FFQ 1-7 ☐ >x1 ☐☐

(don't include alcohol **free** lager etc)

b) When you drank these how many <u>pints</u> did you normally have?

(if range given code mid-point) ☐☐ . ☐☐

5.3 a) How often have you drunk

Beer/Stout/Lager/Cider/Alcopops? FFQ 1-7 ☐ >x1 ☐☐

b) When you drank these how many <u>pints</u> did you normally have?

(if range given code mid-point) ☐☐ . ☐☐

5.4 a) How often have you drunk

Low alcohol wine? FFQ 1-7 ☐ >x1 ☐☐

b) When you drank this how many <u>glasses</u> did you normally have?

(if range given code mid-point) ☐☐ . ☐☐

5.5 a) How often have you drunk

Wine/Sherry/Martini/Cinzano? FFQ 1-7 ☐ > x1 ☐☐

b) When you drank these how many <u>glasses</u> did you normally have?

(if range given code mid-point) ☐☐ . ☐☐

5.5 a) How often have you drunk

Spirits/Liqueurs? FFQ 1-7 ☐ > x1 ☐☐

b) when you drank these how many <u>measures</u> did you normally have?

(if range given code mid-point) ☐☐ . ☐☐

6: SMOKING

6.1 Did you smoke at the time of your last menstrual period? ☐

0. No *go to 6.3*
1. Yes

6.2 How many per day (record maximum stated)? ☐☐

6.3 Are you currently smoking? ☐

0. No *go to 6.5*
1. Yes

6.4 How many per day? (code max) ☐☐

Go to Section 7

6.5 Does anyone smoke regularly **in the same room as you?** ☐

0. No
1. Yes

7: MEDICINES

I would like to ask you now about any <u>medicines</u> you may have taken.

7.1 What, if any, medicines/inhalers/pills, tablets indigestion remedies have you taken <u>since your last menstrual period?</u>

USE BLOCK CAPITALS & COPY NAMES DIRECTLY OFF BOTTLES IF POSSIBLE

1		
2		
3		
4		
5		
6		

7		
8		

8: PREGNANCIES AND ILLNESSES

8.1 Have you had any previous pregnancies of more than 28 weeks?

0. No
1. Yes ☐

I would now like to ask you a few questions about any ILLNESSES you may have suffered from:

If no to 8.1, go to 8.3

8.2 During your previous pregnancies were you ever treated by a doctor for:

a) High blood pressure (treatment includes admission/bed rest/induction)

0. No
1. Yes ☐

b) Diabetes

0. No
1. Yes ☐

c) Anaemia

0. No
1. Yes ☐

d) Were you anaemic after the birth of any of your previous babies?

0. No
1. Yes ☐

8.3 When not pregnant have you ever been treated by a doctor for:

a) High blood pressure (don't include pill associated high BP)

0. No
1. Yes

b) Diabetes

0. No
1. Yes

c) Anaemia

0. No
1. Yes

8.4 **Either** as a child or an adult, have you ever suffered from asthma?

0. No
1. Yes

If Yes a) was this confirmed by a doctor?

0.No
1.Yes

8.5 Have you had wheezing or whistling in the chest in the last 12 months?

0.No *go to 8.7*
1.Yes

8.6 How many attacks of wheezing have you had in the last 12 months?

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

8.7 Did you suffer from eczema in childhood?

0. No
1. Yes

8.8 Have you had eczema affecting the creases of your elbows or knees in the last year?

0. No

1. Yes

8.9 Have you ever had a problem with sneezing, or a runny, or blocked nose when you DID NOT have a cold or 'flu?

0.No *go to section 9*

1.Yes

8.10 Is the nose problem usually accompanied by itchy-watery eyes?

0.No

1.Yes

8.11 In the last 12 months, have you had a problem with sneezing, or a runny, or blocked nose when you DID NOT have a cold or the 'flu?

0.No *go to section 9*

1.Yes

8.12 Have you used any medicines to treat hayfever, rhinitis or any other nasal problems, at any time in the last 12 months (including sprays, solutions, pills, capsules or tablets)?

0.No

1.Yes

9: BABY'S FATHER

Now I would like to ask some questions about the baby's natural father:

9.1 **Either** as a child or an adult, has he ever suffered from asthma?

0. No *go to 9.3*

1. Yes

8. Don't talk about him *go to Section 11*

9.2 If Yes a) was this confirmed by a doctor?

0.No

1.Yes

9. Don't know

9.3 Has he had wheezing or whistling in the chest in the last 12 months?

0.No *go to 9.5*

1.Yes

9. Don't know

9.4 How many attacks of wheezing has he had <u>in the last 12 months?</u>

0. None

1. 1-3

2. 4-12 ☐

3. More than 12

9. Don't know

9.5 Did he suffer from eczema in childhood?

0. No ☐

1. Yes

9. Don't know

9.6 Has he had eczema affecting the creases of his elbows or knees in the last year?

0. No ☐

1. Yes

9. Don't know

9.7 Has he <u>ever</u> had a problem with sneezing, or a runny, or blocked nose when he DID NOT have a cold or 'flu?

0.No *go to 9.11* ☐

1.Yes

9. Don't know

9.8 Is the nose problem usually accompanied by itchy-watery eyes?

0.No ☐

1.Yes

9. Don't know

9.9 <u>In the last 12 months,</u> has he had a problem with sneezing, or a runny, or blocked nose when he DID NOT have a cold or the 'flu?

0.No *go to 9.11* ☐

1.Yes

9.Don't know

9.10 Has he used any medicines to treat hayfever, rhinitis or any other nasal problems, at any time <u>in the last 12 months</u> (including sprays, solutions, pills, capsules or tablets)?

0.No

1.Yes

9. Don't know

9.11 Approximately what is his height?

In feet and inches ☐ ft ☐☐ . ☐ ins

OR in centimetres ☐☐☐ . ☐ cm

9.12 Approximately what is his current weight?

In stones and pounds ☐☐ st ☐☐ lb

OR in kilograms ☐☐☐ ☐ kg

.

9.13 What was his birth weight?

In pounds and ounces ☐☐ lbs ☐☐ oz

OR in grams ☐☐☐☐ grams

9.14 What is his date of birth?

d d	m m	y y
☐☐	☐☐	☐☐

10: BABY'S FATHER'S OCCUPATION

10.1 Was the baby's father in paid employment or self-employed in the week ending last Sunday?

0. No *go to 10.3*

1. Yes

☐

10.2 Was he working full time or part time?

0. Full time (more than 30 hours) *go to 10.6b*

1. Part time (30 hours or fewer)

☐

10.3 Was he going to college full time?

0.No *if working part-time go to 10.6a*
if not working go to 10.5
1.Yes

10.4 *If yes,* what is he studying? _____

If working part time go to 10.7

If not working go to section 11

10.5 *If not working or studying* was he

Unemployed ? (1)

Permanently unable to work because of
long term sickness or disability ? (2)

looking after home or family? (3)

other ? (specify)_____ (4)

10.6a *If **not** working or working part-time*, what was his last **full-time** job?

If only ever part-time give last part time job.

Then if currently working part time go to 10.7, otherwise go to section 11

Job Position _____

Self-employed/manager/foreman/employee

Industry _____

10.6b *If working full-time*, what is his job ? *(Then go to section 11)*

Probe industry & self-employed/ manager/foreman/ employee

Job Position

Self-employed/manager/foreman/employee

Industry _____

10.7 *If working part-time now,* what is his current job?

Job Position _____

Self-employed/manager/foreman/employee

Industry _____

10.8 *If working part time,* how many hours per week does he work?

☐☐ hrs ☐☐ mins

11: BODY MEASUREMENTS

If not done before get consent here

11.1 Pulse (30sec)

(Double the value to give pulse for 1 minute) ☐☐☐

11.2 Which hand do you write with ?

1. Right
2. Left
3. Completely ambidextrous

☐

11.3 Weight ☐☐☐.☐

11.4 How much did you weigh 3-4 months ago, ie. before you became pregnant?

☐☐ st ☐☐.☐ lbs

☐☐☐ kg

11.5 Head circumference ☐☐.☐ cm

MARK AND MEASURE UP THE NON-DOMINANT ARM AND SIDE OF THE BODY

(measure the left if completely ambidextrous)

11.6 Waist circumference ☐☐☐.☐ cm

11.7 Hip circumference ☐☐☐.☐ cm

11.8 Mid-thigh circumference ☐☐.☐ cm

11.9 Calf circumference ☐☐.☐ cm

11.10 Mid-upper arm circumference ☐☐.☐ cm
(non-dominant side)

11.11 Triceps skinfold
(non-dominant side)

☐☐.☐ mm ☐☐.☐ mm
☐☐.☐ mm ☐☐.☐ mm
☐☐.☐ mm

11.12 Biceps skinfold
(non-dominant side)

☐☐.☐ mm ☐☐.☐ mm
☐☐.☐ mm ☐☐.☐ mm
☐☐.☐ mm

11.13 Subscapular skinfold
(non-dominant side)

☐☐.☐ mm ☐☐.☐ mm
☐☐.☐ mm ☐☐.☐

[ ][ ] . [ ] mm

11.14 Upper suprailiac skinfold (non-dominant side)

[ ][ ] . [ ] mm [ ][ ] . [ ] mm

[ ][ ] . [ ] mm [ ][ ] . [ ] mm

[ ][ ] . [ ] mm

11.15 Skinfold calipers used [ ][ ][ ]

11.16 Time (24 hr clock) [ ][ ][ ][ ]

11.17 Room Temperature [ ][ ] . [ ] °C

11.18 Heel ultrasound performed?

0 = No

1= Yes [ ]

11.19 Intramalleolar distance [ ][ ] . [ ] cm

11.20 Soft tissue distance [ ][ ] . [ ] cm

12. BLOOD SAMPLE

Has the woman given her consent?

0. No [ ]

1.Yes

12.1 What time did you finish your last meal or snack? ☐☐☐☐

Time blood sample taken ☐☐☐☐

FINAL CHECK FOR NURSES

Have you left the Baby's Father's Birth Details Form?

0. No

1.Yes ☐

Have you left a food diary?

0. No

1.Yes ☐

THANK YOU VERY MUCH

Local Research Ethics
Committee No 307/97

APPENDIX III : SWS MATERNAL LATE PREGNANCY QUESTIONNAIRE

SWS serial number

LATE PREGNANCY QUESTIONNAIRE

Name: (Forename, Surname) _____

d d m m y y

Date of Birth: | | | | | | |

Have you changed your address or telephone number since you were seen in early pregnancy

0. No
1. Yes | |

If yes, new address/postcode

Address: _____

_____

Postcode: | | | | | | | |

Phone No: | | | | | | | | | | | |

Have you changed your GP since you would seen in early pregnancy

0. No
1. Yes | |

If yes, new GP's name and address

_____

_____

Interviewer: | |

Date of interview: d d m m y y | | | | | | |

1: OCCUPATIONAL ACTIVITY

1.1 Have you had any paid jobs at any time since you became pregnant?

0. No *(go to Section 2)*
1. Yes | |

1.2 Would you please tell me the paid jobs that you have done during your pregnancy and the weeks of your pregnancy in which you have done them?

If started before pregnancy, week started = 0

If job is still ongoing, week finished = 88

Occupation	Week Started	Week Finished
a)		
b)		
c)		
d)		

If not in paid work at around 11 weeks of pregnancy go to 1.6

1.3 At around 11 weeks of pregnancy – when we interviewed you for the first time during pregnancy - how many paid hours in total did you work during an average week?

☐☐ hrs ☐☐ mins

1.4 Did this include working night shifts?

0. No
1. Yes ☐

1.5 At around this time did your paid work involve any of the following activities in an average day at work?

i) Standing or walking for more than four hours in total?

0. No
1. Yes ☐

ii) Kneeling or squatting for more than an hour in total?

0. No
1. Yes ☐

(iii) Standing or sitting with your trunk bent forward (see diagram) for more than an hour in total?

0. No
1. Yes ☐

(iv) Lifting or carrying weights of 56lbs (25kg) (4 stone) or more by hand, (equivalent to a sack of potatoes, a nine year old child, a very heavy suitcase)?

0. No
1. Yes ☐

1.6 *If not in paid work around 19 weeks of pregnancy go to 1.10*

At around 19 weeks of pregnancy – when you came for your routine scan - how many paid hours in total did you work during an average week?

☐☐ hrs ☐☐ mins

1.7 Did this include working night shifts?

0. No
1. Yes ☐

1.8 Were the activities at work on the card, the same at 19 weeks as they were at 11 weeks?

0. No
1. Yes *go to 1.10* ☐

1.9 At around 19 weeks of pregnancy did your paid work involve any of the following activities in an average * day at work?

i) Standing or walking for more than four hours in total?

0. No
1. Yes ☐

ii) Kneeling or squatting for more than an hour in total?

0. No
1. Yes ☐

(iii) Standing or sitting with your trunk bent forward (see diagram) for more than an hour in total?

0. No
1. Yes ☐

(iv) Lifting or carrying weights of 56lbs (25kg) (4 stone) or more by hand, (equivalent to a sack of potatoes, a nine year old child, a very heavy suitcase)?

0. No
1. Yes ☐

1.10 *If not in paid work now, go to 1.14*

How many paid hours a week in total are you working now?

☐☐ hrs ☐☐ mins

1.11 Does this include working night shifts?

0. No
1. Yes ☐

1.12 Are the activities at work on the card, the same now as they were at 19 weeks?

0. No
1. Yes *go to 1.14* ☐

1.13 Does your paid work involve any of the following activities in an average day at work?

i) Standing or walking for at least an hour in total?

0. No
1. Yes ☐

ii) Kneeling or squatting for at least an hour in total?

0. No
1. Yes ☐

(iii) Standing or sitting with your trunk bent forward (see diagram) for at least an hour in total?

0. No
1. Yes ☐

(iv) Lifting or carrying weights of 56lbs (25kg) (4 stone) or more by hand, (equivalent to a sack of potatoes, a nine year old child or a very heavy suitcase)?

0. No
1. Yes ☐

1.14 Have you at any time during your pregnancy left a paid job or changed the type of paid work that you were doing because of a health problem? (Excludes changes simply because pregnant, such as routine maternity leave).

0. No ☐
1. Yes

If yes, please give details of health problems and change and the stage of pregnancy at which they occurred:

_____

_____

_____

_____

2: ACTIVITY AND EXERCISE

Can I now ask you about your activity and exercise patterns over the last three months? As before we would like you to divide up a "typical" day into three types of activity. These are:

(1) sleeping or lying, (2) sitting, (3) standing or walking.

2.1 Over a typical 24 hour day how many hours have you generally spent sleeping or lying with your feet up? ☐☐ hrs ☐☐ mins

(*ask time usually go to bed & wake up, including any at work!*)

This would indicate xx hours sitting or on your feet.

Of those hours how many on a typical day have you spent sitting down? (*e.g. includes sitting at work, mealtimes, driving, reading, watching TV*). ☐☐ hrs ☐☐ mins

2.3 This would mean that you have spent about xx hours a day on your feet. Does this sound about right?

☐☐ hrs ☐☐ mins

2.4 * Out of these xx hours spent on your feet, about how much of the time were you **actively on the move** than standing fairly still)?

1.	Very little	10%
2.	Some	30%
3.	About half	50%
4.	Most	70%
5.	Almost all	90%

☐

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

a) **strenuous exercise** which made your heart beat rapidly **AND** left you breathless e.g. jogging, vigorous swimming or cycling, aerobics.

FFQ categories 1-7 ☐ >x1 ☐

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

b) **moderate exercise** which left you exhausted but not breathless, e.g. brisk walking, dancing, easy swimming or cycling, badminton, sailing.

FFG categories 1-7 ☐ >x1 ☐☐

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

c) **gentle exercise** which left you tired but not exhausted, e.g. walking, heavy housework (including washing windows and polishing), gardening, DIY, golf.

FFQ categories 1-7 ☐ >x1 ☐☐

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

2.6 * Which of the following best describes your walking speed at present?

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

☐

3: DIETARY QUESTIONS

3.1 Now I am going to ask you about the foods you have eaten in the past 3 months. To do this I have a list of foods and I would like you to tell me how often you have eaten each food during the past 3 months. Again the list may include foods you never eat or you may find foods which you eat a lot are missing. These can be added on at the end. *(Define the 3 month period)*

	FOOD DESCRIPTION	FREQUENCY EATEN							
FOOD CODE		Never	Once every 2-3 Months	Once a Month	Once a Fortnight	1-2 Times per Week	3-6 Times per Week	Once a day	More than once a day
1	White Bread	1	2	3	4	5	6	7	☐
	When you ate bread/toast/sandwiches, how many slices/rolls did you eat at a typical meal? ☐☐.☐ *Rolls (count as 2 slices)* *French bread (2" counts as 1 slice)*								
2	Brown and wholemeal bread/rolls	1	2	3	4	5	6	7	☐
	How many slices/rolls did you eat at a typical meal? ☐☐.☐ *Rolls (count as 2 slices)*								

3	Crackers and cheese biscuits	1	2	3	4	5	6	7	☐
4	Wholemeal and rye crackers	1	2	3	4	5	6	7	☐
5	'Bran' breakfast cereals	1	2	3	4	5	6	7	☐

	FOOD DESCRIPTION	FREQUENCY EATEN							
FOOD CODE		Never	Once every 2-3 Months	Once a Month	Once a Fortnight	1-2 Times per Week	3-6 Times per Week	Once a day	More than once a day
6	Other breakfast cereals	1	2	3	4	5	6	7	☐
7	Added bran to foods	1	2	3	4	5	6	7	☐
8	Cakes and gateaux	1	2	3	4	5	6	7	☐
	Buns								☐

9		1	2	3	4	5	6	7	
10	Pastries	1	2	3	4	5	6	7	
11	Biscuits-chocolate, digestive and ginger	1	2	3	4	5	6	7	
12	Other biscuits	1	2	3	4	5	6	7	
13	Fruit puddings	1	2	3	4	5	6	7	
14	Milk based puddings and sauces	1	2	3	4	5	6	7	

	FOOD DESCRIPTION	FREQUENCY EATEN							
FOOD CODE		Never	Once every 2-3 Months	Once a Month	Once a Fortnight	1-2 Times per Week	3-6 Times per Week	Once a day	More than once a day
15	Other puddings	1	2	3	4	5	6	7	

16	Yogurt and fruit fools	1	2	3	4	5	6	7	☐
17	Potatoes – boiled and jacket	1	2	3	4	5	6	7	☐
	When you ate these how many potatoes did you eat at a typical meal? ☐☐.☐ *Large baking (count as 3)/ new (count as 0.5)*								
18	Roast potatoes and chips	1	2	3	4	5	6	7	☐
	When you ate these how many potatoes did you eat at a typical meal? ☐☐.☐								
19	Yorkshire puddings and savoury pancakes	1	2	3	4	5	6	7	☐
20	Brown and white rice	1	2	3	4	5	6	7	☐
	Pasta and dumplings								☐

21		1	2	3	4	5	6	7	
	FOOD DESCRIPTION	FREQUENCY EATEN							
FOOD CODE		Never	Once every 2-3 Months	Once a Month	Once a Fortnight	1-2 Times per Week	3-6 Times per Week	Once a day	More than once a day
22	Tinned vegetables	1	2	3	4	5	6	7	☐
23	Peas and green beans	1	2	3	4	5	6	7	☐
24	Carrots	1	2	3	4	5	6	7	☐
25	Parsnips, swede and turnip	1	2	3	4	5	6	7	☐
26	Sweetcorn and mixed veg	1	2	3	4	5	6	7	☐
27	Beans and pulses	1	2	3	4	5	6	7	☐

28	Tomatoes	1	2	3	4	5	6	7	☐
29	Spinach	1	2	3	4	5	6	7	☐
30	Broccoli, Brussels sprouts and spring greens	1	2	3	4	5	6	7	☐

	FOOD DESCRIPTION	FREQUENCY EATEN							
FOOD CODE		Never	Once every 2-3 Months	Once a Month	Once a Fortnight	1-2 Times per Week	3-6 Times per Week	Once a day	More than once a day
31	Cabbage and cauliflower	1	2	3	4	5	6	7	☐
32	Peppers and watercress	1	2	3	4	5	6	7	☐
33	Onion	1	2	3	4	5	6	7	☐
34	Green salad	1	2	3	4	5	6	7	☐

35	Side salads in dressing	1	2	3	4	5	6	7	
36	Courgettes, marrow and leeks	1	2	3	4	5	6	7	
37	Mushrooms	1	2	3	4	5	6	7	
38	Vegetable dishes	1	2	3	4	5	6	7	
39	Vegetarian foods	1	2	3	4	5	6	7	

	FOOD DESCRIPTION	FREQUENCY EATEN							
FOOD CODE		Never	Once every 2-3 Months	Once a Month	Once a Fortnight	1-2 Times per Week	3-6 Times per Week	Once a day	More than once a day
40	Tinned fruit not including grapefruit, prunes, figs or blackcurrants	1	2	3	4	5	6	7	
41	Cooked fruit not including blackcurrants	1	2	3	4	5	6	7	

42	Dried fruit	1	2	3	4	5	6	7	☐
43	Fresh apples and pears	1	2	3	4	5	6	7	☐
44	Fresh oranges and orange juice	1	2	3	4	5	6	7	☐☐
45	Grapefruit and grapefruit juice	1	2	3	4	5	6	7	☐☐
46	Blackcurrants, ribena and hi-juice blackcurrant drinks	1	2	3	4	5	6	7	☐☐
47	Other fruit juices (not squashes)	1	2	3	4	5	6	7	☐☐
48	Diet Coke and Pepsi not including caffeine free	1	2	3	4	5	6	7	☐☐
	FOOD DESCRIPTION	FREQUENCY EATEN							
FOOD		Never	Once	Once a	Once a	1-2	3-6	Once	More than

CODE			every 2-3 Months	Month	Fortnight	Times per Week	Times per Week	a day	once a day
49	Coke and Pepsi	1	2	3	4	5	6	7	☐☐
50	Soft drinks not including diet drinks (low calorie or low sugar)	1	2	3	4	5	6	7	☐☐
51	Bananas	1	2	3	4	5	6	7	☐
52	Fresh peaches, plums, cherries and grapes	1	2	3	4	5	6	7	☐
53	Strawberries and raspberries	1	2	3	4	5	6	7	☐
54	Fresh pineapple, melon, kiwi and other tropical fruits	1	2	3	4	5	6	7	☐
55	Nuts	1	2	3	4	5	6	7	☐

56	Bacon and gammon	1	2	3	4	5	6	7	☐
57	Pork	1	2	3	4	5	6	7	☐
58	Chicken and turkey	1	2	3	4	5	6	7	☐

	FOOD DESCRIPTION	FREQUENCY EATEN							
FOOD CODE		Never	Once every 2-3 Months	Once a Month	Once a Fortnight	1-2 Times per Week	3-6 Times per Week	Once a day	More than once a day
59	Lamb	1	2	3	4	5	6	7	☐
60	Beef	1	2	3	4	5	6	7	☐
61	Minced meat dishes	1	2	3	4	5	6	7	☐
62	Meat Pies	1	2	3	4	5	6	7	☐

63	Liver and kidney	1	2	3	4	5	6	7	☐
64	Paté and liver sausage	1	2	3	4	5	6	7	☐
65	Faggots and black pudding	1	2	3	4	5	6	7	☐
66	Sausages	1	2	3	4	5	6	7	☐
67	Ham and luncheon meat	1	2	3	4	5	6	7	☐
68	White fish	1	2	3	4	5	6	7	☐

	FOOD DESCRIPTION	FREQUENCY EATEN							
FOOD CODE		Never	Once every 2-3 Months	Once a Month	Once a Fortnight	1-2 Times per Week	3-6 Times per Week	Once a day	More than once a day
69	Fish fingers and fish dishes	1	2	3	4	5	6	7	☐

70	Oily fish	1	2	3	4	5	6	7	☐
71	Shellfish	1	2	3	4	5	6	7	☐
72	Boiled and poached eggs	1	2	3	4	5	6	7	☐
73	Omelette and fried eggs	1	2	3	4	5	6	7	☐
74	Cottage Cheese	1	2	3	4	5	6	7	☐
75	Cheese	1	2	3	4	5	6	7	☐
76	Pizza, quiches and cheese flans	1	2	3	4	5	6	7	☐
77	Soup	1	2	3	4	5	6	7	☐

78	Mayonnaise and salad cream	1	2	3	4	5	6	7	☐

	FOOD DESCRIPTION	FREQUENCY EATEN							
FOOD CODE		Never	Once every 2-3 Months	Once a Month	Once a Fortnight	1-2 Times per Week	3-6 Times per Week	Once a day	More than once a day
79	Pickles, chutney, tomato ketchup and brown sauce	1	2	3	4	5	6	7	☐
80	Chocolate	1	2	3	4	5	6	7	☐☐
81	Other sweets	1	2	3	4	5	6	7	☐☐
82	Ice cream and chocolate desserts	1	2	3	4	5	6	7	☐
83	Cream	1	2	3	4	5	6	7	☐
84	Crisps and savoury snacks	1	2	3	4	5	6	7	☐☐

85	Sweet spreads	1	2	3	4	5	6	7	
86A	Gravy granules and powders	1	2	3	4	5	6	7	
86B	Stock cubes and Marmite	1	2	3	4	5	6	7	

	FOOD DESCRIPTION	FREQUENCY EATEN							
FOOD CODE		Never	Once every 2-3 Months	Once a Month	Once a Fortnight	1-2 Times per Week	3-6 Times per Week	Once a day	More than once a day
87	Drinking chocolate and milk shakes not including McDonald style milkshakes	1	2	3	4	5	6	7	
88	Decaffeinated coffee and tea	1	2	3	4	5	6	7	
89	Tea	1	2	3	4	5	6	7	
	Coffee								

90		1	2	3	4	5	6	7	
93	Spreading fat (1) _____ F	1	2	3	4	5	6	7	☐
94	Spreading fat (2) _____ F	1	2	3	4	5	6	7	☐
95	Spreading fat (3) _____ F	1	2	3	4	5	6	7	☐
96	Frying fat or oil (1) _____ F	1	2	3	4	5	6	7	☐
97	Frying fat or oil (2) _____ F	1	2	3	4	5	6	7	☐
98	Frying fat or oil (3) _____ F	1	2	3	4	5	6	7	☐

	FOOD DESCRIPTION	FREQUENCY EATEN							
FOOD		Never	Once	Once a	Once a	1-2	3-6	Once	More than

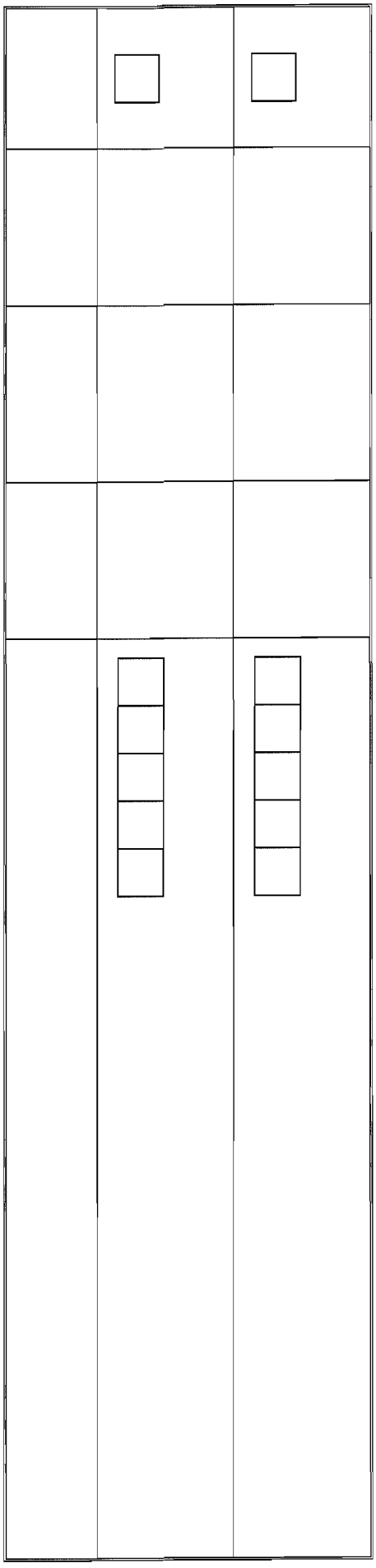
CODE			every 2-3 Months	Month	Fortnight	Times per Week	Times per Week	a day	once a day
99	Other vegetable oil (1) e.g. salad dressings, _____ marinades F ☐☐☐☐☐	1	2	3	4	5	6	7	☐
100	Other vegetable oil (2) e.g. salad dressings, _____ marinades F ☐☐☐☐☐	1	2	3	4	5	6	7	☐

3.2 Are there food or drinks which you have eaten or drunk **once a week or more** which are not on the list? Include breakfast bars such as Nutrigrain and Kellogg's

0. No/1. Yes ☐

IF YES

NAME OF FOOD/DRINK	1-2 times per week	3-6 times per week	Once a day	More than once a day
☐☐☐☐☐				☐
☐☐☐☐☐				☐
☐☐☐☐☐				☐



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

3.3: Which types of milk have you used regularly in drinks and added to breakfast cereals over the last 3 months?

0. None
1. Whole pasteurised
2. Semi-skimmed pasteurised
3. Skimmed pasteurised
4. Whole UHT
5. Semi-skimmed UHT
6 Skimmed UHT
7. Other

Milk 1 ☐ Other (specify)_____

Milk 2 ☐ Other (specify)_____

Milk 3 ☐ Other (specify)_____

3.4 On average over the last 3 months how much

* of each milk have you consumed per day?

Milk 1 ☐.☐☐ pints

Milk 2 ☐.☐☐

Milk 3 ☐ ☐☐ pints

3.5 Have you added sugar to breakfast cereals, tea & coffee, puddings etc.?

0. No *go to 3.7* ☐

1. Yes

3.6 Approximately how many teaspoons of sugar have you added each day? ☐☐

3.7 When you eat meat, how much of the fat have you usually cut off (including chicken skin)? ☐

1. all 100%
2. most 60%
3. some 30%
4. none 0%
9. not applicable

3.8 Just thinking about the **past week** how many servings did you eat of:

vegetables and vegetable-containing dishes (excluding potatoes)?	
fruit and pure fruit juices?	
meat and fish and their dishes?	

4: FOOD SUPPLEMENTS

4.1 <u>During the past three months</u> have you taken any pills, tonics or tablets to supplement your diet? (e.g. vitamins, minerals, iron tablets, folic acid, fish oils) ☐

0. No

1. Yes

If yes, please state which:

(for number per day, record number of tablets/ capsules/ teaspoons per day, as appropriate)

Supplement	Number per day	How many days in the last 90?	Did you start taking this: 1: Less than 1 month ago 2: 1-2 months ago 3: More than 2 months ago
☐☐☐☐			
☐☐☐☐			
☐☐☐☐			
☐☐☐☐			
☐☐☐☐			

5: APPETITE AND NAUSEA DURING PREGNANCY

5.1 Have you experienced any nausea or sickness over the last 3 months?

0. No
1. Yes ☐

If yes, has this been:

1. Mild (nausea only)
2. Moderate (sometimes sick) ☐
3. Severe (regularly sick, can't retain meals)

5.2 **Compared with BEFORE you were pregnant,** are you eating:

1. More
2. The same ☐
3. Less <u>in amount</u>

5.3 If **more,** is this

1. Because you feel more hungry
2. To prevent you feeling sick ☐
3. Because you feel it is best for the baby

(9. Not sure/other reason)

If **less**, is this

1. Because you feel less hungry
2. Because of nausea/sickness
3. Don't want to put on too much weight

(9. Not sure/other reason)

6: ALCOHOL CONSUMPTION

I'd like to ask you a few questions about your drinking and smoking habits.

6.1 Do you ever drink alcohol?

0. No *go to section 7*
1. Yes

During the past three months:

6.2: a) How often have you drunk **Shandy or Low Alcohol Beer/Lager/Cider**? FFQ 1-7 ☐ >x1 ☐☐

(don't include alcohol **free** lager etc)

b) When you drank these how many pints did you normally have? ☐☐.☐☐

(if range given code mid-point)

6.3 a) How often have you drunk **Beer/Stout/Lager/Cider/Alcopops?** ☐ ☐☐

FFQ 1-7 >x1

b) When you drank these how many pints did you normally have?

(if range given code mid-point)

□ . □□

6.4 a) How often have you drunk

Low alcohol wine? FFQ 1-7 □ >x1 □□

b) When you drank this how many glasses did you normally have?

(if range given code mid-point)

□□ . □□

6.5 a) How often have you drunk

Wine/Sherry/Martini/Cinzano? FFQ 1-7 □ > x1 □□

b) When you drank these how many glasses did you normally have?

(if range given code mid-point)

□□ . □□

6.6 a) How often have you drunk

Spirits/Liqueurs? FFQ 1-7 □ > x1 □□

b) when you drank these how many measures did you normally have?

(if range given code mid-point)

□□ . □□

7: SMOKING

7.1 Are you currently smoking?

0. No
1. Yes

□

If Yes, how many per day (code max) □□

If No, *go to Section 8*

8: MEDICINES

I would like to ask you now about any medicines you may have taken.

8.1 What, if any, medicines/inhalers/pills, tablets indigestion remedies have you taken since we administered a questionnaire earlier in the pregnancy?

USE BLOCK CAPITALS & COPY NAMES DIRECTLY OFF BOTTLES IF POSSIBLE

1		
2		
3		
4		
5		
6		
7		
8		

9: BODY MEASUREMENTS

9.1 Pulse (30sec)

(Double the value to give pulse for 1 minute) ☐☐☐

9.2 Which hand do you write with ?

1. Right
2. Left
3. Completely ambidextrous

☐

9.3 Weight ☐☐☐.☐ kg

Mark and measure up the non-dominant arm and side of the body
(measure the left if completely ambidextrous)

9.4 Mid-thigh circumference ☐☐☐.☐ cm

9.5 Calf circumference ☐☐.☐ cm

9.6 Mid-upper arm circumference ☐☐.☐ cm

(non-dominant side)

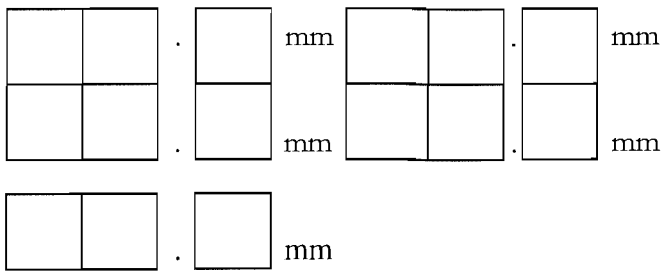
9.7 Triceps skinfold
(non-dominant side)

☐☐.☐ mm ☐☐.☐ mm
☐☐.☐ mm ☐☐.☐ mm
☐☐.☐ mm

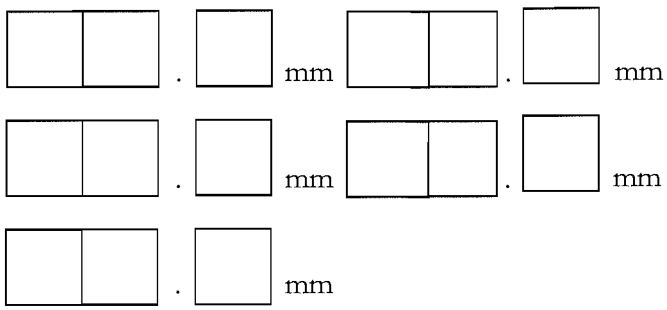
9.8 Biceps skinfold
(non-dominant side)

☐☐.☐ mm ☐☐.☐ mm
☐☐.☐ mm ☐☐.☐ mm
☐☐☐ mm

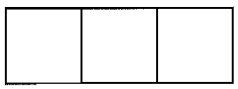
9.9 Subscapular skinfold (non-dominant side)



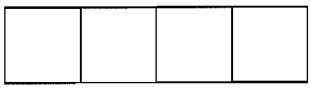
9.10 Upper suprailiac skinfold (non-dominant side)



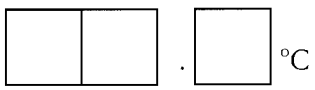
9.11 Skinfold calipers used



9.12 Time (24 hr clock)



9.13 Room Temperature

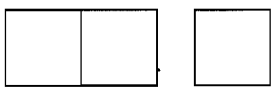


9.18 Heel ultrasound performed?

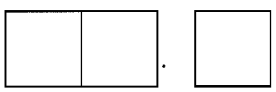
0 = No

1= Yes

9.19 Intramalleolar distance



9.20 Soft tissue distance



10: BLOOD SAMPLE

Has the woman given her consent?

0. No

1. Yes ☐

10.1 What time did you finish your last meal or snack?

Time blood sample taken ☐☐☐☐

APPENDIX IV : SWS CALCANEAL QUS PROTOCOL HEEL ULTRASOUND SCANNING PROTOCOL

Sahara machine is sensitive to error so need to be careful.

QUALITY ASSURANCE WITHOUT LAPTOP

1. **Press *On***. The machine will show *initializing*.
2. When the machine says *"Apply gel"*, **Don't press open. Press *Program*** then ***1*** then ***Enter*.**
3. When the machine *says "Apply gel for QC; Press open"*, **Apply gel to TRANSCDUCERS** (don't touch, pea size, on tip of both transducers, away from you)
4. **Then Press *Open*.**
5. When the machine says *"Insert phantom, press measure"*, Insert phantom (right colour dot for machine, minimize handling by using finger grooves (to lessen temperature fluctuations)
6. **Then Press *Measure.***
7. When the machine says *"Remove phantom, press prep, clean"*, **Remove phantom. Press *Prep.***
8. When the machine says "QC passed", **Don't press ON, Press +/- twice then press *Print.*** Put printout in plastic envelope by printer.
9. To clean, use wet lanolein free tissue for transducers and phantom then dry both with lint free tissue.
10. Leave machine switched on, with phantom upside down and foot rester in place.

How to measure a patient

1. **Press *On***. The machine will show *initializing*
2. Scrub the left heel of the women vigorously with wet wipi to remove any debris

3. When the machine says *"Apply gel; press open"*, **Apply gel** (no touch, pea size, on tip of both transducers, away from you, DON'T APPLY ON PATIENTS HEEL)
4. **Press *Open*.**
5. **Patient position:**
    a. sitting on static chair
    b. place heel right back in machine
    c. ensure line between 2nd and 3rd toe
    d. squeeze foot rest arms together till snug fit
    e. attach shin strap, angle leg so top foam in contact with skin all the way around
    f. push down on middle of rest till clicks stop
    g. ask patient to rest her hands on right knee
    h. ask patient not to speak during scan

5. **Press *Measure***
6. When the instrument says (remove foot…."; **remove foot** and **Press Prep.**
7. When results shown, **Press +/- once till screen says BUA and SOS then press *Print.***
8. **Then just write the sws Id number on print out and put in the plastic envelope by printer.**
11. **Cleaning:** the oil gel will stain clothes so wipe lady's heel with lanolein free wet wipe and dry using lint free tissue. Repeat wet then dry cleaning with transducer probes
12. Leave machine switched on, with phantom upside down and foot rester in place.

APPENDIX V : CALCANEAL QUS REPEATABILITY PROTOCOL

Aims: To Assess using the reproduciblility of the soft tissue and inter-malleolar measurements at the ankle.

SAMPLE: **20 WOMEN AGED 20-35 YEARS (HEALTHY VOLUNTEERS FROM STAFF AT THE MRC EEU AND SGH).**

Definitions:

- Intermalleolar measurement – the distance between the tip of the lateral malleolus to the tip of the medial malleolus as measured using a caliper.
- Soft tissue measurement – the width of the ankle at a height half way between the sole and the level of the malleoli.

Method:

- Each subject provides her name, age, height and weight.
- Measurements are performed using a single set of calipers.
- Measurements are performed with the subjects standing.
- **ON EACH OCCASION, RIGHT THEN LEFT HEEL INTER-MALLEOLAR AND SOFT TISSUE DISTANCES ARE MEASURED BY ONE EXAMINER. THE MEASUREMENTS ARE THEN REPEATED BY A SECOND EXAMINER.**
- Each subject is then re-measured one week later.

APPENDIX VI: SWS MATERNAL NEONATAL DXA INFORMATION SHEET

What happens when my baby is born?

As we discussed with you earlier, we would like to take measurements of your baby's size and length when he/she is born.

How will you know when my baby is born?

The staff on the Labour Ward or your midwife will let us know.

Is there anything you want me to do?

We do not expect you to be thinking of us when your baby is born! However, if you or someone in your family could remember to contact us on the freephone number **0800 7834503** (the same one as on your fridge magnet), it would help make sure that we see your baby soon after the birth.

Is there anything else I will be asked to do for the survey?

We have obtained valuable information on your baby's growth through pregnancy. In order to see how this growth continues we would like to visit you at home when your baby is 6, 12 and 24 months old. This is to ask some questions about your child's health and diet and to measure his/her growth. Subsequently we would also like to make occasional visits.

Is there anything else you want me to do?

Because the information we have collected is so valuable, we may ask if you are willing to take part in one of a number of smaller studies. The aim of these is to identify ways of improving a child's health right through into adult life. If you are selected for these studies we will contact you separately to ask if you are willing to help further.

What are the extra studies?

Two areas that are of interest to us are:

CHEST ILLNESSES AND BREATHING PROBLEMS

Breathing problems are very common in babies and young children and little is known about how to prevent them occurring. Breathing tests done a few weeks after birth can help us to understand more about how these problems arise and how we might prevent them.

Bone density in babies

Osteoporosis, or 'thin' bones is a common problem in older people. It is known that having strong bones early in life makes it less likely that osteoporosis will develop later in life. We are hoping to find out if the mother's diet before and during pregnancy can affect the way in which a baby's bones develop.

What if I don't want to take part?

None of these studies are compulsory and if you do not take part it will not affect the care of your child in any way. We have been extremely grateful for your help in the past and of course would be even more grateful if you would stay with us as we see how your baby grows.

Are there any advantages in taking part in the next set of studies?

By taking part you will have very accurate measurements made of your child and will be able to see how he/she is developing. At birth we will give you a card showing the measurements of your baby at birth to keep with the photographs of the scans that you have received.

You will also know that you are helping to improve the health of future generations by contributing to these studies.

What if I want further information?

We will be happy to answer any queries and are available on our freephone number

0800 7834503

FINALLY, THANK YOU FOR ALL THE HELP YOU HAVE GIVEN US.

****

Local Research Ethics
Committee Nos: 089/99, 153/99

APPENDIX VII : SWS NEONATAL ASSESSMENT AT BIRTH

BIRTH AND INFANCY

INFORMATION

LEAFLET

```
File: X             Maxrec:  0      Rec: 1       Page: 1 of 20          REPLACING
--------------------------------------------------------------------------------
   MRC Number       [     ]     Hospital Number  [       ]

   Mother's Surname [                              ]

   Address          [                                        ]
                    [                                        ]
                    [                                        ]
   Postcode         [          ]

   Telephone number [              ]
                                  Data abstracted by [  ]  Julia    04
                                                           Lyn      07
   Postnatal assessment sheet data                         Valerie  08
   -------------------------------                         Jane     09

   Date of delivery     [      ]

   Time of delivery     [    ]

   Neonatal hypoglycaemia  Yes / No   [ ]
--------------------------------------------------------------------------------
F1: Help   F3: Prev   F4: Next   F5: Goto   F6: Search  F10: Save  Alt-F10: Exit

File: X             Maxrec:  0      Rec: 1       Page: 2 of 20          REPLACING
--------------------------------------------------------------------------------

   Abnormalities        Yes / No    [ ]

   If YES, enter code if on       Details 1 [  ][                            ]
   coding guide or description    Details 2 [  ][                            ]
               if not             Details 3 [  ][                            ]


Were you in paid employment when we saw you at 34 weeks   Yes / No  [ ]
               If YES, on what date did you last work   [      ]

(if stopped more than 1 working day previously) & was this planned,
or was it because of a health problem   [ ]
                                                    1 Planned
                                                    2 Health problem

--------------------------------------------------------------------------------
F1: Help   F3: Prev   F4: Next   F5: Goto   F6: Search  F10: Save  Alt-F10: Exit
```

File: X Maxrec: 0 Rec: 1 Page: 3 of 20 REPLACING

Since we saw you at 34 weeks have you been taking any pills, tonics or tablets to supplement your diet Yes / No [ ]

Supplement name	Suppl code	Amount (over last 6 weeks (42 days)) *
< >	[ ]	[ ]
< >	[ ]	[ ]
< >	[ ]	[ ]
< >	[ ]	[ ]
< >	[ ]	[ ]

* irrespective of gest age at birth

Did you have any antibiotic tablets for a kidney, bladder or urine infection at any stage in the pregnancy [ ] Yes / No

Have you ever had treatment for vaginal candidiasis or thrush [ ] Yes / No

Was the baby delivered directly onto your abdomen [ ] Yes / No / Don't know

Have you decided on the baby's name (Enter X if not decided) [ ] Forenames [ ] Surname

---

F1: Help F3: Prev F4: Next F5: Goto F6: Search F10: Save Alt-F10: Exit

File: X Maxrec: 0 Rec: 1 Page: 4 of 20 REPLACING

What time did the baby's last feed finish [ ]

How has the baby been fed since delivery [ ]

1 Breast 2 Bottle 3 Both 4 NGT - br milk 5 NGT formula
6 NG formula + IVI 7 IVI 8 Oral formulation 0 NGT+Breast+IVI 9 Not Known

Bottle type [ ],[ ]

A Farley's First B Wyeth SMA Gold C C&G Premium D Other
[ ]

How do you intend to feed the baby when you go home [ ] 1 Breast / 2 Bottle

Brand if bottle [ ]

F1: Help F3: Prev F4: Next F5: Goto F6: Search F10: Save Alt-F10: Exit

File: X Maxrec: 0 Rec: 1 Page: 5 of 20 REPLACING

Date of measurement [ ] (To sick / prem to measure enter 888888)
Time of measurement [ ] 24 hr clock

Occipto-frontal	[ ]	[ ]	[ ]
Left mid-upper arm	[ ]	[ ]	[ ]
Upper abdominal	[ ]	[ ]	[ ]
Lower abdominal	[ ]	[ ]	[ ]
Triceps skinfold	[ ]	[ ]	[ ]
Subscapular skinfold	[ ]	[ ]	[ ]
Thigh skinfold	[ ]	[ ]	[ ]

HIP STABILITY

Crown - rump	[ ]	[ ]	[ ]
Crown - heel	[ ]	[ ]	[ ]

F1: Help F3: Prev F4: Next F5: Goto F6: Search F10: Save Alt-F10: Exit

```
File: X             Maxrec:  0      Rec: 1       Page: 6 of 20          REPLACING
--------------------------------------------------------------------------------
        Measurer    [  ]         Helper      [  ]     -  1. Parent   2. KG
                                                         3. Midwife  4. Julia
                                                         5. Sue Beare
                                                         6. Auxillary
                                                         7. Lyn   8. Valerie
                                                         9. Student Midwife

        Hair colour [ ] *  * 1 Blond        2 Pale brown/blond
                             3 Medium brown 4 Dark brown
                             5 Black        6 Redhead

        hair extent [ ]

        Anthropometer used   [ ]
        Min carriage reading [   ]
--------------------------------------------------------------------------------
F1: Help   F3: Prev   F4: Next   F5: Goto   F6: Search  F10: Save  Alt-F10: Exit
```

```
File: X             Maxrec:  0      Rec: 1       Page: 7 of 20          REPLACING
--------------------------------------------------------------------------------
Link card data
--------------

       *                  a     b    c             d       e       f     g
Preg  Date      Place      Dur. Onset S.D.  Bld loss Sex     BWt      I/M  Status
1.  [      ] [          ] [    ] [ ]  [ ]   [    ]   [ ]  [        ] [ ]    [ ]
2.  [      ] [          ] [    ] [ ]  [ ]   [    ]   [ ]  [        ] [ ]    [ ]
3.  [      ] [          ] [    ] [ ]  [ ]   [    ]   [ ]  [        ] [ ]    [ ]
4.  [      ] [          ] [    ] [ ]  [ ]   [    ]   [ ]  [        ] [ ]    [ ]
5.  [      ] [          ] [    ] [ ]  [ ]   [    ]   [ ]  [        ] [ ]    [ ]

* if year only recorded code as midpoint i.e. 1506xx

a wks.days      c 1 S.D.    e Fractions of       g 1 LB        7 Miscarriage
                  2 Inst      oz coded as          2 SB        8 TOP
b 1 Normal        3 C.S.      decimal              3 PND
  2 Induced                                        5 Ectopic
  3 Emerg CS    d M Male    f I Imp (lb.oz)        6 Mole
                  F Female    M Met (Grams)

--------------------------------------------------------------------------------
F1: Help   F3: Prev   F4: Next   F5: Goto   F6: Search  F10: Save  Alt-F10: Exit
```

File: X Maxrec: 0 Rec: 1 Page: 8 of 20 REPLACING

	*		a	b	c		d	e	f	g
Preg	Date	Place	Dur.	Onset	S.D.	Bld loss	Sex	BWt	I/M	Status
6.	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
7.	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
8.	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
9.	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
10.	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]

* if year only recorded code as midpoint i.e. 1506xx

a wks.days
b 1 Normal
2 Induced
3 Emerg CS

c 1 S.D.
2 Inst
3 C.S.

d M Male
F Female

e Fractions of oz coded as decimal
f I Imp (lb.oz)
M Met (Grams)

g 1 LB
2 SB
3 PND
5 Ectopic
6 Mole
7 Miscarriage
8 TOP

Previous history UTI/cystitis No / Yes [ ]
Previous infertility investigations No / Yes [ ]
Family history of hypertension No / Yes / Adopted [ ]
Family history of diabetes No / Yes / Adopted [ ]

F1: Help F3: Prev F4: Next F5: Goto F6: Search F10: Save Alt-F10: Exit

File: X Maxrec: 0 Rec: 1 Page: 9 of 20 REPLACING

Ultra sound	Date	CRL	BPD	FL	AC	HC
1.	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
2.	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
3.	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
4.	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
5.	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]

Placental Position [ ] 0 Not low 1 Low

Anterior/Posterior [ ] 0 Anterior 1 Posterior 2 Fundal 3 Lateral
4 posterior + fundal 5 Posterior + lateral

F1: Help F3: Prev F4: Next F5: Goto F6: Search F10: Save Alt-F10: Exit

File: X Maxrec: 0 Rec: 1 Page: 10 of 20 REPLACING

Data from patient held white notes/ green hospital notes

Urine 0 Nil 1 Trace 2 + 3 ++ 4 +++ 5 ++++ 9 No specimin

Oedema 0 Nil 1 Minimal/slight 2 Present 3 Marked

	Date	Weight	Imp/Metric	Urine alb.	Urine Sug.	B.P.	Oedema
1.	[ ]	[ ]	[ ]	[ ]	[ ]	[ ] [ ]	[ ]
2.	[ ]	[ ]	[ ]	[ ]	[ ]	[ ] [ ]	[ ]
3.	[ ]	[ ]	[ ]	[ ]	[ ]	[ ] [ ]	[ ]
4.	[ ]	[ ]	[ ]	[ ]	[ ]	[ ] [ ]	[ ]
5.	[ ]	[ ]	[ ]	[ ]	[ ]	[ ] [ ]	[ ]
6.	[ ]	[ ]	[ ]	[ ]	[ ]	[ ] [ ]	[ ]
7.	[ ]	[ ]	[ ]	[ ]	[ ]	[ ] [ ]	[ ]
8.	[ ]	[ ]	[ ]	[ ]	[ ]	[ ] [ ]	[ ]
9.	[ ]	[ ]	[ ]	[ ]	[ ]	[ ] [ ]	[ ]
10.	[ ]	[ ]	[ ]	[ ]	[ ]	[ ] [ ]	[ ]

F1: Help F3: Prev F4: Next F5: Goto F6: Search F10: Save Alt-F10: Exit

File: X Maxrec: 0 Rec: 1 Page: 11 of 20 REPLACING

	Date	Weight	Imp/metric	Urine alb.	Urine Sug.	B.P.	Oedema
11.	[ ]	[ ]	[ ]	[ ]	[ ]	[ ] [ ]	[ ]
12.	[ ]	[ ]	[ ]	[ ]	[ ]	[ ] [ ]	[ ]
13.	[ ]	[ ]	[ ]	[ ]	[ ]	[ ] [ ]	[ ]
14.	[ ]	[ ]	[ ]	[ ]	[ ]	[ ] [ ]	[ ]
15.	[ ]	[ ]	[ ]	[ ]	[ ]	[ ] [ ]	[ ]
16.	[ ]	[ ]	[ ]	[ ]	[ ]	[ ] [ ]	[ ]
17.	[ ]	[ ]	[ ]	[ ]	[ ]	[ ] [ ]	[ ]
18.	[ ]	[ ]	[ ]	[ ]	[ ]	[ ] [ ]	[ ]
19.	[ ]	[ ]	[ ]	[ ]	[ ]	[ ] [ ]	[ ]
20.	[ ]	[ ]	[ ]	[ ]	[ ]	[ ] [ ]	[ ]
21.	[ ]	[ ]	[ ]	[ ]	[ ]	[ ] [ ]	[ ]
22.	[ ]	[ ]	[ ]	[ ]	[ ]	[ ] [ ]	[ ]
23.	[ ]	[ ]	[ ]	[ ]	[ ]	[ ] [ ]	[ ]
24.	[ ]	[ ]	[ ]	[ ]	[ ]	[ ] [ ]	[ ]
25.	[ ]	[ ]	[ ]	[ ]	[ ]	[ ] [ ]	[ ]

Urine 0 Nil 1 Trace 2 + 3 ++ 4 +++ 5 ++++ 9 No specimin

Oedema 0 Nil 1 Minimal/slight 2 Present 3 Marked

F1: Help F3: Prev F4: Next F5: Goto F6: Search F10: Save Alt-F10: Exit

File: X Maxrec: 0 Rec: 1 Page: 12 of 20 REPLACING

A/N complications from patient held white notes/green hosp notes

---

Threatened abortion No / Yes / Loss of co-twin [ ]
(vag. bleeding <28 weeks)

Antepartum haemorrage No / Yes [ ]

Amniocentesis performed No / Yes [ ]

Chorconic villus sampling No / Yes [ ]

Clinical suspicion of growth retardation No / Yes [ ]
(SFD / light for dates / placental deficiency)

F1: Help F3: Prev F4: Next F5: Goto F6: Search F10: Save Alt-F10: Exit

File: X Maxrec: 0 Rec: 1 Page: 13 of 20 REPLACING

MSU				
---	0	<10	0	<10,000/skin flora
	1	10-20	1	10,000-100,000
	2	>20	2	>100,000

Date	Pus Cells	Bacteria
[ ]	[ ]	[ ]
[ ]	[ ]	[ ]
[ ]	[ ]	[ ]
[ ]	[ ]	[ ]
[ ]	[ ]	[ ]
[ ]	[ ]	[ ]
[ ]	[ ]	[ ]
[ ]	[ ]	[ ]

Serum AFP [ ] date [ ]
2nd AFP [ ] 2nd date [ ]

Mum's blood group [ ] A / B / AB / O
Rhesus Pos/Neg [ ]

F1: Help F3: Prev F4: Next F5: Goto F6: Search F10: Save Alt-F10: Exit

```
File: X             Maxrec:  0      Rec: 1       Page: 14 of 20        REPLACING
--------------------------------------------------------------------------------
Pregnancy outcome  [ ]   1 Loss <28 wks     Reason if 2/3 <                    >
                         2 SB
                         3 NN Death
                         4 LB
                         5 Delivered elsewhere ? outcome
                         6 Delivered elsewhere liveborn

Mode of delivery   [ ]               Reasons [ ] [ ]  <                    >
1 Normal/spont   2 Forceps   3 LSCS          (See coding guide)
4 Ventouse

Presentation       [ ]
1 Vx/cephalic   2 Breech   3 Other

Duration of labour - length    1st  [  ] hrs  [  ] mins
                               2nd  [  ] hrs  [  ] mins
                               3rd  [  ] hrs  [  ] mins

--------------------------------------------------------------------------------
F1: Help   F3: Prev   F4: Next   F5: Goto   F6: Search  F10: Save  Alt-F10: Exit
```

```
File: X             Maxrec:  0      Rec: 1       Page: 15 of 20        REPLACING
--------------------------------------------------------------------------------
Fetal distress       [ ]  No / Yes
            Evidence [ ]  1 CTG only  2 CTG + FBS   3 CTG + CBpH

Meconium             [ ]  No / Yes

Other Complications  [  ]    [  ]
of labour - see coding guide

Retroplacental clot  [ ] No / Yes
Calcification        [ ]
Infarction           [ ]   No / Yes

Other 1 [ ]   Other 2 [ ] 1 Gritty     3 Clot between membranes   7 looked
                          2 Succ lobe  4 Pale   5 Thin   6 Grey     unhealthy
Placenta weight      [     ]

Membranes            [ ]  0 Complete   1 Doubtful   2 Incomplete   9 Not charted

--------------------------------------------------------------------------------
F1: Help   F3: Prev   F4: Next   F5: Goto   F6: Search  F10: Save  Alt-F10: Exit
```

```
File: X             Maxrec:  0      Rec: 1       Page: 16 of 20        REPLACING
--------------------------------------------------------------------------------
No of Vessels        [ ]
Insertion type       [ ]  0 Central   1 Lateral   2 Battledore   3 Velamentous
                          8 Not recorded
Blood loss (ml)      [    ]
INFANT
Infant sex           [ ]  Male / Female
Birthweight          [    ]
Head circumference   [   ]

Apgar 1              [  ]
Apgar 5              [  ]
                                                      See coding guide
Admitted to SCBU     [ ]  No / Yes     Reason(s) [  ]  [                    ]
                                                 [  ]  [                    ]
                                                 [  ]  [                    ]
Temperature          [   ]

--------------------------------------------------------------------------------
F1: Help   F3: Prev   F4: Next   F5: Goto   F6: Search  F10: Save  Alt-F10: Exit


File: X             Maxrec:  0      Rec: 1       Page: 17 of 20        REPLACING
--------------------------------------------------------------------------------
Labour onset         [ ]  0 Spont / Normal
                          1 Elective CS
                          2 ARM / Other surgical
                          3 IV oxytoin
                          4 Im / Nasal / buccal oxytocin
                          5 Other oxytocin agent
                          6 ARM and oxytocin
                          7 Other induction
                          8 Prostin
                          9 ARM, prostin & oxytocin
                          A Emergency CS

Tear                 [ ]  0 Nil
                          1 1st degree   2 2nd degree   3 3rd degree
                          4 Episiotomy   8 CSection

Placental delivery   [ ]  1 Simple   2 CCT   3 Manual

Placenta             [ ]  1 Complete   2 Incomplete   3 Uncertain

--------------------------------------------------------------------------------
F1: Help   F3: Prev   F4: Next   F5: Goto   F6: Search  F10: Save  Alt-F10: Exit
```

File: X Maxrec: 0 Rec: 1 Page: 18 of 20 REPLACING

Amniotic fluid samples taken [ ] No / Yes

Date placental samples taken [ ] Time [ ]

Date cord blood separated [ ] Time [ ]

Umbilical cord [ ] see coding Comment 1 [ ] <
No / Yes guide Comment 2 [ ] <
Comment 3 [ ] <

F1: Help F3: Prev F4: Next F5: Goto F6: Search F10: Save Alt-F10: Exit

File: X Maxrec: 0 Rec: 1 Page: 19 of 20 REPLACING

Admitted before labour No / Yes [ ] (exclude labour induction for post maturity)

	Date	Main indication	2nd indication	3rd indication
1.	[ ]	[ ]	[ ]	[ ]
2.	[ ]	[ ]	[ ]	[ ]
3.	[ ]	[ ]	[ ]	[ ]
4.	[ ]	[ ]	[ ]	[ ]
5.	[ ]	[ ]	[ ]	[ ]

Clinical evidence of hypertension No / Yes [ ]
(diastolic BP>90 x 2 >4 hrs apart +/- proteinuria)

Max severity BP [ ] 1 91-99 2 100-109 3 >110

Max proteinuria [ ] 0 None 1 Trace 2 + 3 ++ 4 +++ 5 ++++

Date of onset
Raised BP [ ]
Proteinuria [ ]

F1: Help F3: Prev F4: Next F5: Goto F6: Search F10: Save Alt-F10: Exit

File: X Maxrec: 0 Rec: 1 Page: 20 of 20 INSERTING

Maximum 24 hour proteinnuria [ ]

Eclamptic fit [ ] No / Yes

Fetal distress [ ]

0 CTG normal 1 CTG suspicious 2 CTG abnormal 3 2nd stage abnormality only

Lowest scalp Ph [ ]

Cord pH if taken [ ]

F1: Help F3: Prev F4: Next F5: Goto F6: Search F10: Save Alt-F10: Exit

APPENDIX VIII: NEONATAL WHOLE BODY AND LUMBAR SPINE DXA PROTOCOL

SWS NEONATES
DXA NEONATE SCANNING PROTOCOL

1. Turn on PC and printer
2. Enter **DPX** at prompt
3. Quality control: at menu select **F3**, then position phantom under the mat over the picture on the table, with gold on the outside. Press **Esc** to start Qa. Need to press keyboard to confirm that lights function. Qa provides an auto print out.
4. Ensure on correct database (press **F4** then **F6** to check; **PgUp/ PgDn** to change database).
5. Baby scan – total body: at menu press **F6** and select total body using arrow keys. Press **Esc** to return to main menu.
6. Enter baby details – at main menu press **F1** then **F4** to enter new baby's details. Enter all fields using **arrow keys** to toggle between fields. Enter the SWS number under facility. Change date of birth if same as scan date. Height and weight allow 2 s.f. only. Press **Esc** when complete.
7. Press **Esc** to start scanning process
8. Position the wrapped baby ½" below top line, ensure baby is centered and lying on incopad.
9. Check scan mode is paediatric small and width is 300. If not press **F1** to change.
10. Press **Esc** to start scanning
11. Ensure 5 lines scanned before baby's head otherwise abort by pressing **F1** then continue **N**, restart **Y** or **N**.
12. Once scanned 2 lines after feet Press **F1** to abort, continue **N**, restart **N** and save data **Y**.
13. Press **ESC** to enter main menu

14. To start the spine scan Press **F6** and select AP spine
15. Press **F1** to select baby and Press **ESC** to check through details.
16. Press **ESC** to start scanning.
17. Check scan mode is paediatric and width is 90. If not press **F1** to change.
18. Position the scanner so beam is just below the umbilicus/ at level of hips and centered.
19. Press **ESC** to start scan
20. Ensure scan starts on L5 and end on T12, otherwise abort and restart.
21. Once scanned T12, Press **F1** to abort and save data **Y**
22. Weigh baby and enter full weight into comment 1 box (**F1)**
23. To print for the parents press **F2, F1** then print screen on analysis of grey scale

APPENDIX IX : SWS PATERNAL DXA INFORMATION SHEET

31.8.01

PATIENT INFORMATION SHEET

THE RELATIONSHIP BETWEEN THE BONE MASS OF FATHERS AND THEIR CHILDREN.

YOU ARE BEING INVITED TO TAKE PART IN A RESEARCH STUDY. BEFORE YOU DECIDE, IT IS IMPORTANT FOR YOU TO UNDERSTAND WHY THE RESEARCH IS BEING DONE AND WHAT IT WILL INVOLVE. PLEASE TAKE TIME TO READ THE FOLLOWING INFORMATION CAREFULLY AND DISCUSS IT WITH ANYONE YOU WISH. ASK US IF THERE IS ANYTHING THAT IS NOT CLEAR OR IF YOU WOULD LIKE MORE INFORMATION. TAKE TIME TO DECIDE WHETHER OR NOT YOU WISH TO TAKE PART.

What is the purpose of this study?

This study is trying to find out how a baby's bones grow and what may alter the risk of fracture in later life, over 60 years later. Some of these factors are inherited from the parents. However, recent studies have suggested that factors, such as a woman's diet and body build during the pregnancy, may affect the growth of the baby's bones. We now wish to investigate how much of the strength of a baby's bones is determined by the strength of the father's bones.

WHY HAVE I BEEN CHOSEN?

As part of the Southampton Women's Survey your child had a special bone density scan soon after birth and you were given a print-out of the skeleton. This scan provided us with detailed information about the amount of bone growth during the pregnancy. We are writing to invite you to participate in a research study, which will involve a similar scan of your own bone mass. This is so that we can link your measurements to those of your child. In this way we can find out how your bone structure may have influenced your child's bone strength at 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 effect the standard of care you receive.

WHAT WILL HAPPEN TO ME IF I TAKE PART?

If you decide to take part, we shall contact you to arrange a single appointment at the Southampton General Hospital at a time convenient to you. The appointment will last 1 hour and during this time we shall perform the same type of bone mineral density scan which we did for your child, as well as scan your heel using an ultrasound machine and ask you some questions related to your bones.

The bone density scan does not work if you are wearing any metal objects, like rings, buckles or zips, so we do ask you to come in tracksuit bottoms and T shirt if possible.

WHAT ARE THE POSSIBLE DISADVANTAGES AND RISKS IN TAKING PART?

The bone density scan involves you lying on a table and a small scanning arm passing over you, about one foot in the air; it does not touch you. The dose of x-rays is less than spending half a day in Cornwall. The scan will not cause you any pain or harm. We will of course give you results of the scan and provide any medical advice necessary if your bone mineral density values are low.

WHAT ARE THE POSSIBLE BENEFITS OF TAKING PART?

By taking part in this study, you will have an assessment of your bone density and a copy of the results to take with you. The information we get from this study may help us to 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. The results of your bone density will be made available to your GP if your results are found to be low.

WHAT WILL HAPPEN TO THE RESULTS OF THE RESEARCH?

We will link the information from this study with results about your child to help us calculate your contribution to your child's bone strength. These findings will be published in the medical literature. You will not be identified in these reports/ publications in any way.

WHO IS ORGANIZING AND FUNDING THE RESEARCH?

This study is funded by the Medical Research Council and is being organized by the Environmental Epidemiology Unit at Southampton, UK. Is is being conducted as an additional component of the Southampton Women's Survey.

CONTACT FOR FURTHER INFORMATION

For further information please contact Professor C. Cooper or Dr M. K. Javaid at the Medical Research Council Environmental Epidemiology Unit 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

Professor C. Cooper MA DM FRCP FMedSci
Professor of Rheumatology

Local Research Ethics Committee no. 213/01

APPENDIX X : SWS PATERNAL QUESTIONNAIRE

Paternal Lifestyle, body build and skeletal status as determinants of neonatal bone mass.

Paternal Lifestyle Questionnaire

SWS ID _____ Date _____

SURNAME _____ INITIALS ___

Date of birth ___ Day _____ Month _____ Year

Height _____ . _____ metres _____ Weight _____ . Kg

Grip Strength

1. MEDICAL HISTORY

A. HAVE YOU EVER BEEN TOLD BY A DOCTOR THAT YOU SUFFER FROM HYPERTHYROIDISM (OVER ACTIVE GLAND)?

Yes No Don't Know

b. Have you ever been told by a doctor that you suffer from diabetes?

Yes No Don't Know

If YES do you inject insulin?

Yes No Don't Know

2. FRACTURE HISTORY

a. Have you ever suffered from a broken bone (fracture) ?

Yes No Don't Know

If YES, which bone, at what age was your first fracture of that bone, and what was the level of trauma (see glossary)

	Number	Age first fracture	Level of trauma 1-3
Vertebral			
Hip			
Forearm			
Other			

c. Have you ever been told by a doctor that you had osteoporosis (thinning bones) ?

Yes No Don't Know

3. FAMILY HISTORY

Have any of your parents or siblings (brothers or sisters) suffered a hip fracture after the age of 50 years? (leave blank if not applicable see glossary).

a. Mother	Yes	No	Don't Know
b. Father	Yes	No	Don't Know
c. Brother	Yes	No	Don't Know
d. Sister	Yes	No	Don't Know

4. MEDICATION HISTORY

Has your doctor treated you with any of the following treatments?

a. Androgens	YES	NO	DON'T KNOW
b. Calcitonin	YES	NO	DON'T KNOW
c. Calcium	YES	NO	DON'T KNOW
d. Fluoride	YES	NO	DON'T KNOW
e. Vitamin D	YES	NO	DON'T KNOW

5. PHYSICAL ACTIVITY

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

a) **strenuous exercise** which made you heart beat rapidly **AND** left you breathless **e**.g. jogging, vigorous swimming or cycling, aerobics.
Frequency ____

and **on average** about how long did each period of activity last? ___Hrs ___mins

b) **moderate exercise** which left you exhausted but not breathless e.g. brisk walking, dancing, easy swimming or cycling, badminton, sailing.
Frequency ____

and **on average** about how long did each period of activity last? ___Hrs ___mins

c) **gentle exercise** which left you tired but not exhausted e.g. waling, heavy housework (including washing windows and polishing), gardening, DIY, golf.
Frequency ____

and **on average** about how long did each period of activity last? ___Hrs ___mins

d) Which of the following best describes your walking speed at present?

1. very slow
2. stroll at an easy pace
3. normal speed
4. fairly brisk
5. fast

6. SMOKING

a. Do you smoke cigarettes or other forms of tobacco?

Yes now ____ Not now but in the past____ Never____

b. If you have ever smoked cigarettes

1. how old were you when you first started smoking? _____
2. how many on average do/did you smoke a day? _____
3. If you have now stopped, how old were you when you stopped? _____

7. ALCOHOL CONSUMPTION

I'd like to ask you a few questions about your drinking and smoking habits.

a. Do you ever drink alcohol? Yes/ No

If NO go to question 8.

If yes

During the last three months

a) How often have you drunk **Shandy or low alcohol beer/ lager/ cider?** _____
(don't include alcohol **free** lager etc)

b) When you drank these how many pints did you normally have? _____

During the last three months

a) How often have you drunk **Beer/Stout/Lager/ Cider/Alcopops?** _____

b) When you drank these how many pints did you normally have? _____

During the last three months

a) How often have you drunk **Low alcohol wine?** _____

b) When you drank these how many glasses did you normally have? _____

During the last three months

a) How often have you drunk **Wine/Sherry/Martini/Cinzano?** _____

b) When you drank these how many glasses did you normally have? _____

During the last three months

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

b) When you drank these how many measures did you normally have? _____

8. FOOD SUPPLEMENTS.

During the past three months have you taken any pills, tonics or tabletss to supplement your diet?
(e.g. vitamins, iron tablets, folic acid, fish oils etc.)
YES/NO

If YES, please state which

(for number per day, record number of tablets/ capsules/teaspoons per day, as appropriate)

Supplement	Number per day	How many days in the last 90?	Did you start taking this: 1: Less than 1 month ago 2: 1-2 months ago 3: More than 2 months ago

9. DIETARY CALCIUM INTAKE

Think about your usual eating habits over the last year.

1. How much milk do you usually use in an average day? (Probe: Do you have milk delivered? Think about milk used in tea and coffee, on breakfast cereals or puddings, and in cooking.) Give your answer to the nearest 1 pint ___pts

2. Next, I would like to ask you about a number of different foods. Please tell me whether or not you eat the food, how often you have it if you do eat it, and how much you have on the days when you eat the food. (Ring the correct answers and fill in amounts.)

	Not eaten	1/ 3-4wks	1/ 1-2wks	1-2 d/wk	3-5d/wk	6-7d/wk	Amount per day	Milk?	
Tea	1	2	3	4	5	6	___cups ___mugs	1 None 2 Liquid milk* 3 Tinned milk 4 Powdered milk ___g+	
Coffee	1	2	3	4	5	6	___cups ___mugs	1 None 2 Liquid milk*1/4 or less 3 " ; ¼ 4. " ; ½ 5 " : all 6 Tinned milk 7 Powdered milk ___g+ 8 Coffemate	
Other milky drinks (Horlicks, Bournvita, Ovaltine, Hot chocolate, Cocoa,Complan, Build-up etc.)	1	2	3	4	5	6	___cups ___mugs	1 None 2 Liquid milk*1/4 or less 3 " ; ¼ 4. " ; ½ 5 " : all 6 Tinned milk 7 Powdered milk ___g+	1 Horlicks, other milk-powder based drinks 2 Ovaltine, cocoa, other drinks not milk-powder based 3 Complan etc

*Liquid milk: whole, semi-skim, skim, UHT, sterilised, powdered made-up + Level tsp=2g; rounded=3g; heaped=4g

	Not eaten	1/ 3-4wks	1/ 1-2wks	1-2 d/wk	3-5 d/wk	6-7 d/wk	Amount per day	
Milk alone	1	2	3	4	5	6	___small glasses ___large glasses ___cups ___mugs	
Breakfast cereal (Probe for porridge made with milk)	1	2	3	4	5	6	___number of portions	?Milk 1 None 2 small 3 medium 4 large
Bread, toast and rolls	1	2	3	4	5	6	___slices	1 White 2 Brown 3 Wholewheat 1 small 2 large 1 Sliced 2 Unsliced 3 Rolls ---
Cheese	1	2	3	4	5	6	___number of portions	1 small 2 medium 3 large
Cakes, scones and biscuits	1	2	3	4	5	6	___number of portions	Sm Med Lg Cakes + scones 1 2 3 Biscuits 1 2 3
Deserts made with milk	1	2	3	4	5	6	___number of portions	Sm Med Lg Custard, blancmange, milk puddings, yog. 1 2 3 Jelly made with milk, angel delight, mousses 1 2 3 Ice cream

(probe for custard or ice-cream on other desserts)

APPENDIX XI: SWS PATERNAL DXA PROTOCOL

Selection of Subjects:

Fathers of all babies, who had a DXA scan performed in the neonatal period as part of the bone component of the SWS study, will be approached to take part in this study.

A list of eligible subjects will be generated using the Southampton Women's Survey database. Each subject will be contacted by post and telephone, and given written information about the study (Appendix C). They will then be asked if they agree to participate and an appointment will be given to them to attend the Osteoporosis Centre at the SGH.

At the Osteoporosis Centre, each subject will:

1. Complete a 15 minute interviewer administered osteoporosis risk factor questionnaire (Appendix B) to ascertain previous fracture history, previous illnesses and medication, dietary calcium intake, smoking and current exercise.
2. Have their height measured using a stadiometer and their weight measured on electronic scales.
3. Undergo DXA (whole body, lumbar spine, femoral neck) (Hologic QDR 2000) and quantitative ultrasound of the left heel (Hologic Sahara Calcaneal Ultrasound).

In the event of a subject's bone density having a T score <-2.5, a clinical evaluation will be performed by Dr MK Javaid, ARC Clinical Fellow, and communicated to the GP. If necessary protracted care will be continued in the rheumatology out patients department.

The total time for the attendance is estimated at 25 minutes.

APPENDIX XII: PAH1 NINE MONTH QUESTIONNAIRE

THE MRC PRINCESS ANNE COHORT - A FOLLOW-UP STUDY

MRC Environmental Epidemiology Unit, Southampton

QUESTIONNAIRE

Interviewer .....

Date and time ☐☐ ☐☐ ☐☐ ☐☐ ☐☐
day month year hour minute

ID number of child ☐☐☐☐☐☐

Child's first name(s) .....

Child's surname .....

Child's date of birth ☐☐ ☐☐ ☐☐
day month year

Sex (1=boy, 2=girl) ☐

Address.....

.....

.....

.....

Postcode: ☐☐☐☐ ☐☐☐

Telephone number: ☐☐☐☐ ☐☐☐☐☐☐☐

GP: .....

.....

.....

PART 1 HEALTH

How has [name]'s health been in general over the last 12 months? (1= very good, 2=good, 3=fair, 4=bad, 5=very bad) ☐

Does [name] have any long-standing illness or disability? By longstanding we mean anything that has troubled [name] over a period of time, or that is likely to affect him/her over a period of time? (0=no, 1=yes) ☐

If yes

Specify the three most important ones.

Problem 1

.....

Problem 2:

.....

Problem 3:

.....

Does this illness or disability (these illnesses or disabilities) keep [name] from going to school? (0=no, 1=yes) ☐

Does this illness or disability limit [name's] activities in another way? (0=no, 1=yes) ☐

If yes, specify:

.....

All

Now, I'd like you to think about the two weeks ending yesterday. During these two weeks did [name] have to cut down on any of the things that he/she usually does about the house or at school or in his/her free time because of a condition you have just told me about or any other illness or injury? (0=no, 1=yes) ☐

If yes, how many days was this in all during these 2 weeks?

☐☐ days

How many times has [name] been admitted to hospital?

Hospital admission is defined as a stay in hospital for at least one night, exclusive of hospital stay immediately after birth

☐☐ times

If ever admitted

How old was [name] when he/she was admitted for the first time?

☐☐ years ☐☐ months

How old was [name] when he/she was admitted last time?

☐☐ years ☐☐ months

Specify the three most important reasons for these admission(s):

Reason 1

.....

Reason 2:

.....

Reason 3:

.....

All

Does [name] use any medication on prescription at the moment? (0=no, 1=yes) ☐

If yes, copy names directly off bottles or packets.

Name	Total daily dose (with units)	Prescription
1		
.....		
2		
.....		
3		
.....		
4		
.....		
5		
.....		

PART 2 NUTRITION

What kind of milk does [name] usually drink, or use in tea, coffee or cereals? (1= full fat cows' milk, 2=semi-skimmed cows' milk, 3=skimmed cows' milk, 4=soya milk, 5=none) ☐

How much milk does [name] usually drink per day? (0=none, 1= <¼ pint, 2=¼ to ½ pint, 3=½ to 1 pint, 4= >1 pint) ☐

PART 3 ACTIVITY

In the last week (ending yesterday), has [name] done a continuous walk that lasted at least 15 minutes? (0=no, 1=yes) ☐
INCLUDING WALKING TO SCHOOL. SEE PART 4

If yes, on how many days in the last week?

☐☐ days

In the last week, has [name] done any housework or gardening which involved pulling or pushing, like hoovering, cleaning a car, mowing grass or sweeping up leaves for at least 15 minutes a time? (0=no, 1=yes) ☐

If yes, on how many days in the last week?

☐☐ days

In the last week, has [name] done any sports or exercise activities, for at least 15 minutes a time (not counting things done as part of school lessons)? (0=no, 1=yes) ☐

If yes, on how many days in the last week?

☐☐ days

Did [name] do any of these sports or exercise activities for at least 15 minutes a time on Saturday or Sunday of the last week? (0=no, 1=yes) ☐

If yes, was it on Saturday or Sunday or on both days? (1=Saturday, 2=Sunday, 3=both days) ☐

How long did [name] spend *(on Saturday, Sunday, in total on both days)* doing these sports or exercise activities? *See Appendix A* ☐

Still thinking about last week, on how many of the weekdays did [name] do any of these sports or exercise activities for at least 15 minutes *(not counting things done as part of school lessons)?* (0, 1, 2, 3, 4, 5 days in the last week) ☐

On each weekday that [name] did sports or exercise activities, how long did [name] spend? *See Appendix A* ☐

PART 4 TRANSPORT TO SCHOOL

What is the one way distance from home to [name's] school? (0=less than ½ mile, 1=between ½ and 1 mile, 2= between 1 and 2 miles, 3=between 2 and 3 miles, 4=more than 3 miles) *10 minutes walk is about ½ mile* ☐

How does [name] usually get to school?
USE MORE THAN ONE ANSWER IF NECESSARY

On foot (0=no, 1=yes) ☐
By bike (0=no, 1=yes) ☐
By bus (0=no, 1=yes) ☐
By train (0=no, 1=yes) ☐
By car (0=no, 1=yes) ☐

PART 5 HOUSEHOLD COMPOSITION

Definition of household: a group of people (not necessarily related) sharing a kitchen or cooking facilities.

How many people live in the household, including [name].

of 16 years and older? ☐☐ people

younger than 16 years? ☐☐ people

younger than [name] ? ☐☐ people

Which people, living in the household, are responsible for [name]'s upbringing at the moment?

Parent 1 (1=mother, 2=father, 3=stepmother, 4=stepfather, 5= other) ☐

Present during interview? (0=no, 1=yes) ☐

Parent 2 (1=mother, 2=father, 3=stepmother, 4=stepfather, 5= other, 6=no 2nd) ☐

Present during interview? (0=no, 1=yes) ☐

Have the same people, living in the household, been responsible for [name]'s upbringing all his/her life? (0=no, 1=yes) ☐

We want to know if [name] has lived with the same parents all his/her life.

If not, how old was [name] when the first change occurred?

☐ years ☐☐ months

PART 6 BIRTH ORDER

How many children (live born and still born) did the (natural) mother have **before** [name] was born?

Include stillborn children after pregnancy duration of 24 weeks or more.

☐☐ live born child(ren)

☐☐ still born child(ren)

And how many children (live born and still born) did she have **after** [name] was born?

☐☐ live born child(ren)

☐☐ still born child(ren)

Did any of her children die? (0=no, 1=yes) ☐

If yes, in what year?

☐☐☐☐

PART 7 PARENTS' EDUCATION

Answer the questions separately for parent 1 and parent 2.

What is highest degree or professional and vocational qualification of [name]'s parents?

See Appendix B

Parent 1 ☐☐

Parent 2 ☐☐

At what age were the parents last in full time education?

Parent 1 ☐☐ years

Parent 2 ☐☐ years

How many years in total have the parents been in full time education?

Parent 1 ☐☐ years

Parent 2 ☐☐ years

PART 8 PARENTS' SMOKING STATUS

ANSWER THE QUESTIONS SEPARATELY FOR PARENT 1 AND PARENT 2

Parent 1

Has parent **ever** smoked regularly? (0=no,1=yes) ☐

At least once a day for a year or more

If no, go to questions on parent 2.

IF YES

How old was parent when he/she first ever smoked regularly .....years

What is the most that parent ever smoked regularly?

Cigarettes ..... per day

Roll-ups..... per day

Tobacco.....oz per week

Cigars.....per day

Does parent still smoke regularly (0=no, 1=yes) ☐

If yes

How many per day?

Cigarettes ..... per day

Roll-ups..... per day

Tobacco.....oz per week

Cigars.....per day

If no
At what age did parent last smoke regularly? .....years

Parent 2

Has parent **ever** smoked regularly? (0=no,1=yes) ☐
AT LEAST ONCE A DAY FOR A YEAR OR MORE

IF YES

How old was parent when he/she first ever smoked regularly .....years

What is the most that parent ever smoked regularly?

Cigarettes ..... per day

Roll-ups..... per day

Tobacco.....oz per week

Cigars.....per day

Does parent still smoke regularly (0=no, 1=yes) ☐

If yes
How many per day?

Cigarettes ..... per day

Roll-ups..... per day

Tobacco.....oz per week

Cigars.....per day

IF NO

At what age did parent last smoke regularly? .....years

APPENDIX A – Physical Activity

1. Less than 15 minutes
2. 15 minutes, less than 30 minutes
3. 30 minutes, less than 1 hour
4. 1 hour, less than 1½ hours
5. 1½ hours, less than 2 hours
6. 2 hours, less than 2½ hours
7. 2½ hours, less than 3 hours
8. 3 hours, less than 4 hours
9. 4 hours, less than 5 hours
10. 5 hours or more

APPENDIX B - Education

List of qualifications (HSE):

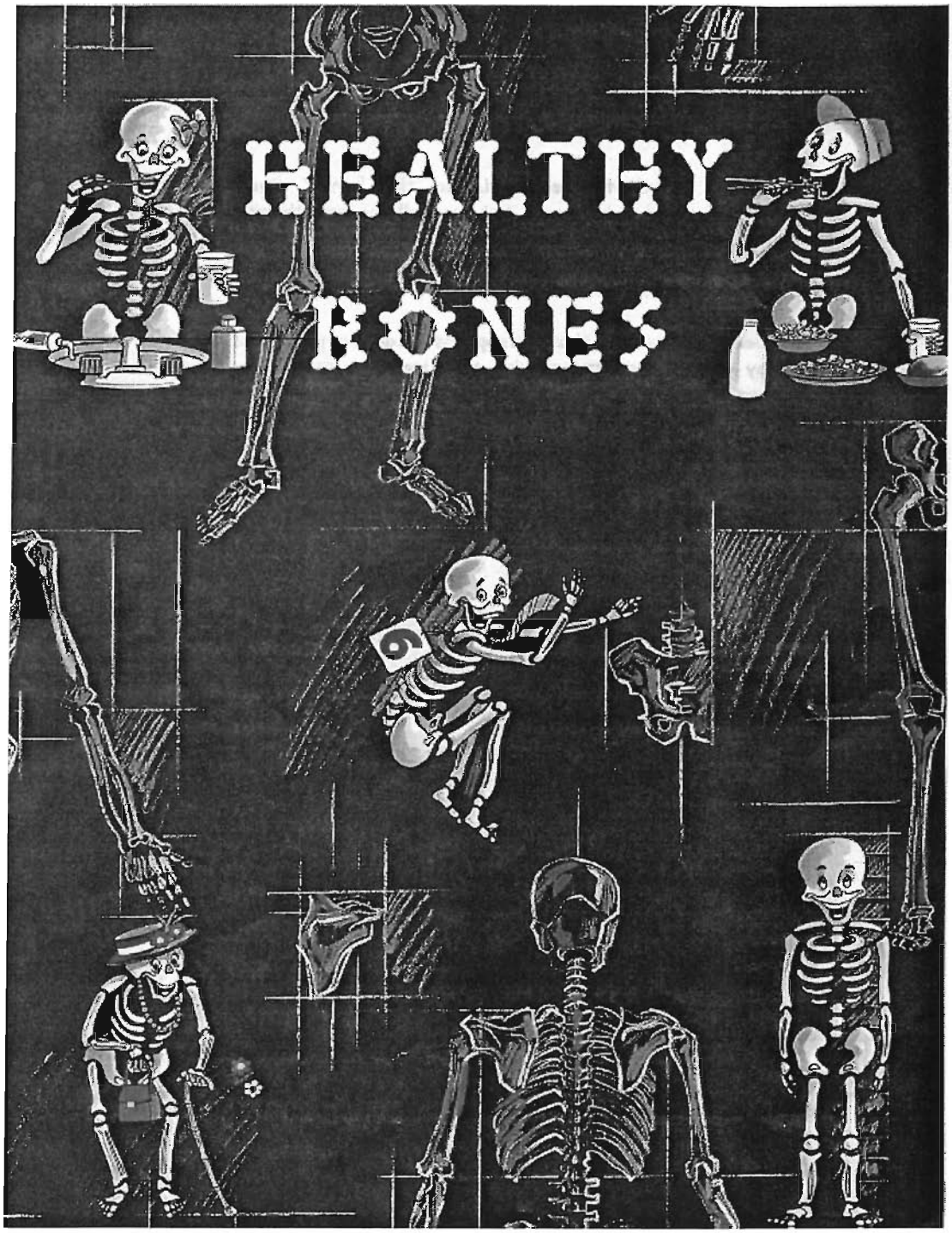
1. Degree/degree level qualification (including higher degree)
2. Teaching qualification
3. Nursing qualifications (SRN SCM SEN RGN RM RHS Midwife)
4. HNC/HND, BEC/TEC higher BTEC Higher/SCOTEC
5. ONC/OND/BEC/TEC/BTEC not higher
6. City and Guilds Full Technological Certificate
7. City and Guild Advanced/Final Level
8. City and Guilds Craft/Ordinary Level
9. A-levels / Higher school Certificate
10. AS level
11. SLC/SCE/SUPE at Higher Grade or Certificate of Sixth Year Studies
12. O-level passes taken in 1975 or earlier
13. O-level passes taken after 1975 or earlier GRADES A-C
14. O-level passes taken after 1975 or earlier GRADES D-E
15. GCSE GRADES A-C
16. GCSE GRADES D-G
17. CSE GRADE 1/SCE BANDS A-C/Standard Grade LEVEL 1-3
18. CSE GRADES 2-5/SCE Ordinary BANDS D-E
19. CSE Ungraded
20. SLC Lower
21. SUPE Lower or Ordinary
22. School Certificate or Matric
23. NVQ Level 5
24. NVQ Level 4
25. NVQ Level 3/Advanced Level GNVQ
26. NVQ Level 2/Intermediate level GNVQ
27. NVQ Level1/Foundation level GNVQ
28. Recognised Trade Apprenticeship completed

29. Clerical or Commercial Qualification (eg typing/book-keeping/commerce)
30. Qualifications outside UK
31. Other qualifications that cannot otherwise be coded
32. NVQ level not specified
33. Nursery Nurse Examination Board Qualification

MORE OVERLEAF

34. Military qualification
35. Foundation courses that cannot otherwise be coded
36. No qualifications

APPENDIX XIII: CHILDREN DXA INFORMATION SHEET


HEALTHY
BONES

WHAT IS OSTEOPOROSIS?

You may have heard of osteoporosis, or "thin bones". Our bones are strongest in our early adult years, but gradually become thinner as we get older. In some people, they become so thin that they break very easily, resulting in broken bones typically at the hip, wrist, or spine. This is the condition known as *osteoporosis*.

It has been discovered that osteoporosis can be prevented to some extent by a good diet and regular exercise throughout life. However, some people will still develop osteoporosis and recent research has suggested this may be partly because of the way their bones grew from before birth to early adulthood. In other words the growth of children's bones may strongly affect the strength of the bones when they are 60 years older.

HOW CAN YOU HELP?

This survey is a unique opportunity to compare results of size measurements at birth and see how the bones have grown after 9 years. Using this information we are aiming to find out more about what causes a child's bones to grow and so be able to give good advice to parents and children.

WHAT IS INVOLVED?

We want to do a special bone scan to see how much they

have grown since birth. This involves a 20 minute scan during which your child will lie on a table. A scanning arm moves over the body at a height of about two feet. The scan will examine the whole body and then look more carefully at the lower back. The scan is not at all uncomfortable and there is a tiny amount of exposure to X-rays (the same as one days natural sunlight or a ½ day trip to Cornwall).

We shall tell you the results of the scan, and will provide you with an information leaflet about healthy bone growth in children.

APPENDIX XIV: CHILDREN WHOLE BODY AND LUMBAR SPINE PROTOCOL

PRINCESS ANNE FOLLOWUP II
9 YEAR OLD SCANNING PROTOCOL

1. Turn on PC and printer
2. Enter **DPX** at prompt
3. Quality control: at menu select **F3**, then position phantom under the mat over the picture on the table, with gold on the outside. Press **Esc** to start Qa. Need to press keyboard to confirm that lights function. Qa provides an auto print out. Qa needs to be performed every 2 days.
4. Ensure on correct database (press **F4** then **F6** to check; **PgUp/ PgDn** to change database)
5. Dress code: Tracksuit bottoms and T shirt (No jumpers or shoes) NO METAL (no jewelry, hair clips, watches, check pockets)
6. Weigh child without shoes. If <30 Kg use paediatric large software. If >=30Kg use adult software/ medium mode
7. Children scan (Total Body Area and Lumbar spine)
8. Enter childs details – at main menu press **F1** then **F4** to enter new baby's details. Enter all fields using **arrow keys** to toggle between fields. Enter the PAH number under facility. Height and weight allow 2 s.f. only. Enter full weight in comments 1. Press **Esc** when complete
9. Total body: at menu press **F6** and select total body. Press **Esc** to return to main menu.
10. Enter Childs details – at main menu press **F1** then **F4** to enter new child's details (dob, height, weight, ethnicity). Enter all fields using **arrow keys** to toggle between fields. Enter the ID number under facility. Height and weight allow 2 s.f. only. Press **Esc** when complete.

11. Press **Esc** to start scanning process.
12. Ensure correct scan mode and width (paediatric large vs medium; scan width = 580)
13. Press **Esc** to start scanning
14. Ensure 5 lines scanned before childs head otherwise abort by pressing **F1** then continue **N**, restart **Y** or **N**.
15. Once scanned 2 lines after feet Press **F1** to abort, continue **N**, restart **N** and save data **Y**.
16. Press **ESC** to enter main menu
17. To start the spine scan Press **F6** and select AP spine
18. Press **F1** to select child and Press **ESC** to check through details.
19. Position child with hips flexed to 75 –90 % using grey box.
20. Ensure correct scan mode and width (paediatric vs medium; scan width = 280)
21. Press **ESC** to start scanning
22. Position the scanner so beam is one inch below the umbilicus
23. Press **ESC** to start scan
24. Ensure scan starts on L5 and end on T12, otherwise abort and restart.
25. Once scanned T12, Press **F1** to abort and save data **Y**.
26. To print for the parents press **F2** then print screen on analysis of grey scale.