UNIVERSITY OF SOUTHAMPTON

FACULTY OF MEDICINE, HEALTH AND LIFE SCIENCES

School of Medicine

Early Life Determinants of Skeletal Growth in Children: A Longitudinal Study

by

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ABSTRACT

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EARLY LIFE DETERMINANTS OF SKELETAL GROWTH IN CHILDREN: A LONGITUDINAL STUDY

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Nicholas Castell William Harvey

Evidence is accruing that factors in early life are associated with the risk of osteoporotic fracture in older age. Thus small epidemiological studies have suggested that maternal lifestyle, body build, physical activity and stores of 25(OH)-vitamin D during pregnancy may influence bone mass in the offspring. The aim of this thesis was to explore these concepts in a unique, large, prospective parent-offspring cohort, the Southampton Women's Survey (SWS), and to assess the relative contribution of parental and childhood factors to bone mass at 4 years old.

Healthy babies were recruited from the SWS. Their mothers had been characterised in detail for body build, lifestyle, physical activity and diet before and during pregnancy. Serum 25(OH)-vitamin D was measured in late pregnancy. 841 children underwent DXA assessment at birth and the remainder of the cohort (n=279), plus a subset of the neonatal DXA group (n=154), underwent DXA assessment at 4 years old. Childhood lifestyle, physical activity, medical history and diet were assessed by questionnaire, and the child was measured and weighed at the visit. Additionally, in a subset of 81 children and their mothers, physical activity was objectively assessed using the Actiheart combined accelerometer and heart rate monitor.

The mutually independent maternal predictors of bone mass at birth included height, parity, and smoking, fat stores and walking speed in late pregnancy. These associations were attenuated by 4 years, with only maternal height still statistically significantly predicting childhood bone mass. Mothers who had low levels of vitamin D in late pregnancy had children with lower bone mass at birth, but this effect was not seen at 4 years. Daily milk intake and Actiheart-measured energy expenditure were the strongest 4-year predictors of bone mass.

These results are consistent with previous findings and suggest that a life course approach to the prevention of osteoporotic fracture is appropriate. Thus public health strategies aimed at optimising the intra-uterine and post-natal environments to maximise bone mineral accrual may reduce the burden of osteoporotic fracture in future generations.

Table of Contents

Figures

Tables

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Glossary

DXA (Dual X-ray absorptiometry) indices

BA:	Bone area
BMC:	Bone mineral content
BMCh:	BMC adjusted for height
aBMD:	Areal bone mineral density
vBMD:	Volumetric bone mineral density
BMAD:	Bone mineral apparent density (calculated)
BMDh:	BMC adjusted (by regression) for BA and height (Horlick method)
WB:	Whole body
LS:	Lumbar spine

Biochemical measurements

IFG-1:	Insulin-like growth factor-1
IFG-2:	Insulin-like growth factor-2
PTH:	Parathyroid hormone
PTHrP:	Parathyroid hormone related protein
25D:	25(OH)-vitamin D

Time points

PP:	Pre-pregnancy
EP:	Early pregnancy (11 weeks)
LP:	Late pregnancy (34 weeks)

Other

TSF:	Triceps skinfold thickness
PBM:	Peak bone mass

1 Background

1.1 Introduction

Osteoporosis has a huge impact on public health, through the increased morbidity, mortality and economic costs associated with resultant fractures. Peak bone mass achieved in early adulthood is a major contributor to risk of osteoporotic fracture in later life. Evidence is accruing that environmental factors in early life have a critical influence on the magnitude of peak bone mass achieved, and on later risk of fracture. These findings of interactions between the genome and the environment are paralleled by evidence of developmental plasticity in the natural world- that is, the ability of one genotype to lead to different phenotypes in response to the prevailing environmental milieu. This work explores the influence of the early environment on bone mineral accrual and body composition in children.

1.2 Osteoporosis

1.2.1 Definition

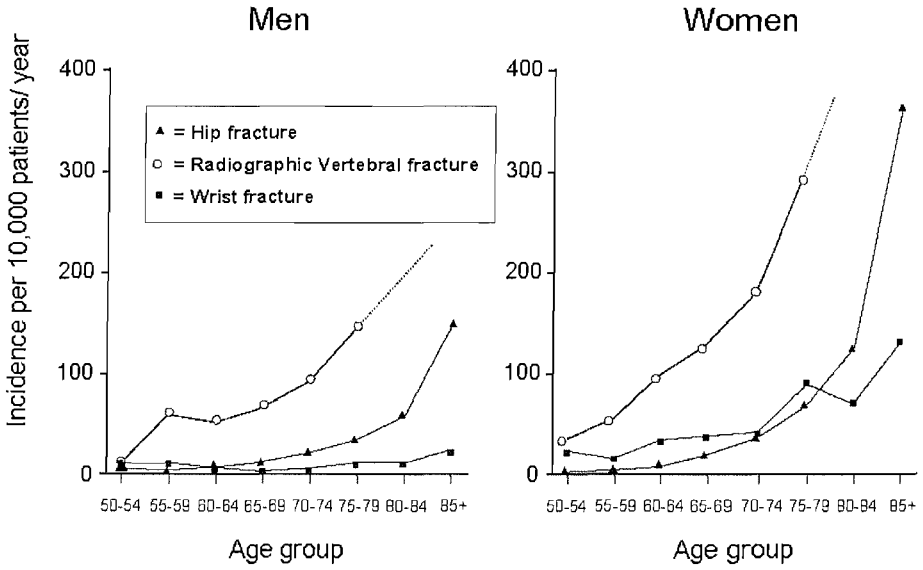
Osteoporosis is defined as a "systemic skeletal disease characterised by low bone mass and micro-architectural deterioration of bone tissue with a consequent increase in bone fragility and susceptibility to fracture"(1). The World Health Organisation (WHO)(2) have defined osteoporosis clinically as a DXA-derived T score of less than –2.5, and it is important to note that this only takes into account the deterioration in mineralization, and does not reflect decline in micro-architecture.

1.2.2 Epidemiology

There were an estimated 1.66 million hip fractures worldwide in 1990(3); 1 197 000 in women and 463 000 in men. Data from the UK suggest that there is an overall fracture incidence of 21.1/1000 per year (23.5/1000 men and 18.8/1000 women)(4), and that there is a bimodal age distribution, with peaks in youth and in the very elderly(5). A study using the General Practice Research Database (UK) demonstrated an overall lifetime risk of fracture for a 50 year old woman as 53.2%, and for a man as 20.7%(6). The corresponding lifetime risks for hip fracture were 11.4% and 3.1% respectively. The ten-year risk for all fractures was 9.8%

and 7.1% in women and men respectively at age 50 years, and increased to 21.7% and 8.0% at age 80 years, consistent with the known pattern of age-related osteoporotic risk (Figure 1).

Figure 1: Incidence of fracture according to age and sex(6;7).



Osteoporosis has been estimated to cost the UK around £1.7 billion per year, mainly through hospitalization for fractures(8). The morbidity and mortality associated with hip and vertebral fractures are considerable- 5-20% of people will die within one year after a hip fracture, and over 50% of survivors will be incapacitated, many needing nursing home care(9).

1.3 Growth from conception to peak bone mass

1.3.1 Overview of growth

The foetus accretes 80% of the required 30g calcium during the last trimester in human pregnancies(10), and then bone mass increases largely as a result of increase in bone size throughout childhood. During the adolescent growth spurt around a further 25% of the final peak bone mass (PBM) is achieved(11). Peak bone mass is reached in the twenties, but the exact timing appears to vary with skeletal site and gender(11). After the achievement of PBM, bone mass starts to decline and this is accelerated after the menopause in women (Figure 2).

The concept of a child remaining in the same position relative to its peers throughout growth to peak is known as "tracking"(12). This has been well described in several studies, which have sought to model the normal range of childhood growth for the purpose of clinical assessment(12;13). The resulting "centile charts" are plots of the average growth curves for healthy children, and allow identification of children who deviate from this normal pattern of growth. Because early attempts to formulate these charts were hampered by lack of separation of children who were breast or bottle-fed, or of different ethnicities, the World Health Organisation undertook a large project to derive the optimal growth standards in normal children(14). Such charts define appropriate height and weight for age and gender; charts for bone mass have thus far not been developed.

Children who experience a temporary restriction in dietary intake respond initially with a reduced growth velocity(15). Thus gain in height and weight is slowed, and noted clinically by their crossing centiles downwards. For an acute restriction, there is usually recovery of the pre-existing growth trajectory of return of adequate nutrition. This may take the form of accelerated growth and a rapid recovery of the original pattern, or of a slower, but more prolonged recovery, with subsequent delay in maturation(15). This process is known as "catch-up growth". The converse may apply after a period of nutritional excess, here known as "catch-down growth". These observations tend to imply that the trajectory of growth is set very early in life, either from genetic inheritance, or environmental factors in utero.

An alternative use of the terms catch-up and catch-down, is to describe growth in infancy relative to peers, taking birthweight as the starting point. Thus a child born small relative to its peers may either stay small, get smaller or increase in size relative to its peers. This latter option may be termed catch-up growth, although there is some disagreement about the correct definition of the term. This thesis will be mainly concerned with catch-up from birth. The pattern of growth over the intrauterine and postnatal periods depends on the interplay of genetic and environmental influences. Thus increase in foetal weight reaches a maximum velocity at around 34 weeks, when it begins to slow, probably because of physical constraint, as a result of maternal pelvis size(16). Thus a child genetically predisposed to be large, as a result of a large father, but carried by a small mother, can be delivered. This is consistent with animal studies of crossbreeding shire horses and Shetland ponies- when the mother is the shire horse, the baby is larger than when the mother is the Shetland pony(17). In this study, both foals became of similar size after a few months, midway between the size of their

parents. Postnatally, human studies have shown that generally the pre-slowing growth trajectory is regained, with accelerated growth in the first few months after birth(16). This reversion to the foetal growth trajectory has been modelled mathematically, and appears sustained over the first year of life(18;18;19).

This leads to the concept that there is a tendency to revert to a growth trajectory that has been determined in foetal life, initially by genetic makeup, but potentially also by environmental factors acting at critical periods in development. A period of undernutrition early in postnatal life in rats may lead to long term poor growth, whereas a similar period later in postnatal life is followed by a recovery of the original growth trajectory(20). Clearly interventions of this sort in the human foetus are not possible. Birthweight is therefore a surrogate for the intrauterine environment, particularly that in the last trimester, but, given the above, it is possible that environmental influences acting in early gestation might alter the growth trajectory, and not become apparent until infancy. Indeed, in clinical settings, it has been noted that the relationship between a child's anthropometry and their final height is weaker when measured at birth than at 2 years(12). Consistent with this is evidence from the observation that the majority of infants cross centile lines during the first two years of life: 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 downwards do so much later, starting between three and 18 months, and are likely to have shorter parents(21).

The implications of different patterns of growth have not been fully elucidated, and definite tracking of bone mass has also thus far not been demonstrated. However, given that height is largely determined by the longitudinal dimension of skeletal size, it seems reasonable that at least skeletal size, if not volumetric density, may follow a similar pattern to overall height. Recent longitudinal studies have been consistent with this notion, at least across the pubertal growth spurt(22). If bone mass does track from prenatal life to peak, then anything in utero which alters this trajectory adversely could result in suboptimal accrual of peak bone mass, and thus increased risk of osteoporosis in later life: Bone mass at any point after achievement of PBM depends in part on the magnitude of PBM achieved, and in part on the rate of subsequent bone loss. Hui et al explored the relative contributions of these two determinants and found that at age 70 years, they each explained 50% of the variance in BMD(23). A more recent modelling study from Hernandez et al demonstrated that PBM was six times more

powerful a predictor of age of onset of osteoporosis than age of menopause or rate of involutional bone loss(24).

A study relating childhood growth to risk of hip fracture in later life (See section 1.4.2.1) suggested several possible routes to this adverse outcome: Thus children who grew poorly and had tall mothers, and especially children who were small at birth and average size by age 7 years had increased risk of hip fracture in later life(25). This could come down to a mismatch between genetic potential and pre and postnatal nutrition, so that a genetically big child, with longer hip axis, is kept small on the one hand; on the other a genetically large individual who was small at birth undergoes excessive catch-up growth and thus longitudinal skeletal growth is in excess of the capacity to mineralise. The pattern of childhood growth may have implications for other diseases in adulthood, such as diabetes and ischaemic heart disease, which tend to be more associated with smallness at birth and then a period of rapid rebound adiposity in late infancy(26).

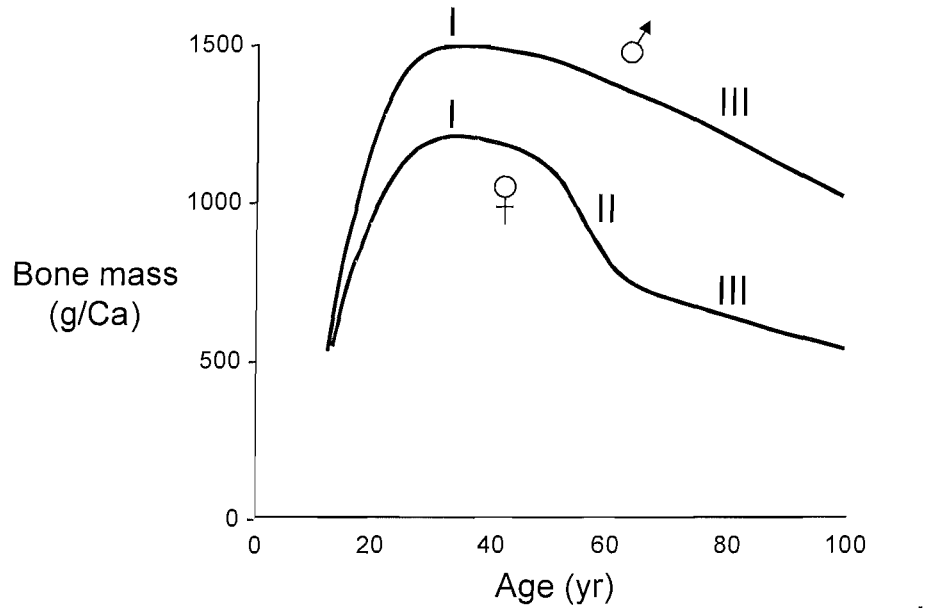


Figure 2: Bone mass by age and sex(27).

1.3.2 Determinants of peak bone mass

Peak bone mass has a multitude of genetic and environmental determinants. The latter act throughout the accrual of bone mass from conception, through infancy, childhood and young adulthood. These data will be described, before moving onto a review of the environmental factors that act in the intrauterine period (intrauterine "programming"(28)), and the possible underlying mechanisms.

1.3.2.1 Genetic contribution

Many different polymorphisms of genes thought to be important in the regulation of bone mass have been studied. These are detailed in the next section. Inheritance studies suggest that between 50 and 80% of the variance in peak bone mass is determined by genotype(29;30). These widely differing estimates may reflect different genetic make-up of different populations, and different study methodologies. Twin and family studies suggest that the inherited component of peak BMD is polygenic, and that environmental factors appear to be the major determinants of bone loss(31). Thus the variance in rate of bone loss explained by heredity is much lower, with postmenopausal bone mass being predicted much less strongly that than before the menopause(29). Additionally, in terms of fracture, which is the most important outcome, there is little increased concordance in monozygotic compared with dizygotic twins in both men and women(32). Indeed the intra-pair difference in bone mineral density between twins was greater for mono- than di-zygotic twins in one study, suggesting that environmental influences in utero, such as differential placentation, may have persisting effects(33).

1.3.2.2 Specific gene involvement

Much work has focused on several putative candidate genes (Table 1): the vitamin D receptor (VDR), collagen 1 alpha 1 (CO1A1), and insulin-like growth factor-1 (IGF-1). There is now evidence concerning interleukin-6 (IL-6)(34), transforming growth factor beta-1 (TGF-beta 1)(35), and LDL-receptor related protein 5 (LRP-5)(36). However, most studies have found small effects, and often with conflicting results. Polymorphisms of the VDR have been studied most, and have been shown to explain a small proportion of the variance of BMD in most studies(37). Since many of the VDR polymorphisms studied appear to be non-functional, it may be that there is linkage with another allele which is actually responsible for

the functional change. Type I collagen is an important constituent of bone matrix, and polymorphisms of the Sp1 binding site of COL1A1 have been shown to be associated with BMD and fracture risk several(38), but not all(39) studies.

Table 1 Summary of studies of individual genes and bone mass.

Gene	Effect	Citation
IL-6	G polymorphism increased previous fracture risk 46% in 964 PM women	Nordstrom et al 2004
TGF-beta-1	TT vs CC genotype, BMD 10% higher at LS and FN in 102 PM women	Hinke et al 2001
LRP-5	Several SNPs related to BMD in PM women	Koay et al 2004
VDR	192 OP, 207 controls. BB and Bb weakly assoc with reduced fractures; bb assoc with higher BMD	Langdahl et al 2000
COL1A1	Yield strength bone higher in Ss than SS subjects.	Mann et al 2001
COL1A1	38 MZ and 40 DZ pre-m twins No assoc Sp1 with fractures or BMD	Hustmyer et al 1999

PM: Post-menopausal; BMD: Bone mineral density; LS: Lumbar spine; FN: Femoral neck; SNP: Single nucleotide polymorphism; MZ: Monozygotic; DZ: Dizygotic; Pre-m: Pre-menopausal.

None of these studies have found that any of these genes account for more than a small proportion of variance in bone mass. Thus it seems likely that the genetic component of BMD is determined by a multitude of different genes. That facts that osteoporosis increases in older age and particularly after the menopause in women, and does not show any classic Mendelian inheritance pattern are all consistent with a polygenic determination. Additionally, non-functional mutations and genetic variation between populations further complicate the issue.

It is unlikely that the genome and environment act independently on the skeleton; in fact there is increasing evidence for an interaction between them, for example between birthweight and the VDR gene(40), calcium intake and the VDR gene(41), and calcium intake and the Col1A1 gene(42). These data will be discussed in more detail later.

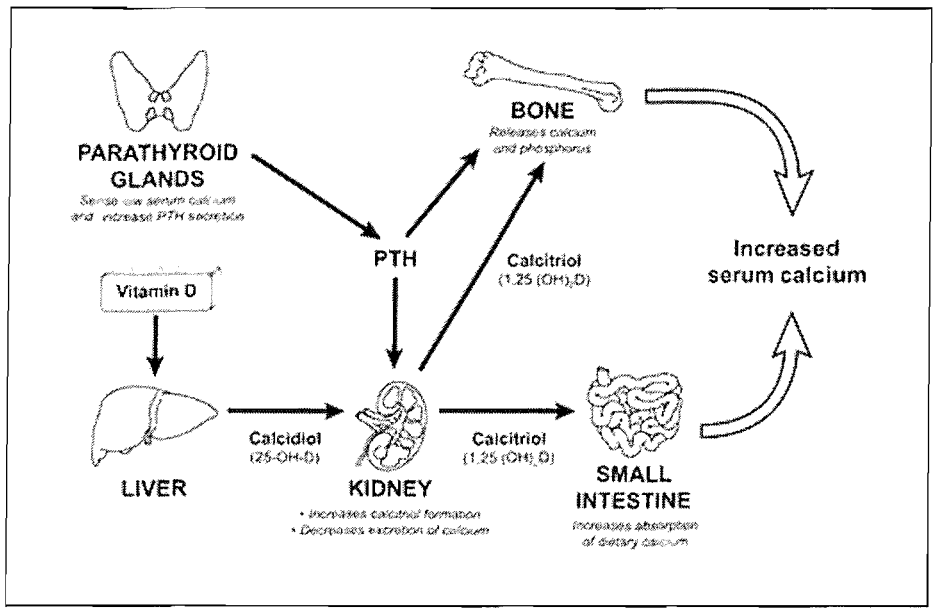
1.3.2.3 Vitamin D

Vitamin D is a secosteroid which is synthesized in the skin by the action of sunlight. It plays a crucial role in bone metabolism and skeletal growth(43). Around 95% is acquired via photosynthesis in the skin, with the minority from the diet. There are two dietary forms: D2, from plants, or D3, from animals; the latter mainly found in oily fish and fortified margarines and breakfast cereals(44).

1.3.2.3.1 Photosynthesis and metabolism

Vitamin D is synthesized from the action of sunlight (wavelengths 290- 315 nm) on cutaneous 7-dehydrocholesterol, converting it to pre-vitamin D3(43;45)(Figure 3). Once formed, pre-vitamin D3 undergoes membrane-enhanced temperature-dependent isomerisation to vitamin D3(43), which is translocated into the circulation where it binds to vitamin D-binding protein (DBP)(45). The main determinant of vitamin D synthesis in the skin is the level of sun exposure. The total amount of energy accrued from sunlight is dependent on duration and extent of skin exposure, but also on latitude and season. Thus pigmented skin and covering reduce synthesis, and using sun-block with a factor higher than 8 almost completely prevents formation of vitamin D(44). At latitudes of 48.5° (Paris, France), the skin is unable to form vitamin D between the months of October through to March(43). In Northern latitudes this results in a seasonal variation in levels of vitamin D, with peaks over the summer months and a trough in the winter(45). Use of sunscreen during the summer may prevent adequate synthesis of vitamin D and subsequent storage in fat for the winter months, thus leading to deficiency(45).

Figure 3 Vitamin D metabolism



Circulating vitamin D is converted in the liver to 25(OH)-vitamin D (calcidiol), which is the main circulating store (Figure 3). This step, which involves the cytochrome P450 system, is not tightly regulated and thus an increase in photosynthesis of vitamin D in the skin will lead to an increase in 25(OH)-vitamin D in the circulation(45;46), bound to DBP. Excess 25(OH)-vitamin D is converted to 24,25(OH)-vitamin D which is thought be relatively metabolically inactive(45). The 25-(OH)-vitamin D-DBP complex enters renal tubule cells by membrane-bound megalin transport, where the enzyme 1-α-hydroxylase converts it to 1,25(OH)2-vitamin D (calcitriol), which is the active compound(46). Although the kidney is the primary site for conversion of circulating 25(OH)-vitamin D, many tissues, such as macrophages, osteoblasts, keratinocytes, prostate, colon and breast express the 1-α-hydroxylase enzyme(43;47;48). Since anephric patients have very low levels of 1,25(OH)2-vitamin D in the blood, it seems likely that these extra-renal sites function at the paracrine level, and do not play a major role in calcium homeostasis(44).

Although D2 and D3 differ minimally in chemical structure, there is some evidence to suggest that D2 is less effective in determining circulating 25-(OH)-vitamin D status(49).

1.3.2.3.2 Actions of vitamin D

1,25(OH)2-vitamin D is around 1000 times more potent than 25(OH)-vitamin D, but is present in much lower concentrations(43). However, it seems to be the predominant active form of the vitamin, acting at a nuclear receptor site. 1,25(OH)2-vitamin D binds to a

cytoplasmic receptor, the VDR, and this complex travels into the nucleus, where it binds to RXR(50;51). This complex binds to the VDRE and this induces the binding of several initiation factors, resulting in transcription of the vitamin D responsive gene(50).

The major physiological function of vitamin D is to maintain calcium concentrations within the appropriate range in the circulation (Figure 3), mainly by regulating absorption from the small intestine. Thus 1,25(OH)2-vitamin D facilitates calcium entry into the small intestine by inducing the epithelial calcium channel. It also induces several other proteins in the small intestine, including calcium binding protein (Calbindin D9K), alkaline phosphatase, low affinity Ca ATPase, brush border actin, and calmodulin(52). There appears to be a biphasic response with a rapid initial increase in calcium flux over a couple of hours, followed by a more sustained increase that begins at 12 hours, suggesting that 1,25(OH)2-vitamin D may have a rapid membrane effect, followed by a slower action on gene expression(52;53).

The net result of vitamin D action in the bowel is an increase in calcium and phosphorus absorption. In the vitamin D deficient state, no more than 10-15% of dietary calcium and 60% of phosphorus is absorbed, but with adequate vitamin D, this fractional absorption may rise to 30-40% for calcium and 80% for phosphorus(52;53). The main role of 1,25(OH)2-vitamin D in bone mineralisation is by maintenance of the calcium x phosphorus product in the blood. If there is inadequate dietary calcium, then the 1,25(OH)2-vitamin D-VDR interaction results in RANKL production by osteoblasts, causing pre-osteoclasts to differentiate into osteoclasts and to resorb bone, thus increasing the blood calcium and phosphorus concentrations(46;54).

1.3.2.3.3 Control of vitamin D axis by PTH

The parathyroid chief cells contain a VDR, and 1,25(OH)2-vitamin D acts on this to decrease PTH synthesis and secretion. The parathyroid glands also contain a calcium sensing receptor (CaSR), which acts to maintain blood calcium concentration within set limits(44). Thus decreased calcium concentrations lead to an increase in PTH in the blood. Parathyroid hormone acts to increase conversion of 25 to 1,25(OH)2-vitamin D by stimulating the renal 1α-hydroxylase, via a increase in serum phosphorus concentration, and also has a direct effect on calcium and phosphorus resorption from bone(44).

1.3.2.3.4 Food sources and recommended intakes

Few foods contain significant amounts of vitamin D. The most effective sources are oily fish (for example salmon, mackerel) and fortified foods such as margarine and breakfast cereal. The amount of vitamin D derived from fish is modest: wild salmon contains around 400 iu per 3.5 oz(45). There is much controversy over the recommended daily intake of vitamin D. Older guidance has suggested 200 iu per day for children and adults up to 50 years old and 400 – 600 iu for older adults(55). However, humans have evolved to synthesise much higher levels of vitamin D in the skin: 30 minutes exposure at midday in the summer sun in a bathing suit will release around 50,000 iu into the circulation within 24 hours in white persons(56). Previous guidelines were not based on any rigorous assessment of the effects of levels and more recent dosing studies have shown that supplementation with 200-400 iu per day is unlikely to maintain levels of 25(OH)-vitamin D over winter months, let alone replenish stores in somebody who is frankly vitamin D deficient(57). Thus a daily maintenance dose of around 1000 iu per day may be more appropriate in people without adequate sunshine exposure(58).

1.3.2.3.5 Physiology of vitamin D in pregnancy

During pregnancy there is an increase in 1,25(OH)2-vitamin D, which may be largely due to an increase in DBP(59). This rise is associated with an increase in intestinal calcium absorption (to around 80% intake), and an absorptive hypercalciuria(59). There does not seem to be a rise in maternal PTH or 25(OH)-vitamin D during pregnancy, suggesting that the rise in 1,25(OH)2-vitamin D may be due to another factor, such as parathyroid hormone-related peptide, which may be secreted by the placenta(60). Studies of maternal bone mass in pregnancy have been conflicting, but most suggest a probable decrease, with a possibly greater decrease in lactation(61-65). The VDR appears to develop after birth in the infant intestine, and thus calcium absorption is a passive process immediately after birth(10). Additionally, foetal mice which lack vitamin D receptors do not show a reduction in calcium concentrations or placental calcium transport, implying that production of 1,25(OH)2-vitamin D is not critical for calcium homeostasis in foetal mice at least(10), although may play a role in rats(66). The foetal kidney does appear to be able to convert 25 to 1,25(OH)2-vitamin D, but the exact timing of commencement of 1α-hydroxylase activity is not known(10). The situation in human pregnancy remains to be clarified.

Although VDR is thought not be expressed in foetal intestine, and there is little direct evidence that 25 or 1,25(OH)2-vitamin D are critical for intrauterine bone mineral accrual in humans, some suggestions have come from recent epidemiological studies: In a study of 198 children, aged 9 years, in Southampton(67), mothers who were deficient (<11ng/ml) in 25(OH)-vitamin D in late pregnancy had children with reduced whole body bone mineral content at 9 years ($r=0.21$, $p=0.0088$). This association appeared to be partly mediated by venous umbilical cord calcium, suggesting that placental calcium transport might be implicated in the underlying mechanism. Mothers who delivered in the winter months had children with lower BMC than those who delivered in the summer, concordant with the expected seasonal variation in levels of 25(OH)-vitamin D. Postnatally, Zamora et al, in a retrospective cohort study of 106 healthy Caucasian girls, demonstrated that vitamin D supplementation in the first year of life was associated with higher areal BMD at the femoral neck ($p=0.02$) at age 8 years, after adjusting for confounders(68). The girls had similar calcium intakes in both previously supplemented and unsupplemented groups at time of the DXA scan, but clearly infant calcium intake is harder to quantify retrospectively. Thus there is evidence that vitamin D supplementation in the first year of life may influence bone mass in later childhood.

Data from the Hertfordshire (UK) cohort indicated an inverse correlation between weight at 1 year and adult 1,25(OH)-vitamin D levels. There was a 19.1% reduction in 1,25-vitamin D levels in the highest compared with the lowest tertile of weight at 1 year(69), such that those individuals with the lowest birthweight had the highest levels of 1,25-vitamin D in later life. The authors speculate that the mechanism might involve programming of renal 1α-hydroxylase, such that poor intrauterine calcium intake would lead to an increase in 1α-hydroxylase activity in an attempt to increase 1,25(OH)2-vitamin D and thus calcium resorption. However, as VDR may not be expressed till postnatal life, an increase in foetal 1,25(OH)2-vitamin D may not have an effect till sometime after birth. Other studies have shown a positive association between birthweight and peak bone mass (see section 1.4.2) and it may be that although adequate stores of 25(OH)-vitamin D are necessary for bone health, a higher set point for 1,25(OH)2-vitamin D production, associated with low birth weight, might result in higher levels of bone turnover and thus greater risk of fracture.

It is not known how levels of maternal vitamin D might affect skeletal development in the foetus. 25(OH)-vitamin D crosses the placenta, but 1,25(OH)2-vitamin D does not, and

potential mechanisms would include an affect on placental calcium transport or a direct effect of 25(OH)-vitamin D on neonatal bone, or an interaction with PTH or PTHrP. This is discussed further in section 1.3.2.4

1.3.2.3.6 Vitamin D supplements in pregnancy

In addition to the effects noted above, maternal deficiency in vitamin D has been associated with neonatal frank rickets and hypocalcaemia(70). Thus several studies have explored the use of vitamin D supplements in pregnancy: Observational studies suggest that reduced sunlight exposure in the last trimester is associated with an increased risk of neonatal tetany(71), and that 25(OH)-vitamin D levels are lower in babies with craniotabes, as well as in their mothers(72). Infants of mothers with low vitamin D intake may have lower calcium levels at day four(73).

There have been several, mainly small, intervention studies examining this issue, but only one has so far examined bone mass at birth. Thus 506 women were supplemented at 12 weeks gestation to 400 iu/day vs 633 placebo(74). Levels of 25(OH)-vitamin D levels were higher in maternal, umbilical cord, and infant serum (day 3 and 6) in the supplemented group. This was not a randomised trial, but supplemented women from one clinic vs placebo in another clinic. Another study compared 59 Asian women, supplemented with 1000 iu from 28 to 32 weeks(75), with 67 controls. Calcium levels were higher in the supplemented mothers, and there was a lower incidence of symptomatic neonatal hypocalcaemia and growth retardation amongst babies of supplemented mothers. Again in an Asian population(76), 25 mothers were randomised to 1200 iu 25(OH)-vitamin D per day, 20 mothers to 600 000 iu twice (7^{th} and 8^{th} month), and 75 mothers to placebo. In this study there was no difference in calcium and alkaline phosphatase levels between mothers taking 1200 iu/day and those taking placebo. However, those taking 600 000 iu twice had higher maternal and cord calcium and lower alkaline phosphatase than placebo. In a second study(77) the same group supplemented 100 Asian-Indian women with 600 000 iu twice (again at 7^{th} and 8^{th} months) vs 100 controls and found again, higher maternal and cord calcium and lower alkaline phosphatase. There have been two studies in French populations: 15 women were randomised to receive 1000 iu per day from 3^{rd} trimester vs 15 controls(78). Day 4 neonatal calcium and 25(OH)-vitamin D levels were higher in the supplemented group. In the second study 21 French women to receive 1000 iu per day in the last trimester, 27 to receive 200 000 iu once during 7^{th} month

and 29 controls(79). Here neonatal calcium at day 2 and 6 was similar in all groups, but maternal serum 25(OH)-vitamin D was greater in both intervention groups than in the controls. In the one study to measure bone mineral at birth(80), there was no difference in radial BMC in offspring of 19 Asian mothers who had taken 1000 iu 25(OH)-vitamin D per day compared with 45 controls. However this lack of observed effect is likely to reflect both the small numbers of subjects and the poor sensitivity of single photon absorptiometry in measuring the tiny amount of bone mineral in the baby's distal radius.

None of these studies has suggested that vitamin D supplementation during pregnancy carries a significant risk. Human beings have evolved to cope with as much as 25 000 iu 25(OH)-vitamin D formation daily in the skin. Although rat studies using the equivalent of 15 000 000 iu per day have resulted in extraskeletal calcifications, there is no evidence that doses below 800 000 iu per day have any adverse effect. 2 studies(81;82) have examined the children of hypoparathyroid women given 100 000 iu daily for the duration of pregnancy and found no morphological or physiological adverse consequences. These children were followed for up to 16 years. Recent work has demonstrated a moderate increase in atopy in children of mothers in the highest quarter of serum vitamin D in pregnancy, where levels were greater than 30 ng/ml (unpublished data). However, in this study the numbers were small with only 6 cases of atopy (asthma, eczema) by 9 years in the top quartile of maternal vitamin D, 4 each in the middle quartiles and 2 in the bottom. These numbers, even in the highest quartile, were actually lower than the figure for the general population. Additionally, in the Southampton Women's Survey, there was no association between maternal 25(OH)-vitamin D status and atopic or non-atopic eczema at 9 months or age (unpublished data). Thus, this finding needs to be further examined in larger studies, but suggests, for safety, that the optimal intervention would be to supplement those mothers found to be deficient in vitamin D, rather than all pregnant mothers.

Overall then, the data suggest that maternal supplementation with 25(OH)-vitamin D is likely to be safe, particularly if it brings them just into the replete level, but the definite benefit of this intervention has yet to be proved.

1.3.2.3.7 Measurement of vitamin D and normal ranges

25(OH)-vitamin D is the major store of vitamin D and is the most appropriate for measurement. 1,25(OH)2-vitamin D is an adaptive hormone, and therefore its level will reflect prevailing conditions such as calcium intake, and thus defining a normal level may not be meaningful(44). The concept of what is the normal range for 25(OH)-vitamin D is highly controversial at the moment. Given that humans have evolved to have a much higher levels of vitamin D than currently, the process of measuring levels in a population and defining a lower cut-off of the distribution as deficient may not be valid. Historically, serum levels have been classed as "replete", insufficient or deficient. The distinction between replete and insufficient has been made on the basis of whether there is a secondary rise in parathyroid hormone, and deficient as a cut off below this level. Previously studies have looked at the level of 25(OH)-vitamin D in populations where a rise in PTH is seen, and this cut off has been around 50 nmol/l(83). However, a proportion of the population do not show a rise in PTH with decreasing 25(OH)-vitamin D levels, as a result of concomitant magnesium deficiency(84). Thus an alternative approach is to explore the relationship between fractional calcium absorption in the bowel and level of 25(OH)-vitamin D. Using this technique there appears to be a threshold where absorption reaches a plateau at levels of around 80 nmol/l of 25(OH)-vitamin D(85;86). Thus the prevailing view currently is that the minimum healthy level of 25(OH)-vitamin D is 80 nmol/l(86). The normal level is pregnancy is difficult to define as there is a rise in DBP and also haemodilution. However, the current view is that the standard adult level of 80 nmol/l should apply here as well, in the absence of any specific data(86).

There are several different methods available to measure 25(OH)-vitamin D. The gold standard is seen to be gas chromatography- mass spectrometry (GC-MS), but this technique is slow, expensive and time-consuming. Most labs use commercial kit assays, which are usually radio-immunometric assays (for example, IDS, Diasorin, Nicholls), although a chemi-luminescence assay also exists (Diasorin Liaison). The assays tend to be less accurate than GC-MS and high-performance liquid chromatography (HPLC), and also discriminate less well between the D2 and D3 forms(87). Comparison of the Diasorin RIA kits with HPLC showed good correlation for D3, but D2 tended to be slightly underestimated(88).

1.3.2.4 Mineralization of the foetal skeleton and maintenance of the materno-foetal calcium gradient

There is a net positive calcium flux from the maternal to foetal circulation across the placenta. A miniature version of the skeleton is laid down in the embryonic period, and primary ossification centres form in the vertebrae and long bones between the 8th and 12th weeks, but it is not until the third trimester that the bulk of mineralization occurs(89). The mechanism of regulation of this process is poorly understood in humans, but it is thought that both parathyroid hormone (PTH) and parathyroid hormone-related peptide (PTHrP) have important and complementary roles(10). Vitamin D has not shown to be critical to this process, and in a mouse model, lack of VDR did not significantly affect placental calcium transport or skeletal mineralisation(10), although in the rat, 1,25(OH)2-vitamin D did seem to influence placental calcium flux(66).

The main determinant of skeletal mineralization in utero appears to be the foetal plasma calcium concentration(10), and this is mainly influenced by foetal parathyroid hormone (PTH) activity, even though this is set at a low level throughout gestation. Lack of parathyroids in foetal mice leads to low foetal calcium levels and decreased skeletal mineralization(90). PTH does not cross the placenta, and maternal hypo- and hyperparathyroidism appear to affect the foetus via decreasing or increasing the calcium load presented to the foetal circulation. In humans maternal hyperparathryoidism may lead to stillbirth or neonatal hypocalcaemia(10), secondary to suppression of foetal PTH. Maternal hypoparathyroidism leads to increased levels of foetal PTH via foetal parathyroid hyperplasia, and generalized skeletal demineralization(10).

The action of PTH seems to be by increasing calcium resorption from the foetal kidney, and possibly bone, to increase calcium concentration. PTH does not seem to influence placental calcium transfer, as injection of PTH into thyroparathyroidectomized foetal sheep does not increase placental calcium flux, in contrast to injection of mid-regions of PTHrP(91). Thus there is increasing evidence that PTHrP is the major determinant of placental calcium transport in animals, and that levels of PTHrP are increased in response to a low foetal plasma calcium level(92;93). PTHrP appears to be produced in the parathyroid glands in some species, but not in others, and this may explain the differing responses of mice and sheep to its removal. This procedure influences placental calcium transport in the latter but not in the former(91;92). It is not definitely known where PTHrP is produced in human

pregnancy, but may be produced in the placenta, and is present in high concentrations in breast milk(10;60).

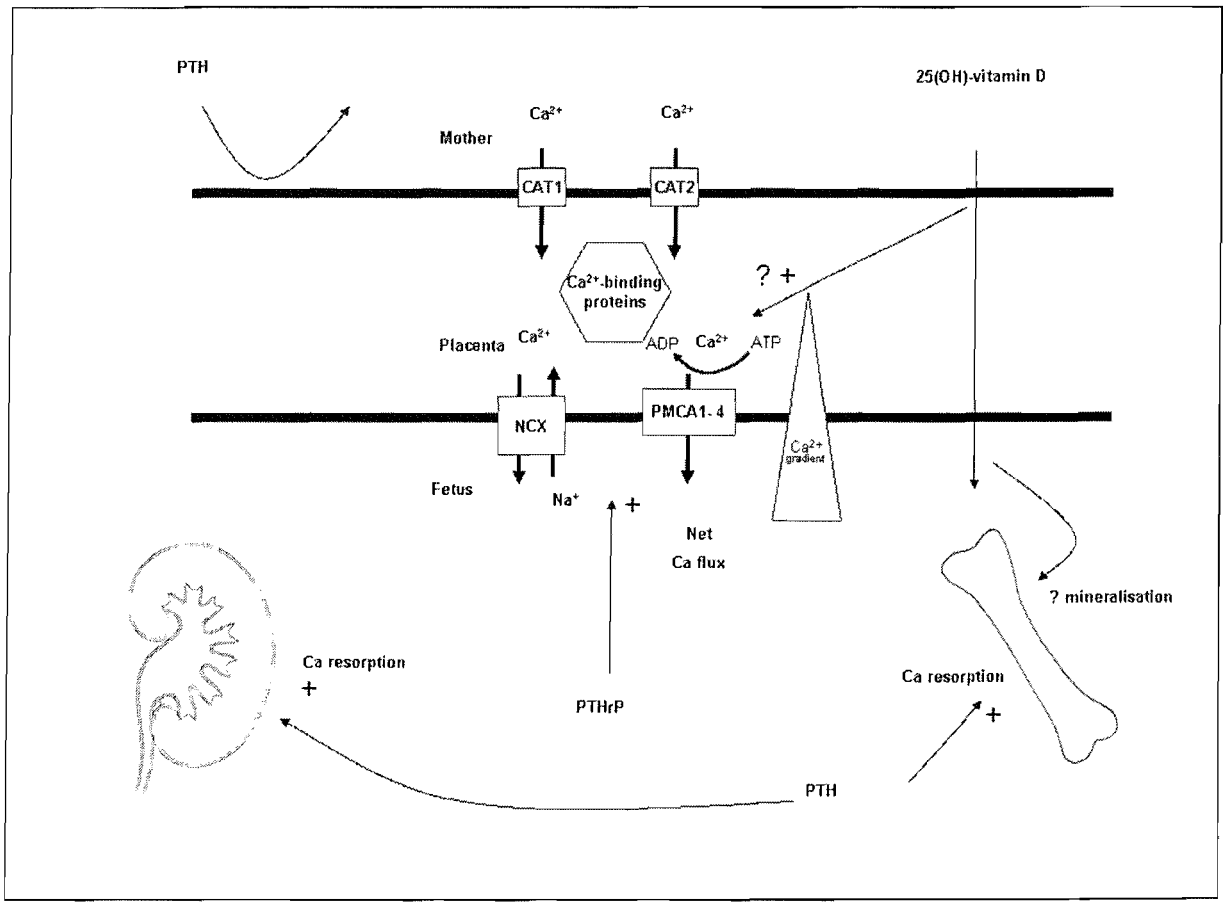
It is unclear how the actions of foetal PTH and PTHrP interact with some of the characterised molecular apparatus in the placenta: Placental calcium transfer occurs in the syncytiotrophoblast and proceeds through a sequence of events consisting of facilitated apical entry through a calcium transport channel, cytosolic diffusion of calcium bound to calbindin and finally, basolateral extrusion of calcium ions through a plasma membrane calcium dependent ATPase(94). This last group of transport channels includes four individual isoforms (PMCA 1-4). These have been previously demonstrated in human placenta, as well as in fetal skeletal muscle and brain. PMCA 1 and 4 are present in most tissues while PMCA 2 and 3 are found in more specialised cell types (95;96). One study in the rat has suggested that a 2-3 fold increase in PMCA gene expression is associated with a 72-fold increase in calcium transport across the placenta during late gestation(97). The regulation of this process is as yet unknown, but at least one of the isoforms of PMCA has been shown to be regulated by vitamin D (98), and in some animals, but not others, 1,25(OH)2-vitamin D appears necessary for maintenance of the maternofoetal calcium gradient (66). Recent work in human subjects has shown that the level of mRNA expression of an active placental calcium transporter (PMCA3), thought to be situated on the basal membrane of the placenta, is positively correlated with whole body BMC in the offspring at birth(99). These observations may suggest a possible mechanism for the influence of maternal vitamin D status on placental calcium transport and intrauterine bone mineral accrual.

In addition to its effects on placental calcium transport, PTHrP influences linear bone growth by acting on prehypertrophic chondrocytes in the foetal growth plate to inhibit differentiation to hypertrophic chondrocytes, and both over and under(100;101) expression of PTHrP or its receptor are associated with short-limbed dwarfism in animals and humans(102-106). Additionally there is evidence that PTH and PTHrP differentially affect mineralization of cortical and trabecular bone(107;108), and thus are attractive candidates for the physiological investigation of programming.

Thus both foetal PTH and PTHrP activity contribute to foetal plasma calcium concentration, but the action of PTH appears to predominate. It is unclear in human pregnancy how

25(OH)- or 1,25(OH)2-vitamin D are involved in this process. The main effect of PTHrP seems to be on placental calcium transport(10). This process is summarised in Figure 4.

Figure 4 Placental calcium transport.



1.3.2.5 Environmental factors in infancy and childhood

The nature of infant feeding has been shown to influence bone mineral accrual, with a positive correlation between mineral content in the feed and infant bone mass(109) (Table 2). Much of this work has been carried out in premature infants, who tend to be small and have reduced BMD. Studies of premature infants randomised to formulas of differing calcium concentrations have shown short-term increases in bone mineral accrual with the higher calcium formulas(110). However, when these children were followed up in later childhood, there was no difference in bone mass when adjusted for body size between the different feeding regimes(111), although they were on average shorter and lighter than children born at term.

There are very few data in term infants. One of the studies, however, found that, although at 6 months infants fed a high calcium formula had greater BMD that those fed breast milk, when they were all put onto normal formula for the next 6 months, the differences disappeared(112), consistent with postnatal tracking along the growth trajectory. Zamora et al found, in a retrospective study, that children given vitamin D supplements in the first year of life had higher bone mass at the radius and femoral neck in later childhood(68), suggesting that vitamin D supplementation early in life might have persisting effect on bone mass, although the children were not followed up over puberty, when a great percentage of final bone mass is accrued(113). 72 term infants aged 6 to 18 months were randomised to a 1-year programme of either gross or fine motor activities, and within this either to high or low calcium intake. There was no effect of either intervention alone, but children in the high activity group with low calcium intakes had poorer accrual of bone mass relative to the other infants(114). The authors suggest that if calcium intake is insufficient to match demand caused by increased loading, poorer mineralisation may result. As the collagen matrix is synthesised first and then subsequently mineralised, this may be a reasonable proposition.

The issue of breast versus formula feeding has been explored in pre-term infants in relation to bone mass in infancy and childhood, finding that breast fed infants had lower bone mineral infancy, but with normal bones for their reduced size in older childhood (compared with term babies), and the studies described above have suggested that there may be a short term benefit to breast milk (115), but with no difference in bone mineralisation in the longer term(111). The comparison between breast and bottle may also depend on the calcium content of the formula(110). There is also evidence that breast-fed babies are leaner than their bottle-fed peers during childhood(116), and may be at reduced risk of obesity in adult life(117).

Table 2 Summary of dietary intervention studies in infants and children.

Age	Intervention	Outcome	Persistence	Citation
Infant (Pre-term)	High calcium formula vs milk	Higher neonatal BMC	No effect in childhood	Bishop et al 1993, Fewtrell et al 1999 244 preterm babies followed to 8-12 years
Infant (Term)	High vs low calcium formula 6/12 then all moderate calcium formula	High Ca group higher BMC 6/12, no difference at 12/12	NA	Specker et al 1997 67 neonates
	High or low calcium, high or low exercise	High exercise and low calcium: lower BMC	NA	Specker et al 1999 72 infants, 6-18 months age, followed for 1 year
Childhood	High or low calcium, high or low exercise	Increased cortical width and bone area in high exercise high calcium	NA	Specker et al 2003 239 children aged 3-5 years
	Milk derived high Ca vs placebo	BMD gain 1 year increased vs placebo	Increased whole body BMC at 3 years vs placebo	Bonjour et al 1997, 2001 149 prepurbertal girls (8 years old)
	Normal or Ca fortified diet	Increased hip and lumbar spine BMD at 2years vs placebo	Equalised after further year with no intervention	Merrilees et al 2000 91 teenage girls

Although it is widely proposed that increasing calcium intake during childhood and adolescence will be associated with greater accrual of bone mass, the evidence relating dietary calcium intake to bone mass among children and young adults has been inconsistent, particularly with regard to persistence of effect(118). Continuing the theme of exercise-

calcium intake interaction, Specker et al randomised toddlers (age 3 to 5 years) to either gross or fine motor exercises and either low or high calcium intake, measuring tibial cortical bone thickness and area by PQCT at 12 months. Consistent with the findings of their study in infants, they found that children in the high exercise, high calcium group had an increase in these parameters, but children on no calcium supplement in the high activity group had lower cortical thickness and area than children in the low activity group(119).

There have been many observational studies of calcium or milk intake and BMD. In 649 Chinese girls, milk intake was statistically significantly associated with forearm BMD ($p<0.05$)(120). However, using a food frequency questionnaire, Rozen et al(121) found no association between calcium intake and BMD at any site in 2000 adolescent Israeli school girls. Children who avoid milk tend to be smaller and have smaller skeletons than those who drink milk(122), and in a study of 45 peri-pubertal girls in Canada, calcium intake was positively associated with whole body BMC at baseline and 2 years later, and also with accrual of bone mineral over this period(123). These studies are difficult to interpret due to the potential confounding by other determinants of bone growth, but have been complemented in recent years by intervention studies. These trials demonstrate that calcium supplementation, either as a salt or in milk, tends to increase bone mass whilst the supplement is taken. In a double-blind, placebo-controlled study, by Bonjour et al(124), 149 healthy prepubertal girls aged 8 years were either allocated two food products containing 850 mg of calcium (phosphate salt derived from dairy products) or not (placebo) on a daily basis for 1 yr. The difference in BMD gain between calcium-supplemented and placebo was greater at radial and femoral sites (7-12 mg/cm^2 per yr) than at the lumbar spine (2 mg/cm^2 per yr). The difference in BMD gains between supplemented and placebo groups was greatest in girls with a spontaneous calcium intake below the median of 880 mg/d. The increase in mean BMD of the 6 sites in the low-calcium consumers was accompanied by increased gains in mean BMC, bone size, and stature. In another study 91 teenage girls were randomised to either a normal diet, or a diet fortified with dairy products to increase daily calcium intake to 1160 mg/day(125). After 2 years of follow-up BMD was increased at the hip (4.6%) and lumbar spine (1.5%), and this was statistically significantly greater than that seen in the unsupplemented group. However, at the end of a 3rd year, when no formal dietary manipulation was imposed, there was no difference in calcium intake or in BMD between the two groups. In contrast, when Bonjour et al(126) reassessed their cohort of children 3 years

after discontinuing calcium supplementation, there was still a significant difference in overall mean BMC ($p=0.031$) and BMD ($p=0.012$) in favour of the supplemented group.

Longitudinal studies of bone mass in children which use DXA are hampered by the technical limitations of the measurement: Because DXA gives a two dimensional assessment of bone mass, areal BMD given by DXA systematically overestimates true volumetric BMD as bone size increases. Additionally, differential changes in fat mass may alter the measurement of bone mass. Increased calcium intake may lead to a filling in and thus reduction of remodelling sites in bone and thus a rapid short term increase in BMD, but without any real long-term increase in bone accrual. However, taken together, the data from existing studies indicate greater bone mineral accrual among children and adolescents whilst receiving calcium supplementation over periods varying from 12 to 36 months(127-132), whether this is from milk or isolated calcium salts. However, the few data that exist pertaining to persistence of effect support the use of milk products. There are reasonable a priori reasons for milk being a good supplement- a large proportion of bone is protein, and milk provides a ready supply. Bone mineral is composed of calcium hydroxyapatite, which contains calcium and phosphate, and milk supplies this particular calcium salt. Additionally milk contains other growth promoting factors such as IGF-1.

Several reports in children and adolescents involved in competitive sport or ballet indicate that intense exercise is associated with an increase in bone mineral accrual at weight-bearing skeletal sites(133-135). In some of these investigations, intense exercise seems to be associated with a greater gain in bone size than volumetric bone mineral density. However, the more relevant issue for public health programmes is the effect of moderate exercise on bone mineral accrual. Some prospective studies have indicated that exercise programmes undertaken in schools may have a positive effect on this outcome(136;137). There have been several studies of the impact of exercise programmes on bone mass in late childhood: Bass et al found that mean bone mineral density (BMD) at weight bearing sites was higher in 45 pre-pubertal gymnasts (mean age 10.4 years) than in sedentary controls. Bone mass was also higher in retired gymnasts than in age matched controls(133). An increase in bone mineral at the femoral neck and lumbar spine was found in a group of 9-10 year olds, allocated to either control, or to a 10 month exercise programme(136). A moderate exercise programme of 30 minute sessions, three times a week for eight months was associated with a significantly greater increase in BMD at the lumbar spine and femoral neck, compared with controls, in

one randomised controlled trial in 10-year old boys(137). A persisting association between bone mass and childhood exercise was found in one study of female American college students. Those who did not participate in high school sports were seven times more likely to have low bone mass in early adulthood than those who did(138).

However, much less is known about the influence of physical activity on bone mass in younger children, and in particular, whether bone mineral accrual varies significantly within the normal range of activity for a healthy population. One study to address this issue examined the association between physical activity measured by accelerometry, and BMD measured by DXA in 368 children aged 4 to 6 years. Statistically significant positive associations were found between physical activity and BMD at each of whole body, hip and lumbar spine; hours of television viewing per day inversely predicted hip BMD in girls ($r=-0.15$, $p<0.01$)(139). Additionally in this cohort femoral neck cross sectional area was positively associated with physical activity(140). The relationship between everyday activity and bone mineral is an important issue, as levels of physical activity may be amenable to modification by public health interventions. It remains uncertain to what extent the greater gains in areal BMD observed in intervention studies translate into long-term increases in bone strength and a reduction in later fracture risk.

Thus the data suggest that children with habitually greater levels of physical activity have higher bone mineral accrual, and that programmes of increased physical activity may increase bone mass temporarily. However, whether these observations translate into long-term benefits or a reduction in fracture risk, either in childhood, or in later adulthood, remains to be seen.

Other factors which influence peak bone mass include cigarette smoking, alcohol consumption, and the presence of anorexia nervosa or exercise-induced amenorrhea. The potential beneficial influence of oral contraceptive use remains uncertain.

1.4 Developmental plasticity and intrauterine programming

1.4.1 Overview

In the natural world there are numerous examples of developmental plasticity, that is, the ability of a single genotype to give rise to several different phenotypes. This allows developing organisms to adapt to the prevailing environmental conditions- for example, if times are hard, maternal undernutrition may act as a signal to the developing foetus, leading to an altered pattern of gene expression, in a way appropriate to the environment likely to be encountered at birth. An example is the water flea *Daphnia*- if the mother is exposed to traces of a predator, the young are then born with a protective "helmet"(141). The problem arises when the developing organism is then exposed to a mismatch between the expected and actual environment: the "protective" helmet of the water flea actually reduces reproductive competitiveness in the absence of the predator. Nutritional plenty, in an organism whose genotype is expecting nutritional constraint, may lead to disease.

Evidence has been accruing that for human diseases such as osteoporosis, which show a dramatic increase in prevalence with age, and for which heredity can only partly explain, there is an interaction between the genome and the environment in the expression of the disease. Thus, given a particular genotype, the environmental factors to which a subject is exposed in early life are a critical determinant of later health and disease. This phenomenon is known as "programming". Programming is defined as "persisting changes in structure and function caused by environmental stimuli acting at critical periods during early development"(28). There is no doubt that the skeleton can be permanently changed by an adverse early environment- rickets is a very visible example. The data supporting this hypothesis will now be reviewed: These include epidemiological studies of BMD and fracture in cohorts where birth details are known, physiological studies, exploration of maternal determinants of childhood growth, and studies of potential underlying mechanisms using animal models.

1.4.2 Epidemiological evidence

Several studies have utilized birth records to evaluate the relationship between growth in early life and later bone mass. These include cohorts of men and women from Bath, Hertfordshire and Sheffield, in whom accurate records of weight in infancy were kept. In a cohort of 153

women in Bath born between 1968 and 1969 and traced at age 21, data on childhood growth were obtained from linked school and health records(142). Statistically significant associations were found between weight at 1 year and BMC (but not BMD) at the lumbar spine and femoral neck ($p<0.01$), independent of adult weight and body mass index. Similar results were obtained for 189 women and 224 men aged 63-73 who were born and remained in Hertfordshire(143). Statistically significant associations between weight at 1 year and BMC at the spine ($p<0.02$) and femoral neck ($p<0.01$) among women, and spine ($p<0.03$) among men were seen. In Sheffield, 143 men and women aged 70-75 underwent assessment with DXA(144). Records of birth weight, birth length, head size and abdominal circumference had been recorded. There were statistically significant ($p<0.01$) positive associations between birth weight and adult whole body bone and lean mass among men and women. There were weaker, but still significant ($p<0.03$) associations between birth weight and BMC at the femoral neck and lumbar spine. These remained significant after adjustment for factors known to influence bone mass.

In more recent work, the relative contributions of pre- and post-natal factors to bone mass in the seventh decade were evaluated(145). 498 men and 468 women, born in Hertfordshire during the period 1931-39 and still living there, were recruited; as before, detailed birth records were available. Birth weight was associated with bone mineral content in both men (proximal femur: $r=0.16$, $p=0.0003$; lumbar spine $r=0.10$, $p=0.03$) and women (proximal femur: $r=0.16$, $p=0.0008$; lumbar spine $r=0.11$, $p=0.03$); relationships with bone mineral density were weaker, and statistically significant at the proximal femur in men only ($p=0.03$). Relationships between weight at one year and bone mineral content were even stronger (proximal femur: men $r=0.22$, $p<0.0001$; women $r=0.14$, $p=0.002$). In men, 18% of the variance in proximal femoral bone area was explained by a model that included birth weight, weight at one year and adult weight, with the relative contributions attributed to each being 2.8%, 6.8% and 8.2% respectively. In women, similar modelling produced figures of 6.7%, 4.2% and 3.9% (overall variance of 15% in proximal femoral bone area). Hence weight at each of these three points in the life course was important in the determination of adult bone mass, with greater contributions of earlier growth to skeletal size than to volumetric bone mineral density.

These finding have been replicated in other countries: Yarborough et al found, in 305 postmenopausal Caucasian women (mean age 70 years)(146), that birth weight was positively

correlated with BMC at the forearm, hip and lumbar spine, and that the age-adjusted mean BMC increased significantly from the lowest to the highest tertile of birth weight. Adjusting for adult weight diminished this association at the forearm and hip, but not at the spine. Adjustment for multiple other covariates, including height, did not materially change these associations. Adult weight and height were significantly correlated with birth weight ($r=0.19$ and $r=0.24$, respectively). Birth weight was not independently correlated with BMD. A similar dichotomy between BMC and BMD, related to birthweight, was found in a cohort of adolescent boys and girls in Sweden(147).

1.4.2.1 Childhood growth and risk of hip fracture in later life

Clinically the most important consequence of osteoporosis is fracture. The correlation between growth in childhood and risk of hip fracture in later life was demonstrated in a longitudinal study in Helsinki, Finland(25). Data were collected on a total of 7086 men and women born between 1924 and 1933. Body size at birth had been recorded and an average of 10 measurements of height and weight made throughout childhood. Incidence of first hip fracture was identified using the national hospital discharge register. After adjusting for age and sex, two major determinants of hip fracture in later life were identified: tall maternal height ($p<0.001$) and a low rate of childhood growth from age 7 to 15 years (height, $p=0.006$; weight, $p=0.01$). The effects of maternal height and slow childhood growth were statistically independent of each other and remained after adjusting for socio-economic status. In addition, fracture subjects were shorter at birth but of average height by age 7 years. Further work in a second Finnish cohort showed a relationship between poor growth in infancy and increased risk of hip fracture in later life(148), with a 6.4 fold increase in risk for those subjects in the lowest quartile of weight gain between 1 and 12 years. These findings are interesting as they suggest several paths to increased fracture risk. Thus a low rate of childhood growth, both early and late in childhood, could lead to poorer mineralization of bone tissue, and/ or decreased bone width and thus lower bending strength. Greater maternal height may act via a longer femoral neck, or faster catch-up growth, particularly in those children who were smaller at birth and of average size by age 7 years, whose skeletal growth may have been pushed beyond its capacity to mineralise. This concept is supported by the observation that fractures in children most frequently happen in early puberty, where linear growth velocity is high and ahead of volumetric mineralisation(149).

1.4.2.2 Maternal influences during pregnancy

Parental birth weight, maternal smoking, body composition and physical activity during late pregnancy have all been demonstrated to affect neonatal bone mass(150): In 145 infants born at term to women in Southampton, UK, the birth weights of both parents and the height of the father were positively correlated with neonatal whole body BMC, independent of the infants' duration of gestation. Mothers who smoked during pregnancy had, on average, babies with a 7.1 g (11%) lower whole body BMC than mothers who did not smoke ($p=0.005$). Smoking at the time of the last menstrual period was not associated with lower BMC. Women who indulged in vigorous activity in late pregnancy, had a faster walking pace, or had lower triceps skin fold thickness (reflecting lower fat stores) had babies with a lower BMC. These influences were independent of placental weight (a marker of the placental capacity to deliver nutrients to the growing foetus). Similar results were found in a more recent mother-offspring cohort, the Southampton Women's Survey(151). In this study of the initial part of the cohort on which this thesis is based, maternal height ($p=0.04$), triceps skinfold thickness ($p=0.004$), smoking ($p=0.05$), and parity ($p=0.01$) were independent predictors of neonatal whole body BMC in 363 subjects. These data therefore provide evidence that environmental modulation in utero, in combination with genetic factors, has an effect on neonatal bone indices. The authors postulated that maternal thinness might reflect lower available nutrients for the foetus; the effect of exercise, competition between mother and foetus for finite resources.

1.4.3 Physiological and mechanistic studies

1.4.3.1 Growth Hormone/Insulin-like Growth Factor-1 axis: Adult birth cohorts

Cohorts where birth records exist have also been used to study the possible physiological mechanisms underlying the phenomenon of programming. In the Hertfordshire (UK) cohort, a study of 34 healthy men, whose serum cortisol was measured every 20 minutes for 24 hours, demonstrated a weak negative correlation between integrated cortisol level and BMD at the lumbar spine ($r=-0.37$, $p<0.05$). Similar relationships were demonstrated at three of five proximal femoral sites(152). There were also significant positive associations between trough cortisol level and rate of bone loss at the lumbar spine ($r=0.38$, $p<0.05$), femoral neck ($r=0.47$, $p<0.001$) and trochanteric region ($r=0.41$, $p=0.02$) over the four year follow up period. The association with rate of bone loss remained significant after adjustment for adiposity, lifestyle factors, and serum testosterone and oestradiol levels. Thus the cortisol axis

appears to have a long-term influence on bone resorption and is a possible candidate for programming in early life. Measurements were also made of the growth hormone (GH) secretory profile, insulin-like growth factor-1 (IGF-1), IGF-binding protein-1 and -3, and GH-binding protein(153). Bone mineral density was measured at the lumbar spine and femoral neck using DXA. There were statistically significant associations between femoral neck BMD and each of GH ($r=0.46$; $p<0.01$) and fasting IGF-1 concentration ($r=0.46$; $p<0.01$). After allowing for the peak GH concentration, median GH was negatively ($p<0.05$) associated with bone mineral density. Weight at 1 year was not related to peak GH, but was strongly related to the median GH concentration ($r=0.42$; $p=0.01$). These observations are consistent with a dual effect of GH secretion on bone density. High peak GH values drive IGF-I production and may maintain bone mineralization in adult life. However, integrated GH secretion, after adjusting for the effect of pulse amplitude, was negatively associated with bone density in adult life. This particular characteristic of the GH secretory profile correlated with growth during infancy and might be programmed by environmental factors during intrauterine or early postnatal life. Finally a study of 38 women found that lumbar spine BMC and BMD were positively associated with all measures of GH concentration, although relationships were strongest for BMC with trough GH ($r=0.47$, $p<0.01$)(154). Associations persisted after adjustment for age, body mass index, lifestyle factors, and osteoarthritis score in multiple regression models. Total (integrated) daily GH concentration tended to increase ($p=0.08$) and IGF-1 concentration fall ($p=0.05$) with rising birth weight, suggesting a role for the GH/IGF-1 axis in the programming of adult bone mass among women. Taken together these studies indicate a possible dichotomy of influence of aspects of GH programming on peak bone mass and rate of bone loss.

1.4.3.2 GH/IGF-1 axis: Venous umbilical cord blood and neonatal bone mass

The GH/IGF-1 axis is an important mediator of postnatal growth and this role has recently been explored in relation to skeletal development using venous umbilical cord blood samples. In a sample of 119 infants from the Southampton cohort (described above(150)), cord serum IGF-1 and insulin-like growth factor binding protein (IGFBP-3) concentrations were related to neonatal body composition measured by DXA(155). There were strong positive associations between cord serum IGF-1 concentration and each of whole body BMC ($r=0.38$, $p<0.001$), lean mass ($r=0.40$, $p<0.001$), and fat mass ($r=0.50$, $p<0.001$) after adjusting for gestational age and sex. There was no association between cord serum IGF-1 and BMC adjusted for bone size. Neither cord serum IGF-1 nor IGFBP-3 explained the relationships

between neonatal bone mass and maternal smoking, fat stores, exercise and birthweight described above. Thus cord serum IGF-1 appeared to be more closely related to the size of the neonatal skeleton than to its degree of mineralization, and seemed to act independently of the previously demonstrated maternal determinants.

1.4.3.3 Leptin

Leptin is a peptide hormone encoded by the obese (ob) gene, and is a candidate for involvement in foetal programming. It is produced by adipocytes and seems to behave as a fat sensor, acting on the hypothalamus. There is recent evidence that adults who had a low birth weight have higher levels of leptin than would be expected from their level of adult obesity(156). Data, again from Herfordshire(157), showed a strong correlation between plasma leptin concentration and bone mineral content ($p<0.001$). However, the negative association with rate of bone loss was significant only at the femoral neck in women ($p<0.01$) and all associations were explained by the association of leptin with adiposity.

1.4.4 Gene-environment interactions

1.4.4.1 Epigenetic mechanisms

The concept of developmental plasticity provides useful insights into potential mechanisms by which the environment may interact with the genome. Although the exact genes inherited cannot be changed, their expression can be modified in several ways. The phenomenon of heritable information not contained in the base-pairs is termed "epigenetics" and two basic mechanisms of epigenetic modification of gene expression have been discovered(158). Firstly, gene regulatory regions (eg promoters, inhibitors) may be methylated or demethylated, and this process may alter gene expression in a graded fashion. Secondly, the structure of the protein-DNA complex may be modified by histone chromatin acetylation. There is widespread demethylation and remethylation during gametogenesis, and also during early embryogenesis just prior to blastocyst development. Further modification may be possible after these times and any modifications are then stable through all subsequent somatic differentiation. Additionally, the methylation status of a particular gene may depend on whether it is maternally or paternally derived, and this is termed "imprinting"(159). There is increasing evidence that environmental factors may influence methylation status during development(158;160;161), thus providing a good candidate mechanism for interactions

between the genome and the environment. Alternative mechanisms would include a direct effect of an environmentally-influenced factor, for example, vitamin D, on the promoter region of a gene.

1.4.4.2 Epidemiological studies of gene-environment interaction

In the first studies to examine the possibility of gene-early environment interactions, an interaction between spine BMD, VDR genotype and birth weight was demonstrated(40). Among individuals in the lowest third of birth weight, spine BMD was higher with the VDR "BB" than "bb" genotype ($p=0.01$) after adjusting for age, sex and adult weight. BMD was reduced ($p=0.04$) in individuals with the same genotype in the highest third of birthweight. A similar study revealed that two single nucleotide variants in the GH gene (coded GHV1 and GHV2) and 1 variant in the IGF-1 gene were analysed. Among men, the GH1V1 2 allele was associated with greater spine bone loss rate ($p=0.03$) and tended to be associated with lower weight at one year. Similarly, men in the GHV2 22 genotype group were, on average, 20 oz lighter at one year than their counterparts ($p=0.04$) in the 11/12 group. In women, the GHV1 2 allele was significantly associated with lower bone mineral content at all sites, and with lower femoral neck bone density and accelerated femoral bone loss rate. Tests for interaction between GHV1 genotype and weight at one year were again significant ($p=0.02$). There was no association between bone mass, early environment and the IGF-1 gene in either sex(162).

1.4.5 Animal models

There is good evidence from animal models that undernutrition during pregnancy may lead to an adverse outcome for the offspring. Widdowson's pioneering work demonstrated that maternal undernutrition led to permanently small offspring in pigs(163), and her work in rats showed that, after a critical post-natal time period, undernutrition no longer led to permanent changes in size(164). Recent work in rats has led to a deeper understand of the possible underlying mechanisms, in addition to providing experimental evidence of programming. Amman et al investigated the effects of four isocaloric diets with varying levels of protein content (15, 7.5, 5, and 2.5% casein) on areal bone mineral density (BMD), bone ultimate strength, histomorphometry, biochemical markers of bone remodeling, plasma IGF-1, and sex hormone status in adult female rats(165). After 16 weeks on a 2.5% casein diet, BMD was significantly decreased at all skeletal sites assessed. Plasma IGF-1 was decreased by 29-34% and no oestrus sign in vaginal smear was observed. Using the same protocol the authors

investigated the effect of protein restriction on ovariectomized and sham operated rats, pair-fed with isocaloric diets containing either 15 or 2.5% casein. Trabecular BMD was decreased by either manipulation, with effects appearing to be additive. Cortical BMD was decreased only in rats on a low-protein diet. This was accompanied by an increased urinary deoxypyridinoline excretion without any change in osteocalcin levels, suggesting an uncoupling of resorption and formation. Isocaloric protein undernutrition decreased bone mineral mass and strength. Thus there is good evidence of the importance of adequate dietary protein in an otherwise energy-replete diet.

Mehta et al explored the effect of maternal undernutrition using a rat maternal protein deficiency model.(166) They found that offspring of protein-restricted mothers had a reduction in bone area ($p=0.04$) and BMC ($p=0.06$), but not BMD, compared to offspring of mothers fed a normal diet during pregnancy. Furthermore, offspring of the protein-restricted mothers had abnormally widened growth plates compared with the offspring of controls ($p<0.001$). Additional work explored the physiological determinants of these observations(167). Dams were fed an 18% casein (control) diet or 9% casein (low protein) diet from conception until the end of pregnancy. The offspring were then fed a normal protein diet until harvest at 8, 12, and 16 weeks after birth. At 8 weeks, total colony forming units- fibroblasts (CFU-F) and alkaline phosphatase-positive CFU-F were significantly ($p<0.01$) reduced in the low protein group compared to controls. At 12 weeks, no significant differences were observed in colony formation. Modulation of osteoblast proliferation and differentiation by IGF-1 and GH was observed ($p<0.01$) in the control group at 8 weeks and the low protein group at 12 weeks. Alkaline phosphatase specific activity was significantly decreased at 8 weeks ($p<0.001$) in the low protein group. At 12 and 16 weeks this was reversed, with significantly increased specific activity in the low protein group. Thus maternal undernourishment in rats appears to modulate skeletal growth in the offspring by delaying maturation of the growth plate, with subsequent "catch up" growth.

1.4.6 DXA measurement in neonates

There have been few studies of the use of DXA in neonates. There are several potential problems with measurement of bone mass in small beings: Babies have a low absolute bone density, and thus detecting the edges of the bones is difficult. Manufacturers have designed software with special detection algorithms for this purpose. Secondly, babies tend to move. In one validation study of piglets(168), it was found that increasing movement during the whole

body scan was associated with higher bone density. However, work on an earlier part of the SWS cohort (Kassim Javaid, personal communication) has demonstrated the opposite association. However in this latter study, babies who moved more had lower BMC than those who moved less, and in the piglet study the same piglets were studied with different amounts of movement. These data do imply however, that neonatal movement may be a source of error. In the piglet study, the animals covered a range of weights, some of which were comparable to that of human neonates, and the DXA- derived BMC was strongly correlated with total ashed calcium content at sacrifice.

1.4.7 Size or density?

Most studies in this field have used DXA as the assessment of bone mineral. DXA is an excellent tool for risk stratification in osteoporosis, but has the important limitation of giving only a two-dimensional measurement of bone mineral. Thus the front-back dimension is missing, so all the contained bone mineral is assumed to be in the same plane. As a result of this, there is systematic overestimation of true volumetric bone mineral density as bone size increases. Thus DXA derived BMD is, in reality, an "areal" density, and is a medical rather than a mathematical concept. There have been several attempts to estimate volumetric density from the two dimensional scan(169), but these are clearly just estimates; true volumetric density can only be measured with a technique that derives the data in three dimensions; an example is peripheral quantitative computed tomography (PQCT). It will be apparent, then, that it is not possible to definitively distinguish effects on bone size from those on volumetric bone density using DXA. However, clues are given by the general pattern seen, such that early life factors are generally associated with bone area and bone mineral content (completely unsize-corrected measures), rather than with areal bone mineral density, which is partially size corrected. Conversely, areal bone mineral density is strongly associated with contemporary factors such as loading and adiposity. This leads to the notion that the size, and probably the geometry, of the skeletal envelope are laid down in early life, as a consequence of environmental modulation of the inherited genetic make-up. In contrast, mineralisation within this envelope is dependent on subsequent environmental influences through childhood to peak.

1.4.8 Influences on body composition

Bone is one of three body compartments, the other two being lean mass (muscle) and fat mass. Whilst there is a body of data pertaining to the relationship between the early environment and bone mass, there are far fewer data for fat or lean mass. Some, but not all, adult cohort studies have suggested a linear relationship between birthweight and adult body mass index (BMI) (170)'(171), and additionally, that low birth weight is associated with altered fat distribution, and higher risk of type 2 diabetes and ischaemic heart disease(172-174). These observations are likely to reflect tracking of body size, and possibly body composition, from birth to adulthood, but more detailed characterisation of these associations is thus far lacking.

1.5 Unanswered questions

The initial evidence from different small mother-offspring studies suggest that maternal body build and lifestyle during pregnancy influence intrauterine bone mineral accrual, and that mothers who are deficient in vitamin D during pregnancy have children with reduced whole body BMC at age 9 years. These data are intriguing, but are from small studies that lack the preconceptional and postnatal data of SWS, and also the sheer depth of characterisation of the mothers and offspring. Thus exploring these issues in the SWS will allow, within a single study structure, replication of these original findings in a much larger cohort, investigation of effects before pregnancy and of their relative magnitude from birth to 4 years, and finally the interplay between maternal and postnatal childhood factors in determining offspring bone mineral accrual.

Thus questions to answer include:

- Do the effects of maternal body build and lifestyle seen at birth persist into childhood?
- Is the previously documented association between maternal 25(OH)-vitamin D levels and offspring bone mass replicated at birth and in early childhood?
- How do childhood and maternal factors interact to influence childhood growth?

The basis of this thesis is a unique cohort of women of childbearing age in Southampton, UK(175). This is described in detail in the next section, but briefly, these women were recruited before they became pregnant, which enabled detailed characterisation of lifestyle,

anthropometry, diet, blood parameters both preconceptionally and during early and late pregnancy. The children are followed up regularly during the first few years of life and similarly characterised. Thus comprehensive data on a cohort of over 2000 mother-child pairs are available, and the SWS is therefore an ideal cohort in which to attempt to answer the questions raised by the previous research.

1.5.1 Summary of specific aims of research

The following hypotheses will be explored:

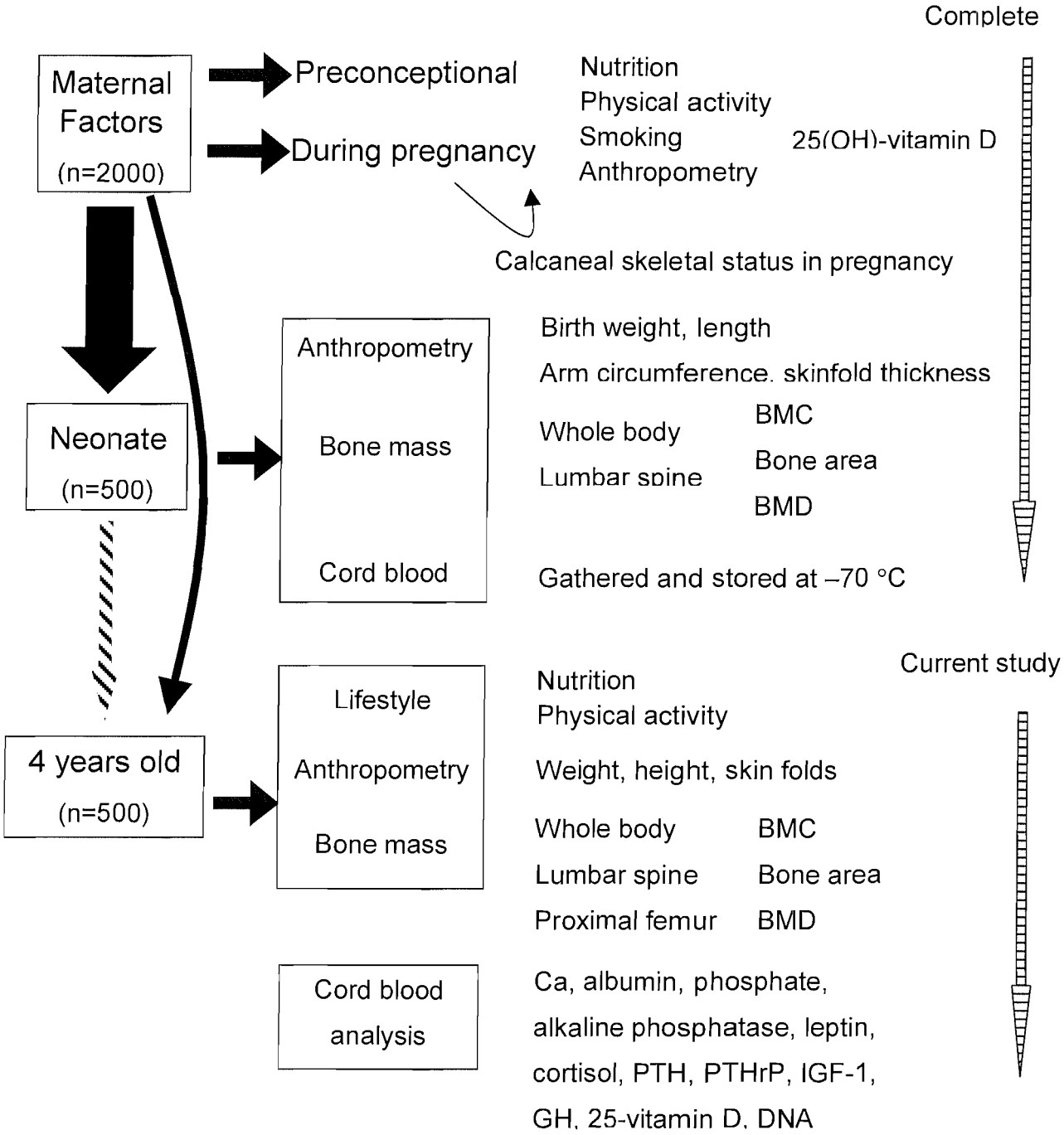
1) a) Parental lifestyle factors (diet, smoking, exercise), body composition and bone mass influence intrauterine and childhood bone mineral accrual.

2) a) Maternal vitamin D status in pregnancy predicts bone mass in the offspring at birth and at 4 years old.

b) Childhood diet and exercise predict contemporary bone mass.

2 Methods

Figure 5: Outline of SWS bone study, from preconception to 4 years.



2.1 Overview of Southampton Women's Survey

The Southampton Women's Survey (SWS) is a well-established prospective cohort of around 12,500 women aged 20- 34 years(175). Run by the Medical Research Council (MRC) and the University of Southampton, the study was set up to assess diet, body composition, physical activity and hormone levels in a large group of non-pregnant women. For those women who became pregnant, the aim was to investigate the influence of these maternal factors on the development of the child throughout its early life.

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

The women underwent an initial interview by a trained research nurse. The information recorded is summarised in Table 3. At this visit, an interviewer administered 100-item questionnaire (FFQ) was used to assess diet. This has been previously validated against food diaries(176), and other factors such as the woman's physical activity, smoking, family background, education, ethnicity, housing, household composition, childcare arrangements, benefits, general health, menstrual and obstetric history, and her own and her partner's occupation. Detailed body composition measurements were taken, including weight, height, waist and hip circumference, and skinfold thickness at four sites (triceps, biceps, subscapular and supra-iliac regions), using Harpenden callipers. Measurements of skinfolds were taken at each site following a standard protocol until three readings within 5% of each other were recorded. The nurses were carefully trained and regular inter-observer variability studies performed to ensure measurements were as accurate as possible. Weight was measured using digital scales (Seca Ltd) which were calibrated 4 times per year, and height using a stadiometer. Venous blood has been collected and stored at -70°C. Protocols for anthropometric measures will be found in Appendix AA.

Table 3 Information recorded on the mothers.

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

The recruited women were asked to inform the study coordinators immediately if they became pregnant, and also to give written consent for their GP or hospital doctor to communicate this information. Pregnant women were then invited to attend interviews in early (11 weeks) and late (34 weeks) pregnancy. At these visits, diet and lifestyle factors were assessed in a similar way to the initial visit. The detailed anthropometry was repeated, and venous blood was collected and stored at -70°C. At birth, the babies were measured (length using a neonatometer, head and abdominal circumference) and weighed, using digital scales (Seca Ltd, again calibrated regularly), and skinfold thickness measured (triceps, sub-scapular and thigh using Harpenden callipers to obtain three measurements within 5% of each other). Samples of cord blood were also collected and again stored at -70°C.

25(OH)-vitamin D was measured in the late pregnancy serum using a radio-immunoassay (Diasorin, Stillwater, USA) which had a CV of <10%.

2.2 Assessment of offspring bone mass at birth

Mothers registered with specific GP practices were invited to participate in the bone component of the SWS. More information on sub-studies within the SWS can be found in Inskip(175). These studies have been based around cohorts of mother and baby pairs from different GP practices to minimise the participation load on any particular mother/child.

After birth, the mother was asked to agree to her baby undergoing assessment of bone mass, within 2 weeks of birth, using a Lunar DPX DXA instrument with specific paediatric software (GE Corporation, Madison, Wisconsin, USA) (Consent form- appendix A). The instrument was located in the Princess Ann Maternity Hospital, Southampton, and underwent daily quality assessment, and was calibrated against a water phantom weekly. The mothers could attend either as an inpatient, or return from home within the two-week time period. At the visit to the scan room, the baby was pacified and fed if necessary, undressed completely, and then swaddled in a standard towel. It was placed on a waterproof sheet in a standard position on the scanner. Whole body measurement was performed first, followed by lumbar spine, using specific software protocols. The baby was kept in position using rice bags placed over the bottom end of the towel for whole body, and either side for the lumbar spine scan. A print out of the whole body scan was given to the mother as a momento of the occasion. The baby was weighed at the end of the visit on calibrated digital scales, and this weight and the previously recorded length were entered into the DXA record on the computer. The manufacturer's short-term and long-term coefficients of variation (CV) of the DXA instrument were 0.8% and 1.4% respectively (for adults). When a spine phantom was repeatedly scanned in the same position 24 times the CV was 0.15%. It was not possible to do repeat scans on the babies to derive a CV for human measurements. The effect of movement artefact was minimised by excluding scans felt by two operators to have excess movement (see below).

The electronic DXA records were transferred regularly to the secure servers at the MRC unit via ZIP disks. Births from August 2002 to the end of 2003 were included in the data set. The whole body scan for each subject was reviewed and analysed using the Lunar software. The regions of interest defined by the automated software were frequently inaccurate, so were placed manually in the optimal positions. The scans were then reviewed again by an experienced DXA operator, and agreement reached on which scans should be excluded because of unacceptable movement artefact. The final data set was amalgamated with maternal and paternal data from pre-, early- and late- pregnancy.
Full ethics approval was granted for this study (appendix B).

2.3 Assessment of fathers

Fathers were invited, at the time of the neonatal DXA assessment, to attend the Osteoporosis Centre at Southampton General Hospital for a DXA scan. After informed written consent was obtained, their height (stadiometer) and weight (calibrated digital scales- Seca Ltd) were measured, and whole body, lumbar spine and hip bone mass, and whole body lean and fat mass, were assessed using a Hologic Discovery DXA instrument (Hologic Inc., Bedford, MA, USA). Data were collected by questionnaire on their lifestyle, exercise, health and previous fractures, and diet, focusing on calcium and vitamin D intake (Table 4). This was similar to the maternal questionnaire, but has not been validated by food diary.

Table 4 Information recorded on fathers.

Demographics	Anthropometry	Lifestyle	Health
Age	Height	Diet	Illness
	Weight	Physical activity	Previous fractures
	Birthweight		

Full ethics approval was gained for this study (appendix C).

2.4 Assessment of the offspring at 4 years old

Whole body DXA scan at 4 years old

Subjects were recruited from the SWS cohort. The mothers of children becoming 4 years old were sent a letter and information sheet telling them about the study and inviting them to take part (Appendix D and E). Soon after this the mother was telephoned at home to see whether she was willing for her child to participate. If the response was positive, a time for the visit to the Osteoporosis Centre at Southampton General Hospital

was organised. A letter confirming this appointment in writing was sent out to the mother, containing a direct contact telephone number in case of problems attending (Appendix F).

At the visit to the Osteoporosis Centre, informed written consent for the DXA scan was obtained from the mother or father (Appendix G). The data obtained at this visit are summarise in Table 5. The child's height (using a Leicester height measurer) and weight (in underpants only, using calibrated digital scales (Seca Ltd)) were measured. A list of weights for standard items of clothing was available to adjust the weight for those children who were not willing to undress. The child was then invited to lie down on the DXA couch. Whole body, lumbar spine and left hip scans were taken, using a Hologic Discovery instrument (Hologic Inc., Bedford, MA, USA). To make this more appealing, a suitably bright sheet with appropriate pictures was laid on the couch first. To help reduce movement artefact, the children were shown a suitable DVD cartoon. The whole body DXA scan took around 5 minutes. After this, the children underwent lumbar spine and left hip scans, each of which took around 20 seconds. The total radiation dose for the scans were as follows; whole body (paediatric scan mode) 4.7 microsieverts, spine (L1-L4) 1.5 microsieverts, hip 7.3 microsieverts (total dose 26.7 microsieverts). This is equivalent to three days background radiation and is significantly less than that for a chest radiograph. The manufacturer's coefficient of variation (CV) for the instrument was 0.75 % for whole body scans (for adults), and the experimental CV when a spine phantom was repeatedly scanned in the same position 16 times was 0.68%. Again it was not possible to repeatedly scan individual children to obtain a CV for the 4-year olds.

Lumbar spine DXA scan at 4 years old

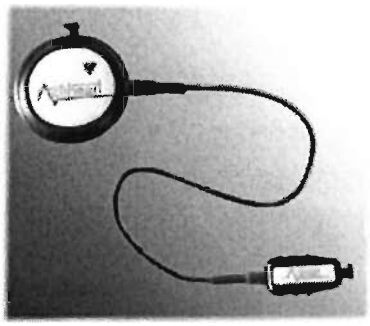
Table 5 Information recorded on children at 4 years.

Demographics	Diet	Lifestyle	Anthropometry
Age	Milk	Physical activity questionnaire	Height
	Other dairy	Physical activity by Actiheart	Weight
	Oily fish	Illness	BMI
	Other calcium and vitamin D containing foods	Medications	Mid-upper arm circumference
		Fractures	Grip strength (bilateral)

After the scan the child's mid-upper arm circumference was measured three times on the left side, and further measurements were taken until three readings within 5% of each other were obtained. Grip strength was measured three times on either side, alternating between sides, with the child's arm in a standard position.

The child's diet (focused on calcium and vitamin D intake), exercise and illnesses (including fractures) were assessed by an interviewer led questionnaire for the mother, father (if mother was absent) or carer (appendix H). This has not been validated, but was based on the maternal FFQ.

In a subset of children and mothers, an Actiheart combined accelerometer and heart rate monitor (Cambridge Neurotechnology Ltd, Cambridge, UK) was fitted to both mother and child. They were asked to wear these continuously for 7 days and then post back in the envelopes supplied. The unit comprised a small disc, 1.5 cm across and 3mm thick, and a short lead. Both of these parts were secured to the skin via clipping onto standard electrocardiograph electrode pads. The disc was positioned in the midline just below the xiphisternum and the lead going out horizontally to the left chest wall.

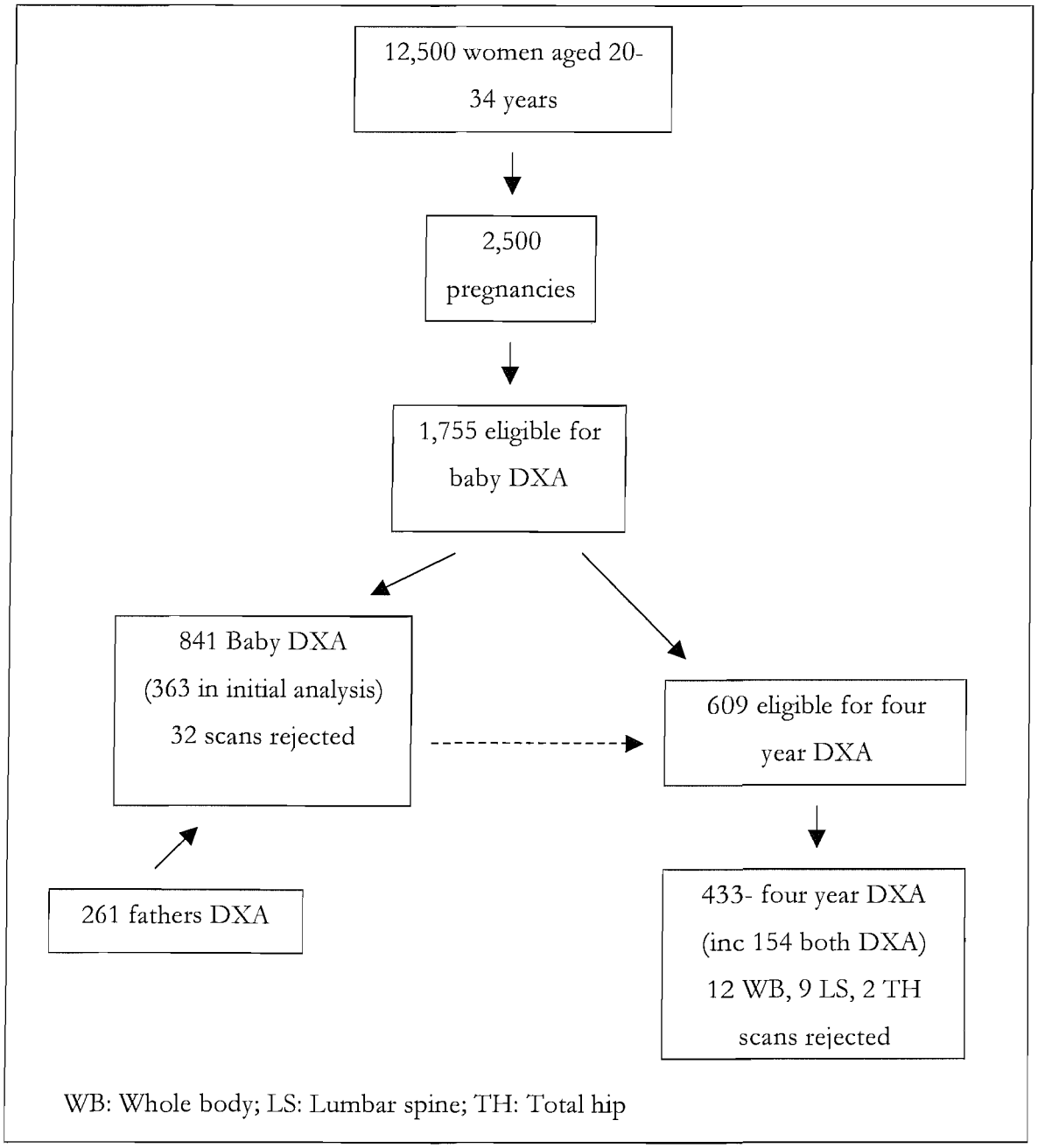


Actiheart instrument

At the end of the visit the child and mother were thanked, and given a copy of the colour print out of the scans to take home (appendix I) The child was also given a certificate for good behaviour (appendix J).

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

Figure 6 Overview of cohorts used in thesis.



2.5 Analysis

Figure 6 gives an overview of the different subsets of children in this study. The raw data were double entered and checked for consistency and to be within range. Inconsistencies were checked and either corrected, accepted or set to missing as appropriate after review of the original records. All statistical analyses were performed using Stata V8.2 (Statacorp, Texas, USA). Variables were checked for normality using the skewness and kurtosis test. Appropriate transformations were made to convert variables to a normal distribution. This was usually by logarithm, but a few variables were better with a square root transformation. Where variables were transformed they were also standardised. Thus outcomes in these cases would be either in sd per unit change in predictor, or in change in outcome per sd change in predictor. Untransformed variables were used as predictors in some regression models, but when used as outcomes, the variable was always transformed.

To compare gender differences in the offspring, Student's T-Test was used on normally distributed and transformed variables, and the means and 95% confidence intervals and significance reported. To assess relationships between maternal predictors and offspring factors, regression and correlation techniques were used. In the chapter on growth, logistic regression was used to relate maternal factors to growth. Throughout, a 95% level of statistical significance has been used, with no correction for multiple testing, as the associations investigated are based on a priori hypotheses.

Power calculations were based on the previous small study from the Princess Ann Hospital(150). To have 90% power at the 5% level, to detect a difference of 0.5 sd in whole body BMC at birth between offspring of mothers in the lowest versus highest quartile of triceps skinfold thickness, would require 100 people in each group. This would therefore need 400 overall. To ensure adequate power and to allow for drop out and unusable scans, 500 mother offspring pairs would need to be recruited.

The Actiheart monitor measured physical activity using a piezo-electric circuit to measure vertical movement, and a heart rate monitor. Thus both activity causing a rise in heart rate, but with little vertical movement, for example cycling, and activity such as jumping, which may not cause much of a rise in heart rate, can be measured. The instrument records over 7 days and give a summary measure for 1-minute intervals. The software gives, initially, total energy expenditure per day, but with further analysis, specific activity counts (relating to

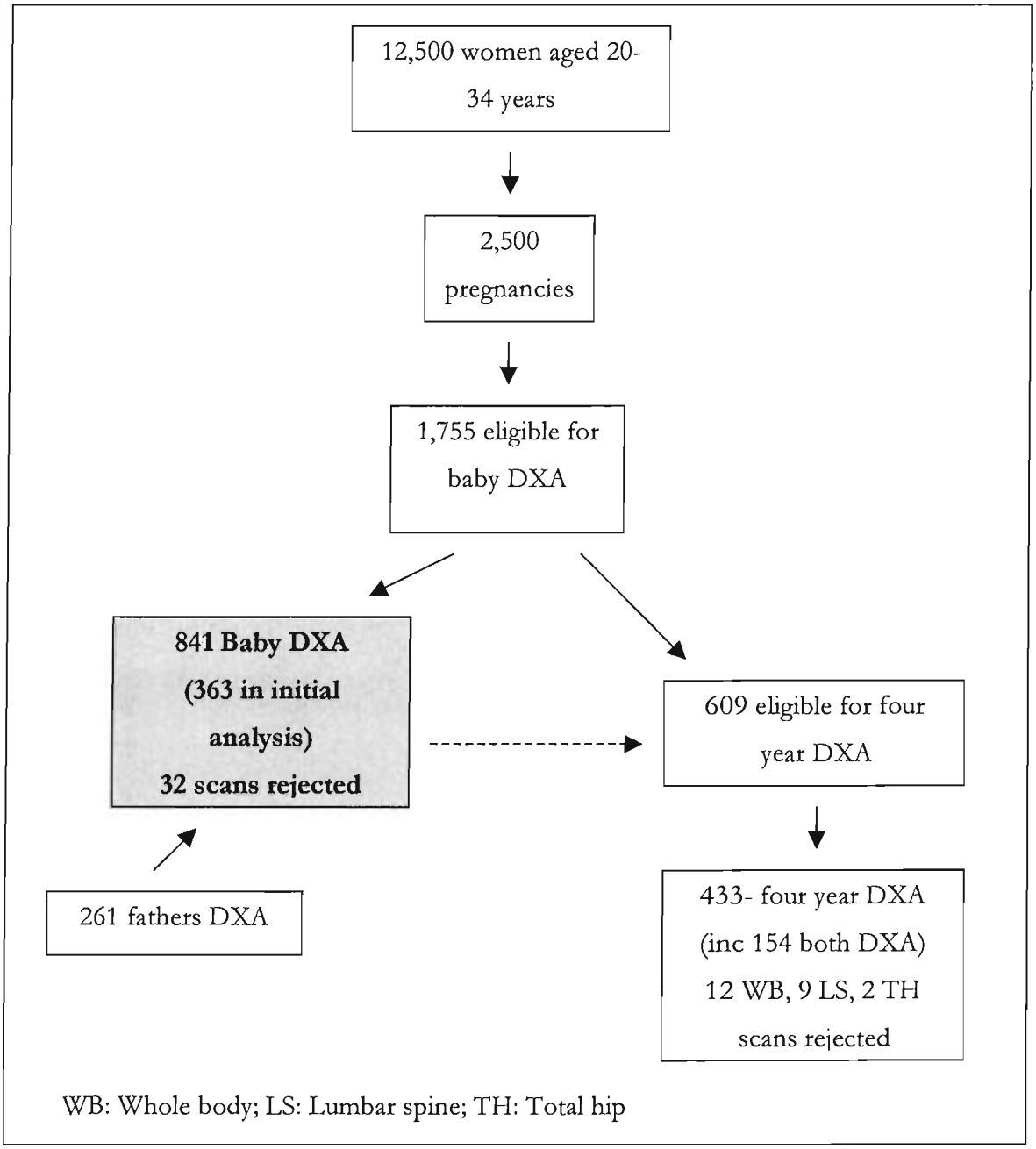
movement) can be derived, and estimates made of time spent at different levels of activity, using sleep to calibrate as a baseline. For this thesis, only total energy expenditure was used, as further work will be undertaken in Cambridge to extract more detailed information. To account for basal metabolic rate, the measures were adjusted for BMI. Recent work has validated the instrument in children at this age: Preliminary results suggest a correlation of $r=0.67$ ($p<0.05$) for energy expenditure between the Actiheart and doubly-labelled water in 10 free-living 4 year-olds (Ulf Ekelund, MRC Epidemiology Unit, Cambridge, UK- personal communication).

Further details of specific analyses are given in subsequent sections.

One SWS baby:

3 Maternal determinants of intrauterine bone mineral accrual and neonatal body composition

Figure 7 Birth DXA cohort (shaded box).



3.1 Aim

To explore the maternal determinants (body build, lifestyle, diet and 25(OH)-vitamin D status) of intrauterine bone mineral accrual and neonatal body composition (bone, lean and fat).

3.2 Summary of methods

The details methods may be found in Section 2 and the cohort is defined in Figure 7. Mothers were invited to attend the Princess Ann Hospital for their baby to undergo assessment of bone mass by DXA, and measurement of weight, within two weeks of birth. This visit may have been whilst still an inpatient, or after the mother and baby had gone home.

3.3 Analysis

Neonatal fat and percentage fat were transformed to normality by square root and then standardised. Analysis was initially performed on the complete neonatal cohort together and then by gender. The analysis was repeated excluding babies contained in the original mother-baby cohort(151), to explore whether these results were reproducible in a separate cohort of babies. Specific analyses are described in the course of the results.

3.4 Results: Description of the mothers whose children had a neonatal DXA assessment

3.4.1 General

1,755 babies were eligible for DXA assessment at birth. Of these 873 were scanned, and after exclusion of 32 scans with excessive movement artefact, data on 841 mothers were available from the initial pre-pregnancy interview (48% of those eligible). Some of these women also had data in early pregnancy (n=683) and in late pregnancy (n=787). The mean (sd, n) age of the mothers was 28.2 years (3.8 years, 841) and 52% were in their first pregnancy (n=438). Social class data were available for 779 mothers and are summarised in Table 6.

3.4.2 Anthropometry

Maternal anthropometric measures before and during pregnancy are summarised in Table 6. Median maternal triceps skinfold thickness increased with each successive time point. Thus median (IQR) initial pre-pregnancy triceps skinfold thickness was 19.5 mm (15.1 to 24.2 mm). In early pregnancy median (IQR) triceps skinfold thickness was 19.7 mm (15.7 to 24.1 mm) and in late pregnancy 20.6 mm (16.6 to 25.4 mm). This increase was not statistically significant however.

3.4.3 Lifestyle/ diet

26.8% mothers were current smokers at the initial interview, whereas 12.2% admitted to smoking in early pregnancy and 13.1% in late pregnancy. 28.4% drank more than 10 units of alcohol per week before pregnancy compared with 4.4% in early and 0.3% in late pregnancy. 77.9% mothers drank up to 6 pints of milk per week before pregnancy, with the corresponding figures for early and late pregnancy being 69.3% and 59.6% respectively. At the initial interview before pregnancy, 54.4% mothers reported habitually walking "fairly briskly" or " fast". At the early pregnancy interview this was 31.8% and in late pregnancy was 5.8%. The percentages of mothers taking more than 1.25 hours of strenuous exercise per week before and in early and late pregnancy were 29.8%, 12.6% and 6.1% respectively. These data are summarised in Table 7 and Table 8.

Table 6 Maternal demographics, anthropometry and smoking before (PP), and in early (EP) and late (LP) pregnancy.

Characteristic			n
Age, years (mean (sd))		28.2 (3.8)	841
Birthweight, g (mean (sd))		3244 (529)	755
Percentage nulliparous		52.0	438
Social class (%)	I	5.9	46
	II	38.9	303
	IIIn	36.3	283
	IIIm	6.8	53
	IV	10.1	79
	V	1.9	15
Height, cm (mean,sd)		163.4 (6.3)	841
PP weight, kg (median, IQR)		65.1 (58.6 to 73.4)	837
PP BMI, kg/m^2 (median, IQR)		24.3 (22.0 to 27.6)	837
Grip strength left hand, kg (mean, sd)		27.8 (5.7)	498
PP triceps skinfold, mm (median, IQR)		19.5 (15.1 to 24.3)	840
EP triceps skinfold thickness, mm (median, IQR)		19.7 (15.7 to 24.1)	683
LP triceps skinfold thickness, mm (median, IQR)		20.6 (16.6 to 25.4)	785
PP smoking, %		26.8	226
EP smoking, %		12.2	83
LP smoking, %		13.1	103

Table 7 Maternal physical activity before (PP), and during early (EP) and late (LP) pregnancy.

Characteristic		n (%) PP	EP	LP
Walking speed	V slow	4 (0.5)	6 (0.9)	125 (15.9)
	Easy pace	54 (6.4)	110 (16.1)	411 (52.2)
	Normal	326 (38.7)	350 (51.2)	205 (26.1)
	Fairly brisk	410 (48.7)	199 (29.1)	44 (5.6)
	Fast	48 (5.7)	18 (2.6)	2 (0.3)
Strenuous exercise, hrs/week	0.00	288 (34.3)	387 (56.7)	593 (75.4)
	to 0.25	156 (18.6)	130 (19.0)	87 (11.1)
	to 1.25	145 (17.3)	80 (11.7)	59 (7.5)
	> 1.25	250 (29.8)	86 (12.6)	48 (6.1)

Table 8 Maternal alcohol and milk intake before (PP) and in early (EP) and late (LP) pregnancy.

Characteristic		n (%) PP	EP	LP
Units alcohol per week	0 to 1.5	208 (24.7)	523 (76.7)	671 (85.4)
	to 4.5	203 (24.1)	100 (14.7)	95 (12.1)
	to 10.0	192 (22.8)	29 (4.3)	18 (2.3)
	> 10.0	239 (28.4)	30 (4.4)	2 (0.3)
Pints milk per week	0 to 2.0	207 (24.6)	196 (28.7)	124 (15.8)
	to 3.5	312 (37.1)	205 (30.0)	241 (30.6)
	to 6.0	136 (16.2)	72 (10.5)	104 (13.2)
	> 6.0	187 (22.2)	210 (30.8)	318 (40.4)

3.4.4 Maternal social class

When examined using χ^2 for trend, there were statistically significant associations between maternal social class and smoking habits, alcohol intake and hours of strenuous exercise per week (Smoking: Table 9, Table 10, Table 11).

Table 9 Maternal social class and pre-pregnancy (PP) smoking (χ^2 for trend, p<0.0001).

	Mother's social class						
PP smoking	**I**	**II**	**IIIn**	**IIIm**	**IV**	**V**	**Total**
No	44	244	206	36	40	8	578
%	95.65	80.53	72.79	67.92	50.63	53.33	74.2
Yes	2	59	77	17	39	7	201
%	4.35	19.47	27.21	32.08	49.37	46.67	25.8
Total	46	303	283	53	79	15	779
	100	100	100	100	100	100	100

Table 10 Maternal social class and early pregnancy (EP) smoking (χ^2 for trend, p<0.0001).

	Mother's social class						
EP smoking	**I**	**II**	**IIIn**	**IIIm**	**IV**	**V**	**Total**
No	34	248	196	36	45	5	564
%	100	94.3	85.22	81.82	72.58	62.5	87.99
Yes	0	15	34	8	17	3	77
%	0	5.7	14.78	18.18	27.42	37.5	12.01
Total	34	263	230	44	62	8	641
	100	100	100	100	100	100	100

Table 11 Maternal social class and late pregnancy (LP) smoking (χ^2 for trend, p<0.0001).

	Mother's social class						
LP smoking	**I**	**II**	**IIIn**	**IIIm**	**IV**	**V**	**Total**
No	40	257	233	43	53	11	637
%	100	90.81	89.27	81.13	69.74	73.33	87.5
Yes	0	26	28	10	23	4	91
%	0	9.19	10.73	18.87	30.26	26.67	12.5
Total	40	283	261	53	76	15	728
	100	100	100	100	100	100	100

Thus before pregnancy 49.4% mothers in social class IV admitted to smoking, compared with 19.5% in social class II. In late pregnancy the corresponding figures were 30.3% and 9.2%. When the analysis was limited to those mothers assessed at all time points, of those who were smoking before pregnancy, 53.2% had given up by early (Table 12) and 55.8% by late pregnancy (Table 13). Of those mothers who smoked in early pregnancy 85.7% were still smoking in late gestation (Table 14).

Table 12 Maternal smoking before and in early pregnancy (χ^2 for trend, $p<0.0001$).

Pre-pregnancy smoking	Early pregnancy smoking		
	No	Yes	Total
No	481	4	485
%	99.18	0.82	100
Yes	83	73	156
%	53.21	46.79	100
Total	564	77	641
%	87.99	12.01	100

Table 13 Maternal smoking before and in late pregnancy for all social classes (χ^2 for trend, $p<0.0001$).

Pre-pregnancy smoking	Late pregnancy smoking		
	No	Yes	Total
No	482	3	485
%	99.38	0.62	100
Yes	87	69	156
%	55.77	44.23	100
Total	569	72	641
%	88.77	11.23	100

Table 14 Maternal smoking in early and late pregnancy (χ^2 for trend, $p<0.0001$).

Early pregnancy smoking	Late pregnancy smoking		
	No	Yes	Total
No	558	6	564
%	98.94	1.06	100
Yes	11	66	77
%	14.29	85.71	100
Total	569	72	641
%	88.77	11.23	100

Table 12, Table 14, and Table 13 include women with complete smoking data at all three time points.

Comparing maternal habits in late gestation with those before pregnancy, smoking was further categorised by social class (59 mothers whose social class was not categorised are excluded): In social class I all mothers who smoked before pregnancy had given up by late gestation. Mothers in higher social classes were more likely to give up smoking by late

gestation, such that 58.2% in class II, 58.5% in class IIIn, 47.1% in class IIIm, 37.8% in class IV and 42.9% in class V of mothers who smoked before pregnancy had given up by the time of late pregnancy.

Maternal alcohol intake also varied weakly by social class before and in late pregnancy (Table 15), but not in early pregnancy. Thus, of the small number of mothers in social class V, 40% drank more than 10 units per week before pregnancy compared with 25% in class II. To allow valid χ^2 testing, social class groups IIIn and IIIm were combined, as well as social class groups V and VI. Alcohol intake was grouped into "- 1.5 units per week" and "> 5 units per week". When the analysis was repeated limiting the subjects to those with data on alcohol consumption at both time points, there was no statistically significant trend shown (p=0.095). Additionally 4 of the 6 mothers in social class V who drank more than 10 units per week before pregnancy were not assessed in early pregnancy and the other two reduced their intake to < 1.5 units per week.

Table 15 Maternal social class and alcohol intake (units per week) in late pregnancy (χ^2 for trend, p=0.095).

LP alcohol (units/ week)	**I**	**II**	**III**	**IV, V**	**Total**
- 1.5	13	134	146	34	327
	61.9	80.72	87.95	80.95	82.78
> 1.5	8	32	20	8	68
	38.1	19.28	12.05	19.05	17.22
Total	21	166	166	42	395
	100	100	100	100	100

The total time spent in strenuous exercise per week also varied significantly across maternal social class before (Table 16) and in early pregnancy, but just failed to reach statistical significance in late pregnancy.

Table 16 Maternal social class and hours of strenuous activity per week before pregnancy (χ^2 for trend, p=0.038).

	Mother's social class						
PP strenuous exercise (hours/ week)	**I**	**II**	**IIIn**	**IIIm**	**IV**	**V**	**Total**
0	12	85	109	22	34	5	267
%	26.09	28.05	38.65	42.31	43.59	33.33	34.41
-0.25	9	52	53	12	13	2	141
%	19.57	17.16	18.79	23.08	16.67	13.33	18.17
1.25	5	67	38	5	14	4	133
%	10.87	22.11	13.48	9.62	17.95	26.67	17.14
> 1.25	20	99	82	13	17	4	235
%	43.48	32.67	29.08	25	21.79	26.67	30.28
Total	46	303	282	52	78	15	776
%	100	100	100	100	100	100	100

3.5 Results: Comparison with mothers whose children did not undergo DXA scanning at birth

There were 1,755 mothers who were eligible for their offspring to have a neonatal DXA assessment. Thus 914 did not undergo DXA assessment. The data for the overall cohort had not all been cleaned, so it was only possible to compare a few variables. Thus, comparing the mothers of those babies who did and did not have a DXA scan at birth, there were no differences in body build, age or smoking habits before pregnancy. However, the mothers of babies who underwent DXA scanning tended to have had fewer previous pregnancies (p=0.057).

Table 17 Comparison of baseline measures on women whose children did or did not have a DXA at birth.

Measurement	**No DXA**	**DXA**
PP age, years	28.1	28.2
Height, cm	163.3	163.4
PP TSF$^+$, mm	20.1	20.2
% nulliparous	48.6	52.0*
% PP smoking	26.8	26.8

PP: Pre-pregnancy; TSF: Triceps skinfold thickness; Table shows mean values or %.

$^+$Geometric means; *p<0.1

3.6 Results: Description of neonates

There were 841 neonates with DXA data. The number of mothers with data for each variable differed by time point and category as some mothers may, for example, have been able to answer a question on smoking, but not able to recall their own birthweight. As not all mothers informed the study coordinators till after 11 weeks, the initial data are most complete, followed by late pregnancy and then early pregnancy. When comparing effects of maternal determinants at these different time points, the analysis has been limited to those mothers with complete data at all time points for the variable in question.

This analysis is based on the group of 841 neonates (the "complete" cohort). Previous work has been performed on the first 363 babies of this "complete" cohort and this is termed the "initial" cohort. The findings in the initial cohort were compared with those in the complete cohort and in the cohort comprising those babies in the complete cohort subsequent to the initial cohort. After characterisation of the neonates, maternal determinants of neonatal bone mass and body composition at each time point (before pregnancy, early pregnancy and late pregnancy), and change in maternal factors between time points were explored.

3.6.1 Obstetric measures

The mean (sd, n) gestational age of the boys was 279.5 days (10.2 days, 438) and for the girls was 280.5 days (10.4 days, 403), p=0.167. 4.6% of the neonates were born before 37 weeks gestation (n=35) and the range of gestational ages was 238 to 303 days. After adjustment for gestational age, the mean (sd, n) birthweight of the boys was 3558.6 g (440.7 g, 432) and of the girls was 3447.6 g (427.9 g, 401), p=0.0002. The mean (sd, n) age of the boys at the time of the DXA scan was 6.14 days (4.93 days, 438) and of the girls 6.03 days (4.92 days, 403), p=0.727.

3.6.2 DXA bone indices

DXA assessments were available for 438 boys and 403 girls, giving measurements of whole body bone area (BA), bone mineral content (BMC), areal bone mineral density (aBMD) and size corrected bone mineral density (BMDh, defined as BMC adjusted for BA by regression). After adjustment for gestational age and age at DXA scan, the boys had a greater mean (sd) whole body bone area than the girls. Whole body bone mineral content and areal bone mineral density were also higher in the boys (Table 18). Whole body BA and BMC were

highly correlated (r=0.98, p<0.0001), and the associations with maternal factors were very similar for both.

3.6.3 DXA body composition indices

Neonatal fat and percentage fat (total fat mass divided by sum of total lean, fat and bone mass) were not normally distributed and were transformed to normality by using the square root. The boys had higher mean total lean mass and percentage lean mass than the girls, but the girls had higher total and percentage fat mass. Percentage bone mass was also greater in the boys than the girls (Table 18).

Table 18 Characteristics of the neonates, by gender.

Characteristic	Boys		Girls		p
		n		n	
Gestational age, days (mean, sd)	279.5 (10.2)	438	280.5 (10.4)	403	0.167
Age at DXA, days (mean, sd)	6.1 (4.9)	438	6.0 (4.9)	403	0.727
Birthweight, g (mean, sd)	3558.6 (440.7)	432	3447.6 (427.9)	401	0.0002
WB BA, cm^2 (mean, sd)	120.0 (22.7)	438	114.1 (21.8)	403	0.0001
WB BMC, g (mean, sd)	64.1 (13.9)	438	60.3 (13.2)	403	<0.0001
WB BMD, g/cm^2 (mean, sd)	0.531 (0.0253)	438	0.526 (0.0255)	403	0.002
Total lean mass, g (mean, sd)	3005.6 (308.9)	438	2849.8 (279.4)	403	<0.0001
Total fat mass, g (median, IQR)	507.7 (365.6 to 645.8)	438	529.8 (404.9 to 687.7)	403	0.004
Percent bone (mean, sd)	1.8 (0.26)	438	1.7 (0.26)	403	0.01
Percent lean (mean, sd)	84.0 (4.3)	438	82.5 (4.3)	403	<0.0001
Percent fat (median, IQR)	14.0 (11.0 to 16.9)	438	15.3 (12.9 to 18.5)	403	<0.0001

3.6.4 Gestational age and neonatal outcomes

The gestational age of the neonate was associated with birthweight and all DXA-derived measurements of bone mass and body composition. The standardised variables are summarised in Table 19 to facilitate comparison. Birthweight increased by 20.8 g (95% CI: 17.9 to 23.7 g) for each day of gestation, with whole body BA, BMC and BMD increasing by 1.1 cm^2 (95% CI: 1.1 to 1.4 cm^2), 0.72 g (95% CI: 0.64 to 0.82 g) and 0.0006 g/cm^2 (95% CI: 0.0005 to 0.0008 g/cm^2) respectively. Although percentage bone and fat mass increased with increasing gestational age, percentage lean decreased by 0.13 g/day (95% CI: 0.10 to 0.16 g/ day decrease).

Table 19 Gestational age, and birthweight, bone mineral and body composition. Outcomes are standardised regression (β) coefficients, so units are sd/day increase in gestational age.

Outcome (sd)	β (sd/day)	95% CI (sd/day)	r	p
Birthweight	0.043	0.037 to 0.049	0.44	<0.0001
Whole body BA	0.048	0.042 to 0.054	0.49	<0.0001
Whole body BMC	0.046	0.041 to 0.052	0.48	<0.0001
Whole body BMD	0.023	0.017 to 0.029	0.24	<0.0001
Total lean mass	0.040	0.034 to 0.046	0.41	<0.0001
Total fat mass	0.036	0.030 to 0.042	0.37	<0.0001
Percentage bone mass	0.036	0.030 to 0.042	0.37	<0.0001
Percentage lean mass	-0.029	-0.035 to –0.022	-0.30	<0.0001
Percentage fat mass	0.029	0.022 to 0.035	0.30	<0.0001

3.6.5 Age at DXA scan and bone outcomes

The baby's age at the time of the DXA scan was associated positively with total and percentage lean, and negatively with BMD and percentage fat and bone mass. The relationship was best modelled with a quadratic term. Table 20 summarises these data. Although the association with WB BA and BMC were not statistically significant, there was a strong relationship between WB BMD and the quadratic term. Since BMC showed no association at all, this finding is likely to be spurious, and may also reflect the influence of fat/ lean ratio on DXA derivation of bone mineral.

Table 20 Age at DXA and neonatal bone mass and body composition. Outcomes are standardised regression (β) coefficients, so units are sd/day^2 increase in age.

Outcome (sd)	β (sd/day^2)	95% CI (sd/day^2)	r	p
Whole body BA	0.0007	-0.0003 to 0.002	0.05	0.18
Whole body BMC	-0.0002	-0.0001 to 0.007	-0.01	0.71
Whole body BMD	-0.003	-0.004 to –0.002	-0.22	<0.0001
Total lean mass	0.005	0.004 to 0.006	0.37	<0.0001
Total fat mass	0.001	-0.0002 to 0.002	0.05	0.13
Percentage bone mass	-0.004	-0.005 to –0.003	-0.25	<0.0001
Percentage lean mass	0.001	0.00004 to 0.002	0.07	0.04
Percentage fat mass	-0.0008	-0.002 to 0.0001	-0.06	0.08

3.7 Results: Maternal anthropometry and neonatal bone mineral

Maternal determinants of neonatal whole body bone mass were explored at each time point. There were strong associations between maternal factors and each of neonatal BA and BMC, but neonatal aBMD showed no statistically significant relationships with any maternal measure. BMDh was only associated with maternal grip-strength ($r=-0.11$, $p=0.01$).
Table 21 summarises the associations between maternal anthropometric measures at each time point and neonatal BA and BMC. Neonatal whole body bone mineral content was positively associated with maternal height, BMI and triceps skinfold thickness (TSF) before, and during early and late pregnancy. TSF was found to be a better predictor than the corresponding measures at the biceps, supscapular or suprailiac regions. The association between maternal TSF and neonatal bone mass strengthened in magnitude and statistical significance from before to late pregnancy. This pattern remained consistent after the analysis was repeated limiting participants to those with complete TSF data at each time point (to remove any effect caused by different numbers of participants between pre and late pregnancy). Change in triceps skinfold thickness, however, was not associated with BMC at birth.

Maternal weight gain (early to late pregnancy) was positively correlated with neonatal BMC ($r=0.22$, $p<0.001$, $n=638$), and this persisted after baby's birthweight was subtracted from maternal weight gain (as a crude measure of intrinsic maternal weight gain rather than gain due to growth of the foetus). Maternal left handed grip strength was positively associated with BMC, such that BMC increased by 0.3 g (95% CI: 0.1 to 0.5 g, $p=0.005$) for each 1 kg increase in grip strength. Maternal birthweight was available for 755 participants. BMC increased by 6.4 g for each 1 kg increase in maternal birth weight. Paternal height showed a borderline statistically significant association with BMC ($r=0.11$, $p=0.09$).

Table 21 Maternal anthropometry and neonatal WB BA and BMC.

Predictor		Mean (sd) BA (cm^2)	p	Mean (sd) BMC (g)	p	n
Maternal PP	to 26.4	116.7 (22.4)	0.77	62.3 (13.8)	0.49	277
age (yrs)	to 30.2	119.0 (21.7)		63.6 (13.5)		276
	> 30.2	115.9 (22.6)		61.3 (13.4)		288
Maternal PP	to 160	113.2 (22.0)	<0.001	59.9 (13.2)	<0.001	256
height (cm)	to 166	118.6 (22.9)		63.4 (14.2)		304
	> 166	119.3 (21.5)		63.3 (13.1)		280
Maternal PP	to 22.9	114.8 (20.5)	<0.001	61.1 (12.4)	<0.001	283
BMI (kg/m^2)	to 26.0	115.4 (22.8)		61.0 (13.5)		270
	> 26.0	121.5 (22.9)		64.8 (14.4)		283
Maternal PP	to 16.5	114.8 (21.1)	0.002	60.7 (12.6)	0.002	279
TSF (mm)	to 22.5	115.5 (22.3)		61.5 (13.3)		284
	> 22.5	121.5 (22.9)		64.7 (14.5)		276
Maternal EP	to 17.2	115.7 (22.1)	0.003	61.4 (13.3)	0.003	233
TSF (mm)	to 22.2	115.6 (20.3)		61.4 (12.2)		217
	> 22.2	120.7 (22.8)		64.4 (14.2)		232
Maternal LP	to 17.8	113.1 (22.0)	<0.001	59.9 (13.1)	<0.001	263
TSF (mm)	to 23.8	117.6 (21.7)		62.4 (13.1)		256
	> 23.8	120.8 (22.3)		64.4 (14.0)		266
Maternal EP-	- 5.7	113.0 (22.1)	<0.001	59.8 (13.4)	<0.001	211
LP weight	- 8.8	116.9 (20.6)		62.1 (12.3)		210
gain (kg)*	> 8.8	122.3 (22.2)		65.4 (13.7)		210
Maternal	to 3100	113.5 (23.3)	<0.001	60.2 (14.2)	<0.001	214
birthweight	to 3400	116.3 (19.7)		61.6 (11.8)		177
(g)	> 3400	121.8 (21.9)		65.0 (13.4)		220
Maternal left	to 25.5	112.8 (21.8)	0.001	60.1 (13.0)	0.005	173
handed grip	to 30.0	118.8 (21.0)		63.2 (13.2)		170
strength (kg)$^+$	>30.0	119.7 (21.3)		63.4 (12.9)		154

* Maternal pregnancy weight gain minus birthweight of infant; $^+$ Grip strength measured at 19 weeks

3.8 Results: Maternal lifestyle and neonatal bone mineral

Mothers who had had fewer previous pregnancies gave birth to children with lower whole body BMC (Spearman r=0.13, p=0.0001) than those who had had more. Parity was negatively correlated with BMDh (Spearman r=-0.13, p=0.0001), but not aBMD. There were no other statistically significant determinants of aBMD or BMDh. Social class did not predict bone mass in the offspring (Table 22). BMC was 3.9 g (95% CI: 0.8 to 6.9 g) lower in the offspring of mothers who smoked in early pregnancy compared with those who did not, and 4.2 g (95% CI: 1.4 to 7.0 g) lower in the offspring of mothers who smoked in late pregnancy compared with those who did not (Table 23). These patterns remained consistent after the analysis was repeated limiting participants to those with complete smoking data at each time point.

Alcohol and milk intake at any time did not predict bone mineral in the neonate. After grouping the walking speeds "fairly briskly" and "fast" together (as there were only 2 mothers in the latter group), BMC decreased by a mean of 1.8 g (95% CI: 0.6 to 3.0 g) as category of late pregnancy walking speed increased (Table 24). No other measures of maternal physical activity showed statistically significant relationships with neonatal bone mass.

Figure 8 represents the maternal determinants of neonatal whole body bone area and bone mineral content.

Table 22 Maternal social class and parity, and neonatal WB BA and BMC.

Predictor		**Mean (sd) BA (cm^2)**	**p**	**Mean (sd) BMC (g)**	**p**	**n**
Social class	I	113.9 (22.9)	0.65	60.2 (14.5)	0.56	46
	II	118.3 (21.8)		63.0 (13.4)		302
	IIIn	115.6 (22.0)		61.2 (13.2)		283
	IIIm	115.0 (22.4)		60.8 (13.8)		53
	IV	119.5 (22.7)		64.0 (13.9)		79
	V	120.0 (27.1)		64.2 (16.2)		15
Previous children	0	114.1 (21.8)	<0.001	60.8 (13.4)	<0.001	437
	1	119.3 (22.4)		63.2 (13.6)		285
	2	122.5 (22.9)		64.8 (14.1)		92
	3 +	127.6 (18.7)		68.2 (12.5)		27

Table 23 Maternal smoking, alcohol and milk intake and neonatal WB BA and BMC.

Predictor		Mean (sd) BA (cm^2)	p	Mean (sd) BMC (g)	p	n
PP smoking	No	116.9 (22.3)	0.55	62.0 (13.7)	0.43	615
	Yes	118.0 (22.2)		62.9 (13.4)		226
EP smoking	No	118.2 (21.7)	0.007	62.8 (13.3)	0.013	599
	Yes	111.3 (83)		59.0 (13.3)		83
LP smoking	No	118.2 (21.9)	0.001	62.8 (13.3)	0.004	684
	Yes	110.5 (23.2)		58.7 (14.3)		103
PP alcohol	to 1.5	117.3 (21.3)	0.40	62.5 (12.9)	0.32	217
(units/ week)	to 4.5	118.0 (23.7)		62.7 (14.6)		204
	to 10.0	118.3 (21.7)		63.0 (13.4)		192
	> 10.0	115.4 (22.5)		61.1 (13.5)		228
EP alcohol	to 1.5	117.2 (22.2)	0.59	62.6 (13.5)	0.12	671
(units/ week)	to 4.5	118.8 (21.6)		63.6 (13.7)		95
	to 10.0	112.1 (24.9)		60.1 (10.7)		18
	> 10.0	96.3 (25.6)		58.0 (11.0)		2
LP alcohol	to 1.5	117.2 (22.2)	0.59	62.3 (13.6)	0.52	671
(units/ week)	to 4.5	118.8 (21.6)		63.0 (13.0)		95
	to 10.0	112.1 (24.9)		59.4 (14.5)		18
	> 10.0	96.3 (25.6)		50.2 (16.2)		2
PP milk	to 2.0	116.5 (22.9)	0.88	61.7 (14.0)	0.76	207
(pints/ week)	to 3.5	116.9 (21.1)		62.2 (12.9)		311
	to 6.0	120.6 (24.2)		64.3 (14.5)		136
	> 6.0	115.8 (22.0)		61.6 (13.5)		187
EP milk	to 2.0	117.1 (22.7)	0.75	62.1 (13.4)	0.99	196
(pints/ week)	to 3.5	117.8 (20.7)		62.4 (12.8)		205
	to 6.0	120.2 (20.6)		64.8 (13.5)		72
	> 6.0	116.2 (22.8)		61.9 (13.8)		209
LP milk	to 2.0	117.7 (23.5)	0.98	62.6 (14.0)	0.85	124
(pints/ week)	to 3.5	116.0 (20.2)		61.4 (12.4)		241
	to 6.0	121.2 (21.7)		64.6 (13.0)		104
	> 6.0	116.7 (23.3)		62.1 (14.2)		318

Table 24 Maternal physical activity and neonatal WB BA and BMC.

Predictor		Mean (sd) BA (cm^2)	p	Mean (sd) BMC (g)	p	n
PP walking	V slow	132.4 (43.3)	0.37	72.1 (30.4)	0.26	4
speed	Easy	119.8 (21.4)		64.3 (13.1)		54
	Normal	117.0 (22.2)		62.2 (13.5)		326
	F. brisk	116.8 (22.5)		62.1 (13.6)		409
	Fast	117.2 (20.1)		61.8 (12.4)		48
EP walking	V slow	118.7 (36.1)	0.78	64.2 (25.8)	0.63	6
speed	Easy	117.0 (23.1)		62.1 (13.7)		110
	Normal	117.3 (21.2)		62.3 (12.8)		349
	F. brisk	117.3 (22.3)		62.5 (13.7)		199
	Fast	120.4 (20.6)		65.3 (12.9)		18
LP walking	V slow	120.1 (25.1)	0.003	64.1 (15.3)	0.004	125
speed	Easy	118.3 (21.7)		62.8 (13.1)		411
	Normal	114.8 (20.8)		61.1 (12.8)		205
	Br./Fast	111.0 (23.3)		58.3 (13.8)		46
PP strenuous	0.0	118.9 (22.8)	0.11	63.4 (14.1)	0.075	288
exercise	to 0.25	116.9 (22.3)		62.2 (13.7)		156
(hours/	to 1.25	116.6 (21.3)		62.0 (12.8)		145
week)	> 1.25	115.8 (22.3)		61.3 (13.4)		249
EP strenuous	0.0	117.2 (22.7)	0.98	62.3 (13.8)	0.98	387
exercise	to 0.25	118.2 (19.8)		63.0 (11.8)		130
(hours/	to 1.25	116.9 (21.1)		62.0 (13.2)		80
week)	> 1.25	117.1 (22.4)		62.5 (14.0)		85
LP strenuous	0.0	117.3 (22.3)	0.21	62.3 (13.7)	0.23	593
exercise	to 0.25	120.4 (21.8)		64.1 (13.0)		87
(hours/	to 1.25	116.4 (22.3)		62.0 (13.1)		59
week)	> 1.25	111.2 (21.1)		58.6 (12.5)		48

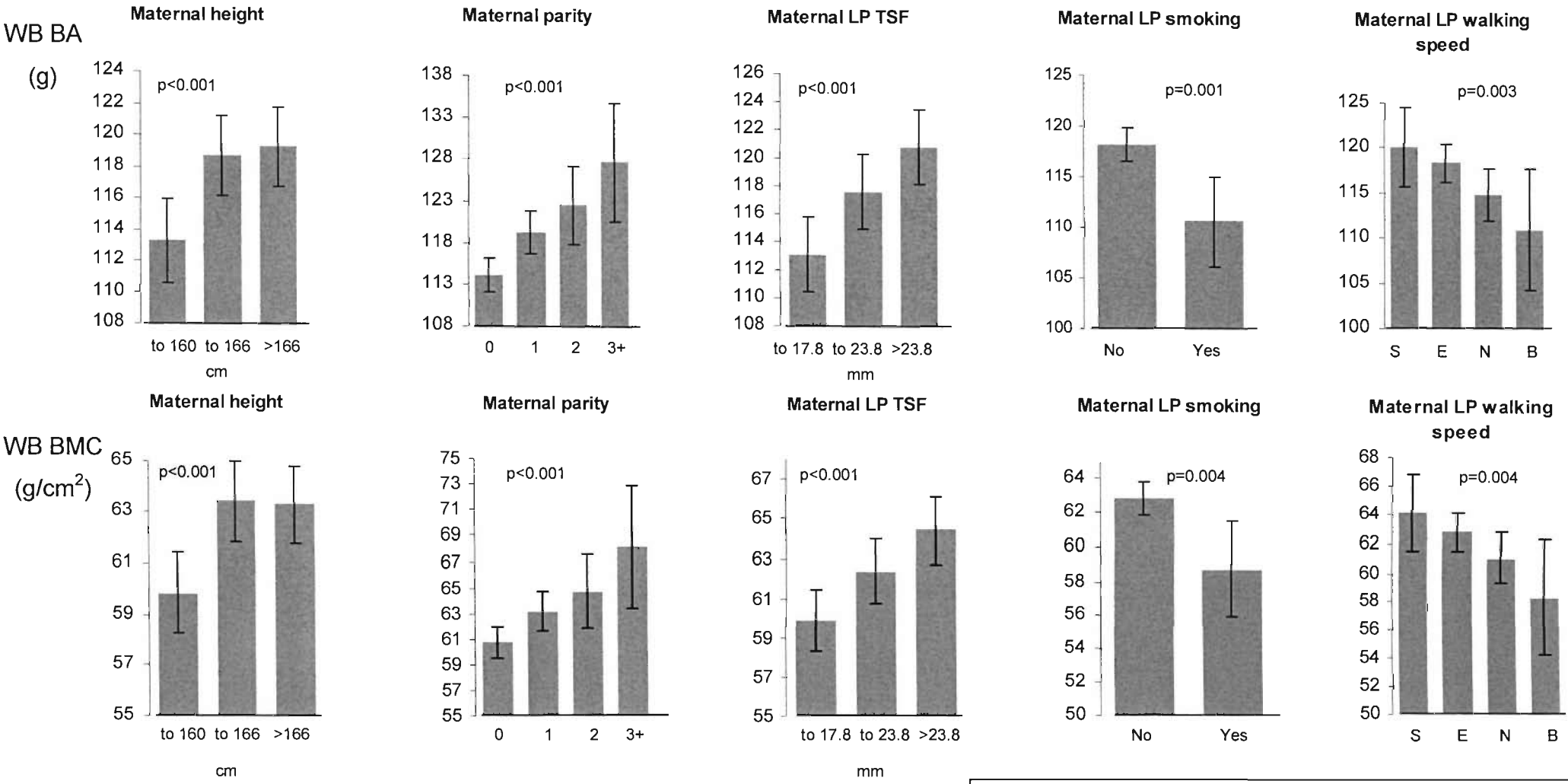


Figure 8 The maternal determinants of neonatal whole body BA and BMC.

Walking speed: S=Slow, E=Easy, N=Normal, B=Brisk/fast

3.9 Results: Mutually independent maternal determinants of neonatal bone mass

Maternal height and parity were recorded at the initial pre-pregnancy interview, and formed the basis of multivariate models for the predictors of neonatal whole body BMC. Owing to the lack of associations between any maternal measures and neonatal aBMD and BMDh, no models are presented here. The independent maternal determinants of neonatal BMC were explored at each of the three time points and then combined to form a model encompassing all time points. Again models for BMC and BA were similar.

The associations between neonatal bone mass and each of maternal smoking, TSF and walking speed became of greater magnitude and statistical significance at each subsequent time point, and this persisted after limiting the analysis, for each variable, to those participants with data for each variable at each time point. Thus the late pregnancy variables were used for these three measures. The mutually adjusted maternal predictors of neonatal BA and BMC are shown in Table 25 and Table 26, and included maternal height, birthweight, parity, LP triceps skinfold thickness, smoking and walking speed.

Table 25 Mutually independent maternal predictors of neonatal WB BA, (R^2=10.0%, n=704)

Predictor	β	p	95 % CI
Maternal birthweight (g)	0.01	<0.001	0.003 to 0.009
Height, cm	0.40	0.003	0.14 to 0.66
Parity, 4 groups	4.82	<0.001	2.80 to 6.85
LP TSF, sd	1.75	0.032	0.15 to 3.35
LP smoking Y/N	-9.26	<0.001	-13.93 to -4.59
LP walking speed, 4 groups	-2.95	0.005	-5.02 to -0.88

LP: Late pregnancy; TSF: Triceps skinfold thickness

Table 26 Mutually independent maternal predictors of neonatal WB BMC, (R^2=8.1%, n=704).

Predictor	β	p	95 % CI
Maternal birthweight (g)	0.003	0.001	0.001 to 0.005
Height, cm	0.22	0.006	0.06 to 0.38
Parity, 4 groups	2.44	<0.001	1.20 to 3.68
LP TSF, sd	1.16	0.020	0.18 to 2.15
LP smoking Y/N	-4.85	0.001	-7.71 to -1.98
LP walking speed, 4 groups	-1.75	0.001	-3.02 to -0.48

LP: Late pregnancy; TSF: Triceps skinfold thickness

3.10 Results: Maternal determinants of neonatal bone mineral by gender

Generally the relationships between bone mineral and maternal factors were similar in both genders (Table 27), although the associations between maternal triceps skinfold thickness and neonatal bone mineral did not reach statistical significance in the girls. The associations with maternal height, parity and walking speed became of borderline significance. Similarly attenuated associations were seen between BMC in the boys and maternal height and walking speed. For all these relationships, β-coefficients were similar to the original model however, suggesting that this is likely to have been a statistical artefact; indeed the interaction terms for gender with the maternal factors were not statistically significant.

Table 27 Mutually independent maternal determinants of neonatal whole body bone mineral content by gender.

	Boys			Girls			Both		
	β	p	95% CI	β	p	95% CI	β	p	95% CI
Birthweight (g)	0.003	0.039	0.0001 to 0.005	0.004	0.002	0.002 to 0.007	0.003	0.001	0.001 to 0.005
Height (cm)	0.23	0.060	-0.009 to 0.46	0.20	0.079	-0.023 to 0.42	0.22	0.006	0.06 to 0.38
Parity	3.61	<0.001	1.73 to 5.48	1.48	0.080	-0.18 to 3.14	2.44	<0.001	1.20 to 3.68
LP TSF (sd)	1.63	0.024	0.21 to 3.05	0.83	0.232	-0.54 to 2.21	1.16	0.020	0.18 to 2.15
LP smoking (Y/N)	-4.56	0.033	-8.75 to -0.36	-5.26	0.009	-9.22 to -1.30	-4.85	0.001	-7.71 to -1.98
LP Walking speed (4gps)	-1.56	0.093	-3.39 to 0.26	-1.76	0.053	-3.53 to 0.02	-1.75	0.001	-3.02 to -0.48

LP: Late pregnancy; TSF: Triceps skinfold thickness

3.11 Results: Replication of original study

Similar analysis has been performed on an initial cohort, comprising the first 363 babies of the cohort described above(151). The analysis was repeated in the complete cohort excluding the initial 363 babies, effectively allowing replication in a new, separate, group of babies. Table 28 shows the mutually adjusted maternal predictors of neonatal WB BMC in the initial cohort (n=363).

Table 28 Maternal predictors for neonatal whole body BMC in the initial cohort.

Maternal characteristic	β, p
Height (cm)	0.25, 0.04
LP TSF (sd)	1.9, 0.004
LP Smoking (Y/N)	-4.3, 0.05
Parity (4 groups)	3.8, 0.01

This model is consistent with the findings in the complete cohort described in the first part of this chapter. When this analysis was repeated in the complete cohort, excluding the initial 363 babies, these factors remained statistically significant predictors of neonatal bone mineral.

LP: Late pregnancy; TSF: Triceps skinfold thickness

Table 29 Independent maternal predictors of neonatal WB BMC, excluding babies in original cohort (R^2=7%, n=455).

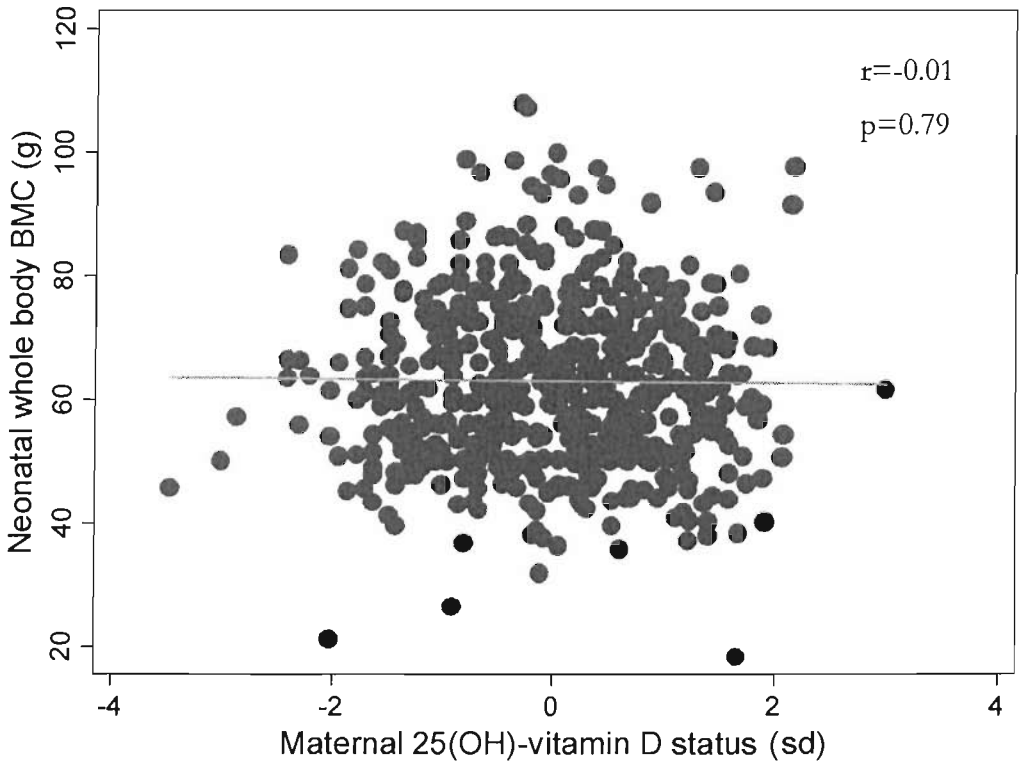
Predictor	**β**	**p**	**95% CI**
Height (cm)	0.30	0.002	0.11 to 0.49
LP TSF (sd)	1.43	0.022	0.21 to 2.65
LP smoking (Y/N)	-5.28	0.005	-8.96 to -1.60
Parity (4 groups)	3.04	<0.001	1.49 to 4.58

Maternal walking speed in late pregnancy made a small contribution to the model, but was of borderline significance (p=0.082), similar to the original finding.

3.12 Results: Maternal vitamin D status and neonatal bone mineral

Maternal 25(OH)-vitamin D (25D) was measured in late pregnancy in 555 women. As it was not normally distributed, it was log-transformed. The median (IQR) late pregnancy serum 25D level in the mothers was 64 nmol/l (46 to 90 nmol/l). The range was 12 to 262 nmol/l. There was seasonal variation such that measurements in winter months were lower than those in summer. When included as a continuous variable, there were no statistically significant associations between maternal 25D status and birthweight, BA, BMC, aBMD or BMDh when the boys and girls were combined or examined separately (Figure 9).

Figure 9 Maternal 25(OH)-vitamin D status in late pregnancy and whole body BMC in all infants.



Comparing bone mineral in offspring of mothers who were deficient (27.5 nmol/l) in 25D in pregnancy with those of mothers who were replete, there was a trend towards lower BA and BMC in the former, although this was not statistically significant. When the genders were analysed separately, the association became of borderline statistical significance in the girls (BA: deficient: 106.5 cm^2, replete: 119.4 cm^2, p=0.083; BMC: deficient 55.6 g, replete 63.5, p=0.076) but not boys (BA: deficient: 120.9 cm^2, replete: 117.8 cm^2, p=0.66; BMC: deficient

64.5 g, replete 62.5, p=0.65). However, power was limited by the small number of mothers (10 for each gender) who were deficient in 25D by this cut off. A higher cut off of 33 nmol/l was found to be more discriminatory. Thus the mean BA of female offspring of mothers whose 25D was less than 33 nmol/l was 111.4 cm^2 vs 119.9 cm^2 in those of mothers with 25D more than 33 nmol/l (p=0.045). There was a similar association for BMC (p=0.046), but not for BMD (p=0.210). For boys the values for BA were 119.0 cm^2 and 117.8 cm^2 respectively (p=0.79). However the gender-25(OH)-vitamin D interaction term was not statistically significant. The associations for BA, BMC and BMD are shown in Table 30.

Table 30 Maternal 25(OH)-vitamin D status in late pregnancy and neonatal bone mineral by gender.

		WB BA (cm^2)	WB BMC (g)	WB BMD (g/cm^2)
Girls	< 33 nmol/l	111.4 (21.0)*	58.7 (12.3)*	0.523 (0.024)
	> 33 nmol/l	119.9 (23.1)*	63.8 (14.0)*	0.529 (0.026)
Boys	< 33 nmol/l	119.0 (22.9)	63.4 (14.0)	0.528 (0.021)
	> 33 nmol/l	117.8 (21.9)	62.5 (13.4)	0.528 (0.024)

* p<0.05, by Student's T-Test

n: Girls deficient 33, replete 237; boys deficient 24, replete 261

Figure 10 Maternal 25(OH)-vitamin D status in late pregnancy and neonatal bone mineral in girls (n=270).

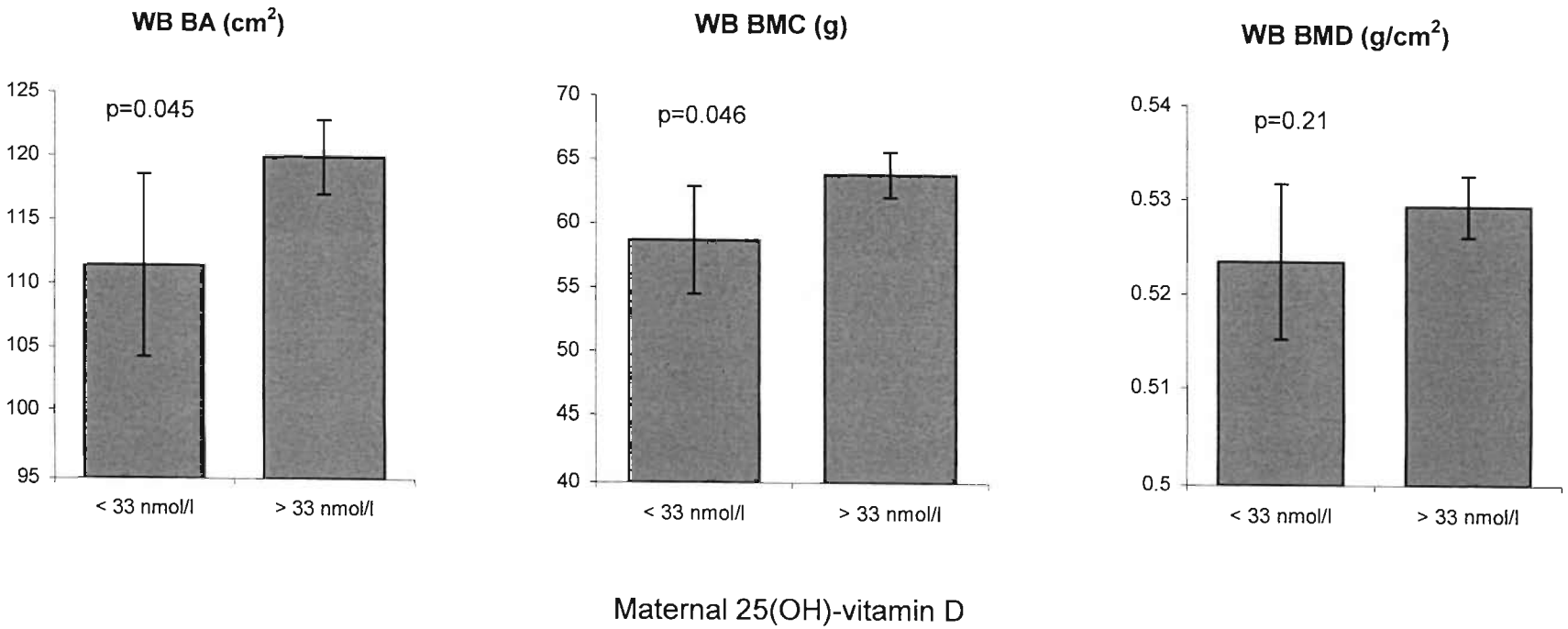


Figure 10 summarises these relationships in the girls alone. The association between maternal 25D status and neonatal BA and BMC remained significant after inclusion of any of maternal late pregnancy TSF, smoking or walking speed in bivariate models. After inclusion of maternal social class, the number of participants in the analysis dropped to 248 due to missing values and the association became statistically non-significant. Although there was no association between maternal height and 25D status, after inclusion of both these factors, the association between 25D and neonatal BA ($p=0.084$) and BMC ($p=0.087$) became of borderline statistical significance.

3.13 Results: Maternal determinants of neonatal body composition

There were statistically significant associations between measures of neonatal body composition and each of maternal height, triceps skinfold thickness, smoking, alcohol intake, walking speed, parity and birthweight. Figure 11, Table 31, Table 32, and Table 33 summarise these relationships. Whereas maternal height strongly predicted neonatal lean mass, there were no associations with fat mass or proportionate body composition. Maternal triceps skinfold thickness predicted all of these outcomes. Babies of higher birth order had greater birthweight, lean and fat mass, and greater percentage fat, but lower percentage lean. The associations with triceps skinfold thickness and smoking strengthened in magnitude and statistical significance at each successive time point, and this was preserved when the participants were limited to those with complete data for either variable at each stage. Maternal late pregnancy walking speed (in 4 groups with "fairly brisk" and "fast" categories combined) was positively correlated with neonatal percentage lean, but negatively with total fat and lean mass, birthweight and percentage fat mass. Walking speed in early or late pregnancy showed no significant associations with these outcomes. Offspring of mothers who smoked in pregnancy had offspring with reduced birthweight, lean and fat mass, and percentage fat, but increased percentage lean. There were weak trends towards lower fat mass, lower percentage fat and increased percentage in lean in the offspring of mothers who drank more. Offspring birthweight decreased as maternal alcohol consumption in early pregnancy increased, but there were no statistically significant associations with late pregnancy alcohol intake.

Table 31 Maternal height and triceps skinfold thickness, and neonatal body composition.

Predictor		Mean (sd) Lean mass (g)	p	Median (IQR) Fat mass (g)	p	Mean (sd) % lean	p	Median (IQR) % fat	p	n	Mean (sd) birthweight (g)	p	n
Height (cm)	to 160	2853.5 (297.9)	<0.001	501.6 (379.3,648.9)	0.013	83.3 (4.4)	0.39	14.5 (11.8,17.7)	0.36	256	3396.3 (440.4)	<0.001	255
	to 166	2942.1 (286.6)		524.4 (390.4,661.1)		83.4 (4.2)		14.6 (12.1,17.4)		304	3516.1 (414.5)		298
	> 166	2989.3 (287.4)		521.0 (408.4,667.7)		83.2 (4.2)		14.4 (12.2,17.4)		280	3594.0 (429.9)		279
PP TSF	to 16.5	2916.4 (299.7)	0.035	482.9 (376.4,618.5)	<0.001	84.1 (4.1)	<0.001	13.6 (11.4,16.4)	<0.001	279	3432.4 (405.3)	<0.001	277
(mm)	to 22.5	2896.2 (286.9)		505.4 (383.8,651.8)		83.2 (4.3)		14.5 (12.0,18.0)		284	3488.4 (448.5)		279
	> 22.5	2983.2 (291.2)		549.4 (435.1,691.7)		82.6 (4.2)		15.1 (12.8,18.1)		276	3599.2 (432.0)		275
EP TSF	to 17.2	2934.5 (304.2)	0.086	496.0 (368.3,633.0)	<0.001	84.0 (4.0)	<0.001	13.8 (11.4,16.8)	<0.001	233	3471.5 (423.7)	0.001	231
(mm	to 22.2	2907.0 (302.0)		483.6 (383.4,627.5)		83.7 (4.2)		14.0 (11.6,17.1)		217	3464.1 (434.8)		215
	> 22.2	2966.1 (279.4)		561.7 (436.8,691.7)		82.3 (4.3)		15.3 (12.8,18.1)		232	3610.7 (435.1)		229
LP TSF	to 17.8	2895.6 (298.2)	0.046	470.5 (361,598.8)	<0.001	84.3 (3.8)	<0.001	13.6 (11.1,16.2)	<0.001	263	3409.4 (415.0)	<0.001	262
(mm)	to 23.8	2948.3 (300.1)		504.9 (389.7,648.7)		83.5 (4.1)		14.1 (11.8,17.4)		256	3519.3 (435.9)		251
	> 23.8	2957.4 (282.3)		552.7 (437.2,682.8)		82.4 (4.4)		15.1 (12.9,18.1)		266	3578.8 (436.9)		264

PP: Pre-pregnancy; EP: Early pregnancy; LP: Late pregnancy; TSF: Triceps skinfold thickness

Table 32 Maternal parity and walking speed, and neonatal body composition.

Predictor		Mean (sd) Lean mass (g)	p	Median (IQR) Fat mass (g)	p	Mean (sd) % lean	p	Median (IQR) % fat	p	n	Mean (sd) birthweight (g)	p	n
Parity	0	2869.0 (290.8)	<0.001	473.9 (362.9,606.1)	<0.001	84.0 (4.3)	<0.001	13.6 (11.2,16.6)	<0.001	437	3424.9 (433.5)	<0.001	433
(Previous	1	2989.2 (281.8)		534.0 (431.8,692.7)		82.8 (4.1)		14.8 (12.6,18.2)		285	3564.0 (408.9)		282
children)	2	3025.7 (297.2)		589.5 (448.2,690.1)		82.3 (3.8)		15.8 (13.4,18.1)		92	3661.4 (452.6)		91
	3 +	2993.1 (281.6)		643.6 (464.0,736.6)		81.3 (4.6)		17.0 (13.0,20.8)		27	3658.2 (377.6)		27
EP walking	V slow	2938.3 (345.1)	0.83	521.7 (376.4,562.6)	0.23	83.4 (4.2)	0.24	16.4 (10.6,16.6)	0.19	6	3527.9 (518.7)	0.55	6
speed	Easy	2939.7 (337.2)		541.0 (409.7,720.9)		82.7 (4.5)		15.1 (12.7,18.4))		110	3540.5 (464.7)		107
	Normal	2937.7 (271.7)		508.5 (393.6,637.1)		83.4 (4.2)		14.5 (11.8,17.1)		349	3518.8 (427.6)		346
	F. brisk	2932.8 (309.4)		429.7 (307.3,654.8)		83.6 (3.9)		14.2 (12.0,17.5)		199	3492.7 (439.4)		198
	Fast	2933.1 (340.0)		578.4 (564.1,592.9)		82.8 (5.5)		13.9 (11.6,17.6)		18	3580.7 (375.5)		18
LP walking	V slow	2942.2 (307.7)	0.109	559.7 (408.7,731.8)	<0.001	82.5 (4.7)	<0.001	15.4 (12.3,18.5)	<0.001	125	3564.1 (504.9)	0.002	124
speed	Easy	2947.8 (288.3)		525.5 (402.3,641.2)		83.3 (4.1)		14.7 (12.1,17.3)		411	3522.3 (425.7)		405
	Normal	2917.0 (290.4)		491.8 (379.2,604.0)		84.0 (3.8)		14.0 (12.0,16.6)		205	3460.9 (407.5)		204
	Br./fast	2871.7 (328.6)		463.9 (311.0,641.8)		84.5 (4.3)		13.5 (10.5,16.7)		46	3357.5 (447.2)		46

EP: Early pregnancy; LP: Late pregnancy

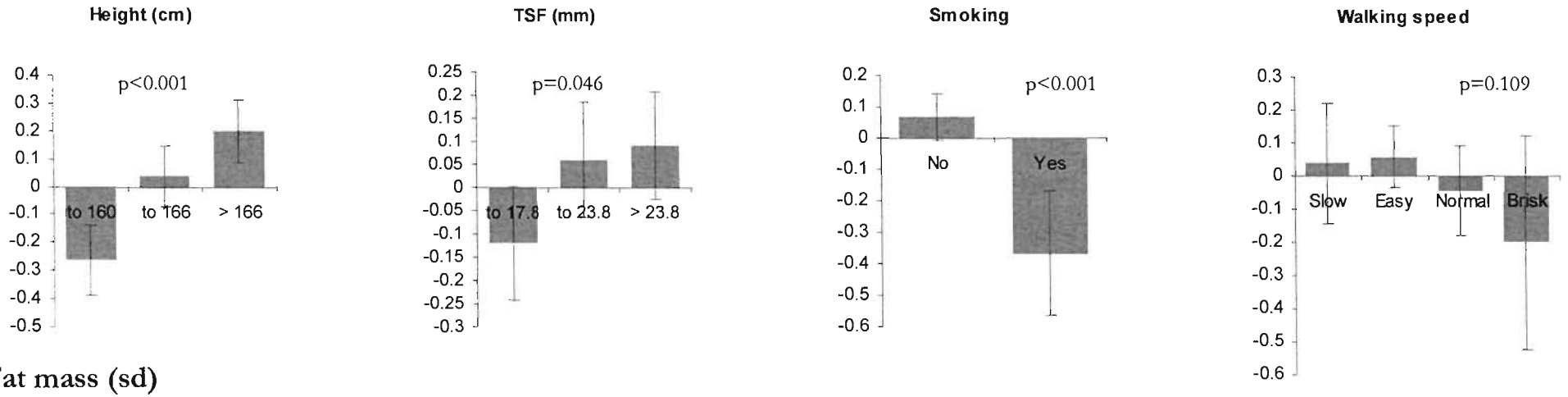
Table 33 Maternal smoking and alcohol intake, and neonatal body composition.

Predictor		Mean (sd) Lean mass (g)	p	Median (IQR) Fat mass (g)	p	Mean (sd) % lean	p	Median (IQR) % fat	p	n	Mean (sd) birthweight (g)	p	n
EP	No	2952.9 (291.0)	<0.001	522.6 (402.4,667.2)	0.004	83.2 (4.2)	0.052	14.6 (12.1,17.6)	0.043	599	3544.0 (424.0)	<0.001	595
smoking	Yes	2817.9 (305.3)		457.1 (312.2,641.6)		84.2 (4.2)		13.5 (10.6,17.4)		83	3310.6 (468.7)		80
LP	No	2951.3 (290.3)	<0.001	521.2 (402.4,667.2)	0.001	83.2 (4.2)	0.012	14.6 (12.1,17.5)	0.009	684	3535.9 (421.5)	<0.001	678
smoking	Yes	2822.7 (299.5)		441.8 (330.5,597.3)		84.4 (4.0)		13.4 (10.7,16.6)		103	3285.8 (460.8)		101
EP alcohol	to 1.5	2942.7 (290.3)	0.303	520.8 (398.0,667.7)	0.086	83.3 (4.1)	0.13	14.7 (12.1,17.6)	0.11	522	3529.3 (438.0)	0.04	517
(units/	to 4.5	2929.0 (315.7)		512.1 (398.8,647.7)		82.9 (4.3)		14.1 (12.3,17.6)		100	3514.2 (431.5)		98
week)	to 10.0	2901.0 (304.5)		487.5 (369.8,606.1)		83.7 (5.1)		13.8 (11.5,16.6)		29	3480.6 (409.7)		29
	> 10.0	2902.1 (318.4)		525.3 (356.1,560.7)		85.0 (3.6)		13.4 (11.1,15.7)		30	3345.9 (421.1)		30
LP alcohol	to 1.5	2930.2 (290.7)	0.821	510.3 (392.0,660.5)	0.31	83.3 (4.3)	0.28	14.4 (12.0,17.6)	0.36	671	3506.8 (439.9)	0.20	666
(units/	to 4.5	2992.6 (301.3)		509.9 (406.8,612.6)		83.7 (3.5)		13.8 (12.0,16.6)		95	3520.1 (400.3)		94
week)	to 10.0	2835.5 (351.3)		481.4 (384.6,573.6)		84.0 (3.6)		14.4 (12.3,16.6)		18	3340.7 (389.5)		16
	> 10.0	2464.5 (30.5)		403.2 (284.3,542.9)		84.3 (5.5)		13.8 (10.5,17.6)		2	2951.7 (136.6)		2

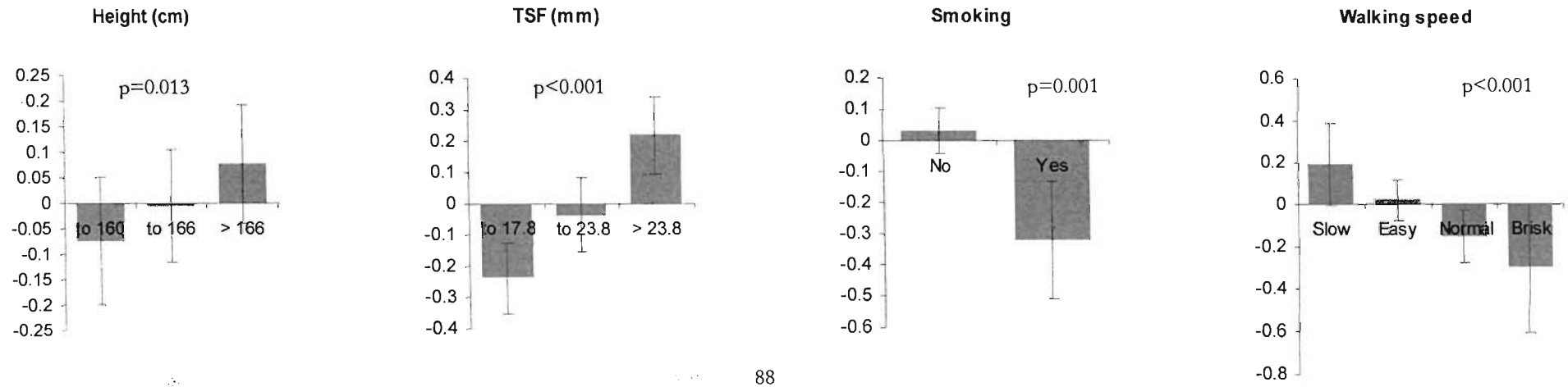
EP: Early pregnancy; LP: Late pregnancy

Figure 11 Maternal height, triceps skinfold thickness, smoking and walking speed and neonatal fat and lean mass (g). Outcomes in standard deviations.

Lean mass (sd)



Fat mass (sd)



3.13.1 Mutually independent maternal predictors of neonatal body composition

The mutually independent predictors of neonatal birthweight (Table 34), lean mass (Table 35), fat mass (Table 36), percentage fat (Table 37) and percentage lean (Table 38) included maternal height, parity, and triceps skinfold thickness, smoking and walking speed in late pregnancy. Whilst triceps skinfold thickness remained a strong determinant of neonatal fat mass in the multivariate model, it did not retain a statistically significant association with lean mass. For percentage fat and lean, maternal height became statistically non-significant, but parity, triceps skinfold thickness, walking speed and smoking remained although the associations were in opposite directions. Thus smokers and faster walkers had offspring with less percentage fat and more percentage lean, whereas mothers with increased adiposity and parity had offspring with greater percentage fat and less percentage lean.

Table 34 Mutually independent maternal predictors of birthweight (g) (R^2=0.16, n=776).

Predictor	β	p	95% CI
Height (cm)	14.9	<0.001	10.3 to 19.5
Parity (4 groups)	121.5	<0.001	85.7 to 157.3
LP TSF (sd)	50.9	0.001	22.2 to 79.6
LP smoking (Y/N)	-260.1	<0.001	-344.5 to -175.7
LP walking speed (4 groups)	-58.8	0.002	-95.5 to -22.2

LP: Late pregnancy; TSF: Triceps skinfold thickness

Table 35 Mutually independent maternal predictors of neonatal lean mass (g) (R^2=0.12, n=786).

Predictor	β	p	95% CI
Height (cm)	9.8	<0.001	6.6 to 13.0
Parity (4 groups)	87.2	<0.001	62.6 to 111.8
LP smoking (Y/N)	-139.2	<0.001	-197.2 to -81.3
LP walking speed (4 groups)	-25.5	0.044	-50.3 to -0.63

LP: Late pregnancy

Table 36 Mutually independent maternal predictors of neonatal fat mass (g) (R^2=0.12, n=784).

Predictor	β	p	95% CI
Height (cm)	0.017	0.002	0.007 to 0.028
Parity (4 groups)	0.288	<0.001	0.205 to 0.371
LP TSF (sd)	0.145	<0.001	0.078 to 0.212
LP smoking (Y/N)	-0.145	0.001	-0.230 to -0.059
LP walking speed (4 groups)	-0.386	<0.001	-0.581 to -0.190

LP: Late pregnancy; TSF: Triceps skinfold thickness

Table 37 Mutually independent maternal predictors of neonatal % lean (R^2=0.09, n=785).

Predictor	β	p	95% CI
Parity (4 groups)	-0.943	<0.001	-1.300 to -0.586
LP TSF (sd)	-0.682	<0.001	-0.969 to -0.396
LP smoking (Y/N)	1.253	0.003	0.417 to 2.088
LP walking speed (4 groups)	0.546	0.003	0.181 to 0.910

LP: Late pregnancy; TSF: Triceps skinfold thickness

Table 38 Mutually independent maternal predictors of neonatal % fat (sd) (R^2=0.09, n=785).

Predictor	β	p	95% CI
Parity (4 groups)	0.238	<0.001	0.153 to 0.322
LP TSF (sd)	0.156	<0.001	0.088 to 0.223
LP smoking (Y/N)	-0.128	0.003	-0.214 to -0.042
LP walking speed (4 groups)	-0.310	0.002	-0.507 to -0.113

LP: Late pregnancy; TSF: Triceps skinfold thickness

3.14 Summary discussion

3.14.1 Neonatal bone mineral

The results of this work form part of a consistent pattern incorporating 3 different cohorts of babies(150;151). Thus this study has replicated previous findings that maternal height, adiposity, physical activity and smoking are determinants of intrauterine bone mineral accrual. Additionally, maternal birthweight was again positively associated with neonatal bone mass.

Although the influence of maternal smoking, triceps skinfold thickness and walking speed appeared to strengthen from before pregnancy through to early and then late gestation, the change in triceps skinfold thickness did not predict bone mass in the neonate. This suggests that foetal growth may be more sensitive to perturbations in the environmental milieu in late pregnancy than in early. However, most of the late pregnancy measurement was explained by that before conception, suggesting that pre-pregnancy nutritional status is important.

Increasing birth order was associated with increasing bone mass and birthweight. It is not clear why this should be so, but may involve increasing maturity of the uterine vasculature or epigenetic modulation of expression of genes involved in placental nutrient flux.

The overall patterns in the boys and girls were similar. Although the associations between BMC and skinfold thickness or walking speed did not reach statistical significance in the girls, the interaction terms were not statistically significant, suggesting that these findings were due to statistical artefact rather than real differences. This is in contrast with the findings for paternal bone mineral, where a more significant gender dichotomy was apparent in the relationship with offspring bone mass.

3.14.2 Maternal 25(OH)-vitamin D

These data are consistent with previous findings that mothers who are deficient in 25D in pregnancy give birth to offspring who have decreased bone mineral in childhood(67). The association was independent of maternal lifestyle, but was weakened by maternal height. This seems likely to be a spurious finding, as there was no significant association between height and 25D status, and it is difficult to think of a plausible biological explanation for this. Previous work has shown that the main determinants of maternal 25D status are hours of sunshine per day and use of 25D supplements(67). These data are not yet available in this

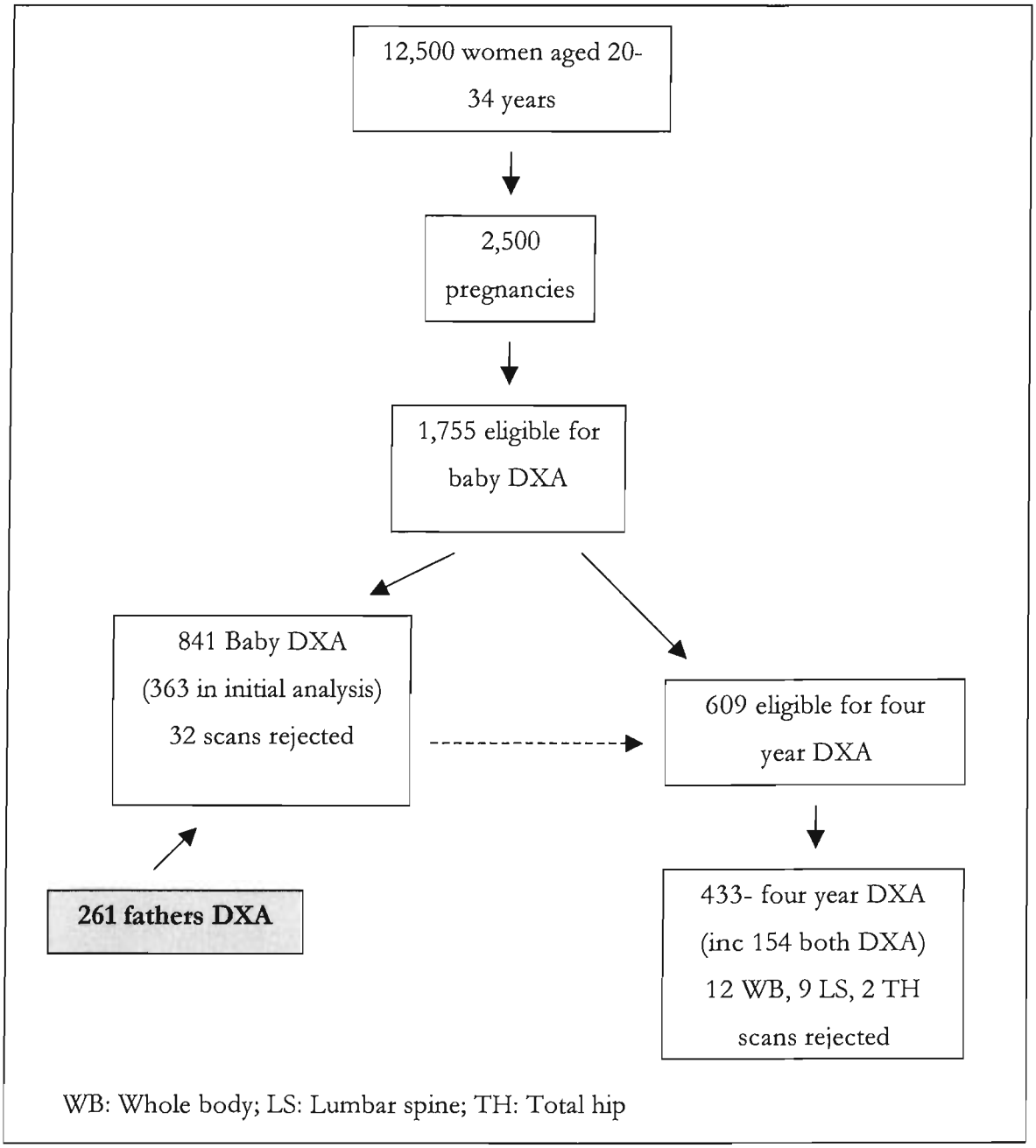
cohort, but future work may be able to include them. The association was only present in the girls, again consistent with previous work. Since the gender-25(OH)-vitamin D interaction term did not attain statistical significance, it is possible that in this SWS cohort there either was not a real gender difference, or there was insufficient statistical power to demonstrate it.

3.14.3 Neonatal body composition and birthweight

Neonatal birthweight and body composition were influenced by maternal height, parity, smoking, adiposity and walking speed. Maternal triceps skinfold thickness remained a strong predictor of neonatal fat mass, but not lean mass, in multivariate modelling, suggesting either a genetic association, or that maternal nutritional status tends to affect neonatal fat mass primarily. Mothers who smoked had offspring with lower birthweight, lean and fat mass, and this is consistent with previous findings. Smoking has a detrimental effect on placental blood flow and toxins from inhaled smoke may directly inhibit foetal growth. Maternal physical activity, assessed by walking speed, was associated negatively with birthweight, lean mass, fat mass, and percentage fat mass, but positively with percentage lean mass, possibly reflecting competition between mother and foetus for finite resources.

4 Paternal predictors of neonatal body composition

Figure 12 Paternal cohort (shaded box).



4.1 Aim

To explore the paternal determinants of neonatal bone mineral accrual and body composition.

4.2 Summary of methods

Details of the methods are given in Section 2. Briefly, the fathers were invited to attend for assessment of bone mass and body composition by DXA, diet, lifestyle and health by questionnaire, and anthropometric measures.

4.3 Analysis

Paternal weight, fat mass and percentage fat mass were skewed and thus transformed to normality by log-transformation. Pearson correlation was used to compare the strength of association between paternal and neonatal DXA measures, and regression methods to estimate the slope of the associations.

4.4 Results

After matching to neonatal scans and maternal initial data, there were 261 father-mother-neonate triplets. The mean height (sd) of the fathers was 178.3 cm (6.6 cm). Father's weight was not normally distributed so was log-transformed and standardised. The median weight was 82 kg (IQR 75-92 kg). There was a significant negative association between father's age and height (β=-0.24 cm/year, p=0.002), and thus paternal height was adjusted for age at DXA assessment. Table 39 summarises the anthropometric and DXA measures in the fathers.

Table 39 Paternal age, height, weight and body mass index (BMI).

Characteristic	Summary	n
Age, years (mean, sd)	33.9 (5.2)	261
Height, cm (mean, sd)	178.3 (6.5)	261
Weight, kg (median, IQR)	82 (75 to 92)	261
BMI, kg/m^2 (median, IQR)	26.0 (23.6 to 28.7)	261

4.4.1 Paternal anthropometry, and neonatal bone mass and body composition

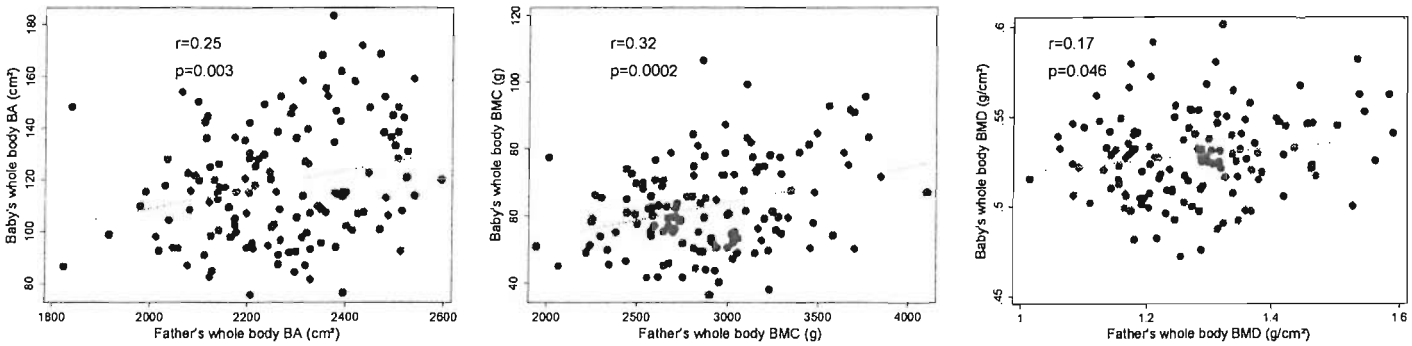
Paternal height strongly predicted birthweight of the offspring, such that birthweight increased by 11g for each 1 cm increase in paternal height (p=0.008). There was a trend towards greater neonatal BA as paternal height increased (β=0.4 cm^2/cm, p=0.056). Paternal BMI did not predict birthweight or any of the DXA indices in the offspring. When maternal height and fat stores (measured by triceps skinfold thickness) were added into the regression models, the paternal-offspring relationships remained robust. Maternal and paternal height predicted neonatal birthweight similarly (β for mother's height=10.5 g/cm, p=0.010 vs 11.0 g/cm, p=0.008), but neonatal BMC was more strongly related to maternal than paternal height (β for maternal height to BMC=0.33 g/cm, p=0.008 vs 0.21 g/cm, p=0.11 for paternal height). This may have been due to lower power as a consequence of the lower number of fathers than mothers in the sample.

4.4.2 Paternal DXA indices and neonatal bone mass and body composition

When the neonates were analysed together, there were statistically significant associations between the paternal and the corresponding neonatal DXA indices. Thus paternal BA predicted neonatal BA (r=0.15, p=0.01), and paternal BMC and BMD were associated with neonatal BMC and BMD (r=0.19 and 0.13, p=0.001 and 0.027 respectively). However, when these associations were explored by offspring gender, it was apparent that the father-daughter relationships accounted for these associations, and that the father-son relationships did not reach statistical significance (Table 40 and Figure 13). The gender-BMC interaction term was of borderline statistical significance (p=0.064), and limiting the analysis to the lowest 75% of birthweight (in case the observation was due to the lower mean birth size of the girls) did not change the relationships. Thus, in the female offspring, increasing paternal BA, BMC and BMD were associated with increasing skeletal size, and also increasing lean and fat mass. Paternal skeletal size negatively predicted percentage lean mass in the offspring and positively percentage fat mass and bone mass. Paternal percentage bone mass was positively associated with neonatal percentage bone mass, and paternal lean mass positively predicted neonatal lean mass. However, there were no other statistically significant associations between measures of paternal and neonatal body composition.

When mother's height, triceps skinfold thickness, smoking, and milk intake were included in multivariate regression models of father's DXA indices, the associations for paternal variables remained statistically significant (BA: p=0.02, BMC: p=0.001, BMD: p=0.02).

Figure 13 Paternal and neonatal DXA indices for female offspring.



4.5 Summary discussion

Paternal skeletal size and density were positively associated with intrauterine bone mineral accrual among female offspring. These associations were independent of the mother's body build. The strongest associations were between corresponding paternal and neonatal DXA indices, and the pattern suggests the possibility of discordance between bone size and density: Paternal BA, BMC and BMD predicted neonatal BA and BMC more strongly that BMD, which is a partly size corrected measure. However, to fully investigate the influences on volumetric density, a different measurement technique such as pQ CT would be needed. The strong gender discordance in the associations raises intriguing mechanistic possibilities and these are considered further the final discussion.

Table 40 Paternal and neonatal bone mineral and body composition by neonatal gender (Pearson correlation coefficient and *p value*; statistically significant associations in bold).

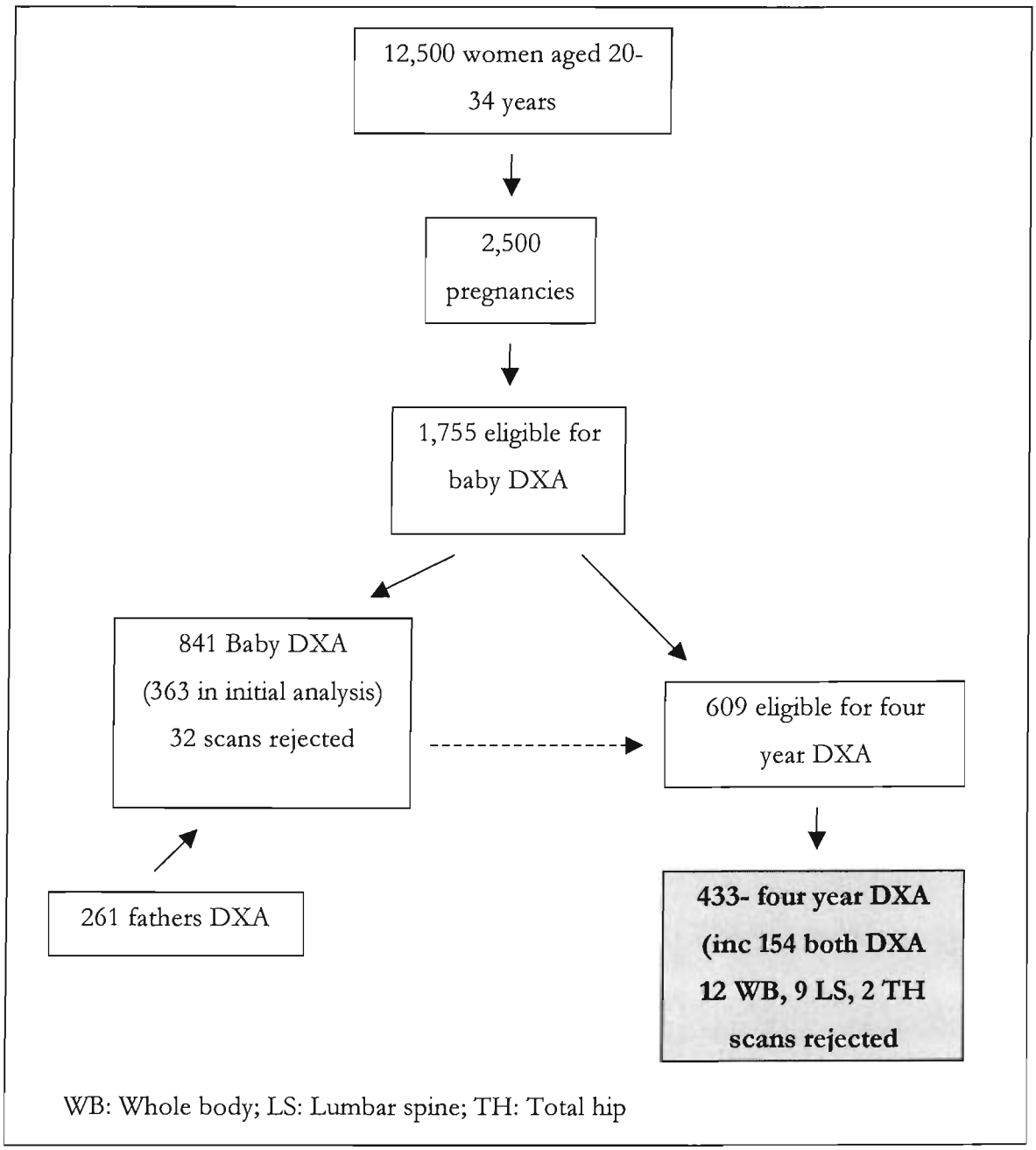
	Baby boys							
Father	**BA**	**BMC**	**BMD**	**%BMC**	**Lean**	**%Lean**	**Fat**	**%Fat**
BA	0.06	0.05	-0.03	-0.03	0.15	0.04	0.006	-0.04
	0.48	*0.55*	*0.75*	*0.70*	*0.085*	*0.63*	*0.94*	*0.62*
BMC	0.07	0.07	0.05	0.03	0.13	0.04	-0.004	-0.05
	0.43	*0.41*	*0.57*	*0.75*	*0.11*	*0.60*	*0.96*	*0.58*
BMD	0.06	0.07	0.11	0.07	0.09	0.03	-0.008	-0.03
	0.48	*0.38*	*0.21*	*0.38*	*0.27*	*0.70*	*0.93*	*0.68*
%BMC	0.07	0.09	0.08	0.04	0.11	0.01	0.01	-0.02
	0.38	*0.31*	*0.32*	*0.61*	*0.18*	*0.87*	*0.88*	*0.79*
Lean	-0.012	-0.02	-0.03	-0.05	0.04	0.03	-0.01	-0.03
	0.89	*0.82*	*0.71*	*0.56*	*0.66*	*0.74*	*0.89*	*0.74*
%Lean	-0.03	-0.03	-0.03	-0.08	0.02	0.02	-0.02	-0.03
	0.73	*0.72*	*0.78*	*0.33*	*0.81*	*0.80*	*0.86*	*0.69*
Fat*	0.02	0.01	-0.01	0.05	-0.02	-0.01	0.009	0.02
	0.85	*0.89*	*0.94*	*0.56*	*0.86*	*0.89*	*0.91*	*0.79*
%Fat*	0.02	0.02	0.01	0.07	-0.03	-0.02	0.01	0.03
	0.81	*0.81*	*0.89*	*0.39*	*0.73*	*0.80*	*0.88*	*0.70*

	Baby girls							
Father	**BA**	**BMC**	**BMD**	**%BMC**	**Lean**	**%Lean**	**Fat**	**%Fat**
BA	**0.25**	**0.23**	0.003	0.11	**0.27**	-0.13	**0.19**	0.13
	0.003	***0.008***	*0.98*	*0.19*	***0.001***	*0.13*	***0.027***	*0.12*
BMC	**0.33**	**0.32**	0.12	**0.22**	**0.25**	**-0.18**	**0.22**	0.17
	0.0001	***0.0002***	*0.17*	***0.009***	***0.0037***	***0.034***	***0.009***	*0.052*
BMD	**0.29**	**0.30**	**0.17**	**0.25**	0.16	**-0.17**	**0.19**	0.15
	0.0005	***0.0003***	***0.046***	***0.0037***	*0.057*	***0.048***	***0.029***	*0.091*
%BMC	0.16	0.16	0.099	**0.17**	0.01	-0.14	0.12	0.12
	0.065	*0.058*	*0.25*	***0.049***	*0.91*	*0.11*	*0.17*	*0.15*
Lean	**0.18**	0.17	0.001	0.02	**0.27**	-0.08	0.15	0.08
	0.03	*0.054*	*0.99*	*0.78*	***0.002***	*0.36*	*0.089*	*0.36*
%Lean	-0.001	-0.004	-0.02	-0.05	0.002	-0.06	0.06	0.07
	0.99	*0.96*	*0.78*	*0.54*	*0.98*	*0.46*	*0.46*	*0.44*
Fat*	0.06	0.06	0.01	0.05	0.08	0.02	-0.003	-0.02
	0.49	*0.52*	*0.87*	*0.55*	*0.36*	*0.79*	*0.97*	*0.78*
%Fat*	-0.01	-0.008	0.02	0.04	-0.003	0.07	-0.07	-0.07
	0.90	*0.92*	*0.85*	*0.66*	*0.97*	*0.41*	*0.42*	*0.41*

* log-transformed.

5 Four year follow up of children in the Southampton Women's Survey: Aims and Methods

Figure 14 Four-year DXA cohort (shaded box).



5.1 Aims

- To test the hypothesis that that maternal lifestyle, physical activity and body build are still associated with bone mass in the offspring at 4 years old.
- To test the hypothesis that maternal vitamin D status in late pregnancy predicts bone mineral in her child at four years old.
- To determine whether contemporary childhood factors, such as milk intake and physical activity, are associated with childhood bone mineral.

5.2 Methods

Detailed methods are described in section 2.4. Briefly, the mothers were invited by letter and then telephone to bring their child at 4 years old to the Osteoporosis Centre in Southampton General Hospital for a DXA scan at whole body, lumbar spine an hip sites. The child's diet, lifestyle and physical activity were assessed by questionnaire, and they underwent measurement of height, weight, mid-upper arm circumference and grip strength. In a small sub-group of mothers and children, physical activity was objectively measured over a 7-day period using a combined heart rate and activity monitor.

5.3 Analysis

The DXA data were transferred regularly to secure servers at the MRC unit via CD. The scans were analysed at the visit by a trained DXA technician, using the automated paediatric software (Vertec Scientific Ltd, Reading, UK). The data were amalgamated with parental pre-, early and late pregnancy data. 4 year fat and percentage fat mass were not normally distributed, and it was found that, in this case, a square-root transformation was most appropriate. These variables were then standardised, giving a measurement in sd. The early life determinants of 4-year old bone mass explored using linear regression techniques. The predictors of catch-up growth were explored using logistic regression. It was apparent that there was some unreliability in the DXA software analysis of measurements of bone mineral at the femoral neck, trochanter and Ward's triangle sites. Measurement at the total hip site appeared more uniformly reliable, and so this site was used exclusively. Movement artefact was most apparent at the head, and head bone mineral accounts for a high percentage of whole body BMC, but not much to linear growth. Thus at 4 years, the whole body measurements are for whole body minus head. Total daily energy expenditure, measured by

Actiheart, was available for a minority of children to give a more objective measure of physical activity.

6 Results: Characterisation of the mothers and children who attended childhood DXA assessment at 4 years

6.1 General

This chapter contains the detailed characterisation of the mothers and children comprising the subset defined by the child undergoing DXA assessment at 4 years old. The text gives an overview of the important findings, whilst the details are contained in the tables. 609 four-year olds were eligible for assessment, and owing to difficulties with contacting their mothers, and refusals/ non-attendance, the number scanned was 433 (Figure 14). 12 whole body, 9 lumbar spine and 2 total hip scans were found to be unusable because of movement artefact and were excluded.

6.2 Maternal characteristics

Data on 433 mother-child pairs were available. The mean (sd) age of the mothers was 28.4 years (3.7 years) and 46% were in their first pregnancy. Social class data were available for 411 mothers and are summarised in Table 41.

6.2.1 Anthropometry

Maternal anthropometric measures before and in early and late pregnancy are summarised in Table 41. Median maternal triceps skinfold thickness increased with each successive time point, however this increase was not statistically significant.

6.2.2 Lifestyle and diet

23.3% (n=433) mothers smoked before pregnancy and 14% in early (n=345) and late (n=416) pregnancy. Mothers in late pregnancy tended to drink more milk and less alcohol than mothers in early gestation or before pregnancy. There was also a trend towards decreased levels of physical activity from before to early to late pregnancy (Table 42 and Table 43).

Table 41 Maternal demographics, anthropometry and smoking before (PP), and in early (EP) and late (LP) pregnancy.

Characteristic			n
Age, years (mean (sd))		28.4 (3.7)	433
Birthweight, g (mean (sd))		3235.2 (541.9)	306
Percentage nulliparous		46	433
Social class (%)	I	5.4	22
	II	41.6	171
	IIIn	36.8	151
	IIIm	6.1	25
	IV	9.3	38
	V	1.0	4
Height, cm (mean,sd)		163.4 (6.8)	429
PP weight, kg (median, IQR)		65.4 (59.3 to 73.2)	430
PP BMI, kg/m^2 (median, IQR)		24.2 (22.2 to 27.5)	429
PP triceps skinfold, mm (median, IQR)		19.0 (15.0 to 25.0)	427
EP triceps skinfold thickness, mm (median, IQR)		19.3 (15.7 to 25.1)	345
LP triceps skinfold thickness, mm (median, IQR)		21.0 (16.8 to 26.1)	416
PP smoking, %		23.3	101/433
EP smoking, %		14.2	49/345
LP smoking, %		13.9	58/416

Table 42 Maternal physical activity before (PP), and during early (EP) and late (LP) pregnancy.

Characteristic		PP	EP	LP
Walking speed (%)	V slow	3 (0.7)	1 (0.3)	69 (16.6)
	Easy pace	28 (6.5)	50 (14.5)	224 (53.9)
	Normal	170 (39.3)	187 (54.2)	102 (24.5)
	Fairly brisk	215 (49.7)	90 (26.1)	20 (4.8)
	Fast	17 (3.9)	17 (4.9)	1 (0.2)
Strenuous exercise, hrs/week (%)	0.00	173 (40.3)	203 (58.8)	306 (73.6)
	to 0.25	69 (16.1)	52 (15.1)	47 (11.3)
	to 1.25	60 (14.0)	41 (11.9)	36 (8.7)
	> 1.25	127 (29.6)	49 (14.2)	27 (6.5)

Table 43 Maternal alcohol and milk intake before (PP) and in early (EP) and late (LP) pregnancy.

Characteristic		PP	EP	LP
PP units alcohol per week (%)	0 to 1.5	128 (29.6)	276 (80.5)	345 (82.9)
	to 4.5	102 (23.6)	43 (12.5)	61 (14.7)
	to 10.0	102 (23.6)	15 (4.4)	8 (1.9)
	> 10.0	101 (23.3)	9 (2.6)	2 (0.5)
PP pints milk per week (%)	0 to 2.0	96 (22.2)	94 (27.3)	48 (11.5)
	to 3.5	165 (38.1)	99 (28.7)	126 (30.3)
	to 6.0	68 (15.7)	46 (13.3)	65 (15.6)
	> 6.0	104 (24.0)	106 (30.7)	177 (42.6)

6.2.3 Maternal social class

As expected, there were statistically significant associations between maternal social class and each of smoking habits and alcohol intake. (Table 44 and Table 48).

Table 44 Maternal social class and late pregnancy (LP) smoking (χ^2 for trend, p=0.0002).

	Mother's social class						
LP smoking	**I**	**II**	**IIIn**	**IIIm**	**IV**	**V**	**Total**
No	20	153	117	21.0	27	3	341
%	95.2	92.2	82.4	84.0	71.1	75	86.1
Yes	1	13	25	4.0	11	1	55
%	4.8	7.8	17.6	16.0	29.0	25	13.9
Total	21	166	142	25.0	38	4	396
	100	100	100	100.0	100	100	100

Thus, in late pregnancy 4.8% and 7.8% mothers in social classes I and II smoked respectively, compared with 29.0 % in class IV. When the analysis was limited to those mothers assessed at all time points, of those who were smoking before pregnancy, 42.5% had given up by early (Table 45) and 45.2% by late pregnancy (Table 47). Of those mothers who smoked in early pregnancy 85.1 % were still smoking in late gestation (Table 46).

Table 45 Maternal smoking before and in early pregnancy (χ^2, p<0.001).

Pre-pregnancy smoking	**Early pregnancy smoking**		
	No	Yes	Total
No	255	5	260
%	98.1	1.9	100
Yes	31	42	73
%	42.5	57.5	100
Total	286	47	333
%	85.9	14.1	100

Table 46 Maternal smoking before and in late pregnancy (χ^2, p<0.001).

Pre-pregnancy smoking	**Late pregnancy smoking**		
	No	Yes	Total
No	256	4	260
%	98.5	1.5	100
Yes	33	40	73
%	45.2	54.8	100
Total	289	44	333
%	86.8	13.2	100

Table 47 Maternal smoking in early and late pregnancy (χ^2, $p<0.001$).

Early pregnancy smoking	Late pregnancy smoking		
	No	Yes	Total
No	282	4	286
%	98.6	1.4	100
Yes	7	40	47
%	14.9	85.1	100
Total	289	44	333
%	86.8	13.2	100

As there were very low numbers of participants in social class V, and very few participants at higher levels of alcohol intake, social classes IV and V were merged, and also the highest categories of alcohol intake. After these adjustments, maternal alcohol intake also varied by social class before and in late pregnancy (Table 48), although there was not a particularly striking pattern.

Table 48 Maternal social class and alcohol intake (units per week) in late pregnancy (χ^2, $p=0.032$).

	Mother's social class					
LP alcohol (units/ week)	**I**	**II**	**IIIn**	**IIIm**	**IV, V**	**Total**
- 1.5	13	134	124	23	34	328
%	61.9	80.7	87.3	92.0	81.0	82.8
≥ 4.5	8	32	18	2	8	59
%	38.1	19.3	12.7	8.0	19.1	14.9
Total	21	166	142	25	38	396
%	100	100	100	100.0	100	100

A small number of participants at in class I taking up to 0.25 hours of strenuous exercise per week and in class IV taking more than 1.25 hours per week led to a statistically modest difference in levels of strenuous activity across social class, but when the highest levels of activity were grouped together, there was no statistical difference across the group. After grouping the "fairly brisk" and "fast" walkers together in late pregnancy (as there was only 1 participant in the latter category), again there was no difference across social class.

6.2.4 Comparison with mothers whose children underwent neonatal DXA assessment

There were two, overlapping, groups of mothers in this study: Those mothers whose children underwent DXA at birth and those whose children underwent DXA at 4 years, with a subset of children attending both visits. It was therefore difficult to compare the two groups directly, and so two groups were defined firstly with the overlap included in the birth DXA group and secondly in the 4-year DXA group. Comparison of the two groups defined in either way demonstrated no significant differences in the measurements reported between them, apart from parity. Children who underwent the 4 year scan were thus on average of higher birth order than those who did not. Their mothers seemed to smoke slightly less, but this difference was not statistically significant, using the χ^2 test. Table 49 summarises the pre-pregnancy findings in the different groups, including the two birth cohorts. There were no differences in pregnancy anthropometric, lifestyle or physical activity measures between the groups.

Table 49 Comparison of mothers in different cohorts

Measurement	Initial (363)	Complete-initial	All 4 year scans	Birth scan only	All birth scans	4 year scan only
Age	28.5	28.0	28.3	28.1	28.2	28.2
Height	163.2	163.5	163.4	163.2	163.4	163.1
% nulliparous	54.9	47.5	43.9	51.7	51.7	43.9
PP smoking %	27.5	26.5	24.9	27.0	27.0	24.9
PP TSF, mm	19.0	19.0	19.2	19.0	19.0	19.2
LP smoking %	14.5	12.1	13.3	14.4	13.4	15.8
LP TSF, mm	19.0	19.0	20.7	20.5	20.6	20.7

PP: Pre-pregnancy; LP: Late pregnancy

6.3 Characterisation of 4-year old children

6.3.1 Introduction

This chapter summarises the baseline characteristics of the children at 4 years, by gender. There were 433 children with 4-year DXA data, and of these 154 also had undergone DXA assessment at birth. Not all 4-year old children were happy to have a whole body scan, so the

available data at 4 years are either whole body, lumbar spine and total hip scans or a combination of these components, shown in Table 50.

Table 50 Scan numbers at each site

Site	Number	%
WB, LS, Hip	397	91.7
WB only	9	2.1
WB & LS	3	0.7
WB & Hip	7	1.6
LS & Hip	15	3.5
LS only	1	0.2
Hip only	1	0.2

WB: Whole body; LS: Lumbar spine

6.3.2 Anthropometric and DXA measures

As at birth, there were statistically significant positive associations between gestational age and 4-year height (β=0.04 cm/day, p=0.003), weight (β=0.03 kg/day, p=0.011) and measures of bone mineral at 4 years (Table 51). Although the relationships between age at DXA and bone mineral did not attain statistical significance, it was felt that age standardisation was appropriate.

Table 51 Gestational age, age at DXA and whole body, lumbar spine and total hip bone mineral at 4 years.

	BA, cm^2	BMC, g	BMCh, g/cm	BMD, g/cm^2
Whole body				
Gestational age (days)	0.48, 0.011	0.51, 0.003	0.07, 0.555	0.0004, 0.005
Age at DXA (years)	13.5, 0.722	33.9, 0.330	9.0, 0.689	0.04, 0.158
Lumbar spine				
Gestational age (days)	0.0002, 0.983	0.009, 0.272	-	0.0003, 0.111
Age at DXA (years)	2.1, 0.297	1.3, 0.421	-	0.01, 0.754
Total Hip				
Gestational age (days)	0.02, <0.001	0.02, 0.001	-	0.0003, 0.142
Age at DXA (years)	-1.0, 0.411	-0.3, 0.754	-	0.03, 0.569

Table shows β, p value. BA: Bone area; BMC: Bone mineral content; BMCh: BMC for height; BMD: Bone mineral density

Table 52 shows the anthropometric and DXA measures of the children at 4 years (DXA measures, height and weight adjusted for gestational age and age at DXA scan). The boys had

higher birthweight, but there was no difference in age, height or weight at the 4-year visit. In contrast to the neonatal patterns, the girls had higher whole body BA and BMC, with the boys showing a statistically non-significant trend towards higher BMD. The boys did continue to have higher lean and percentage lean, and lower fat and percentage fat than the girls. Left sided mid-upper arm circumference seemed slightly higher in girls than boys, but this did not attain statistical significance. Grip strength for both right and left hand was greater in the boys. Lumbar spine BA was higher in the boys, but because lumbar spine BMC was not also significantly higher, BMD at this site was greater in the girls. Although total hip BA was similar in bother genders, the higher BMC in the boys led also to higher BMD.

Table 52 Anthropometric and DXA measures of the children at 4 years.

Characteristic	Boys	n	Girls	n	p diff.
Gestational age, days	277.9 (276.3 to 279.4)	238	278.6 (276.5 to 280.7)	194	0.579
Birthweight, kg	3.54 (3.48 to 3.60)	233	3.38 (3.32 to 3.44)	194	0.0002
Age at DXA, years	4.13 (4.13 to 4.14)	239	4.13 (4.12 to 4.14)	194	0.344
Height, cm	104.1 (103.4 to 104.6)	221	103.5 (102.8 to 104.1)	179	0.148
Weight, kg*	17.5 (17.2 to 17.8)	221	17.1 (16.8 to 17.5)	179	0.090
BMI, kg/m²*	16.2 (16.0 to 16.4)	220	16.0 (15.8 to 16.2)	179	0.277
Left MUAC, mm*	17.2 (17.1 to 17.4)	233	17.4 (17.2 to 17.6)	191	0.168
Right grip strength, kg	7.9 (7.6 to 8.1)	231	7.2 (6.9 to 7.5)	189	0.0005
Left grip strength, kg	7.3 (7.1 to 7.6)	232	7.0 (6.7 to 7.2)	189	0.036
WB BA, cm²	750.8 (744.5 to 757.2)	223	763.3 (755.6 to 771.0))	191	0.013
WB BMC, g	374.3 (368.5 to 380.1)	223	375.6 (49.6)	191	0.78
WB BMCh, g/cm	373.2 (369.0 to 377.5)	208	373.3 (369.1 to 377.5)	177	1.00
WB BMD, g/cm²	0.497 (0.492 to 0.502)	223	0.490 (0.485 to 0.496)	191	0.059
Total lean mass, kg	10.5 (10.3 to 10.6))	223	9.6 (9.4 to 9.8)	191	<0.0001
Total fat mass, kg*	3.8 (3.6 to 3.9)	223	4.4 (4.2 to 4.5)	191	<0.0001
Percent bone	2.57 (2.54 to 2.59)	223	2.61 (2.58 to 2.64)	191	0.037
Percent lean	71.2 (70.6 to 71.9)	223	66.5 (65.7 to 67.2)	191	<0.0001
Percent fat*	0.77 (0.68 to 0.87)	223	1.36 (1.18 to 1.57)	191	<0.0001
LS BA, cm²	27.9 (27.5 to 28.2)	229	26.5 (26.2 to 26.9)	186	<0.0001
LS BMC, g	13.4 (13.1 to 13.7)	229	13.0 (12.7 to 13.4))	186	0.110
LS BMD, g/cm²	0.480 (0.473 to 0.486)	229	0.490 (0.482 to 0.498)	186	0.044
Hip BA, cm²	11.6 (11.4 to 11.9)	232	11.6 (11.3 to 11.8)	187	0.551
Hip BMC, g	6.7 (6.5 to 6.8)	232	6.3 (6.1 to 6.5)	187	0.001
Hip BMD, g/cm²	0.0.572 (0.564 to 0.580)	232	0.539 (0.530 to 0.547)	187	<0.0001
TEE, kCal/day	1641 (1596 to 1686)	52	1468 (1411 to 1525)	35	<0.0001

Table shows mean and 95% CI; * geometric mean and 95% CI
BMI: Body mass index; MUAC: Mid-upper arm circumference; WB: Whole body; BA: Bone area; BMC: Bone mineral content; BMD: Areal bone mineral density; BMCh: BMC for height; LS: Lumbar spine; TEE: Total energy expenditure per day.

6.3.3 Diet and lifestyle at 4 years

Daily milk and questionnaire measures of physical activity were not normally distributed, and were therefore re-grouped into tertiles. Daily milk intake (a sum of all milk intake per day including each different type of milk drunk) was slightly greater in the boys than the girls (Table 53). There were no differences in the time spent watching television (Table 54), with the majority of children watching between 1 and 3 hours per day. Although time spent sitting down (Table 55), on feet (Table 56), or in moderate physical activity (Table 57) was similar in both genders, the boys did spend significantly more time than the girls in strenuous physical activity (Table 58). Thus 34.0% boys and 22.1% girls spent more than 3 hours per day at this level of activity (p=0.022). However, there was a marked difference in total daily energy expenditure, measured using the Actiheart instrument, with the boys expending significantly more energy per day than the girls. There were no statistically significant associations between maternal social class and any of these measures except for time spent in strenuous activity (p=0.024), although no clear pattern was evident here.

Table 53 Daily milk intake at 4 years (pints). p trend = 0.030

Daily milk intake, pints	Boys	Girls	Total
- 0.5	89 (39.4)	91 (47.9)	180 (43.3)
- 1.0	108 (47.8)	85 (44.7)	193 (46.4)
> 1.0	29 (12.8)	14 (7.4)	43 (10.3)
Total	226 (100.0)	190 (100.0)	416 (100.0)

Table shows number (percent).

Table 54 Hours spent watching television per day. p trend = 0.521

Hours television per day	Boys	Girls	Total
> 3.0	28 (12.0)	19 (9.9)	47 (11.1)
2.0 – 3.0	89 (38.2)	72 (37.5)	161 (37.9)
1.0 – 2.0	90 (38.6)	79 (41.2)	169 (39.8)
< 1.0	26 (11.2)	22 (11.5)	48 (11.3)
Total	233 (100.0)	192 (100.0)	425 (100.0)

Table shows number (percent).

Table 55 Hours spent sitting down per day at 4 years. p trend = 0.243

Hours sitting per day		Boys	Girls	Total
	- 3.5	75 (32.5)	50 (26.7)	125 (29.9)
	- 5.0	95 (41.1)	82 (43.9)	177 (42.3)
	> 5.0	61 (26.4)	55 (29.4)	116 (27.8)
	Total	231 (100.0)	187 (100.0)	418 (100.0)

Table shows number (percent).

Table 56 Hours spent on feet per day at 4 years. p trend = 0.414

Hours on feet per day		Boys	Girls	Total
	- 7.5	82 (34.9)	74 (38.5)	156 (36.5)
	- 9.0	92 (39.2)	73 (38.0)	165 (38.6)
	> 9.0	61 (26.0)	45 (23.4)	106 (24.8)
	Total	235 (100.0)	192 (100.0)	427 (100.0)

Table shows number (percent).

Table 57 Hours spent in moderate activity per day at 4 years. p trend = 0.241

Hours in moderate activity per day		Boys	Girls	Total
	- 4.5	80 (34.2)	57 (29.8)	137 (32.2)
	- 6.0	89 (38.0)	72 (37.7)	161 (37.9)
	> 6.0	65 (27.8)	62 (32.5)	127 (29.9)
	Total	234 (100.0)	191 (100.0)	425 (100.0)

Table shows number (percent).

Table 58 Hours spent in strenuous activity per day at 4 years. p trend = 0.022

Hours in strenuous activity per day		Boys	Girls	Total
	- 1.5	55 (23.4)	53 (27.9)	108 (25.4)
	- 3.0	100 (42.6)	95 (50.0)	195 (45.9)
	> 3.0	80 (34.0)	42 (22.1)	122 (28.7)
	Total	235 (100.0)	190 (100.0)	425 (100.0)

Table shows number (percent)

6.4 Summary discussion

The mothers in the four-year cohort were similar to those in the birth cohort, and thus showed the expected associations between social class and smoking. However, the girls and boys at 4 years were more similar than at birth, with no statistically significant differences in height, weight or bone mass, but the girls having higher fat mass, and boys spending more

time in vigorous activity per day, and having a higher total energy expenditure per day. Additionally the boys drank a little more milk per day than the girls.

7 Results: Childhood determinants of 4-year old bone mineral

7.1 Introduction

Since it is likely that childhood factors such as diet, activity and body build will be determinants of contemporary bone mineral, these measures were explored before the maternal factors were examined. The aim of this strategy was to identify factors which might confound or obscure the relationships between maternal measurements and childhood bone mineral at 4 years. All DXA indices were adjusted for gestational age, age at DXA and gender (see preceding chapter). Birthweight was adjusted for gestational age and gender. Where mid-upper arm circumference, current weight and BMI were used as predictors, they were untransformed. When used as outcome variables, they were transformed to normality (inverse for MUAC and log for weight and BMI) and standardised. Whole body DXA measures were whole body minus the head. BMC for height was used as a method of size correction.

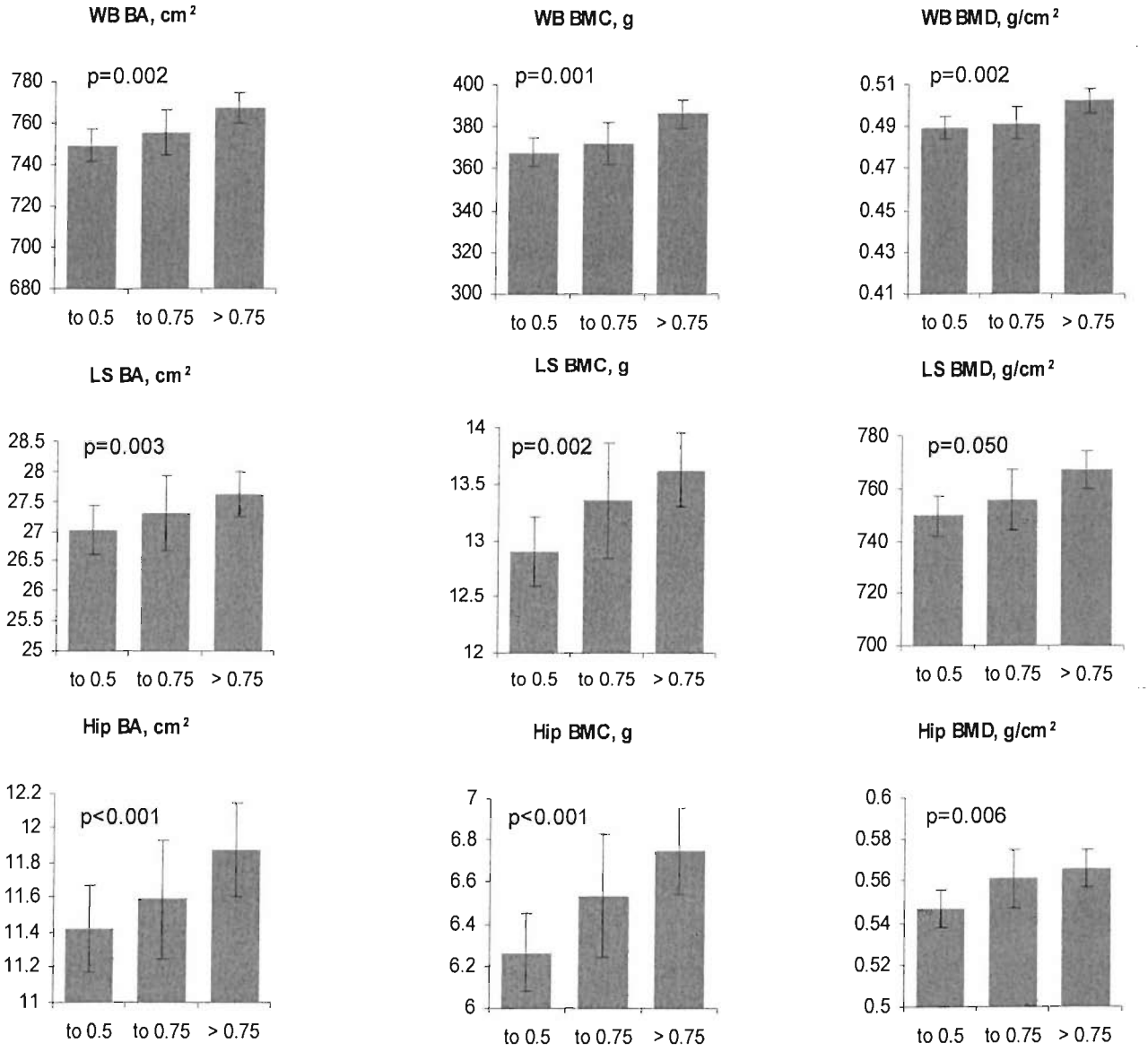
7.2 Childhood dietary influences

Detailed dietary data were not available in time for this thesis, so total milk intake was used a surrogate for calcium intake. Total milk intake per day was strongly associated with whole body BA, BMC and areal BMD, but not BMC for height at 4 years. BA, BMC and areal BMD at the lumbar spine and hip were also strongly associated with milk intake (Table 59 and Figure 15).

Table 59 Total milk intake at 4 years and whole body, lumbar spine and total hip bone mass.

	BA, cm^2		**BMC, g**		**BMD, g/cm^2**	
	β (95% CI) cm^2/pt	p	β (95% CI) g/pt	p	β (95% CI) g/cm^2/pt	p
Whole body						
Daily milk (pts)	19.4 (7.0 to 31.9)	0.002	20.3 (8.7 to 31.9)	0.001	0.015 (0.005 to 0.024)	0.002
Lumbar spine						
Daily milk (pts)	1.0 (0.4 to 1.7)	0.003	0.8 (0.3 to 1.4)	0.002	0.01 (0.00 to 0.03)	0.050
Hip						
Daily milk (pts)	0.7 (0.3 to 1.1)	<0.001	0.7 (0.3 to 1.0)	<0.001	0.021 (0.006 to 0.037)	0.006

Figure 15 Child's milk intake and bone mass at 4 years.



Milk intake in pints per day; BA: Bone area; BMC: Bone mineral content; BMD: Bone mineral density; LS: Lumbar spine

7.3 Childhood anthropometric influences

4-year height, weight, BMI, birthweight, mid-upper arm circumference and grip strength were all positively associated with BA, BMC and BMD at all three sites (Table 60). The relationship between height and weight and whole body BMC is shown in Figure 16.

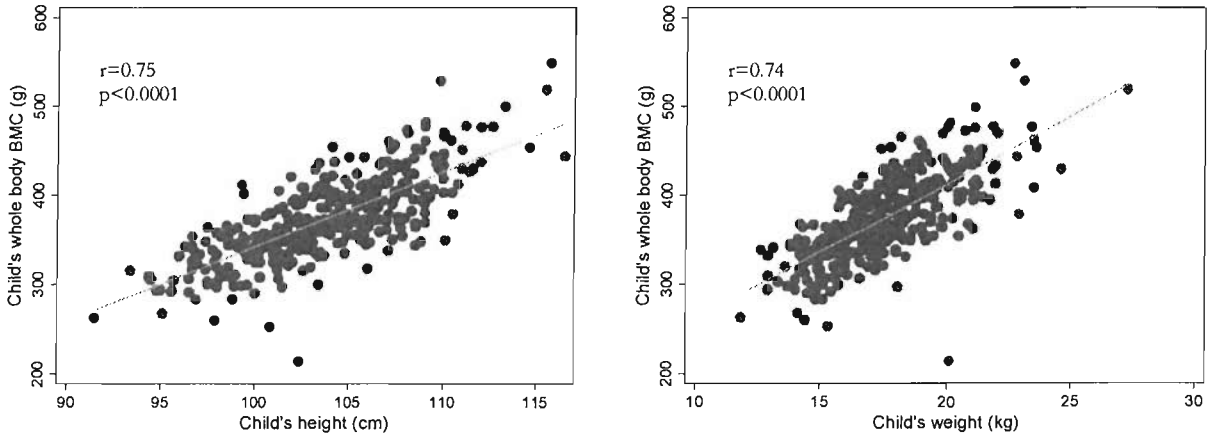


Figure 16 Four-year height and weight and whole body bone mineral content.

One child was found to have very low whole body BMC but normal weight and height. In a sensitivity analysis, inclusion of this subject did not significantly change the associations reported above.

Table 60 Anthropometric measures at 4 years and whole body, lumbar spine and total hip bone mass.

Whole body	BA		BMC		BMCh		BMD	
	β (95% CI) cm^2/	p	β (95% CI) g/	p	β (95% CI) g/cm/	p	β (95% CI) g/cm^2/	p
Height (cm)	8.2 (7.3 to 9.1)	<0.001	8.4 (7.7 to 9.1)	<0.001	-	<0.001	0.006 (0.005 to 0.006)	<0.001
Weight (kg)	12.7 (10.7 to 14.6)	<0.001	15.2 (13.8 to 16.6)	<0.001	3.9 (2.6 to 5.1)	<0.001	0.012 (0.010 to 0.012)	<0.001
BMI (kg/m^2)	7.3 (3.8 to 10.9)	<0.001	12.6 (9.5 to 15.6)	<0.001	8.7 (6.7 to 10.6)	<0.001	0.012 (0.009 to 0.014)	<0.001
MUAC (cm)	15.3 (12.0 to 18.6)	<0.001	19.0 (16.2 to 21.8)	<0.001	7.8 (5.7 to 9.9)	<0.001	0.015 (0.013 to 0.017)	<0.001
Birthweight (kg)	22.1 (13.3 to 30.9)	<0.001	20.1 (12.0 to 28.1)	<0.001	4.4 (-1.0 to 10.0)	0.110	0.00001 (0 to 0.00002)	<0.001
R grip strength (kg)	9.6 (6.8 to 12.3)	<0.001	111.5 (9.0 to 13.9)	<0.001	3.6 (1.8 to 5.3)	<0.001	0.009 (0.007 to 0.011)	<0.001
L grip strength (kg)	7.2 (4.5 to 10.0)	<0.001	9.6 (7.1 to 12.2)	<0.001	1.4 (-0.4 to 3.3)	0.121	0.008 (0.006 to 0.010)	<0.001

Lumbar spine	BA		BMC		BMD	
	β (95% CI) cm^2/	p	β (95% CI) g/	p	β (95% CI) g/cm^2/	p
Height (cm)	0.37 (0.32 to 0.42)	<0.001	0.34 (0.30 to 0.38)	<0.001	0.006 (0.005 to 0.007)	<0.001
Weight (kg)	0.55 (0.45 to 0.66)	<0.001	0.58 (0.50 to 0.65)	<0.001	0.011 (0.009 to 0.013)	<0.001
BMI (kg/m^2)	0.32 (0.12 to 0.51)	0.054	0.44 (0.29 to 0.59)	<0.001	0.010 (0.007 to 0.014)	<0.001
MUAC (cm)	0.46 (0.26 to 0.66)	<0.001	0.62 (0.47 to 0.76)	<0.001	0.014 (0.010 to 0.018)	<0.001
Birthweight (kg)	9.92 (0.53 to 1.45)	<0.001	0.72 (0.35 to 1.09)	<0.001	0.000 (0.000 to 0.018)	0.073
R grip strength (kg)	0.32 (0.16 to 0.48)	<0.001	0.41 (0.29 to 0.53)	<0.001	0.009 (0.006 to 0.012)	<0.001
L grip strength (kg)	0.41 (0.26 to 0.57)	<0.001	0.40 (0.29 to 0.52)	<0.001	0.007 (0.004 to 0.010)	<0.001

Table 60 continued

	BA		BMC		BMD	
Hip	β (95% CI) cm^2/	p	β (95% CI) g/	p	β (95% CI) g/cm^2/	p
Height (cm)	0.20 (0.16 to 0.23)	<0.001	0.17 (0.15 to 0.20)	<0.001	0.005 (0.004 to 0.006)	<0.001
Weight (kg)	0.34 (0.27 to 0.40)	<0.001	0.31 (0.26 to 0.36)	<0.001	0.010 (0.008 to 0.012)	<0.001
BMI (kg/m^2)	0.25 (0.14 to 0.37)	<0.001	0.26 (0.17 to 0.35)	<0.001	0.011 (0.006 to 0.014)	<0.001
MUAC (cm)	0.39 (0.27 to 0.51)	<0.001	0.36 (0.27 to 0.45)	<0.001	0.013 (0.009 to 0.017)	<0.001
Birthweight (kg)	0.63 (0.34 to 0.91)	<0.001	0.49 (0.27 to 0.72)	<0.001	0.013 (0.000 to 0.024)	0.016
R grip strength (kg)	0.26 (0.16 to 0.36)	<0.001	0.26 (0.19 to 0.34)	<0.001	0.010 (0.006 to 0.014)	<0.001
L grip strength (kg)	0.26 (0.16 to 0.35)	<0.001	0.25 (0.18 to 0.33)	<0.001	0.009 (0.006 to 0.012)	<0.001

BMI: Body mass index; MUAC: Mid-upper arm circumference (left)

7.4 Childhood lifestyle influences

There was a weak negative association between time spent in vigorous activity and whole body BA (β=-2.7 cm^2/hour, p=0.104). However, there was no similar association with BMC or BMD, although time on feet per day was positively associated with whole body areal BMD (β=0.002 g/cm^2/hour, p=0.089). No other questionnaire measures of physical activity were associated with whole body bone mineral. At the lumbar spine there were no associations between bone mineral and measures of physical activity, but at the hip, BA was negatively associated with time spent sitting (β=-0.15 cm^2/hour, p=0.004) and positively with time spent on feet per day (β= 0.13 cm^2/hour, p=0.006), with similar findings for BMC (β=-0.08 g/hour, p=0.038 for sitting and β=0.09 g/hour, p=0.020 for standing). However there were no statistically significant associations with areal BMD.

Total daily energy expenditure, however, was statistically significantly positively associated with bone mineral at all sites, and with total fat and lean (Figure 17, Figure 18 and Figure 19). The relationship with percentage fat was positive and with percentage lean negative, but these associations did not reach statistical significance.

Figure 17 Total daily energy expenditure and whole body bone mineral and body composition at 4 years.

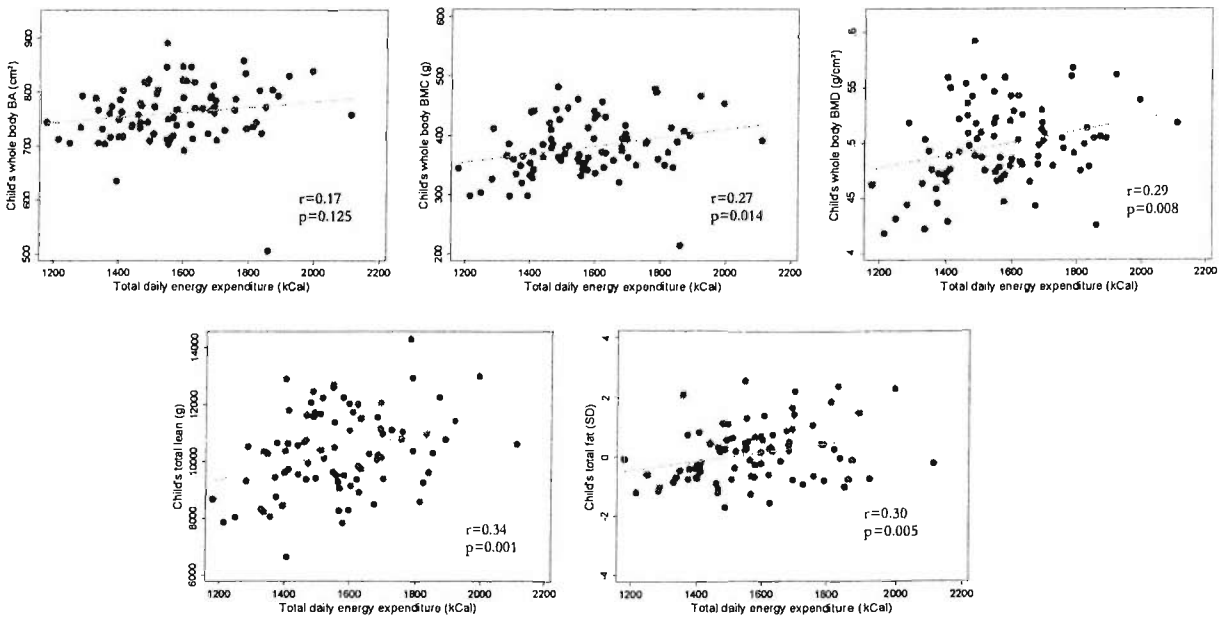


Figure 18 Daily total energy expenditure and lumbar spine bone mineral at 4 years.

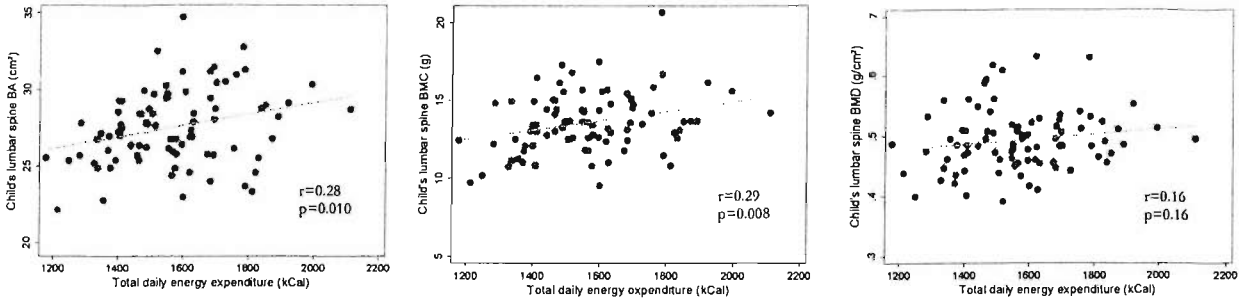
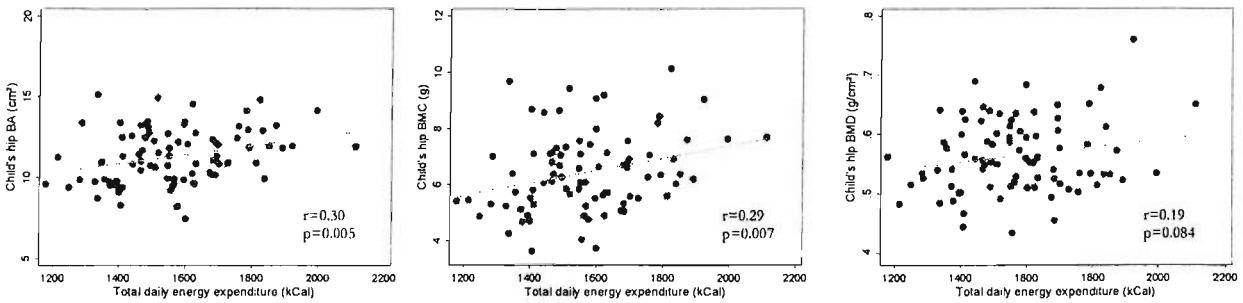


Figure 19 Daily total energy expenditure and total hip bone mineral at 4 years.



Hours spent watching television per day, having previously broken a bone, having a family history of low trauma fracture, taking a vitamin supplement, using fluoridated toothpaste, time outdoors per day, overall (maternally rated) health status and previous use of corticosteroids showed no robust associations with any measures of bone mineral at any site at 4 years.

7.5 Mutually independent childhood determinants of bone mineral at 4 years

The factors above which showed statistically significant associations with 4-year old bone mineral were explored in multivariate models, with the main aim of finding out which variables explained most of the associations, so that these could be considered when examining the maternal determinants.

It was apparent that current height and weight were the strongest determinants of 4-year bone mineral at all sites, and so other 4-year measures were explored separately and then together with these two variables. Of the remaining measures, the trio of left hand grip

strength, total milk intake per day and mid-upper arm circumference were independently and positively associated with whole body BA (R^2=23%), BMC (R^2=39%) and areal BMD (R^2=37%) at 4 years (Table 61). The associations with milk became statistically non-significant when current height was added to the model. When current weight was added as well, the only remaining statistically significant association at any site was between grip strength and areal BMD, and mid-upper arm circumference and BA. Thus, overall, current body size was the strongest predictor of 4-year whole body bone mineral. BMC for height was only positively associated with mid-upper arm circumference (R^2=17%) and current weight (R^2=10%), with the latter becoming statistically non-significant when combined in a bivariate model with the former. Table 61 shows the statistically significant, mutually adjusted determinants of whole body bone mineral at 4 years.

The relationships at the lumbar spine were similar (Table 62), with left-hand grip strength, mid-upper arm circumference and total daily milk intake being positively associated with BA (R^2=12%) and BMC (R^2=25%), although the associations with milk were weaker than for whole body. Indeed total daily milk intake failed to achieve statistical significance for its relationship with lumbar spine areal BMD; grip strength and mid-upper arm circumference accounted for 17% of the variance in this measure. Again the associations were removed by adding in current height and weight, and only current height was statistically significantly associated with lumbar spine BA (R^2=34%).

Total hip BA, BMC and BMD (Table 63) were again positively associated with left-hand grip strength, mid-upper arm circumference and total daily milk intake (R^2=15%, 22% and 12% respectively). Again these associations became statistically non-significant after adding in current height and weight.

Levels of questionnaire-derived physical activity or any of the other lifestyle determinants did not influence these relationships at any skeletal site, and, owing to the small numbers with measurements, total daily energy expenditure was not included in the multivariate analysis.

Table 61 Mutually independent contemporary influences on whole body bone mineral at 4 years.

Whole body	BA (β (95%CI))	BMC (β (95%CI))	BMD (β (95%CI))
R^2 for model, %	23.3	39.2	36.7
Milk intake, pts/day	13.2 (2.3 to 24.1)*	13.1 (3.9 to 22.3)**	0.009 (0.002 to 0.017)*
MUAC, cm	13.6 (10.4 to 16.8)***	16.9 (14.2 to 19.6)***	0.013 (0.011 to 0.015)***
Grip strength, kg	5.4 (2.9 to 7.9)***	6.6 (4.4 to 8.7)***	0.005 (0.003 to 0.007)***

BA: Bone area; BMC: Bone mineral content; BMD: Areal bone mineral density; MUAC: Mid-upper arm circumference; *p<0.05; **p<0.01; ***p<0.001

Table 62 Mutually independent contemporary influences on lumbar spine bone mineral at 4 years.

Lumbar spine	BA (β (95%CI))	BMC (β (95%CI))	BMD (β (95%CI))
R^2 for model, %	12.4	22.1	14.0
Milk intake, pts/day	0.78 (0.14 to 1.41)*	0.54 (0.07 to 1.02)*	0.007 (-0.006 to 0.019)
MUAC, cm	0.33 (0.15 to 0.51)***	0.48 (0.34 to 0.62)***	0.012 (0.008 to 0.015)***
Grip strength, kg	0.37 (0.22 to 0.51)***	0.32 (0.21 to 0.42)***	0.005 (0.002 to 0.008)**

BA: Bone area; BMC: Bone mineral content; BMD: Areal bone mineral density; MUAC: Mid-upper arm circumference; *p<0.05; **p<0.01; ***p<0.001

Table 63 Mutually independent contemporary influences on total hip bone mineral at 4 years.

Total hip	BA (β (95%CI))	BMC (β (95%CI))	BMD (β (95%CI))
R^2 for model, %	14.9	21.6	11.9
Milk intake, pts/day	0.30 (0.19 to 0.41)***	0.48 (0.19 to 0.77)**	0.015 (0.0004 to 0.029)*
MUAC, cm	0.54 (0.15 to 0.93)**	0.19 (0.12 to 0.25)***	0.006 (0.003 to 0.010)***
Grip strength, kg	0.20 (0.11 to 0.29)***	0.28 (0.20 to 0.37)***	0.010 (0.006 to 0.014)***

BA: Bone area; BMC: Bone mineral content; BMD: Areal bone mineral density; MUAC: Mid-upper arm circumference; *p<0.05; **p<0.01; ***p<0.001

7.6 Influence of birthweight on 4-year bone mineral

The child's birthweight was strongly positively associated with whole body BA, BMC and areal BMD ($p<0.001$) and BMCh ($p=0.036$). However these associations did not retain statistical significance when current height and weight were added into the models. Inclusion of grip strength, mid-upper arm circumference and daily milk intake did not change these relationships. In contrast, for lumbar spine BA and hip BA and BMC, weak associations remained with birthweight after inclusion of current body size. Lumbar spine BA, BMC and areal BMD were all positively associated with birthweight, and for total hip BA ($p=0.039$) and BMC ($p=0.033$) there remained weak associations after inclusion of current body size. The association between birthweight and lumbar spine BA also remained significant ($p=0.017$); indeed current weight became non-significant, leaving birthweight and current height as determinants.

Table 64 Birthweight and bone mineral at 4 years (values are β coefficients and R^2).

	Whole body				Lumbar spine			Total hip		
	BA	BMC	BMCh	BMD	BA	BMC	BMD	BA	BMC	BMD
	cm^2	g	g/cm	g/cm^2	cm^2	g	g/cm^2	cm^2	g	g/cm^2
Birthweight (kg)	33.0	29.9	7.1*	0.02	1.4	1.1	0.01*	0.9	0.7	0.02**
R^2 (%)	8.3	8.0	0.9	4.8	5.7	4.7	1.0	6.1	6.2	2.0

BA: Bone area; BMC: Bone mineral content; BMCh: BMC for height; BMD: Bone mineral density; All $p<0.001$ except * $p<0.05$ and ** $p<0.01$

After inclusion of 4-year daily energy expenditure derived from the Actiheart assessment, birthweight was no longer statistically significantly associated with bone mineral at 4 years, in contrast to daily energy expenditure, which remained statistically robust. However, the number of participants in the model dropped from 407 to 84, thus dramatically reducing statistical power.

7.7 Summary discussion

Total daily milk intake, grip strength, mid-upper arm circumference and current height and weight were all positively associated with bone mineral at whole body, lumbar spine and hip at 4 years. The relationships appeared similar for both skeletal size (BA and BMC) and the partly size-corrected measure of areal BMD. Birthweight remained a significant determinant

of bone mineral at 4 years, although the effect was attenuated by inclusion of current body size, consistent with the notion that birthweight is a surrogate for factors which primarily determine skeletal size rather than volumetric density.

Height and BA will always tend to be strongly related because height is determined by the vertical dimension of skeletal size. Weight is closely related to height, and fatness is related to bone mineralization in many studies. Thus it is not surprising that current height and weight explain much of the variance in skeletal size and density. However, because bone mineral is so closely related to these factors, any maternal determinant which increases skeletal size is likely to also increase height and weight. Thus childhood height and weight are probably best viewed as outcomes rather than confounders. Given that grip strength is dependent on muscle bulk and also strongly related to height ($r=0.40$, $p<0.0001$), and that mid-upper arm circumference is also strongly related to weight ($r=0.83$, $p<0.0001$), these factors also are probably better regarded as outcomes when related to maternal factors. In contrast, milk intake, being purely environmental, is appropriately included as a confounding factor in these explorations.

8 Results: Maternal determinants of bone mineral in the offspring at 4 years

8.1 Introduction

This chapter explores the maternal anthropometric, dietary and lifestyle factors, and levels of 25(OH)-vitamin D in pregnancy, and their relationships with bone mineral in the offspring at 4 years old.

8.2 Maternal anthropometric determinants of 4-year bone mineral

As at birth, maternal height (Figure 20) measured before pregnancy, was strongly associated with whole body, lumbar spine and total hip bone mineral at 4 years. Maternal BMI, however, showed no statistically significant relationships with 4-year DXA measures apart from total hip BMD, and only maternal triceps skinfold thickness in pregnancy was statistically significantly associated with whole body bone area (Table 65, Table 66, Table 67).

Table 65 Maternal anthropometry and whole body bone mineral at 4 years.

Predictor	BA, cm^2	BMC, g	BMCh, g/cm	BMD, g/cm^2
Height, cm	2.4 (1.7 to 3.0)***	2.3 (1.7 to 3.0)***	-0.1 (-0.5 to 0.3)	0.0015 (0.001 to 0.002)***
BMI, sd	0.16 (-5.19 to 5.50)	1.4 (-3.6 to 6.3)	2.8 (-0.4 to 6.0)	0.002 (-0.002 to 0.006)
PP TSF, sd	3.45 (-1.58 to 8.48)	2.2 (-2.5 to 6.9)	1.6 (-1.5 to 4.7)	0.001 (-0.003 to 0.004)
EP TSF, sd	5.5 (0.2 to 10.8)*	3.0 (-1.9 to 8.0)	0.9 (-2.4 to 4.1)	0.0004 (-0.004 to 0.004)
LP TSF, sd	4.8 (0.06 to 9.6)*	3.7 (-0.8 to 8.2)	0.6 (-2.4 to 3.6)	0.002 (-0.002 to 0.005)

Table shows β (95%CI). BMI: Body mass index; PP: Pre-pregnancy; EP: Early pregnancy; LP: Late pregnancy; TSF: Triceps skinfold thickness; *p<0.05; **p<0.01; ***p<0.001

Table 66 Maternal anthropometry and lumbar spine bone mineral at 4 years.

Predictor	BA, cm^2	BMC, g	BMD, g/cm^2
Height, cm	0.10 (0.07 to 0.14)***	0.08 (0.05 to 0.11)***	0.001 (0.0005 to 0.002)**
BMI, sd	-0.07 (-0.35 to 0.21)	0.05 (-0.17 to 0.28)	0.003 (-0.003 to 0.009)
PP TSF, sd	0.01 (-0.26 to 0.27)	-0.02 (-0.23 to 0.19)	-0.001 (-0.006 to 0.005)
EP TSF, sd	-0.04 (-0.32 to 0.24)	0.03 (-0.19 to 0.26)	0.002 (-0.004 to 0.007)
LP TSF, sd	0.14 (-0.11 to 0.40)	0.05 (-0.15 to 0.26)	-0.001 (-0.006 to 0.004)

Table shows β (95%CI). BMI: Body mass index; PP: Pre-pregnancy; EP: Early pregnancy; LP: Late pregnancy; TSF: Triceps skinfold thickness; *p<0.05; **p<0.01; ***p<0.001

Table 67 Maternal anthropometry and total hip bone mineral at 4 years.

Predictor	BA, cm^2	BMC, g	BMD, g/cm^2
Height, cm	0.06 (0.04 to 0.08)***	0.05 (0.03 to 0.06)***	0.001 (0.0001 to 0.002)*
BMI, sd	0.02 (-0.15 to 0.20)	0.09 (-0.05 to 0.23)	0.001 (0.0005 to 0.01)*
PP TSF, sd	0.04 (-0.12 to 0.20)	0.06 (-0.07 to 0.19)	0.003 (-0.003 to 0.009)
EP TSF, sd	0.11 (-0.07 to 0.29)	0.10 (-0.04 to 0.24)	0.003 (-0.003 to 0.010)
LP TSF, sd	0.09 (-0.07 to 0.25)	0.08 (-0.04 to 0.20)	0.002 (-0.004 to 0.008)

Table shows β (95%CI). BMI: Body mass index; PP: Pre-pregnancy; EP: Early pregnancy; LP: Late pregnancy; TSF: Triceps skinfold thickness; *p<0.05; **p<0.01; ***p<0.001

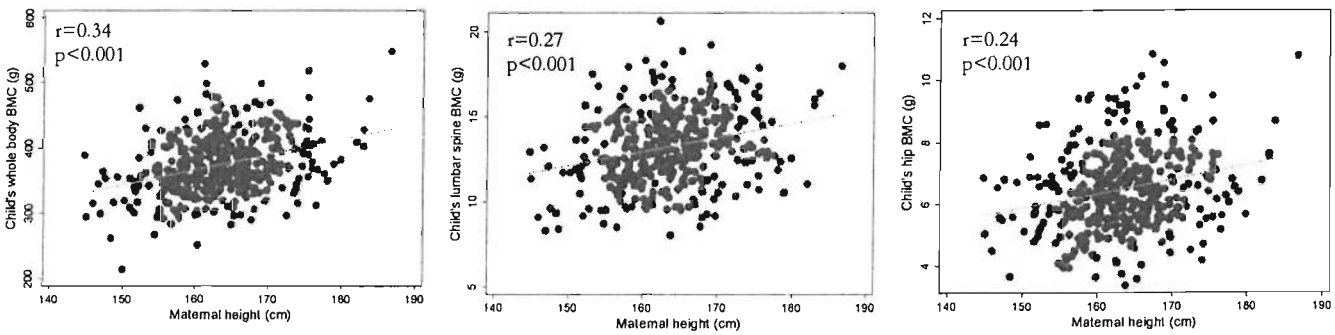


Figure 20 Maternal height and BMC at whole body, lumbar spine and hip in the child at 4 years.

8.3 Maternal lifestyle determinants of 4-year bone mineral

In contrast to the associations seen at birth, maternal parity was not statistically significantly associated with any measure of 4-year bone mineral except for whole body BMCh and lumbar

spine BA. Mothers who smoked in late pregnancy had a trend towards having children with lower whole body BA (p=0.160), but with higher whole body BMD (0.013), with no difference in BMC. Children of mothers in numerically higher social classes had lower lumbar spine BA. Owing to low numbers of participants in the "slow" category of walking speed before and in early pregnancy, this category was combined with "easy" for both time points. There were also very few participants in the "fast" category in late pregnancy and so this was combined with "fairly brisk". Thus walking speed was then in 4 groups. There were no statistically significant associations between this measurement and 4-year bone mineral at any site. Table 68, Table 69 and Table 70 summarise these associations.

Table 68 Maternal lifestyle and whole body bone mineral at 4 years.

Predictor	BA, cm^2	BMC, g	BMCh, g/cm	BMD, g/cm^2
Social class, 6 gps	-1.3 (-6.1 to 3.6)	-0.3 (-4.7 to 4.0)	1.5 (-1.4 to 4.5)	0.0002 (-0.003 to 0.004)
Parity, 4 gps	5.1 (-1.1 to 11.2)	2.0 (-3.7 to 7.7)	6.2 (2.4 to 10.0)**	-0.001 (-0.004 to 0.004)
PP smoking, Y/N	-4.9 (-16.5 to 6.7)	3.6 (-7.1 to 14.3)	5.2 (-1.9 to 12.3)	0.008 (-0.001 to 0.016)
EP smoking, Y/N	-15.2 (-30.7 to 0.3)	0.9 (-13.5 to 15.3)	4.3 (-5.3 to 13.9)	0.01 (-0.001 to 0.02)
LP smoking, Y/N	-10.0 (-24.3 to 4.3)	2.4 (-11.1 to 15.8)	5.0 (-3.9 to 14.0)	0.01 (0.001 to 0.02)*
PP walking speed, 4 gps	1.2 (-5.9 to 8.3)	-0.11 (-6.6 to 6.4)	-1.9 (-6.2 to 2.5)	-0.001 (-0.006 to 0.004)
EP walking speed, 4 gps	-5.7 (-12.8 to 1.5)	-5.3 (-11.9 to 1.3)	-2.1 (-6.5 to 2.4)	-0.004 (-0.009 to 0.002)
LP walking speed, 4 gps	1.1 (-5.6 to 7.7)	-0.1 (-6.1 to 6.0)	-1.1 (-5.1 to 2.9)	-0.0009 (-0.006 to 0.004)

Table shows β (95%CI). PP: Pre-pregnancy; EP: Early pregnancy; LP: Late pregnancy; *p<0.05; **p<0.01

Table 69 Maternal lifestyle and lumbar spine bone mineral at 4 years.

Predictor	BA, cm^2	BMC, g	BMD, g/cm^2
Social class, 6 gps	-0.30 (-0.55 to –0.05)*	-0.08 (-0.28 to 0.12)	0.002 (-0.003 to 0.007)
Parity, 4 gps	0.12 (-0.21 to 0.44)	-0.03 (-0.29 to 0.23)	-0.003 (-0.009 to 0.004)
PP smoking, Y/N	0.25 (-0.35 to 0.86)	0.32 (-0.16 to 0.81)	0.007 (-0.005 to 0.019)
EP smoking, Y/N	0.10 (-0.71 to0.91)	0.35 (-0.29 to 1.00)	0.01 (-0.01 to 0.03)
LP smoking, Y/N	0.12 (-0.63 to 0.87)	0.43 (-0.17 to 1.03)	0.01 (-0.002 to 0.03)
PP walking speed, 4 gps	0.19 (-0.18 to 0.56)	0.06 (-0.24 to 0.35)	-0.001 (-0.009 to 0.006)
EP walking speed, 4 gps	-0.08 (-0.46 to 0.29)	-0.25 (-0.55 to 0.05)	-0.007 (-0.02 to 0.0004)
LP walking speed, 4 gps	-0.04 (-0.38 to 0.30)	-0.06 (-0.34 to 0.21)	-0.001 (-0.008 to 0.006)

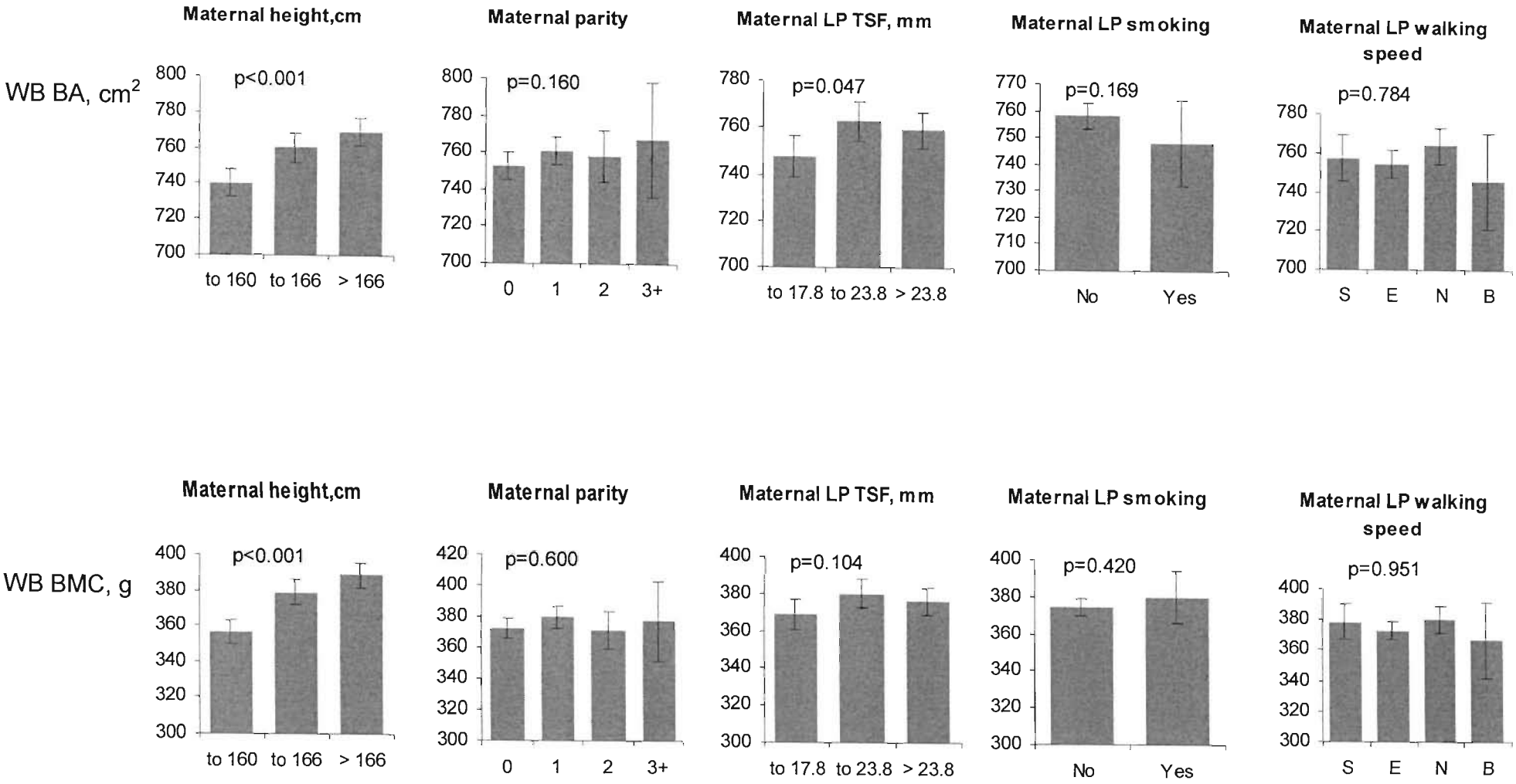
Table shows β (95%CI). PP: Pre-pregnancy; EP: Early pregnancy; LP: Late pregnancy; *p<0.05

Table 70 Maternal lifestyle and total hip bone mineral at 4 years.

Predictor	BA, cm^2	BMC, g	BMD, g/cm^2
Social class, 6 gps	0.02 (-0.14 to 0.17)	-0.01 (-0.14 to 0.11)	-0.002 (-0.008 to 0.004)
Parity, 4 gps	0.14 (-0.06 to 0.34)	0.08 (-0.08 to 0.23)	0.0003 (-0.007 to 0.008)
PP smoke, Y/N	0.15 (-0.23 to 0.53)	0.19 (-0.11 to 0.49)	0.010 (-0.004 to 0.024)
EP smoke, Y/N	0.14 (-0.38 to 0.66)	0.04 (-0.37 to 0.45)	-0.003 (-0.021 to 0.016)
LP smoke Y/N	0.17 (-0.29 to 0.64)	0.22 (-0.14 to 0.59)	0.011 (-0.006 to 0.028)
PP walking speed, 4 gps	-0.09 (-0.32 to 0.15)	-0.09 (-0.27 to 0.09)	-0.004 (-0.013 to 0.004)
EP walking speed, 4 gps	-0.20 (-0.44 to 0.04)	-0.14 (-0.33 to 0.05)	-0.003 (-0.012 to 0.006)
LP walking speed, 4 gps	0.06 (-0.16 to 0.27)	-0.02 (-0.18 to 0.15)	-0.003 (-0.011 to 0.005)

Table shows β (95%CI). PP: Pre-pregnancy; EP: Early pregnancy; LP: Late pregnancy; Nil statistically significant

Figure 21 Maternal determinants of whole body bone area (BA) and bone mineral content (BMC) in the offspring at 4 years old.



Walking speed: S=Slow, E=Easy, N=Normal, B=Brisk/fast

8.4 Maternal dietary determinants of 4-year bone mineral

Maternal milk intake, recorded at the initial pre-pregnancy interview, was positively associated with whole body BMC and BMD, and, at a greater level of statistical significance, with lumbar spine BA and BMC. Table 71, Table 72 and Table 73 summarise these findings.

Table 71 Maternal diet and whole body bone mineral at 4 years.

Predictor	BA, cm^2	BMC, g	BMCh, g/cm	BMD, g/cm^2
PP milk, pts/wk	3.9 (-0.6 to 8.4)	5.0 (0.9 to 9.1)*	1.3 (-1.5 to 4.0)	0.004 (0.001 to 0.007)*
EP milk, pts/wk	1.4 (-3.2 to 5.9)	1.3 (-2.9 to 5.6)	2.6 (-0.2 to 5.4)	0.008 (-0.003 to 0.004)
LP milk, pts/wk	1.9 (-2.8 to 6.4)	3.2 (-1.0 to 7.4)	1.8 (-1.0 to 4.6)	0.003 (-0.0002 to 0.006)
PP alcohol, units/wk	0.1 (-4.2 to 4.5)	1.6 (-2.4 to 5.6)	0.7 (-1.9 to 3.3)	0.002 (-0.001 to 0.005)
EP alcohol, units/wk	4.8 (-3.4 to 12.9)	4.4 (-3.1 to 12.0)	1.7 (-3.5 to 6.8)	0.003 (-0.003 to 0.009)
LP alcohol, units/wk	1.9 (-8.7 to 12.5)	3.0 (-6.7 to 12.7)	3.2 (-3.2 to 9.5)	0.003 (-0.004 to 0.01)

Table shows β (95%CI). PP: Pre-pregnancy; EP: Early pregnancy; LP: Late pregnancy; *$p<0.05$

Table 72 Maternal diet and lumbar spine bone mineral at 4 years.

Predictor	BA, cm^2	BMC, g	BMD, g/cm^2
PP milk, pts/wk	0.36 (0.13 to 0.59)**	0.26 (0.08 to 0.45)**	0.003 (-0.002 to 0.008)
EP milk, pts/wk	0.02 (-0.22 to 0.26)	-0.03 (-0.22 to 0.16)	-0.002 (-0.007 to 0.003)
LP milk, pts/wk	0.02 (-0.22 to 0.26)	0.04 (-0.15 to 0.23)	0.001 (-0.004 to 0.006)
PP alcohol, units/wk	-0.03 (-0.25 to 0.20)	0.04 (-0.14 to 0.22)	0.002 (-0.002 to 0.007)
EP alcohol, units/wk	0.01 (-0.40 to 0.42)	0.05 (-0.28 to 0.38)	0.002 (-0.006 to 0.011)
LP alcohol, units/wk	-0.006 (-0.55 to 0.54)	0.13 (-0.30 to 0.57)	0.006 (-0.005 to 0.017)

Table shows β (95%CI). PP: Pre-pregnancy; EP: Early pregnancy; LP: Late pregnancy; **$p<0.01$

Figure 21 summarises the associations between maternal factors found to influence intrauterine bone mineral accrual and bone mass at 4 years. The overall pattern is one of attenuated relationships, but with trends in similar directions (compare with Figure 8).

Table 73 Maternal diet and total hip bone mineral at 4 years.

Predictor	BA, cm^2	BMC, g	BMD, g/cm^2
PP milk, pts/wk	0.14 (-0.004 to 0.29)	0.11 (-0.005 to 0.23)	0.003 (-0.002 to 0.008
EP milk, pts/wk	-0.05 (-0.20 to 0.10)	-0.03 (-0.15 to 0.09)	0.0004 (-0.005 to 0.006)
LP milk, pts/wk	0.11 (-0.4 to 0.26)	0.05 (-0.07 to 0.17)	-0.0005 (-0.006 to 0.005)
PP alcohol, units/wk	0.06 (-0.08 to 0.20)	0.06 (-0.05 to 0.17)	0.002 (-0.003 to 0.008)
EP alcohol, units/wk	0.10 (-0.16 to 0.37)	0.11 (-0.10 to 0.32)	0.005 (-0.005 to 0.015)
LP alcohol, units/wk	0.26 (-0.08 to 0.60)	0.15 (-0.12 to 0.41)	0.001 (-0.011 to 0.013)

Table shows β (95%CI). PP: Pre-pregnancy; EP: Early pregnancy; LP: Late pregnancy; Nil statistically signficant

8.5 Mutually adjusted maternal determinants of offspring bone mineral at 4 years

It was apparent from the univariate analysis that there was not a consistent pattern of associations for variables at different time points. There is no convincing a priori reason why the time point (before, early or late pregnancy) of the strongest associations should change from birth to 4 years, and thus it was felt appropriate to focus the multivariate analysis on maternal factors found to be associated with offspring bone mass at birth (summarised in univariate form in Figure 21). Additionally, one of the hypotheses was that these influences would persist into childhood. Thus the mutually independent maternal determinants of 4 year whole body bone area included just height and late pregnancy triceps skinfold thickness, whilst for BMC, only maternal height remained, although there was still a trend towards a positive association with triceps skinfold thickness (p=0.102). In contrast, in addition to the association with maternal height, BMD was positively associated with maternal smoking in late pregnancy. BMC for height (BMCh) was associated with maternal parity but not height. Table 74 shows all these mutually adjusted maternal determinants for 4 year whole body BA, BMC and BMD. Note that with the other predictors included, the association between triceps and BA drops to p=0.063. All measures of bone mass at the lumbar spine and total hip were positively associated with maternal height only. Apart from the association between maternal height and total hip BMD, which became of borderline statistical significance (p=0.058), these associations were retained after inclusion childhood milk intake.

Table 74 Mutually adjusted maternal factors and offspring bone mass at 4 years, based on determinants of bone mass at birth.

Predictor	**BA, cm^2** (n=394, R^2=10.4%)		**BMC, g** (n=394, R^2=11.5%)		**BMD, g/cm^2** (n=394, R^2=9.2%)	
	β, 95%CI	p	β, 95%CI	p	β, 95%CI	p
Height, cm	2.2 (1.5 to 2.9)	<0.001	2.3 (1.7 to 2.9)	<0.001	0.0016 (0.0011 to 0.0021)	<0.001
Parity, 4 gps	5.2 (-0.6 to 11.1)	0.081	2.1 (-3.3 to 7.6)	0.445	-0.001 (-0.004 to 0.004)	0.819
LP TSF, sd	4.3 (-0.2 to 8.9)	0.063	3.6 (-0.7 to 7.8)	0.100	0.002 (-0.002 to 0.005)	0.287
LP smoking, Y/N	-8.7 (-22.3 to 4.8)	0.206	6.7 (-5.9 to 19.3)	0.295	0.014 (0.004 to 0.024)	0.006
LP walking speed, 4 gps	0.4 (-5.8 to 6.6)	0.904	-1.1 (-6.8 to 4.7)	0.717	-0.002 (-0.006 to 0.003)	0.495

LP: Late pregnancy; TSF: Triceps skinfold thickness

8.6 Maternal 25(OH)-vitamin D status in late pregnancy and 4-year bone mineral

Maternal 25(OH)-vitamin D level in late pregnancy was not normally distributed, requiring log-transformation to achieve this distribution. It was then standardised so is expressed in sd. The median level (IQR) was 63 nmol/l (44 to 88 nmol/l) and the range was 12 to 440 nmol/l. There were two participants with values much higher than the next highest value, and these two were excluded from the analysis. This left 334 children (189 boys) at 4 years whose mothers had had 25(OH)-vitamin D assessed in late pregnancy.

When the children were analysed all together, there was a trend towards a positive association between maternal 25(OH)-vitamin D status and lumbar spine bone area, but this just failed to attain statistical significance (β=0.25cm^2/sd, p=0.077). Figure 22 shows the relationship between maternal 25(OH)-vitamin D status and whole body and lumbar spine BA at 4 years. The vitamin D levels were dichotomised into two groups, firstly using a cut off of 33 nmol/l and then at 27 nmol/l, to explore whether there was a threshold effect. When the children were analysed together, no statistically significant associations were found.

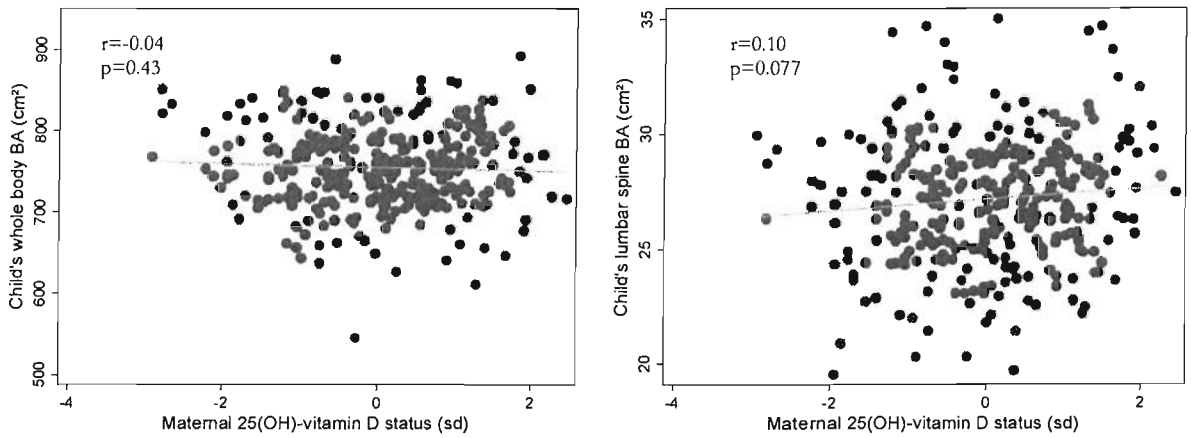


Figure 22 Maternal 25(OH)-vitamin D status in late pregnancy, and bone area at whole body and lumbar spine in the offspring at 4 years.

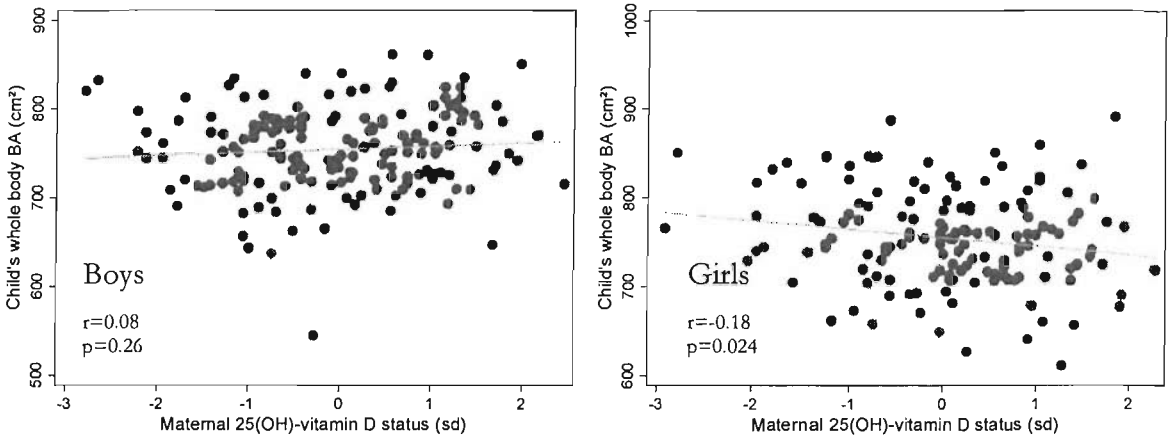


Figure 23 Maternal 25(OH)-vitamin D status and whole body bone area at 4 years in the offspring (boys and girls separately).

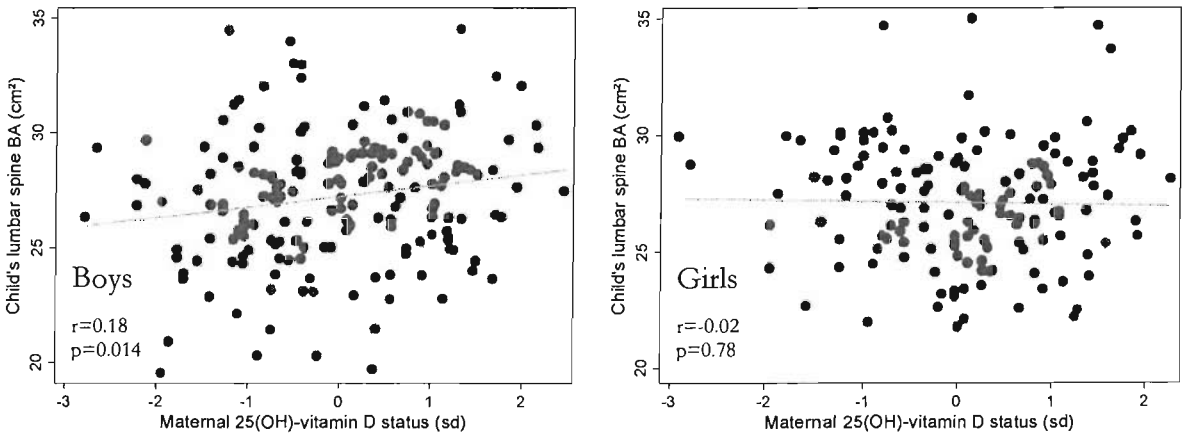


Figure 24 Maternal 25(OH)-vitamin D status and lumbar spine bone area at 4 years in the offspring (boys and girls separately).

When the continuous and dichotomised measures of vitamin D were explored by offspring gender, some statistically significant associations emerged at each DXA site (shown for whole body BA in Figure 23 and lumbar spine BA Figure 24). However, these were generally positive in the boys and negative in the girls, but of low magnitude and significance.

There was a weak negative association between late pregnancy smoking and vitamin D levels, but when smoking, triceps skinfold thickness, parity were included in the regression models, the associations remained statistically significant. When maternal height was included as a covariate, the association with whole body became non-significant, but the regression coefficient did not change very much. Levels of vitamin D were statistically significantly and

negatively related to whole body fat mass at 4 years (r=-0.20, p=0.0002), and after addition of this factor into the regression models, the associations in girls became non-significant, but those in the boys did not change. The gender-fat mass interaction term with whole body bone area was p=0.110, showing a trend towards a significant interaction.

8.7 Paternal anthropometric determinants of 4-year bone mineral

Paternal height and BMI were available for 69 participants. Despite the low sample size, there were trends towards positive associations with bone mineral at 4 years (Table 75).

Table 75 Paternal body build, and whole body, lumbar spine and total hip bone mineral in the offspring at 4 years.

Predictor	BA, cm^2	BMC, g	BMCh, g/cm	BMD, g/cm^2
Whole body				
Height, cm	1.4 (-0.1 to 2.9)*	1.6 (0.2 to 3.0)**	-0.03 (-1.2 to 1.2)	0.001 (0 to 0.002)*
BMI, kg/m^2	-7.7 (-18.3 to 3.0)	-8.6 (-18.7 to 1.5)	0.05 (-8.5 to 8.6)	-0.006 (-0.015 to 0.003)
Lumbar spine				
Height, cm	0.16 (0.06 to 0.26)**	0.08 (-0.001 to 0.15)	-	0.00001 (-0.002 to 0.002)
BMI, kg/m^2	-0.70 (-1.4 to –0.01)**	-0.44 (0.96 to 0.09)	-	-0.003 (-0.016 to 0..010)
Total hip				
Height, cm	0.07 (0.005 to 0.13)**	0.05 (0.005 to 0.10)**	-	0.002 (-0.001 to 0.004)
BMI, kg/m^2	-0.45 (-0.87 to –0.03)**	-0.40 (-0.73 to –0.07)	-	-0.01 (-0.03 to 0.001)*

Table shows β (95%CI). *p<0.1, **p<0.05; BMI: Body mass index

8.8 Summary discussion

The most robust associations observed were between maternal height and 4-year bone mineral. The maternal lifestyle and anthropometric characteristics which were associated with bone mineral at birth showed a similar, but greatly attenuated, pattern of relationships with these indices 4 years later (Figure 21). Maternal triceps skinfold thickness and parity still showed some association with BA or BMC at 4 years, in contrast to childhood milk intake which was associated with measures of bone size and areal density. These relationships are consistent with size of the skeletal envelope being influenced by factors in utero, and then genetic and childhood environmental factors modifying subsequent mineralisation. The positive association between maternal smoking in late pregnancy and whole body areal BMD

is difficult to explain, but did seem to persist after adjustment for percentage fat mass, so was not due to obesity in children of smoking mothers. It may just be statistical artefact as whole bone BA at 4 years was lower in children of smokers, but BMC was not, thus BMD (BMC/BA) is likely to show an opposite relationship to BA.

The associations seen with maternal 25(OH)-vitamin D status are not as consistent as at birth; indeed the direction of the association is negative for many measures in girls, and positive in boys. Inclusion of fat mass at 4 years attenuated the associations in the girls but not in the boys. These associations are discussed further in section 10.

9 Results: Growth from birth to 4 years

9.1 Introduction

In this chapter the growth (anthropometry and bone mineral) of the child from birth to 4 years will be described. The relationships between neonatal and 4-year DXA indices, and the influence of maternal and childhood factors on growth and growth relative to the peer group are also explored.

9.2 Anthropometry from birth to 4 years

Neonatal crown-heel length was compared with the child's height at 4 years. Birthweight was compared with weight at 4 years (Table 76). Because 4-year weight was not normally distributed, it was transformed to normality by taking the logarithm, and was then standardised. For ease of comparison in Table 76, height is also standardised. Thus, with every 1 cm increase in birth length, height at 4 years increased by 0.15 sd (95% CI: 0.10 to 0.19 sd, $p<0.001$), and weight increased by 0.10 sd (95% CI: 0.06 to 0.15 sd, $p<0.001$). With every 1 kg increase in birthweight, 4-year height increased by 0.45 sd (95% CI: 0.26 to 0.64 sd, $p<0.001$) and weight increased by 0.5 sd (0.3 to 0.6 sd, $p<0.001$). When birthweight was divided into tertiles, there was a step increase in 4-year height and weight for middle tertile of birthweight compared with the lowest, and the highest compared with the middle. Thus the babies in the lowest tertile of birthweight were still shorter and lighter at 4 years than the babies in the highest tertile.

Table 76 Birth size and 4-year height and weight.

	4-year height, sd	**4-year weight, sd**
Birthweight gp2	0.21 (-0.03 to 0.45)*	0.29 (0.05 to 0.53)**
Birthweight gp3	0.51 (0.27 to 0.74)***	0.60 (0.37 to0.83)***
Birth length gp2	0.40 (0.17 to 0.63)**	0.26 (0.03 to 0.49)**
Birth length gp3	0.81 (0.56 to 1.05)***	0.58 (0.33 to 0.82)***

Figures are mean difference from lowest tertile (95%CI)

*p<0.10; **p<0.05, ***p<0.01

9.3 Birth size and 4-year bone mineral

Again for whole body and total hip bone mineral there was an increase in the DXA measure at 4 years with each increasing tertile of birthweight (Table 77 and Table 79). For lumbar spine bone mineral, the difference was more apparent comparing the highest with the lowest tertile (Table 78).

Table 77 Birth size and whole body bone mineral at 4 years.

	BA, cm^2	BMC, g	BMCh, g/cm	BMD, g/cm^2
Birthweight gp2	16.7 (4.7 to 28.6)**	12.7 (1.7 to 23.7)**	6.2 (-1.2 to 13.7)	0.006 (-0.003 to 0.015)
Birthweight gp3	28.2 (16.5 to 39.8)***	26.2 (15.5 to 36.9)***	6.7 (-0.5 to 13.9)*	0.016 (0.007 to 0.024)***
Birth length gp2	11.1 (-0.4 to 22.7)*	14.2 (3.7 to 24.8)**	-1.0 (-8.2 to 6.2)	0.01 (0.003 to 0.020)**
Birth length gp3	30.0 (17.8 to 42.2)***	27.4 (16.2 to 38.5)***	-1.3 (-8.9 to 6.4)	0.02 (0.01 to 0.03)***

Figures are mean difference from lowest tertile (95%CI); *p<0.10; **p<0.05, ***p<0.01

Table 78 Birth size and 4-year lumbar spine bone mineral.

	BA, cm^2	BMC, g	BMD, g/cm^2
Birthweight gp2	0.41 (-0.21 to 1.03)	0.39 (-0.11 to 0.89)	0.007 (-0.005 to 0.020)
Birthweight gp3	1.23 (0.63 to 1.83)***	1.00 (0.51 to 1.49)***	0.015 (-0.003 to 0.027)*
Birth length gp2	0.27 (-0.34 to 0.87)	0.47 (-0.02 to 0.95)*	0.012 (-0.001 to 0.024)*
Birth length gp3	1.52 (0.88 to 2.16)***	1.03 (0.52 to 1.54)***	0.011 (-0.002 to 0.024)

Figures are mean difference from lowest tertile (95%CI); *p<0.10; **p<0.05, ***p<0.01

Table 79 Birth size and 4-year total hip bone mineral.

	BA, cm^2	BMC, g	BMD, g/cm^2
Birthweight gp2	0.59 (0.20 to 0.97)**	0.53 (0.23 to 0.84)**	0.019 (0.004 to 0.03)**
Birthweight gp3	0.73 (0.35 to 1.11)***	0.58 (0.28 to 0.88)***	0.015 (0.001 to 0.029)**
Birth length gp2	0.32 (-0.06 to 0.71)	0.42 (0.12 to 0.73)***	0.021 (0.007 to 0.034)***
Birth length gp3	0.45 (0.04 to 0.86)**	0.41 (0.09 to 0.73)**	0.013 (-0.002 to 0.027)*

Figures are mean difference from lowest tertile (95%CI); *p<0.10; **p<0.05, ***p<0.01

9.4 Bone mineral at birth and 4 years

There were 147 children at 4 years old who had undergone neonatal and 4-year DXA assessment. There were strongly statistically significant associations between bone mineral at birth and in childhood, although the amount of variation in the 4-year measures explained by those at birth was at most 12% (Table 80). After BMC was adjusted for height at 4 years (BMCh), just 3.2% of whole body BMC was explained by BMC at birth. The strongest associations were between un-size-corrected DXA indices at birth (BA and BMC) and BMC and areal BMD at 4 years, with weaker associations for these neonatal measures with BA at 4 years.

When 4-year old total milk intake was included in bivariate models, these associations remained robust, despite the drop in participants to 120 for whole body, 121 for spine and 124 for hip DXA. These associations were attenuated by the addition of current weight to the models, but did retain statistical significance. Height at 4 years had a less marked influence on the associations (Italics in Table 80), with statistically significant relationships retained between neonatal whole body BA and BMC, and whole body BMC, BMCh and BMD, and hip BMD at 4 years. Neonatal BMC was associated with lumbar spine BMC and BMD at 4 years.

Table 80 Neonatal and 4-year bone mineral (n=147).

Neonate	4-year whole body				4-year lumbar spine			4-year total hip		
	BA, cm^2	BMC, g	BMCh, g/cm	BMD, g/cm^2	BA, cm^2	BMC, g	BMD, g/cm^2	BA, cm^2	BMC, g	BMD, g/cm^2
BA, cm^2	0.4	*0.5*	*0.2*	*0.0004*	0.02	0.02	0.0006	0.01	0.01 (0.006 to	*0.0006*
	(0.1 to 0.6)**	*(0.3 to 0.7)****	*(0.02 to 0.37)**	*(0.0002 to 0.0006)****	(0.004 to 0.03)*	(0.01 to 0.03)***	(0.0003 to 0.0008)***	(0.0004 to 0.02)*	0.02)**	*(0.0003 to 0.0009)****
R^2 (%)	5.4	*12.1*	*2.8*	*12.1*	3.6	11.8	8.7	2.1	7.1	*8.8*
BMC, g	0.6	*0.8*	*0.3*	*0.0007*	0.03	*0.04*	*0.001*	0.02	0.02	*0.001*
	(0.2 to 1.0)**	*(0.5 to 1.2)****	*(0.01 to 0.6)**	*(0.0004 to 0.001)****	(0.01 to 0.06)*	*(0.02 to 0.06)****	*(0.0005 to 0.0014)****	(0.001 to 0.03)*	(0.01 to 0.03)**	*(0.0005 to 0.002)****
R^2 (%)	5.2	*11.6*	*2.3*	*11.6*	3.6	*12.4*	*9.6*	2.4	7.1	*8.4*
BMD, g/cm^2	190.8	269	49	0.2	11.0	18.5	0.5	9.4	9.3	0.4
	(-63.9 to 445.5)	(35 to 502)*	(-126 to 225)	(0.03 to 0.4)*	(-4.5 to 26.6)	(7.0 to 29.9)**	(0.2 to 0.8)**	(0.1 to 18.6)*	(1.9 to 16.8)*	(0.03 to 0.7)*
R^2 (%)	0.8	2.8	0.0	2.7	0.6	5.7	5.7	1.9	3.2	2.3

*p<0.05, **p<0.01, ***p<0.001. Associations in italics are those for which p<0.05 after inclusion of height at 4 years.

Values are β-coefficient and 95%CI.

9.5 Childhood growth relative to peers over complete distribution of birthweight

Birthweight and length, 4-year height, weight and DXA measures were all converted to Z scores: That is, the distributions were transformed such that the mean was zero and the standard deviation equal to one. The units of measurement then become sd and allow comparison between variables, with the Z score being the number of sd away from the mean of zero. Change in Z score from birth to 4 years was derived by subtracting the birth measure from the 4-year measure, and then adjusted for the birth measure, to try to reduce the effect of "regression to the mean". The childhood and maternal influences on change in Z score were then explored.

9.5.1 Childhood diet and lifestyle, and childhood growth relative to peers

After adjustment for birth size or DXA measurement, there were strongly statistically significant associations between milk intake at 4 years and change in Z score for all DXA whole body measures from birth to 4 years (Table 81). Change in height and weight Z scores from birth to 4 years was again positively related to 4-year milk intake. No other questionnaire-derived factors at 4 years were statistically significantly associated with change in Z score from birth to 4 years.

Table 81 Daily milk intake (pts) at 4 years and change in height, weight and whole body DXA indices Z scores from birth to 4 years.

	β (sd/pt)	95%CI	p	R^2, n
ΔHeight, sd	0.61	0.36 to 0.85	<0.001	7.0%, 307
ΔWeight, sd	0.55	0.28 to 0.82	<0.001	4.5%, 315
ΔBA, sd	0.52	0.17 to 0.87	0.004	6.2%, 120
ΔBMC, sd	18.6	0.36 to 36.8	0.046	2.5%, 120
ΔBMD, sd	0.54	0.04 to 1.03	0.033	3.0%, 120

Change in Z scores adjusted for neonatal measurement

Children with greater total energy expenditure per day, measured by the Actiheart instrument (See section 5.2), grew taller (β=0.002 sd/kCal, 95%CI: 0.0005 to 0.003 sd/kCal, p=0.004)

and fatter (β=0.002 sd/kCal, 95%CI: 0.001 to 0.004 sd/kCal, p<0.001) relative to their peers. However, the low numbers of participants meant that further analysis was not possible as there were only 31 children who had had Actiheart and both neonatal and 4-year DXA assessments.

9.5.2 Maternal diet, lifestyle and anthropometry, and childhood growth relative to peers

After adjustment for birth size, children of mothers who were taller had greater gains in height and weight relative to their peers than those children of mothers who were shorter. Children of mothers who smoked in pregnancy tended to grow more in weight, but not height, relative to their peers from birth to 4 years (Table 82) compared with children of mothers who did not. After adjustment for the corresponding DXA measure at birth, there were weak associations between maternal height and gain in whole body bone area Z score (β=0.02, p=0.064), but an opposite trend with whole body bone mineral content (β=-1.0, p=0.067). Early pregnancy walking speed was positively associated with gain in whole body bone mineral density Z score (β=0.36, p=0.012). However, the numbers of participants with maternal data and both neonatal and 4-year DXA data were small (147), limiting statistical power.

Table 82 Change in height and weight Z scores from birth to 4 years, and maternal height and smoking.

		β	95%CI	p	R^2, n
Maternal height, cm	ΔHeight, sd	0.05	0.04 to 0.07	<0.001	15.1%, 380
	ΔWeight, sd	0.03	0.02 to 0.05	<0.001	5.5%, 390
Maternal LP smoking, Y/N	ΔHeight, sd	0.14	-0.14 to 0.41	0.325	0.3%, 371
	ΔWeight, sd	0.48	0.20 to 0.75	0.001	2.7%, 380

BMI: Body mass index; LP: Late pregnancy

ΔHeight adjusted for birth length; ΔWeight adjusted for birthweight.

Whilst the association between maternal height and change in childhood Z score for height remained statistically significant when total daily energy expenditure was included in the

model, the other associations did not. However, the number of participants included in these models dropped from around 380 to around 80, thus limiting statistical power.

9.6 Catch-up growth in the lowest tertile of birthweight

9.6.1 Birthweight, and change in Z score for height, weight and DXA indices

Table 83 Change in Z score for body size and DXA indices in lowest and highest versus middle birthweight tertiles.

Birthweight	Height	Weight	BA	BMC	BMD
Lowest tertile	0.35**	0.76***	0.35	0.39	0.51*
	(0.09 to 0.61)	(0.50 to 1.02)	(-0.06 to 0.76)	(-0.02 to 0.80)	(0.10 to 0.93)
Highest tertile	-0.39**	-0.65***	-0.43*	-0.46*	-0.56**
	(-0.66 to -0.13)	(-0.91 to -0.39)	(-0.83 to -0.04)	(-0.86 to -0.07)	(-0.96 to -0.16)

*p<0.05; **p<0.01; ***p<0.001 Values are mean (95%CI) difference from middle tertile.

Table 83 demonstrates that children born in the lowest third of birthweight tended to "catch-up" in terms of body size and bone mineral relative to their higher birthweight peers by 4 years. Conversely those in the highest third tended to "catch-down". That is, those children born small relative to their peers showed an increase in Z score for body size and DXA indices from birth to 4 years, and those born heavy showed the opposite pattern.

Children in the lowest third of birthweight were further examined by categorising them as either those who were low and caught up (change in height or weight Z score >0) or those who stayed low (change in height or weight Z score ≤ 0) relative to their peers (Table 84). Logistic regression was used to see if catch-up growth was associated with maternal or childhood factors. Owing to low numbers, it was not possible to examine this issue with regard to DXA measurements, so associations for height and weight only will be described. It should be noted that since the prevalence of the outcome (catch-up growth) is high, the odds ratios do not give a reliable estimate of the magnitude of the associations, so should be interpreted with caution.

Table 84 Numbers (%) of children in the lowest third of birthweight grouped according to subsequent gain or lack of gain in height or weight Z score.

	Height (%)	Weight (%)
No gain	52 (40)	47 (34.3)
Gain	78 (60)	90 (65.7)

9.6.2 Characteristics of children in lowest tertile of birthweight in relation to catch-up growth

Children who were born in the lowest third of birthweight and did not catch up for height or weight were of lower gestational age but higher birthweight than those who did. However, these associations did not attain statistical significance, and did not change after adjustment for gender. Children who did not catch up from low birthweight tended to be longer at birth, and the association with birth length become statistically significant after adjustment for gestational age and gender. Thus children born light who then showed catch-up growth tended to have a longer gestation but be smaller at birth. Table 85 summarizes these associations.

Table 85 Gestational age, birth size and growth relative to peers for children born in the lowest tertile of birthweight.

	Height			Weight		
Birth to 4 years:	No gain	Gain	p	No gain	Gain	p
Gestational age, days	270.1	273.7	0.164	269.6	273.1	0.199
Birthweight, g	2969	2918	0.331	2967	2915	0.341
Birth length, cm	48.4	47.8	0.062	48.2	47.9	0.290
Adj birthweight*, g	2987	2924	0.240	2984	2921	0.264
Adj birthlength,* cm	49.5	48.5	0.0002	49.4	48.6	0.006

* Adjusted for gestational age and gender. Table shows mean values in those born light who did not catch up (No gain) vs those who did (Gain).

9.6.3 Childhood factors and catch up growth in lowest tertile of birthweight

Children born in the lowest third of birthweight were more likely to become taller relative to the rest of the lowest birthweight group if they drank more milk daily, and less likely if they watched more television per day (Table 86). Children with these habits were also more likely to gain weight relative to their low birthweight peers, although the association with television watching did not attain statistical significance. There were no statistically significant relationships with any other measures of childhood lifestyle or activity.

Table 86 Childhood milk intake and television watching, and odds ratios for catch-up growth from birth to 4 years, in lowest birthweight tertile.

	Height, sd		Weight, sd	
	OR (95%CI)	**p, n**	**OR (95%CI)**	**p, n**
Milk intake, pts/day	4.7 (1.5 to 15.1)	0.010, 108	14.0 (3.5 to 56.3)	<0.001, 113
TV watch, hrs/day	0.56 (0.34 to 0.91)	0.020, 110	0.75 (0.47 to 1.19)	0.223, 115

OR: Odds ratio

9.6.4 Maternal factors and catch-up growth in the lowest tertile of birthweight

Only maternal height at the initial interview was associated with the probability of children in the lowest third of birthweight catching up for height (Table 87). Maternal triceps skinfold thickness, walking speed, alcohol or milk consumption at any time point were not associated with the chance of catch up growth. Catch-up in weight, in contrast, was associated with maternal pre-pregnancy height, and smoking before and during pregnancy (Table 88).

Table 87 Maternal height and BMI, and odds ratio for catch-up in **height** from birth to 4 years, in lowest birthweight tertile.

	Odds ratio	**95%CI**	**p**	**n**
Height, cm	1.11	1.04 to 1.18	0.001	129

Table 88 Maternal height and smoking, and odds ratios for catch-up in **weight** from birth to 4 years, in lowest birthweight tertile.

	Odds ratio	95%CI	p	n
Height, cm	1.08	1.02 to 1.15	0.006	136
PP smoking, Y/N	3.6	1.8 to 8.0	0.002	136
EP smoking, Y/N	3.9	1.5 to 10.1	0.006	106
LP smoking, Y/N	3.8	1.6 to 9.4	0.004	126

When these factors were explored in multivariate logistic regression models with 4-year total milk intake and hours of daily television watching, only maternal height and 4-year total daily milk intake remained statistically significant determinants of the chance of height catch-up from birth to 4 years (Table 89). The chance of weight catch-up was also determined by maternal smoking in late pregnancy (Table 90), although the association with childhood milk intake just failed to achieve statistical significance (p=0.058). When birth size was included in the regression models, the association between childhood milk intake and catch-up for weight became statistically significant (p=0.035), and the other associations for height and weight remained robust.

Table 89 Mutually adjusted childhood and maternal factors, and odds ratios for catch-up in **height** from birth to 4 years, in lowest birthweight tertile.

n=123	Odds ratio	95%CI	p
4 yr milk intake, pt/day	5.8	1.9 to 17.5	0.002
Maternal height, cm	1.1	1.0 to 1.2	0.001

Table 90 Mutually adjusted childhood and maternal factors, and odds ratios for catch-up in **weight** from birth to 4 years, in lowest birthweight tertile.

n=122	Odds ratio	95%CI	p
4 yr milk intake, pt/day	2.7	1.0 to 7.5	0.058
Maternal height, cm	1.13	1.05 to 1.21	0.001
LP smoking, Y/N	54.9	1.8 to 13.3	0.002

LP: Late pregnancy

9.7 Summary discussion

These findings suggest that children who are born light tend to catch-up and children born heavy tend to catch-down relative to their peers, by age 4 years. However, in this population, these changes were not enough, on average, to move children from the lowest tertile of size at birth to a higher tertile by 4 years. Thus, despite relative catch-up in small babies and catch down in large ones, the general pattern overall was that small babies became small 4-year olds and large babies became large 4-year olds.

Greater catch-up for height was associated positively with maternal height, and 4-year total daily energy expenditure and milk intake. Greater catch-up for weight was also positively associated with maternal smoking in pregnancy. It is likely the mother's height is a marker of an inherited genetic drive towards tallness, and that greater 4-year milk intake may help accrual of calcium in the skeleton and thus growth. The positive effect of maternal smoking was present even after adjustment for birthweight, suggesting that the effect is not all due to rebound from a low starting point. Mothers who smoke are probably more likely to feed their children a highly energy dense diet (eg fast food) which will promote gain in weight rather than height. The chance of catching up or not relative to peers, within the lowest tertile of birthweight, was again associated with maternal tallness, and childhood milk intake for height and additionally maternal smoking for weight. The implications of these patterns of growth will be evaluated in the next section.

10 Final Discussion

10.1 Principal findings

- Maternal height, parity, and triceps skinfold thickness, smoking and walking speed in late pregnancy were associated with intrauterine bone mineral accrual in the offspring.
- Mothers with reduced stores of 25(OH)-vitamin D in late pregnancy had offspring with lower whole body bone mineral at birth in female infants.
- Paternal height was associated with offspring height at birth and 4 years.
- Paternal bone mineral was positively associated with neonatal bone mineral in the female infants only.
- The associations with maternal factors were much attenuated at 4 years, with only maternal height a consistent determinant of childhood bone mineral.
- Infants born small tended to show catch up growth; those born heavy tended to catch down. However, overall, small babies became small children at 4 years.
- Catch up growth in those born small was associated positively with maternal height and smoking in pregnancy, and childhood milk intake.
- The child's birthweight was associated with bone mineral at 4 years, but these relationships were removed by the addition of current height and weight.
- Childhood milk intake and daily energy expenditure were the strongest childhood determinants of bone mineral at 4 years, and were independent of maternal determinants.

10.2 Neonatal bone mineral

10.2.1 Maternal factors

Consistent with earlier findings, maternal height, parity, birthweight, triceps skinfold thickness, physical activity and smoking were all mutually independently associated with whole body bone area and mineral content of the neonate. As in the previous cohorts(150;151), maternal birthweight remained a significant determinant of intrauterine bone mineral accrual. Whilst the Princess Ann study found a negative association between high levels of physical activity (measured by questions relating to amount of activity that is

"enough to make you short of breath") and neonatal bone mineral, this was not found in the SWS cohort. Rather there was an association with habitual walking speed. This may reflect different questionnaire design or the very low frequency of very high levels of physical activity in our study, possibly reflecting secular trends in physical activity over the last decade.

Although the associations with maternal body build strengthened from before through to late pregnancy, the measurement before conception explained most of the measurement in late pregnancy. This suggests that the relationship between maternal fat stores and foetal bone mineral accrual may be most relevant in the last trimester, and that the biological effect of maternal fat stores is greatest here. However, the late pregnancy measurement of triceps skinfold thickness was almost completely determined by the measurement before pregnancy, and the increase in thickness did not predict infant bone mass. Thus it seems likely that body build before pregnancy is important to set the mother up for adequate supply to the foetus in late pregnancy, which implies that women should try to optimise their nutritional state before conception.

The influence of maternal height is likely to be genetic, although it is possible that taller mothers might have increased capacity to supply nourishment to the foetus and so act directly on foetal growth. There is also the issue of physical constraint due to maternal pelvic size(16), and taller mothers may tend to be larger in this respect(177). There appears to be maternal adaptation to pregnancy, which seems to increase the efficiency of placental nutrient transfer with successive deliveries, as babies of higher birth order had greater bone mineral than those of lower birth order. It is unclear how this happens, but could involve maturation of the uterine vasculature, or epigenetic modification of genes involved in placental calcium transport, thus upregulating their expression in future pregnancies. It is well documented that mothers who smoke have lighter babies (178). The mechanism for the foetal growth restriction in smoking mothers is poorly understood but may include reduced placental function, foetal hypoxia from increased carbon monoxide or a direct toxic effect on foetal growth (179). The associations with maternal fat stores and physical activity may reflect decreased ability to supply adequate nutrition, and a competition between the mother and foetus for finite resources. Since these measures were independently associated with neonatal bone mineral, the effect of physical activity could not be explained by an association with maternal thin-ness.

The associations discussed above are between maternal factors and neonatal whole body bone area and bone mineral content. These indices give a two dimensional measurement of skeletal size (the front to back distance is not assessed). Bone mineral density, as measured by DXA, is partly size corrected, although still not a measure of true volumetric density (see section 1.4.7). Thus, consistent with previous studies relating birthweight to bone mineral at peak and in older age, it seems that these intra-uterine factors primarily affect skeletal size rather than volumetric density. Given this relationship, it is not surprising that current height and weight account for much of the relationship. It could be argued that a large individual will need a larger skeleton, and the associations seen merely reflect an effect on overall body size. However, height and to a lesser extent weight are determined strongly by skeletal size, and so anything that leads to a larger skeleton will inevitably lead to greater overall body size. Conversely, larger body size requires a larger skeleton for support. Height and weight can be measured more precisely and accurately than bone mineral, and thus even an equivalently robust relationship will be seen more clearly with these anthropometric variables than with those derived from DXA assessment.

10.2.2 Paternal factors (height, weight and DXA indices)

The influence of the father on skeletal development in the offspring is necessarily a genetic one, in contrast to the combined genetic and environmental influence of the mother. Maternal and paternal genetic influences may be altered as a result of epigenetic modification of the genome early in development(158). This may result in a phenomenon known as imprinting, where only the maternal or the paternal allele are expressed in the foetus, allowing one parent, rather than both, to determine a particular trait(159;160).

Such epigenetic phenomena may also possibly explain the striking discordance between associations in male and female offspring with paternal bone mass. The borderline statistically significant interaction term implies that this gender disparity is not just a consequence of low sample size in the gender subgroups. Furthermore, the lower mean birthweight in the girls (perhaps allowing greater scope for paternal influence) did not explain the findings. It is possible that there might be a gender/imprinting interaction, such that the paternal allele of a gene influencing skeletal growth tends to be expressed in girls but not boys, for example on the paternally inherited X chromosome. The gene for PMCA3, a member of the PMCA family of plasma membrane calcium transporters is localised to the X chromosome (www.ensemble.org). PMCA3 may play a role in modulating placental calcium flux(99), so is a

possible candidate, as the placental genotype is foetal in origin. Thus far, there is evidence that another plasma membrane calcium transporter, PMCA1, undergoes epigenetic modification(180), but further work in this area will be needed to elucidate these mechanisms further. A different explanation could be that another gender-dependent factor, such as oestrogen/ androgen balance, might modify the genetic relationship.

10.3 Four year bone mineral

Although the associations between parental height and offspring bone mineral remained robust at 4 years, the relationships with other factors were largely attenuated compared with those at birth. Bone mineral at each site was largely explained by body size at 4 years, but total daily milk intake and daily energy expenditure were the strongest environmental childhood determinants, and were independent of maternal factors. As discussed in 10.2.1, the finding that current body size accounted for much of the variation in bone mineral is to be expected. At the neonatal DXA assessment, the baby has had at most two weeks of postnatal influence on its bone mineral, after spending 9 months in the intrauterine environment. As the child grows from birth to 4 years, there is great opportunity for exposure to a variety of environmental benefits, eg high milk intake, and disadvantages, eg inadequate diet, which may modify the skeletal growth trajectory. Since BA was predicted by maternal height, smoking and triceps skinfold thickness, but relationships were weaker with BMC (partly size dependent) and disappeared with BMD (more size dependent), this suggests that intra-uterine influences primarily influence the size of the skeletal envelope. Thus childhood factors, such as milk intake, may influence both longitudinal growth and volumetric bone density, so blurring the effects seen at birth.

For this analysis, childhood dietary data were limited to milk intake as a surrogate for calcium intake, and assessment of physical activity was measured by questionnaire. These limitations meant that it was not possible to fully characterise the influence of the childhood environment on skeletal growth. However, the strong association of bone mineral with current milk intake, and the suggestions of relationships between measures of sedentary-ness (television watching and sitting) and bone mineral support the notion that contemporary childhood environmental factors may modify the skeletal growth trajectory determined by genetic and the intrauterine environment. Results from the subset who underwent Actiheart assessment suggested that greater daily energy expenditure is associated with greater skeletal

size and areal density, height, and weight at 4 years. Greater physical activity may lead to greater loads on the skeleton and thus greater mineralisation. The total energy expenditure measured by the Actiheart is derived partly from heart rate and partly from accelerometry. Thus much of this measure must be due to physical activity rather than different basal metabolic rates. Additionally, the associations persisted after adjustment for measures of childhood size, supporting the notion that greater physical activity is associated with greater skeletal mineralisation independently of overall body size. Further detailed analysis of the Actiheart data will allow further dissection of these issues.

10.4 Maternal vitamin D stores and placental calcium transport

10.4.1 Overview of effects at birth and 4 years

In the PAH cohort of 9 year old children, mothers with reduced stores of 25(OH)-vitamin D in late pregnancy had offspring with reduced whole body BA, BMC and, more weakly, BMD. At birth, in this current thesis, there were similar associations in the girls only, albeit only weakly statistically significant. This pattern is similar to that found in the PAH study(67): Here the associations were statistically significant in the girls, but not boys, but the effect was strong enough to be seen in the cohort as a whole. It is difficult to compare the two cohorts thoroughly as different assays were used to measure vitamin D in the two groups. (IDS in the older study and Diasorin in SWS). These were both radio-immunoassays, and therefore both likely to underestimated D2). However, the proportion of mothers who were deficient (<11 ng/ml) in 25(OH)-vitamin D in the PAH study was 18% and that of mothers who were insufficient (11-20 ng/ml) was 31%. In the SWS group, the figures were 3.6% deficient and 27.8% insufficient. Thus the SWS group had higher levels of vitamin D and so in this cohort there was relatively reduced power to see an effect, particularly when blurred by contemporary diet, lifestyle and vitamin D levels through childhood. This may partly explain the very weak effects found at 4 years. However, although the associations were of borderline statistical significance, they were consistently positive in boys and negative in girls, so that increasing late-pregnancy vitamin D levels in the mothers were associated with decreasing bone mass in girls and increasing bone mass in boys at 4 years old. This is clearly the opposite pattern to that seen at birth and in the previous study.

It is difficult to explain this finding. There was a weak relationship between maternal vitamin D levels and smoking in pregnancy, but addition of smoking, triceps skinfold thickness or

parity did not remove the associations. Addition of maternal height did attenuate the relationships, but regression coefficients did not change dramatically. Inclusion of childhood fat mass, measured by DXA, removed the associations between maternal vitamin D level and whole body BA in girls, but not boys. Indeed, all the negative associations in the girls became non-significant after inclusion of fat mass, whereas the positive associations in the boys were unaffected. The fat mass-gender interaction term showed a trend towards statistical significance. Clearly girls have higher fat mass than boys, and it is know that fat cells have vitamin D receptors(44;47), and may be active in bone metabolism. Timing of adiposity rebound and the rate of accrual of fat mass may also play a part in this. Whilst it is difficult to put this all together, it raises intriguing possibilities about differential mechanisms in the two genders. It is possible that some other, unmeasured, factor was related to maternal vitamin D levels in pregnancy but also to an adverse effect on childhood bone mass.

It is not clear from this study, or previous work, when in pregnancy it is most important to have adequate levels of vitamin D. Since most bone mass is accrued in the last trimester, it would be reasonable to hypothesize that this might be the critical period, but it is important to note that to ensure that levels are adequate by this point, supplementation would have to begin some time earlier in gestation.

10.4.2 Possible mechanisms for relationship between maternal vitamin D stores and offspring bone mass

It is possible that mothers who have higher levels of vitamin D in pregnancy generally lead healthier lifestyles and thus have children who are also healthier and have higher levels of vitamin D themselves. This "inherited environment" effect was felt to be unlikely as a cause for the associations in the previous study, as no other aspects of maternal diet, lifestyle or social class explained the associations(67). It is very unlikely to be a major factor in the association with bone mass at birth. However, one reason for the conflicting results at 4 years could be an association with some other factor which influences skeletal growth in childhood, and had differential effects in pregnancy and in postnatal life.

There are several possibilities for how maternal 25(OH)-vitamin D might influence intrauterine bone mineral accrual:

Firstly, there might be a direct effect on foetal bone after crossing the placenta. There is evidence that 25(OH)-vitamin D can act as a ligand at the VDR and cause activation(44), albeit with lower efficacy than 1,25(OH)2-vitamin D. However, mouse models showing no detriment to bone development in VDR-null mice are against this, although skeletal growth in foetal mice may be somewhat removed mechanistically from that in humans.

A second possibility would be that 25(OH)-vitamin D might act to modulate expression of genes involved in placental calcium transport, thus indirectly influencing bone growth via an increase in calcium flux into the foetal circulation. Placental mRNA expression of one member of the active PMCA calcium transporter family (PMCA), PMCA3, has been shown to positively correlate with bone mineral content in the neonate at birth in the SWS(99), and other members of this family are known to regulated by vitamin D(98). Again, however, animal studies generally implicate PTHrP as the main determinant of placental calcium transport, although one study has suggested an effect of 1,25(OH)2-vitamin D in rats(66), but confirmation of this involvement in humans is thus far lacking.

Thirdly, rather than just being converted to the active form, 25(OH)-vitamin D might alter the regulation of the 1α-hydroxylase gene in the foetal kidney. It is not clear when expression of this gene commences in development, but there is evidence that it is before birth(10). Although the VDR does not appear to be necessary for calcium homeostasis in foetal mice(10), 1,25(OH)2-vitamin D does seem to influence placental calcium flux in some species(66). Work in an adult human cohort indicated that low birthweight is associated with increased 1α-hydroxylase activity(69). Thus low maternal vitamin D might lead to upregulation of the 1α-hydroxylase in attempt to normalise levels of 1,25(OH)2-vitamin D in utero, but with still without being able to fully normalise the system, resulting in lower bone mass at birth. However, if this upregulation were permanent, higher rates of bone turnover might result in later life, such that rate of bone loss and risk of fracture were increased in older age. Where one ended up in this mechanism might depend on postnatal levels of vitamin D, coming back to the concept of a mismatch between pre and postnatal environment being the important determinant of disease.

Finally, vitamin D might interact with adipose tissue, and this is discussed in the next section.

10.4.3 Gender specific effects of vitamin D

Males and females differ both at the genetic level, most obviously in the XX female and XY male karyotype, and also in hormonal status and anatomical construction. Thus there are several possibilities as to how a maternal environmental factor could influence bone mass in one sex rather than both. Girls have higher oestrogen levels than boys, and also greater stores of adipose tissue. As mentioned above, there is increasing evidence that fat may function as an endocrine organ, and expresses VDR(44). Fat also absorbs and sequesters circulating 25(OH)-vitamin D(44), and 1,25(OH)2-vitmain D is known to suppress differentiation of adipocytes(181). Thus there is the possibility that circulating 25(OH)-vitamin D could interact with bone via adipose tissue.

Epigenetic modification of expression of genes involved in placental calcium transport, such as PMCA3, might offer another mechanism for maternal 25(OH)-vitamin D to influence offspring bone mass. However, it is difficult to see how this would be gender specific. The placenta is of the offspring's genotype, but, although male infants have to inherit their X chromosome from their mother, female infants will gain one from both father and mother. Thus, in contrast to a paternally imprinted gene on the paternally inherited X chromosome in female infants, which could explain a genetic influence from father to daughter, it is difficult to use this mechanism to explain an environmental effect from mother to daughter.

Currently it is just not known where vitamin D fits into the mechanisms of placental calcium transport and intra-uterine bone mineral accrual and why there is a gender disparity. Physiological studies directly examining these mechanisms will be needed to elucidate these issues more completely.

10.5 Growth

The predominant pattern of growth from birth to 4 years was of catch-up relative to peers in those children who were born light (lowest tertile of birthweight) and catch-down in those who were born heavy (highest tertile of birthweight). This is consistent with previous studies of infant growth, and the notion that foetal growth velocity decreases prior to birth to enable a genetically large baby to be delivered by a small mother, followed by postnatal reversion to the original foetal growth trajectory(16). However, when repeated measurements are performed, initially very high or very low measurements tend to become nearer the mean on

subsequent measurement, and so part of this observed change could be due to this phenomenon of "regression to the mean". The changes persisted after adjustment for the baseline values, which makes this less likely. Despite birthweight-dependent catch-up and down, those born in the lowest third of birthweight were still smaller, lighter and with lower bone mineral at 4 years compared with those born in the highest. This implies that although there was a tendency to cross centiles, these were not of great enough magnitude to result, on average, in crossing from one tertile of outcome to that above. This does suggest that whatever has influenced birth size in the intrauterine period, the subsequent childhood growth trajectory may be modified by childhood environmental factors and a tendency to revert to genetically determined growth pathway. These two sorts of influences were apparent in this study, with children growing more relative to their peers (moving up the centiles) if their mother was taller, and also if the child themselves drank more milk.

There are very few data pertaining to the determinants of catch-up growth from birth, other than the observation that children born small tend to gain and those born large tend to lose. A study of 90 middle-class children found that two thirds of children crossed centiles in infancy. Examination of these children and comparison with parental height suggested that birth length was primarily related to maternal size, but that height at two years was determined mainly by mid-parental height; catch-up or catch-down occurred when there was a disparity between these two factors(21). Maternal height was again found to be a determinant of catch-up growth in a study of 449 Japanese infants born small for gestational age(182), but the environmental modifiers during infancy remain to be elucidated. In this thesis, it was found that higher levels of childhood milk intake were associated with increased catch-up: This could have been because increased nutrition was driving the growth, or because children growing more demanded higher levels of milk intake. Further work using the SWS should be able to shed more light on this issue, when detailed anthropometric and dietary measurements become available through infancy.

Gain in weight relative to peers was additionally associated with maternal smoking, which may actually reflect the postnatal environment as much as that in utero. Unfortunately the number of participants who were in the lowest tertile of birthweight, with DXA measures at 4 years, was not enough to explore these associations with sufficient statistical power to reveal any significant relationships for bone mass.

10.6 Implications of different patterns of intrauterine and childhood growth

It is apparent that there are a variety of influences on skeletal growth from pre-conception through childhood, and some of these may have persisting effects into later life. This thesis has demonstrated that a baby's size, relative to its peers, may change through infancy. Where the child ends up will depend on the interplay between these influences, both genetic and environmental, throughout the life-course.

At conception the child inherits a genetic tendency to a particular growth trajectory from each parent. Findings from this work have indicated that the magnitude of this association is similar for both mother and father. This trajectory is the baseline to which the child will tend to revert, all other influences being equal. Birthweight itself, however, is largely determined by the foetus's ability to extract nourishment from the mother and, in turn, her ability to supply this, and physical constraint due to pelvis size(16;17;183). Therefore, birth size is modified by local environmental factors in utero. Hence mothers who smoke, have reduced fat stores, have higher levels of physical activity and have lower levels of 25(OH)-vitamin D have smaller babies with reduced bone mineral. The question is whether this alters the subsequent growth trajectory. The associations were attenuated at 4 years, and this attenuation increased with increasing adjustment for body size, suggesting that these intrauterine factors still had modest influence on childhood skeletal size, but that volumetric density was determined more by contemporary factors. This implies that, although growth is fastest in the last trimester, and then undergoes slowing before birth, factors which influence intrauterine growth may not just affect birth size- despite the post-natal catch up, there appears to be a longer term influence. Whether this is because of these factors acting in early pregnancy or preconception, or an effect in late pregnancy over and above the immediate effect on birth size, is unclear.

Given that small babies turn into small 4-year olds, albeit with some catch up, these data are consistent with the notion that low birthweight is a risk factor for lower bone mineral at peak and in older age. How they relate to risk of hip fracture itself is less clear. The first Finnish study(25) suggested that poor childhood growth from age 7 to 15 years and greater maternal height were independently associated with increased risk of hip fracture in older age. In addition, participants who were shorter at birth but of average height by age 7 years were also at increased risk. In the second cohort(148), in which infant growth data were available,

children who grew poorly from 1 to 12 years were at increased risk of hip fracture in older age. Thus it is possible that there are various routes to a common outcome. Given that hip fracture is multifactorial (bone size, density, geometry, turnover are all important) this is not an unexpected finding. This thesis suggests that a child who is born light and then does not catch up will be smaller at age 4 years than one who does catch up, despite the latter group being slightly smaller still at birth. Children of taller mothers were more likely to show catch up growth. Taller adults have a higher risk of fracture(184), possibly because of a longer femoral neck length, or a greater tendency to fall. During childhood, risk of fracture is greatest at entry to puberty(149), where longitudinal growth is accelerated, and where there may be a resulting discordance between skeletal size and the body's capacity to mineralise. Thus children of taller mothers who are small and grow excessively quickly may end up with relatively undermineralised bones, and also adverse femoral geometry, and thus an increased risk of fracture. Additionally, as taller mothers tend to have larger babies, possibly because of larger pelvis size, if the baby is born small, this is more likely to have been because of adverse intrauterine influences rather than natural physical constraint; thus these babies may be starting with sub-optimal bones. This may also suggest that the cause of catch up growth is important- if it is genetically driven by a taller mother, in the absence of adequate nutrition, poorer skeletal mineralisation may result. In contrast, if the catch up is driven by nutrition, then healthier bones may result. The practical difference here may be that the genetic tendency is permanent, whereas the influence of nutrition may only persist as long as the environmental factor continues. Since children tend to end up at the mid-parental height(15;21), it is likely that the difference in parental height will also be important. For example, a tall father and a short mother will be likely to have a small baby at birth, but which shows catch-up growth. Since the cause of the smallness of birth is likely to be natural physical constraint rather than undernutrition, it is possible to speculate that this might be a healthier outcome than if the baby were born small to a large mother. Further work in the SWS may be able to clarify this issue.

This leads to the question of whether, given the intrauterine environment and genetic make-up, and individual's postnatal growth trajectory can be modified by environmental influences such as diet and exercise. The tendency of children to revert to their original growth trajectory after a period of illness or nutritional constraint(12;15) would suggest that short-term interventions may have limited value in improving childhood growth. Indeed studies of calcium supplementation have generally showed only short-term benefits. It is difficult to be

absolutely sure about long-term benefits because of the difficulty in making accurate repeated measurements of bone mass in growing children. However, chronic malnutrition does seem to lead to small peak stature(12;15), and it is likely that good nutrition and lifestyle are needed throughout growth to ensure attainment of optimal peak bone mass.

To put all this together, children who are born small and do not gain relative to their peers, and children of taller mothers who are born small and catch up in excess of their capacity to mineralise their skeleton may be at increased risk of fracture in later life. Contemporary diet and lifestyle at any stage of growth may modify the growth trajectory in the short-term, but the long-term impact of these factors remains to be demonstrated.

10.7 Further work

There are two aspects to future work in this area. Firstly, a greater understanding of the underlying mechanisms in these associations needs to be gained. Secondly, there needs to be translation of this work from observational studies to intervention-based investigation. The effect of 25(OH)-vitamin D supplementation in pregnancy for women found to be deficient in this hormone could be investigated in a randomised controlled trial with sufficient power to come to a conclusion. A lifestyle based intervention, optimising maternal diet, body build and lifestyle during pregnancy and then similar measures in the offspring through childhood might be examined across a range of GP practices, for example in a cluster-randomised trial setting, assigning an entire GP catchment area to intervention or control. A design of this sort would be appropriate here, as lifestyle interventions could easily be confounded by the pregnant mothers talking to each other about the study. Thus geographically separate areas are needed. There are problems with cluster-randomisation though, as the "n" of the study becomes the number of GP practices, and thus greatly reduces statistical power.

In terms of analysis based on this thesis cohort, there are many potential areas of investigation:

- The detailed dietary and growth data from birth to 4 years may be explored further to investigate which nutrients are important at which time point for postnatal growth. This would allow a more longitudinal approach to childhood nutrition, for example, asking whether milk intake at 1 year is associated with bone mass at 4 years. The interplay between maternal and paternal height could also be explored. Additionally

this might suggest possible public health interventions, and allow a more in-depth description of longitudinal childhood growth patterns in relation to bone mineral and activity.

- Regional body composition at 4 years: the whole body DXA scans should allow investigation of the maternal and childhood factors which influence differential adiposity. Regional muscle mass, excluding abdominal organ mass, can also be investigated.
- More detailed investigation of the relationships between maternal and childhood physical activity measured objectively using the Actiheart, and childhood bone mineral.
- Hip structure analysis at 4 years using the DXA hip scan. This software enables calculations of mechanical parameters such as bending strength of the femoral neck from the standard hip DXA scan.

As the children grow older, the aim will be to assess them again by DXA and PQCT at age 6 years. This will give opportunities for further longitudinal characterisation of skeletal growth, as well as measurement of true volumetric bone density for the first time, helping to distinguish factors which influence bone size from those which influence mineralisation.

10.8 Implications for public health strategy

These data reinforce the concept of a life-course approach to preventing disease in late adulthood. Although the effect of maternal factors on bone mass at birth was attenuated at 4 years, the data suggest an effect on bone size, being blurred by genetic and post-natal influences on size and volumetric density. Given the suggestion that catch-up growth in the absence of adequate nutrition may be unhelpful for skeletal mineralisation, optimising birthweight may be beneficial. So recommending that mothers do not smoke, (or drink excessive alcohol) or take excessive exercise in pregnancy, and ensure that they are adequately nourished before conception would seem to be sensible advice. Additionally, ensuring that they are replete in 25(OH)-vitamin D may also be helpful. Postnatally, these data suggest that adequate milk intake is important to help optimise the postnatal growth trajectory. It is likely that other elements of diet are important, but these data were not available for this analysis. Given the evidence from other studies, it is unclear whether a temporary dietary intervention at any time may have long term effects on skeletal growth, and thus a healthy calcium rich

diet, with adequate vitamin D, throughout childhood, in addition to moderate amounts of physical activity, would be reasonable to ensure optimal accrual of peak bone mass.

10.9 Limitations to interpretation of the study

This study utilised a prospective cohort, with comprehensive assessment of the mothers before and during pregnancy. However there were a number of limitations:

10.9.1 General recruitment issues

Mothers were invited to participate via their GPs and by an advertising campaign. Thus they were self-referred, and will tend to be healthier than women who did not volunteer. People who take part in studies are generally also better motivated and more compliant than those who do not. Thus the cohort of mothers, fathers and children is likely to be healthier than a random cross-section of the general population at this age range. It was certainly apparent that the babies who underwent DXA assessment were of higher birthweight than those who did not. However, this would only change the results if the relationships between maternal factors and offspring measurements differed in these populations. It is unlikely that this would be the case, but it is likely that this factor will have made it more difficult to show a relationship, biasing towards the null hypothesis. For example, smoking may be less prevalent in the study cohort than in the general population, so making it more difficult to show an effect of smoking on offspring bone mass. For the four year follow up the children need to survive to this age, so any children who died in infancy will have been lost to the study, as have those who moved away. These were the minority and again, this is likely to make any associations more difficult to demonstrate. Finally, participants may modify their behaviour because they are being studied (Hawthorne Effect). This could be either a real modification or a misrepresentation of true habits (for example denying smoking when they, in reality, do). This will depend on the participants' preconceptions of what habits are beneficial to health, but would generally tend to blur associations and bias towards the null hypothesis. Unfortunately all studies are beset with the problem of recruitment bias and the Hawthorne effect, but the SWS has an advantage in long-term follow-up and detailed data collection, allowing checks on internal consistency of information.

A second general problem is that associations may be mediated by another third factor, which is associated with both demonstrated factors. Thus, as an example, it might be possible to see

a positive association between maternal smoking and maternal bone mass, when this association is actually because increased milk intake is associated with higher bone mass and mothers who smoke drink more milk. This is termed "confounding". Attempts to reduce this sort of error have been made by considering a priori hypotheses, the biological plausibility of associations and exploration in multivariate models.

10.9.2 Parental data

Lifestyle factors such as alcohol intake, smoking and exercise may have been influenced as a result of women tending to under-report behaviour associated with poorer health outcomes and over-report beneficial habits. This would have different effects depending on the direction of the bias and the particular variable. Food intake by the food frequency questionnaire has been validated previously(176), and regular training and updating of the research nurses carried out. Likewise the nurses underwent regular anthropometry assessment and retraining if needed.

The study involves two overlapping subsets of women. These two populations were very similar in the measurements used for the analysis.

10.9.3 Maternal serum samples

Maternal blood samples were collected in late pregnancy and spun down within 24 hours, and serum separated, before being stored at –70°C. 25(OH)-vitamin D should be stable over this period. Any degradation of the samples is likely to be random rather than systematic. 25(OH)-vitamin D was measured using a RIA with high specificity and sensitivity, although there could have been underestimation of the D2 form, which is a problem with all radioimmunoassay techniques. This is probably most relevant in participants who were vegetarians, as they are likely to have higher levels of the D2 form. However, it was not possible to separate out these women for this thesis.

10.9.4 Neonatal anthropometry and bone mineral measurement

Regular assessment of the research nurses' measurements were made, with re-training if needed. However, neonates have a tendency to move, so limiting accuracy of anthropometric measures. However, this is likely to attenuate any findings, as it is unlikely that there is any systematic relationship between maternal factors and neonatal movement. Movement of the

neonate on the DXA scanning table has been shown to affect measurement of BA, BMC and BMD, with a systematic overestimation of bone mass in those subjects with higher movement. In this study movement was graded and those babies with excess movement excluded; the movement artefact across the remaining cohort was reasonably uniform. Again there is no reason to expect a relationship between neonatal movement during the DXA assessment and maternal factors. Edge detection of bones is more difficult in neonates, due to the lower absolute BMD. However, specific paediatric software was utilised, with increased sensitivity for edge detection. Given the variability in the position of the neonates within the DXA software-defined regions of interest, it was not possible to investigate regional bone mass or body composition. Thus in the assessment of body composition, it was not possible to measure truncal obesity from the DXA assessment. Whole body lean mass, measured by DXA, includes a significant contribution from the intra-abdominal organs, so represents these in addition to muscle mass.

10.9.5 Neonatal and infant feeding

Detailed data on neonatal and infant feeding have been collected, but were not available for this thesis. It is likely that these factors will be important, and may well confound the maternal-offspring relationships. When these data are available, it will be possible to explore this and also look longitudinally at feeding and childhood bone mass.

10.9.6 Four year follow-up

Although this thesis has made some inferences of causation, for example that milk intake positively influences bone mineral accrual, it must be remembered that these are cross-sectional data at 4 years, and thus causation cannot be proved. It seems likely however, that, in general, children's habits will be similar over the year preceding the assessment.

10.9.6.1 Questionnaire

Dietary intake was limited to foods containing calcium and vitamin D, and these questions were derived from the previously validated FFQ. However, the selection of questions used at 4 years has not been formally validated against a food diary or measured intakes. Unfortunately the nutrient derivations were not available by the time of writing, so it was not possible to use detailed dietary information. Milk intake was used a crude marker of calcium

intake, but it is expected that valuable information would be gained when more detailed dietary analysis is possible.

10.9.6.2 Actiheart

Only preliminary data for daily total energy expenditure were available, and thus further work will be needed to explore how this relates to physical activity per se, and also to body size and composition. Mathematical algorithms are being developed in Cambridge to optimally clean the data (to account for times when data is lost).

10.9.6.3 Anthropometry

Anthropometric measurements were performed by three people only, and regularly training was undertaken, to optimise accuracy and repeatability, with assessment of inter-observer differences.

10.9.6.4 DXA measurements

Movement artefact was much less of a problem at 4 years, with the majority of the children able to lie still. Artefact was most significant at the head and so whole body minus head bone mineral and body composition measures were used to reduce this. Additionally this reduces the large influence head bone mineral has on the overall measurement. Owing to the lack of lumbar spine and hip measures at birth, it was only possible to compare whole body bone mass from birth to childhood, and the number of participants with scans at both time points was a minority of the cohort, so limiting power. The two instruments measured bone mass using different techniques: the Lunar instrument used a pencil beam and the Hologic a fan beam. These, plus concerns with differential change in body composition and bone size, mean that reporting of absolute change in bone mass from birth to 4 years is not possible, but that change relative to peers, and correlation between the measures, should be valid.

10.10 Conclusions

Maternal lifestyle, anthropometric measurements and levels of 25(OH)-vitamin D during pregnancy were associated with intrauterine bone mineral accrual, but these associations, apart from maternal height, were greatly attenuated by the time the children were 4 years old. Childhood milk intake and daily total energy expenditure were strong determinants of

childhood growth. Together with maternal height and smoking, these factors were associated with variation in the childhood growth trajectory relative to peers from birth to 4 years. These observations suggest that a life-course approach, starting in the preconception period, is appropriate for the prevention of osteoporotic fracture.

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One participant and her mother prepare to meet HRH Duchess of Cornwall during her visit to the Osteoporosis Centre and official opening of the MRC Epidemiology Resource Centre.

Appendix AA:

Protocols for anthropometric measurements

Protocol for measurement of skinfold thickness

a protocol for fieldworkers at the MRC Environmental Epidemiology Unit, University of Southampton

Introduction

The assessment of body composition, that is how much fat, lean tissue and muscle a person has, is essential in evaluating their nutritional status. There is evidence that, for example, a mother's ability to nourish her fetus, is influenced by her level of fatness before she becomes pregnant. There are numerous methods for measuring the amount of fat someone has, but many require expensive equipment and cannot be used in people's homes. Skinfold thickness measurements are simple and non invasive and require relatively inexpensive equipment.

The measurement of a skinfold is a direct measure of a double thickness of skin and subcutaneous fat.. The purpose of measuring skinfolds is twofold:

1. They can be used to assess how much fat someone has and this reflects nutritional status. Low amounts of body fat show that the individual is undernourished. In this country, too much fat is more common. The problems associated with this are well known. We use a series of equations that convert skinfold thicknesses into an amount of fat, or fat mass (see page 7).

2. It is now realised that *where* the fat is stored on the body is important. Different patterns of fat distribution are known to predict the risk of developing coronary heart disease and diabetes. Central fat (on the trunk) appears to be more unfavourable than peripheral fat (on the limbs). We think that the metabolic differences in fat stores at different places in the body may also influence fetal growth.

We measure four standard skinfold sites: triceps, biceps, subscapular and upper suprailiac. These sites were selected from 93 sites originally assessed.[1] They are easily located in relation to bony landmarks, the skinfold can be raised from the underlying tissue and measurements are reproducible.

Apparatus

There are three types of skinfold calipers are in common use in this country: the one we use is the Harpenden 'John Bull' model with external springs. They come with a plastic carrying case and should always be kept and transported in their case. They are delicate precision instruments.

The blades of the calipers are $90mm^2$ and open to 50mm. The large dial reads up to 20mm, and a smaller scale on the dial registers whether you have already gone once or twice round the scale. They exert a constant pressure of $10g/mm^3$. Divisions on the dial are every 0.2mm, but it is usually possible to read to the nearest 0.1mm. There is a screw adjuster on the side of the dial. Loosening this screw allows you to move the dial face relative to the needle, so that you can adjust the instrument to 0 with the blades closed. However, you should only let the appointed person for your study adjust your calipers.

Calipers can be tested and calibrated using machined metal blocks to check the readings about once a month. They can be sent back to the manufacturers to be calibrated, but this is costly and should not be necessary if they are treated carefully.

Technique

1. There is no international consensus as to which side of the body should be used for skinfold measurements. Some use the non-dominant side, others always the right or always the left.[2] There is no statistically significant difference between measurements made on different sides of the body, even when there are considerable differences in muscularity, as in a tennis player.[3,4] However, as we are also interested in muscularity and will be measuring mid upper arm circumference,

We make all measurements on the non dominant side.

2. For the technique of picking up the skinfold: I quote from Noel Cameron: "the skinfold is often described as a "pinch", but the action to obtain it is to sweep the index or middle finger and thumb together over the surface of the skin from about 6 to 8 cm apart and to collect the subcutaneous tissue pushed away from the underlying muscle fascia by this action. To "pinch" the subject suggests a very small and painful pincer movement of the fingers, and this is not the movement made. Firstly, the measurement of skinfolds should not cause undue pain to the subject..... Secondly, a pincer or pinching action does not collect the quantity of subcutaneous tissue normally measured".[5] It is easier to use both hands initially, to massage up a tube of skin with the thumb and fingers of both hands. One hand then remains holding the skinfold throughout the measurement of the skinfold, and the measurer picks up and uses the calipers with the other hand.

3. The positioning of the blades of the caliper on the skinfold will vary with the size of the fold of skin, but in general should be at least one blade-breadth in from the apex of the skinfold. Be careful not to twist the calipers while striving to read the dial.

4. There are different techniques for timing the readings. Some say you should take the reading after 2, 4 or 5 seconds after closing the blades. Others think that that you should wait until the needle on the dial has stopped moving.[5-8] Experienced measurers say they have no difficulty

counting 2 seconds in a reproducible way, but there is no doubt that at 2 seconds you are often trying to read a rapidly moving target, and this is likely to produce differences between measurers.

We therefore use the 5 seconds rule.[8]

By doing this we measure **compressed** fat thickness. This may be important as people vary in the compressibility of their fat. Female fat is more compressible than male fat. However, it is important that we all follow the same convention.

The calipers should be released fully before beginning to count to 5. This is particularly important if a measurer has small hands because it is possible that some pressure will be maintained on the lever of the calipers not allowing them to exert full pressure. The dial should be read at 5 seconds even if it is still moving.

5. Do not drag the calipers off the fold at the end of the measurement, as this is uncomfortable and may damage the calipers. Consciously open the jaws to remove them.

6. Generally at least three measurements are taken at each site, releasing the skinfold and picking it up again each time. Some people keep measuring until three readings very close together are obtained. Some people use the average of three measurements, others use the minimum or the maximum!

We make three readings and use the average. In some studies, the computer analyses the three values and decides if they match closely enough. If they don't, you may have to make up to two further readings.

7. The technique and bony landmarks used are the same in men and women.

Triceps skinfold[5-7]

The subject stands with their back to the measurer, arms hanging by their sides. The tip of the acromion (the point of the shoulder) is palpated and marked. With the subject's arm flexed at 90°, the olecranon (tip of the elbow) is palpated. Put the tape measure on the mark on the acromion and drop it down to the elbow, by the side of the arm. Read the exact distance as if you had drawn an imaginary horizontal line from the bottom most point of the elbow to your tape measure. Mark a point on the arm halfway between the acromion and elbow. This marks the vertical level at which the circumference will be measured. It is important that this measurement is made with the arm flexed, otherwise the tape takes an oblique course across the upper arm, and the mid-point is too high up. The subject is then asked to relax, with the arm hanging by their side. This is important as a very different reading may be obtained if the arm is not fully relaxed.

The tape is placed around the upper arm with the upper border of the tape at the level of the mark, as if to measure mid-upper arm circumference. With the tape in position a horizontal line is drawn on the skin posteriorly and anteriorly at the level of the first mark. The posterior line is used for the triceps fold and the anterior line for the biceps fold. To determine the side-to-side position at which the skinfold is measured, you must "eyeball" the mid-point and the most dorsal (ie the part which sticks out furthest posteriorly) part of the upper arm at the level of your horizontal mark. Make a vertical mark to form a cross.

The skinfold is picked up in a vertical "tube", with two hands, at least 1cm. above and below the cross. The skinfold calipers are applied at the level of the cross, with the cross on the apex of the fold.

It has been shown that the precise site is important, and that very different readings can be obtained, especially by displacement laterally and especially in obese subjects.

Biceps skinfold[5,6]

The subject faces the measurer with their arms hanging down and the (non dominant side) palm facing forward. An anterior horizontal line already marks the level at which the skinfold will be measured. As with the triceps skinfold, you need to "eyeball" the point along this line where the arm bulges forward the most - the mid point of the belly of the biceps muscle. Mark a vertical line here to form a cross. There is sometimes a prominent blood vessel visible here, but you can ignore it: it will not be damaged by the calipers. The skinfold is picked up vertically and the calipers are applied at the level of the cross, with the cross on the apex of the fold.

Subscapular skinfold[5-7,9]

The subject stands with the shoulders and arms relaxed. The lowermost tip of the scapula is identified. This is easy in a slim subject but may be difficult in the obese. It may help to follow the medial border of the scapula downwards until the inferior angle is felt. Alternatively, you can make the scapula stand out by asking the subject to put their arm behind their back, in a half-nelson. Once it is located, however, the subject must relax their arm again before you mark the skin with a cross, immediately below the lowermost tip of the scapula. The skinfold is picked up obliquely, in the natural cleavage of the skin and the calipers are applied at the level of the cross, with the cross on the apex of the fold.

Upper suprailiac skinfold[6,7]

Stand behind the subject. They should stand straight and relaxed with their arms folded in front of them. Locate the iliac crest, the large curving pelvic bone, just below the waist. In obese subjects, you need to palpate firmly, and in all subjects, it helps to feel both sides together. Draw a horizontal line just above the crest at the side. Next find the mid axillary line: ask the subject to lift up their arm. The apex of the axilla is at the lowest point of the axillary "hollow", just behind the thick fold made by the pectoral muscle. Drop an imaginary vertical line down from the apex of the axilla. This is the mid axillary line. Draw a line where this imaginary vertical line meets the horizontal line.. Pick up the fold in the natural creases of the skin and apply the calipers at the level of the cross, with the cross on the apex of the fold. It may help to ask the subject to tilt towards you to ease the tension on the skin while picking up the skinfold.

Calculating percentage body fat[10]

The most common way of assessing how much fat someone has is by expressing it as a proportion of their total body weight. Some people may have had this done when they join a health club or gym. Women who have less than 15% body fat are thought to be underweight. At the age of 25 years, a normal amount would be 25%.[11]

The equations of Durnin and Womersley, reproduced here, have been widely used to calculate percent body fat from skinfold thicknesses. These were based on measurements carried out in a group of men and women between the ages of 16 to 72 years.

When using predictive equations to derive percentage body fat, it is essential that the same skinfold sites and measurement techniques are used as in the original paper and they should not be extrapolated to other populations or to pregnancy.

Calculations

1. total skinfolds = average triceps skinfold + average biceps skinfold + average subscapular skinfold + average upper suprailiac skinfold

2. *density* = c - [m x log total skinfolds]

where c and m are found according to subject's age from this table

Age	16-19	20-29	30-39	40-49	50+
c	1.1549	1.1599	1.1423	1.1333	1.1339
m	0.0678	0.0717	0.0632	0.0612	0.0645

3. $$\text{body fat \%} = \left\{\frac{4.95}{\text{density}} - 4.5\right\} \times 100$$

Sarah Duggleby/Caroline Fall. February 1998

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Protocol For Anthropometric Measurements

Height[1-3]

Place the base-plate on the floor, selecting as firm and level a surface as possible, and preferably near a perpendicular, such as a door architrave, which helps the eye to ensure that the tape is vertical. Ask the subject to remove her shoes and stand on the base-plate with her back to the tape. She should be told to stand as tall and straight as possible with feet together and arms held loosely at the side and shoulders relaxed (to avoid lordosis). She should stand far enough forward on the base-plate such that the tape is not distorted when pulled to vertical. Check that the tape is inserted correctly into the base plate. Raise the tape vertically and place the head plate on the top of the subject's head, using the spirit level to check that the plate is horizontal.

The head should be placed in the Frankfurt Plane, such that an imaginary line joining the upper margin of the external auditory meatus and the lower border of the orbit of the eye is horizontal.

If a short person is measuring a very tall person, considerable error can be introduced when reading off the height scale: their eyes will read the scale at an angle (parallax effect). Measurers should be aware of this and aim to read the scale from as level a position as possible. If there is a lot of height disparity the measurer should try to get level with the scale, by standing on, for example, telephone directories. Read the height to the nearest 0.1cm and beware of digit preference. Make one measurement of height.

Weight

Take time to place the weighing scales on a level surface. Ensure the scales are at zero when they are switched on. Weigh without shoes. Aim for subject to be wearing shirt and trousers/skirt only, and to remove heavy items of clothing and heavy jewellery if possible. Weight to nearest 0.1kg. Make one measurement of weight.

Waist circumference[3]

This should be measured over bare skin. Apply the tape mid-way between the lower rib margin (costal margin) and the iliac crest, both palpated at the side. **Ensure the tape is horizontal all round.** Ask the woman to relax, ie. not to deliberately hold herself in or out, and to look straight ahead with **arms relaxed at her sides.** Make the measurement at the end of expiration. Make sure the tape is not pulled too tight: should be resting on the skin but not indenting it. Measure to the nearest 0.1cm and beware of digit preference. Make one measurement of waist circumference.

Hip circumference[3,4]

Apply the tape at the **widest** part, usually between the greater trochanter (top of the thigh bone) and the lower buttock level, with the legs together. **Ensure tape is horizontal all round.** Measure to the nearest 0.1cm and beware of digit preference. Make several measurements of hip circumference and record a single maximum reading.

Mid upper arm circumference[1,3,4]

The subject stands with her back to the measurer, arms hanging by her sides. The tip of the acromion (the point of the shoulder) is palpated and marked. Then with the subject's arm flexed at 90°, the olecranon (tip of the elbow) is palpated. Put the tape measure on the mark on the shoulder and drop it down to the tip of the elbow, by the side of the arm. Read the exact distance as if you had drawn an imaginary horizontal line from the bottom most point of the elbow to your tape measure. Mark a point on the arm halfway between the acromion and olecranon. This marks the vertical level at which the circumference will be measured. It is important that this measurement is made with the arm flexed, otherwise the tape takes an oblique course across the upper arm, and the mid-point is too high up. The subject is then asked to relax, with the arm hanging by her side. This is important as a very different reading may be obtained if the arm is not fully relaxed. The tape is placed around the upper arm, with the upper border of the tape on the mark. **Ensure tape is horizontal all round.** Make sure the tape is not pulled too tight: it should rest on the skin, but not indent it. Read the tape to the nearest 0.1cm and beware of digit preference. Make one measurement of mid upper arm circumference.

Leg Length[5]

Leg length will be measured from the iliac crest to the floor in the Southampton Women's study. The subject should stand in a relaxed position, with her weight evenly distributed on both feet and with her feet about 25cm apart. The iliac crest will have been marked already to measure the suprailiac skinfold. Check that this point is the highest point of the iliac crest. Ask the subject to stand with their (non dominant side) foot on the stadiometer base plate. Place the top plate at the mark on the iliac crest, ensuring that the tape is vertical and not distorted. Read the tape to the nearest 0.1cm and beware of digit preference. Make one measurement of leg length.

Mid thigh circumference[4]

The subject should stand in a relaxed position, with her weight evenly distributed on both feet. Thigh circumference can be measured over a thin layer of clothing such as tights or thin trousers. Do not measure over jeans. If the subject is wearing a skirt, locate the mid point over the skirt, but mark and measure with the skirt raised. Locate and mark the greater trochanter (top of the thigh bone) - the point at which the bone protrudes most at the top of the leg. Palpate this at the side of the body. Mark the proximal border of the patella (the top of the knee cap) at the side of the leg. Mark a point on the leg halfway between the greater trochanter and the proximal border of the patella. The subject is then asked to relax. Place the tape around the thigh. **Ensure tape is horizontal all round.** Make sure the tape is not pulled too tight: it should rest on the skin, but not indent it. Read the tape to the nearest 0.1cm and beware of digit preference. Make one measurement of mid thigh circumference.

Maximal calf circumference[1,3,4]

The subject stands with her feet about 25cm apart in a relaxed position, with her weight evenly distributed on both feet. Calf circumference can be measured over a thin layer of clothing such as tights or thin trousers. If possible, however, roll up the trousers, as long as it does not compress the area where you measure. Do not measure over jeans. The tape is placed around the calf at the widest part. **Ensure tape is horizontal all round.** Move the tape up and down to locate the maximum circumference. Measure to the nearest 0.1cm and beware of digit preference. Make several measurements of calf circumference and record a single maximum reading.

Sarah Duggleby. February 1998

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Protocol for grip strength measurement in Southampton Women's Survey

Measurement of grip strength.

1. Sit the subject comfortably in the standard chair with arms.
2. Ask them to rest their forearm on the arms of the chair with their wrist just over the end of the arm of the chair – wrist in a neutral position.
3. Demonstrate how to use the dynamometer to show that gripping very tightly registers the best score.
4. Start with the right hand
5. Position the hand so that the thumb is round one side of the handle and the four fingers are around the other side. The instrument should feel comfortable in the hand: alter the position of the handle if necessary.
6. Support the dynamometer lightly at the bottom with one hand and not at the top.
7. Encourage the subject to squeeze as long and as tightly as possible or until the needle stops rising.
8. Record the result to the nearest 1kg on the paper data entry form.
9. Repeat measurement in the left hand
10. Do 2 further measurements in each hand alternating sides to give 3 readings in total for each side.

Appendix A:

Consent form for neonatal DXA study

SOUTHAMPTON Women's SURVEY

SWS number:

CONSENT FORM – FETAL GROWTH AND NEONATAL BONE MASS

Thank you for agreeing to take part in a study of nutrition and health in relation to the development of a baby's bones. Taking part involves my baby having a DXA scan which takes about 15 minutes to perform, during which he/she will be lying wrapped in a blanket, on a table, with a scanning arm moving about two feet above. A very small dose of x-rays is used during the examination. The scan does not cause any pain, and will not harm the baby. All the results will be kept securely and will only be seen by the researchers involved in the study.

Having discussed the procedure with the nurse, I agree for my baby to take part.

I understand that I am free to withdraw from the study:

At any time

Without having to give a reason for withdrawing

And without affecting my future medical care

Signature of participant ..... Date .....

Signature of researcher ..... Date .....

Appendix B:

Ethics committee approval for neonatal DXA study

Southampton
University
Hospitals
NHS Trust

Southampton & S.W. Hants
Joint Research Ethics Committee
Trust Management Offices
Mailpoint 18
Southampton General Hospital
Tremona Road
Southampton SO16 6YD

Tel 01703 794912
Fax 01703 798678

Ref: JM/CPW

29 June 1999

Prof. C Cooper
MRC Epidemiology Unit
Mailpoint 95
SGH

Dear Prof. Cooper

Submission No: 153/99 - Fetal growth and neonatal bone mass.

Following the decision to withhold approval, I am pleased to confirm **full approval** having received annexe B which was missing from the original application.

This approval was considered by the Committee at their meeting on 23 June.

May I draw your attention to the enclosed conditions of approval which **must be complied with.** In particular: it is mandatory that **ALL correspondence, information sheets, consent forms, adverts etc, carry the LREC submission number.**

This committee is compliant with the International Committee on Harmonisation/Good Clinical Practice (ICH) Guidelines for the Conduct of Trials involving the participation of human subjects as they relate to the responsibilities, composition, function, operations and records of an Independent Ethics Committee/Independent Review Board. To this end it undertakes to adhere as far as is consistent with its Constitution, to the relevant clauses of the ICH Harmonised Tripartite Guideline for Good Clinical Practice, adopted by the Commission of the European Union on 17 January 1997.

The composition of the committee is enclosed for your files and confirms which members were present at the meeting. Most pharmaceutical companies request this information and we would be grateful if you could forward this to them if appropriate.

Yours sincerely,

C Wilkinson

Clair Wilkinson (Ms)
Administrator

Appendix C:

Ethics committee approval for paternal DXA study

SOUTHAMPTON & SOUTH WEST HANTS
LOCAL RESEARCH ETHICS COMMITTEE

Chairman: Dr A Kermode

Manager: Mrs Clair Wright
Trust Management Offices
Southampton General Hospital
Tremona Road
Southampton
SO16 6YD

Tel: (023) 8079 4912
Fax: (023) 8079 8678

1

Ref: CPW

3 October 2001

Professor C Cooper
MCR Epidemiology Unit
Mailpoint 95
SGH

Dear Professor Cooper

Submission No:213/01 – Paternal lifestyle, body build and skeletal status as determinants of neonatal bone mass.

The Ethics Committee considered your recent response in accordance with the decision to withhold approval for the above study at the meeting on 26th September 2001 and I am pleased to inform you that approval is now granted.

May I draw your attention to the enclosed conditions of approval which must be complied with. YOU SHOULD BE AWARE THAT A SUBSTANTIAL RANDOM PROPORTION OF RESEARCH PROJECTS ARE AUDITED ANNUALLY.

The data protection officer for the Trust/University is to be notified of the study.

This committee is fully compliant with the International Committee on Harmonisation/Good Clinical Practice (ICH) Guidelines for the Conduct of Trials involving the participation of human subjects as they relate to the responsibilities, composition, function, operations and records of an Independent Ethics Committee/Independent Review Board. To this end, it undertakes to adhere as far as is consistent with its constitution, to the relevant clauses of the ICH Harmonised Tripartite Guideline for Good Clinical Practice, adopted by the Commission of the European Union on 17 January 1997.

The composition of the committee is enclosed for your files and confirms which members were present at the meeting. Most pharmaceutical companies request this information and we would be grateful if you could forward this to them if appropriate.

Should any unforeseen problem of either an ethical or procedural nature arise during the course of this research and you feel the Joint Ethics Committee may be of assistance, please do not hesitate to contact us.

Yours sincerely,

CWright

Clair Wright
LREC Manager

Appendix D:

4-year follow up invitation letter

SOUTHAMPTON WOMEN'S SURVEY
University of Southampton
Biomedical Sciences Building
Mailpoint 731
Bassett Crescent East
Southampton S016 7PX

Bone Study Hotline: 023 8076 4022

«Address1»
«Address2»
«Address3»
«Address4»
«Postcode»

Tuesday, 26 September 2006

Dear «Title» «Surname»,

You have in the past kindly assisted us in the Southampton Women's Survey, in which we are studying the relationship between women's diet and health before and during pregnancy, and the size of their babies. Now that «Babys_forename» is approaching 4 years old we would like to invite him to take part in the next part of the study. This would involve a short visit to the Osteoporosis Centre at Southampton General Hospital.

At the visit, he would be measured and weighed, and we would also check his grip strength, by seeing how hard he can squeeze a special measuring device. You would be asked to complete a short questionnaire, asking about your child's health, diet and activity levels, and he would have bone density scan. This scan is very similar to one he had at birth: It involves lying on a couch for about 6 minutes, with a scanning arm passing about 2 feet over him. The scan does not cause any pain, and the scanning arm will not touch him. It involves a tiny exposure to X-rays (the equivalent of three day's natural background X-rays). The whole visit should take no more than 45 minutes. Your child will be given a certificate, and picture of his skeleton to take home. As an additional part of the study at the end of the visit, if you agree, we will also fit both you and your child with a small activity monitor, which will measure activity levels in both you and your child for one week. We will ask you to post the monitors back to us in a pre-paid envelope at the end of this time.

The enclosed information sheet gives more details of the study.

One of the researchers will telephone in the next week to discuss this with you, and will arrange a time for the visit, if you would like to continue to take part.
Your continuing help with this important work is greatly appreciated.
With many thanks.

Yours sincerely,

N C W Harvey

Dr Nick Harvey
Clinical Research Fellow

Hazel Inskip

Dr Hazel Inskip
Coordinator, SWS

Local Research Ethics
Committee No: 005/03/t

Appendix E:

4-year follow up information sheet

SOUTHAMPTON WOMEN'S SURVEY
University of Southampton
Biomedical Sciences Building
Bassett Crescent East
Southampton S016 7PX

Bone Study Hotline: 023 8076 4022

01/08/05

INFORMATION SHEET

A study to help identify the factors that determine how a child's skeleton grows.

Both you and your child are being invited to take part in the next stage of the Southampton Women's Survey. 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.

Thank you for reading this.

What is the purpose of this study?

This study is trying to find out how a child'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 pregnancy, may affect the growth of her baby's bones.

We now wish to investigate how much of the strength of a child's bones is determined by factors during pregnancy and in the first few years of life, in particular the amount of exercise that your child has. We would also like to see whether the amount exercise you have influences your child's exercise levels.

Why have I been chosen?

As part of the Southampton Women's Survey you and your child have provided much useful information about your pregnancy and your child's growth. Now that your child is 4 years old, we would like to perform a bone density test (DXA scan), and assess their activity levels using a small activity monitor recorder. We would also like to assess your activity levels at the same time in the same way. This will give us important information about how your child's bones have grown since birth.

Do I have to take part?

It is up to you to decide whether or not to take part. If you decide to take part you are still free to withdraw at any time and without giving a reason. This will not affect the standard of care you receive.

What will happen to me if I take part?

We will contact you, and if you decide to take part, we will arrange a single appointment at Southampton General Hospital Osteoporosis Centre. The appointment will last, at most, 45 minutes. During this time we will measure your child's height and weight, arm size, and grip strength, ask you to complete a short questionnaire about your child's diet, activity and health, and perform a bone density scan for your child. The bone density scan does not work if your child is wearing any metal objects like buckles, zips or rings, so we do ask you to bring your child wearing tracksuit bottoms and a T-shirt, if possible.

At the end of the visit we will ask you if you are happy for us to set up activity monitors on you and your child. The monitor is a small plastic instrument that is about the same size as a fifty pence piece. If you agree, we will place a monitor on your child's and your chest with self-adhesive pads. The monitors are water proof and very strong! They will be worn for 7 days and then at the end of this time we would like you to post them back to us in the pre-paid padded envelope. **We will also give you a short questionnaire to fill in at home about your child's activity, and this can be sent back in the envelope with the activity monitors.**

What are the possible disadvantages and risks in taking part?

The bone density scan involves your child lying on a table and a small scanning arm will pass over, about two feet in the air; it does not touch your child. The dose of x-rays is equivalent to about three day's natural background x-rays. The scan will not cause any pain or harm.

The activity monitor is very safe. There may very occasionally be some skin irritation from the adhesive pads.

What are the possible benefits of taking part?

By taking part in this study, your child will have an assessment of bone density, a picture of his/her skeleton, and a certificate to take home with them. We will of course provide any medical advice necessary if the bone mineral density values are found to be low. 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 and only be made available to researchers in the study. In the event of your child being found to have low bone density, we will inform you and we may also make the results of the scan available to your child's GP if you are happy for us to do so.

What will happen to the results of the research?

We will see how your child's activity levels are related to their bone density, which will enable us to see how your, and their, diet and lifestyle has influenced these measurements. These findings will be published in the medical literature. They may also be summarised on the SWS website and the local and national press. 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 Epidemiology Resource Centre, and University of Southampton, UK. It 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 Nick Harvey at the Medical Research Council Epidemiology Resource Centre at Southampton on 023 8077 7624
This information sheet is for you to keep and you will also be given a copy of your signed consent form should you agree to take part.

Thank you for reading this

Professor C. Cooper MA DM FRCP FmedSci
Professor of Rheumatology

Dr Hazel Inskip BSc MSc PhD
Coordinator, SWS

Local Research Ethics Committee no. 005/03/t

Appendix F:

4-year visit confirmation letter

SOUTHAMPTON WOMEN'S SURVEY
University of Southampton
Biomedical Sciences Building
Mailpoint 731
Bassett Crescent East
Southampton S016 7PX

Bone Study Hotline: 023 8076 4022

Tuesday, 26 September 2006

Dear,

Thank you for agreeing to attend the Osteoporosis Centre at Southampton General Hospital for 's 4-year follow up.

As discussed on the telephone, the time is 1300, Tuesday 24th August 2004.

The Osteoporosis Centre is on "C"-level of the hospital. Enter via the main entrance and walk towards Burger King. When you get to the corridors leading off to the right and left, take the corridor to the right and go past pharmacy on the right, and the Rheumatology department on the left. Walk past the lifts and stairs, and continue down the corridor ahead. Take the first corridor on the right (sign-posted Osteoporosis Centre). The Osteoporosis Centre reception is on the right, before you get to the end of the corridor. If you get lost, ask for directions to the IDU (infectious diseases unit). The Osteoporosis Centre reception is just across the corridor from this.

It is important for the scan that all the children wear clothes with no metal buttons, zips or jewellery. I would be grateful if your child could be dressed in t-shirt and shorts or tracksuit bottoms (plus underwear) for the scan.

Before we do the scan, you will be asked to give your permission by signing a consent form. If you have any further questions about the visit, please either phone the number above, or ask staff at the Osteoporosis Centre when you arrive.

Your continuing help with this important work is greatly appreciated.

With many thanks.

Yours sincerely,

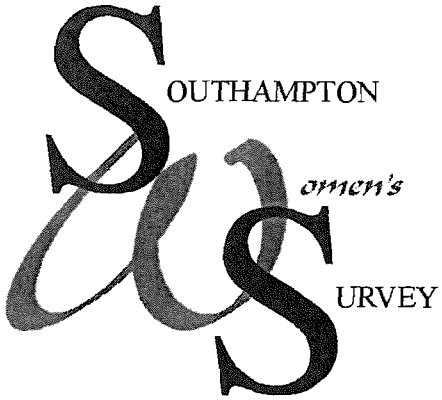
N C W Harvey

Dr Nick Harvey
Clinical Research Fellow

Local Research Ethics
Committee No: 005/03/t

Appendix G:

4-year visit consent forms for DXA and Actihearts



SOUTHAMPTON WOMEN'S SURVEY
University of Southampton
Biomedical Sciences Building
Bassett Crescent East
Southampton S016 7PX

FREEPHONE: 0800 7834503

SWS serial no:

Determinants of skeletal growth in early life: a longitudinal study

CONSENT FORM – FOUR YEAR CHILD BONE MASS MEASUREMENT

Please initial box:

1) I confirm that I have read and understand the information sheet dated 01/08/05 for the above study and have had the opportunity to ask questions ☐

2) I understand that my child's participation is voluntary and that I am free to withdraw my child at any time, without giving any reason, and without our medical care or legal rights being affected ☐

3) I agree for my child to take part in the study and have a repeat DXA scan ☐

Name of child.....

.....	.....	.....
Name of parent giving consent	Date	Signature
.....	.....	.....
Name of person taking consent	Date	Signature
.....	.....	.....
Name of researcher	Date	Signature

Local Research Ethics Committee no. 005/03/t

SOUTHAMPTON WOMEN'S SURVEY
University of Southampton
Biomedical Sciences Building
Bassett Crescent East
Southampton S016 7PX

FREEPHONE: 0800 7834503

SWS serial no:

Parental determinants of skeletal growth- a longitudinal study.

CONSENT FORM – FOUR YEAR CHILD ACTIVITY MEASUREMENT.

Please initial box:

1) I confirm that I have read and understand the information sheet dated 01/08/05 for the above study and have had the opportunity to ask questions ☐

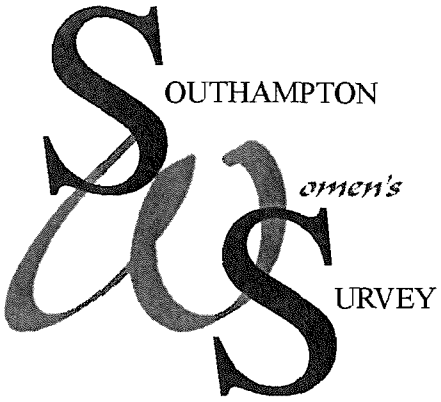
2) I understand that my child's participation is voluntary and that I am free to withdraw my child at any time, without giving any reason, and without our medical care or legal rights being affected ☐

3) I agree for my child to take part in the study, and undergo activity measurement using the Actiheart instrument ☐

Name of child.....

.....	.....	.....
Name of parent giving consent	Date	Signature
.....	.....	.....
Name of person taking consent	Date	Signature
.....	.....	.....
Name of researcher	Date	Signature

Local Research Ethics Committee no. 005/03/t



SOUTHAMPTON WOMEN'S SURVEY
University of Southampton
Biomedical Sciences Building
Bassett Crescent East
Southampton S016 7PX

FREEPHONE: 0800 7834503

SWS serial no:

Parental determinants of skeletal growth- a longitudinal study.

CONSENT FORM – MATERNAL ACTIVITY MEASUREMENT.

Please initial box:

1) I confirm that I have read and understand the information sheet dated 01/08/05 for the above study and have had the opportunity to ask questions ☐

2) I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, and without my medical care or legal rights being affected ☐

3) I agree to take part in the study, and undergo activity measurement using the Actiheart instrument ☐

.....	.....	.....
Name of person giving consent	Date	Signature
.....	.....	.....
Name of person taking consent	Date	Signature
.....	.....	.....
Name of researcher	Date	Signature

Local Research Ethics Committee no. 005/03/t

Appendix H:

4-year questionnaire

SWS Serial Number

SOUTHAMPTON Women's Survey

4 YEAR CHILD QUESTIONNAIRE

Version 1 26/09/2006

Mother's forename only: _____

Child's forename only: _____

[Interviewer to ensure child's name is correct, and record any changes thereon. Also to request additional telephone number, for tracing purposes if family move]

Child's date of birth

d	d	m	m	y	y

Sex M=Male
F=Female

Date of interview

d	d	m	m	y	y

Interviewer

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

Why was the mother not available?

1. *Has left the family home*
2. *Still lives in family home, but was unavailable for interview*
3. *Has died*
4. *Is ill or in hospital*
8. *Other, specify* _____
9. *Don't know*

Who was interviewed?

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

1. FOOD FREQUENCY

Now I am going to ask you about **a few** of the **foods** your child has eaten in the **past 3 months**. I will ask you how often he/she has eaten certain foods and also the amount of food eaten. For some foods, I will show you drawings and models to help you estimate the amount of food. Your child may sometimes have eaten food away from home. If you know the type of food and approximate amount eaten at these times please include them.

Explain the use of spoons, cups, bowl and diagrams.

1.1

	food	never	less than once per month	1-3 times per month	number of times per week							more than once per day	no. of times per day	average amount per serving
					1	2	3	4	5	6	7			
bread and crackers														
1.1	**white bread, rolls, toast**	0	0.3	0.5	1	2	3	4	5	6	7	8	☐	no. of slices ☐☐.☐ *(1 roll/bagel/croissant = 2 slices bread)* *(if all crusts gone=0.7 slice)*
1.2	**brown bread, rolls, toast**	0	0.3	0.5	1	2	3	4	5	6	7	8	☐	no. of slices ☐☐.☐
1.3	**cakes, scones biscuits**	0	0.3	0.5	1	2	3	4	5	6	7	8	☐	no. of portions ☐☐.☐ *(1 portion = 2 biscuits, 1 scone, 1 slice of cake)*
1.4	**breakfast cereals**	0	0.3	0.5	1	2	3	4	5	6	7	8	☐	no. of tbsp ☐☐ *(1 weetabix = 10 tbsp 1 minibix = 1 tbsp)*
1.5 **What are the main types of breakfast cereal used?**		type							brand					☐☐☐☐☐
		type							brand					☐☐☐☐☐
		type							brand					☐☐☐☐☐

	food	never	less than once per month	1-3 per month	number of times per week 1	2	3	4	5	6	7	more than once per day	no. of times per day	average amount per serving
1.6	**oily fish**	0	0.3	0.5	1	2	3	4	5	6	7	8	☐	no. of portions ☐☐.☐ *1 sm can tuna = 2* *med= 4; 1 tbsp= 0.25 portions* *salmon in sandwich = 1*
1.7	**eggs**	0	0.3	0.5	1	2	3	4	5	6	7	8	☐	no. of eggs ☐☐.☐ *yoke= 0.4, white= 0.6*
1.8	**cheese**	0	0.3	0.5	1	2	3	4	5	6	7	8	☐	☐☐.☐ *tbsp grated = 0.5* *small triangle = 1* *cheese per slice = 1 see drawing*
1.9	**baked beans**	0	0.3	0.5	1	2	3	4	5	6	7	8	☐	no. of small tins ☐☐.☐ *small tin = 200g*
1.10	**yoghurt & fromage frais**	0	0.3	0.5	1	2	3	4	5	6	7	8	☐	no. of grams ☐☐☐ *tiny pot 50g,* *bigger 100g*
1.11	**ice-cream**	0	0.3	0.5	1	2	3	4	5	6	7	8	☐	no. of tablespoons ☐☐ *1scoop = 4* *1 choc ice/Fab/Mars i/c etc = 4*
1.12	**custard and sweet white sauce**	0	0.3	0.5	1	2	3	4	5	6	7	8	☐	no. of tablespoons ☐☐
1.13	**butter & margarine**	0	0.3	0.5	1	2	3	4	5	6	7	8	☐	no. of teaspoons ☐.☐ *1 sl bread = 1.5 tsp* *1 pat =2 tsp*
1.14	**What are the main types of spread?**	1/			2/				3/					1 ☐☐☐ 2 ☐☐☐ 3 ☐☐☐
1.15	**milky drinks**	0	0.3	0.5	1	2	3	4	5	6	7	8	☐	no. standard beakers ☐.☐
1.16	**What are the main types of milky drinks?**	1/			2/				3/					1 ☐☐☐ 2 ☐☐☐ 3 ☐☐☐

Now I would like to ask in more detail about your child's milk intake. (Include all milk from 1.15)

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

0. None
1. Whole pasteurised
2. Semi-skimmed pasteurised
3. Skimmed pasteurised
4. Whole UHT
5. Semi-skimmed UHT
6. Skimmed UHT
7. Breast milk
8. Other

Milk 1 ☐ If "Other", *specify*_____

Milk 2 ☐ If "Other", *specify*_____

Milk 3 ☐ If "Other", *specify*_____

1.18 On average over the last 3 months how much of each milk has he/she consumed per day? *(1 average beaker = 0.35 pints; 1 pint = 20oz)*

Milk 1 ☐.☐☐ pints

Milk 2 ☐.☐☐ pints

Milk 3 ☐.☐☐ pints

1.19 During the past 3 months have you given him/her any vitamins or minerals, including vitamin D, iron and fluoride drops or tablets?

0. No *go to 1.21*
1. Yes ☐

1.20 Please state which:

Supplement Name	Code	How many days in the last 90?	Is it: 1) tablet 2) drops 4) liquid 3) other? *(state)*	No. of stated units per day
	☐☐☐☐			
	☐☐☐☐			
	☐☐☐☐			
	☐☐☐☐			

1.21 Does your child use fluoridated toothpaste?

0. No – *go to 1.24*
1. Yes ☐

1.22 How many times per day does your child use fluoridated toothpaste?

1. Once
2. Twice
3. Three times or more ☐

1.23 Does your child swallow the toothpaste?

0. no
1. varies
2. yes ☐

1.24 Does your child use sun cream in sunny weather?

0. no
1. yes ☐

1.25 On an average day, how many hours does your child spend outdoors? ☐☐ • ☐

1.26 On average over the last 3 months how many beakers of water has your child drunk each day? Include plain water and drinks made mostly from water, such as squash, tea and coffee.

Number of beakers per day ☐☐ • ☐

1.27 Is this water mainly: 1/ Tap water, 2/ Filtered tap water,
3/ Ordinary mineral water, 4/ Mineral water with added calcium (eg Danone variety),
5/ Other (please specify) _____ ☐

2. SLEEP, ACTIVITY AND EXERCISE

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

[We are trying to get figures that eventually total approximately 24hrs, so rounding to nearest hour is OK – best guess is acceptable.]

2.1 What time does the study child generally go to sleep at night? (24hr clock) ☐☐☐☐

2.2 How many times **per night** does he/she generally wake for any reason?
Please answer this in relation to the last month? ☐☐ . ☐ per night

If '0', go to 2.4

2.3 In total, how long is he/she generally awake? ☐☐ hrs ☐☐ mins per night
(Only record if regularly over 30mins)

2.4 What time does he/she generally wake up in the morning? (24hr clock) ☐☐☐☐

*[From responses to 2.1 to 2.4, calculate **approximately** how many hours are spent asleep]*

2.5 **This means that he/she sleeps for about ☐☐ hrs ☐☐ mins each night.**

2.6 How many **days per week** does he/she take a daytime nap? ☐
Please answer this in relation to the last month?
If "0", deduct 2.5 from 24 & insert at 2.9

2.7 On the days he/she naps, what is the **total time** spent napping during the day?

☐ **hrs** ☐☐ **mins**

Using responses to 2.6 & 2.7, consult "Daily averages" grid

2.8 Average daily nap time ☐ **hrs** ☐☐ **mins**

Add 2.5 to 2.8 & deduct from 24.

2.9 **This would indicate that he/she is awake for about ☐☐ hrs ☐☐ mins on average each day?**

We are now going to try and divide the hours your child is awake between time sitting down and other activities.

2.10 During the *{number from 2.9}* hours he/she is awake, how much of the day is he/she **sitting**, eg reading books, watching TV/video/computer, eating meals, playing quietly with toys, in a pushchair/car, or similar?

☐☐ **hrs** ☐☐ **mins each day**

[Deduct 2.10 from 2.9]

2.11 This would indicate that he/she is on his/her feet for around
Does that sound about right?

☐☐ **hrs** ☐☐ **mins each day**

We are now going to try and divide the hours your child is on his/his feet between moderately energetic and very energetic activities.

[If necessary, consult "Daily averages" grid to work out how much weekly activities contribute to an average day]

2.12 During the *{number from 2.11}* hours he/she is on his/her feet, how many hours is he/she **standing or walking**, eg walking inside and outside, helping you in the house, 'pottering' about inside and in the garden, ie **moderately energetic**?

☐☐ **hrs** ☐☐ **mins each day**

[Deduct 2.12 from 2.11]

2.13 This would indicate that he/she is actively on the move for around
Does that sound about right?

☐☐ **hrs** ☐☐ **mins each day**

eg ball games, gym club, cycling, swimming, general tearing about, inside and outside or similar, ie **very energetic**?

2.14 On a typical day, how many hours does he/she 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

☐

3. CHILD'S HEALTH

3.1 How is the study child's health in general? Would you say it was:

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

☐

3.2 Does he/she have any long-standing medical condition? By long-standing I mean anything that has troubled him/her over a period of time, or that is likely to effect him/her over a period of time.

0. No- *go to 3.6*
1. Yes

☐

3.3 What is this condition?

3.4 Does this condition limit his/her activities in any way? ☐
0. No- *go to 3.6*
1. Yes

3.5 If "yes", in what way does it limit his/her activities?

_____

3.6 Does your child take any regular medicines (either from the chemist, doctor, or alternative therapies)? Please include inhalers for asthma.

0. No- *go to 3.7* ☐
1. Yes- please list them in the table below

Medicine Name	**Code**	**How many days in the last 90?**	**Is it: 1) tablet 2) drops 4) liquid 3) other? *(state)***	**Dose per day**
	☐☐☐☐			
	☐☐☐☐			
	☐☐☐☐			
	☐☐☐☐			

3.7 Has your child ever needed a course of steroid tablets or steroid inhaler?

0. No- *go to 3.11* ☐
1. Yes

3.8 How long ago did your child need steroids? ☐☐ months

3.9 Did your child take steroid tablets or a steroid inhaler? ☐
1. Inhaler
2. Tablets
3. Both

3.10 For how long? a/ inhaler ☐☐ days

b/ tablets ☐☐ days

3.11 Has your child ever broken any bones? ☐

0. No – *go to 3.14*
1. Yes

3.12 When and how did your child break a bone or bones, and which bones were broken?

Date	Bones broken	What happened?

3.13 Were any of these fractures low trauma (as judged by investigator)? ☐
0. No
1. Yes

3.14 Is there a family history of low trauma fractures? ☐
0. No – *go to 4.1*
1. Yes

3.15 Which family members?

_____

_____

_____

3.16 Which bones? (Please state which family members broke which bones, and how old they were when they first started to fracture)

Family member	**Which bones?**	**Age when started to fracture**

4. CHILD EXAMINATION

4.1 Measurement Date — d d m m y y

4.2 Time (24 hr clock)

4.3 Measurer

4.4 Helpers (Parent = 90)

4.5 Left mid-upper arm circumference (arm straight) — cm / cm / cm

Wriggling
0. No
1. Yes

4.6 Height (barefoot) (LHM) — cm / cm / cm

Wriggling
0. No
1. Yes

4.7 Stadiometer used

4.8 Child's weight (preferably in underwear only) ☐☐.☐☐ kg

4.9 Approx weight of any clothes (except underwear) ☐.☐☐ kg

4.10 Scales used ☐☐☐☐

4.11

	RIGHT SIDE	LEFT SIDE
GRIP STRENGTH **(Record to nearest 0.5kg)**	☐☐ • ☐	☐☐ • ☐
	☐☐ • ☐	☐☐ • ☐
	☐☐ • ☐	☐☐ • ☐

4.12 Which hand does your child mostly use to write or hold a pencil with ? left ☐ right ☐

Ambidextrous ☐
(Writes with both hands)

Appendix I:

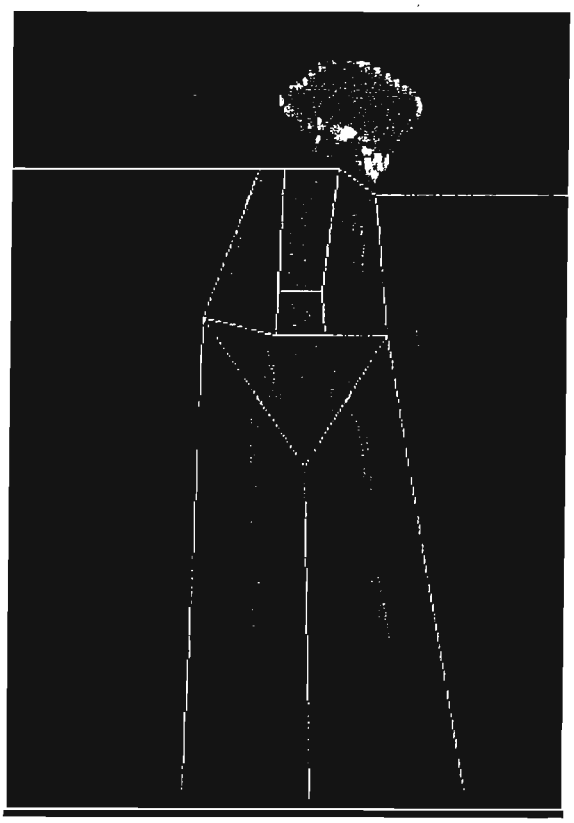
Sample DXA output from 4-year visit

Osteoporosis Centre
Southampton General Hospital
Level C West Wing

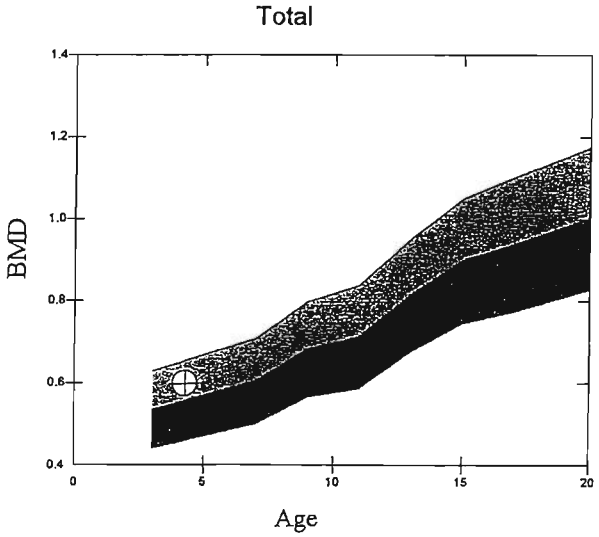
Telephone: 02380794696 E-Mail: pat.taylor@suht.swest.nhs.uk Fax: 02380798995

Name:	Sex: Female	Height: 94.4 cm
Patient ID:	Ethnicity: Pediatric	Weight: 12.9 kg
DOB:		Age: 4

Referring Physician:



318 x 92



Scan Information:

Scan Date: 02 November 2004 ID: A1102040F
Scan Type: a Whole Body
Analysis: 02 November 2004 14:07 Version 12.1
Auto Whole Body
Operator:
Model: Discovery W (S/N 80019)
Comment:

DXA Results Summary:

Region	Area (cm²)	BMD (g/cm²)	T - Score	PR (%)	Z - Score	AM (%)
L Arm	41.55	0.328				
R Arm	58.25	0.360				
L Ribs	44.66	0.483				
R Ribs	50.87	0.431				
T Spine	58.63	0.500				
L Spine	18.64	0.611				
Pelvis	98.63	0.550				
L Leg	125.42	0.513				
R Leg	111.83	0.531				
Subtotal	608.48	0.488				
Head	199.98	0.935				
Total	**808.46**	**0.598**	**-4.6**	**60**	**0.9**	**10**

Sub-Region	Area (cm²)	BMD (g/cm²)

TBAR2695

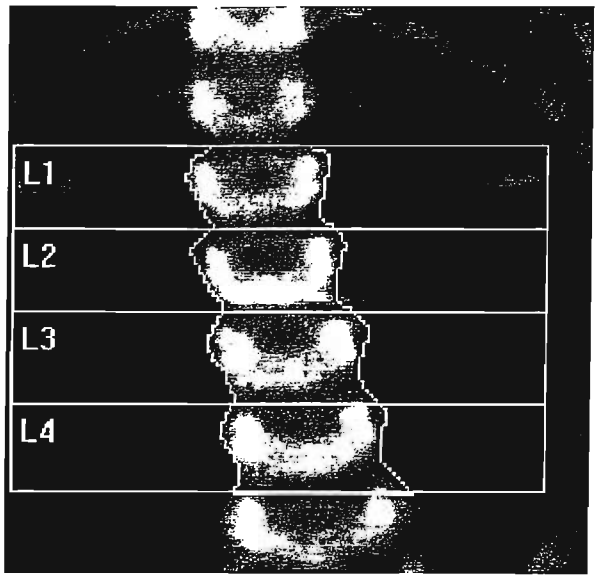
HOLOGI

Osteoporosis Centre
Southampton General Hospital
Level C West Wing

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

Name:	Sex: Female	Height: 94.4 cm
Patient ID	Ethnicity: Pediatric	Weight: 12.9 kg
DOB:		Age: 4

Referring Physician:



116 x 76

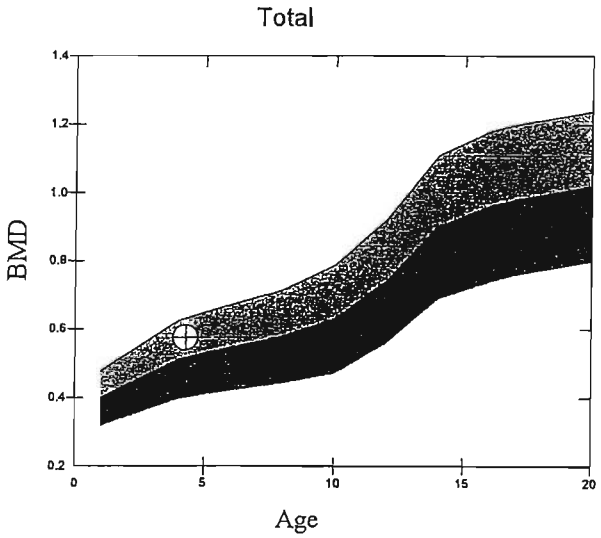
Scan Information:

Scan Date: 02 November 2004 ID: A1102040G
Scan Type: x Lumbar Spine
Analysis: 02 November 2004 14:06 Version 12.1
Lumbar Spine (auto low density)
Operator:
Model: Discovery W (S/N 80019)
Comment:

DXA Results Summary:

Region	Area (cm^2)	BMD (g/cm^2)	T - Score	PR (%)	Z - Score	AM (%)
L1	4.95	0.484	-4.0	52		
L2	5.35	0.595	-3.9	58		
L3	6.32	0.584	-4.5	54		
L4	6.90	0.618	-4.5	55		
Total	**23.53**	**0.576**	**-4.3**	**55**	**1.0**	**11**

Total BMD CV 1.0%



Reference curve and scores matched to Pediatric Female

Source: Hologic, Inc

Physician's Comment:

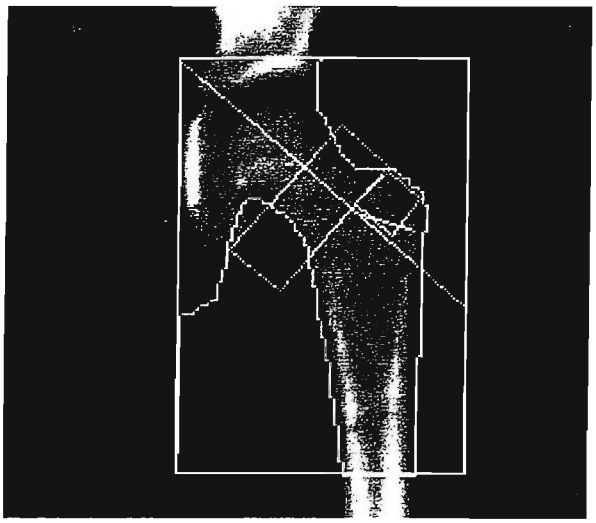
HOLOGI

Osteoporosis Centre
Southampton General Hospital
Level C West Wing

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

Name:	Sex: Female	Height: 94.4 cm
Patient ID:	Ethnicity: Pediatric	Weight: 12.9 kg
DOB:		Age: 4

Referring Physician:



63 x 91

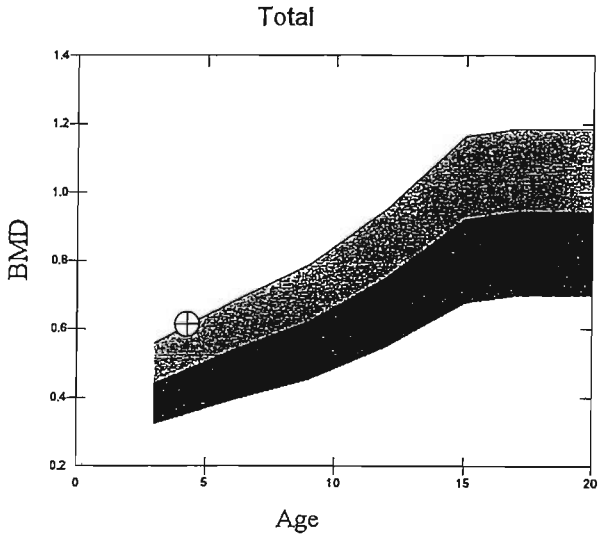
Scan Information:

Scan Date: 02 November 2004 ID: A1102040H
Scan Type: x Left Hip
Analysis: 02 November 2004 14:06 Version 12.1
Left Hip (low density)
Operator:
Model: Discovery W (S/N 80019)
Comment:

DXA Results Summary:

Region	Area (cm²)	BMD (g/cm²)	T - Score	PR (%)	Z - Score	AM (%
Neck	2.93	0.481	-3.3	57	-0.1	9
Troch	1.59	0.629	-0.7	90	4.0	16
Inter	11.06	0.647	-2.8	60	2.0	12
Total	**15.58**	**0.614**	**-2.7**	**65**	**2.1**	**12**
Ward's	1.11	0.457	-2.4	62		

Total BMD CV 1.0%



Reference curve and scores matched to Pediatric Female

Source: Hologic, Inc.

Physician's Comment:

HOLOGI

Osteoporosis Centre
Southampton General Hospital
Level C West Wing

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

Name:	Sex: Female	Height: 94.4 cm
Patient ID:	Ethnicity: Pediatric	Weight: 12.9 kg
DOB:		Age: 4

Scan Information:

Scan Date: 02 November 2004 ID: A1102040F
Scan Type: a Whole Body
Analysis: 02 November 2004 14:07 Version 12.1
Auto Whole Body
Operator:
Model: Discovery W (S/N 80019)
Comment:

DXA Results Summary:

Region	BMC (g)	Fat (g)	Lean (g)	Lean+BMC (g)	Total Mass (g)	% Fat
L Arm	13.61	164.1	208.2	221.8	385.9	42.5
R Arm	20.95	231.8	324.5	345.5	577.3	40.2
Trunk	138.47	1147.9	4274.8	4413.3	5561.1	20.6
L Leg	64.29	594.3	1135.8	1200.1	1794.3	33.1
R Leg	59.43	550.5	1066.3	1125.8	1676.2	32.8
Subtotal	296.75	2688.5	7009.7	7306.4	9995.0	26.9
Head	186.99	487.7	1891.3	2078.3	2566.0	19.0
Total	**483.74**	**3176.2**	**8901.0**	**9384.8**	**12561.0**	**25.3**

Sub-Region	BMC (g)	Fat (g)	Lean (g)	Lean+BMC (g)	Total Mass (g)	% Fat

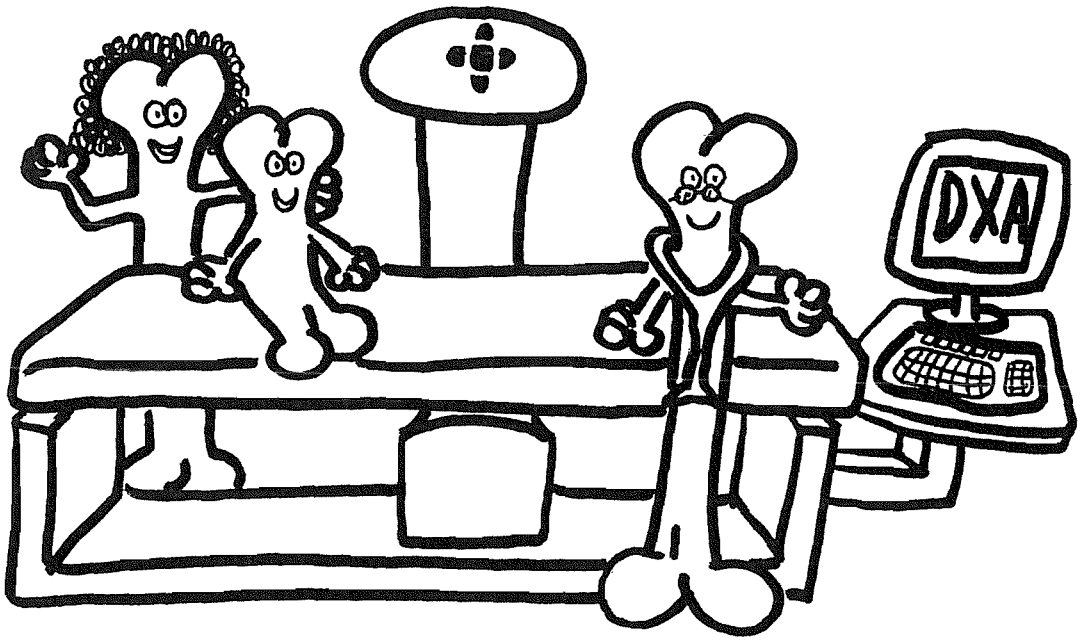
TBAR2695

HOLOGI

Appendix J:

Certificate for good behaviour at 4-year visit

Certificate of Achievement



This is to certify that

.....

was very good and stayed still for the
DXA scan

on .....

Appendix K:

4-year follow up ethics committee and hospital R and D approval

Southampton NHS
University Hospitals NHS Trust

Research & Development
Trust Management Offices
Mailpoint 18
Southampton General Hospital
Tremona Road
Southampton
SO16 6YD

Tel: 023 8079 5078
Fax: 023 8079 8678

5 February 2004

Dr Nicholas Harvey
MRC Environmental Epidemiology Unit
SGH

Dear Dr Harvey

Re: Final R&D Approval Confirmation
R&D No. RHM MED0544
Title: Parental determinants of skeletal growth in early life: A longitudinal study.

Thank you very much for submitting the final ethics approval letter to R&D. Your project is now fully approved on the R&D database and you may refer to it in correspondence by its database number as above.

This letter provides the formal SUHT approval required for your project to commence and confirms that SUHT indemnity for negligence is in place.
Please note: If this is a commercial research project, you must not start until our Director of R&D or his deputy has signed the ABPI-type indemnity provided by the sponsor.

The conditions of this approval and indemnity require you as Principal Investigator to ensure the following:

- There should be a 12 week interval between studies for patients/volunteers unless exemption from this policy has been obtained from R&D.
- All staff involved in the project are familiar with the Research Governance Framework for Health and Social Care and the SUHT Data Protection policy.
- All staff that will be involved with SUHT NHS patients must be aware of the appropriate SUHT policy and procedures and seek the appropriate training if required.
- All staff that will be involved with SUHT NHS patients and/or have access to identifiable patient data have honorary SUHT contracts.
- All data must be collected and stored in accordance with ICH GCP and/or MRC Guidelines for GCP in clinical trials.
- All essential documents are to be stored and maintained in the project files provided by R&D.
- All serious adverse events are to be reported in writing to the Ethics Committee and copied to the R&D Directorate within 7 days.
- The project file issued by R&D is to be maintained and updated by you during the lifespan of the project.

Please note that this trust approval (and your ethics approval) only applies to the current protocol. Any changes to the protocol can only be initiated following further approval from the Ethics Committee via a protocol amendment. All correspondence to the Ethics Committee must be copied to R&D in order to maintain your SUHT R&D approval and indemnity status.

Should any of your team require training in the above policies and procedures please do not hesitate to contact us.

Any breaches of the above may constitute non-compliance with the Research Governance Framework and the project may need to be suspended until such issues are resolved.

Please do not hesitate to contact us should you require further information.

Kind regards

Eric Brown
Research Project Manager

Hampshire and Isle of Wight NHS
Strategic Health Authority

Ref: CPW/HPH

SOUTHAMPTON & SOUTH WEST HAMPSHIRE
LOCAL RESEARCH ETHICS COMMITTEES
1ST Floor, Regents Park Surgery
Park Street, Shirley
Southampton
SO16 4 RJ

21 March 2003

Dr M K Jarvaid
ARC Clinical Research Fellow to Professor C Cooper
MRC Environmental Epidemiology Unit
MP 95
SGH

Tel: 023 8036 2466
023 8036 3462
Fax: 023 8036 4110

General Enquiries: sharon.atwill@gp-j82203.nhs.uk
clair.wright@gp-j82203.nhs.uk

Dear Dr Jarvaid,

Submission No: 005/03/t – Parental determinants of skeletal growth: a longitudinal study.

Following conditional approval and in response to your letter dated 4th March 2003, I am please to confirm full approval having responded satisfactorily to the committees concerns.

The following document(s) were re-considered:

- Letter dated 4th March 2003
- Information Sheet
- Consent Form

Please note that all paperwork i.e. Information Sheet etc. should be on departmental headed paper and must carry identification version number and date.

This approval was granted under Chairman's action by Vice Chairman Mr Mervyn Griffiths, and will be recorded by the Committee at their meeting in April.

This committee is fully compliant with the International Committee on Harmonisation/Good Clinical Practice (ICH) Guidelines for the Conduct of Trials involving the participation of human subjects as they relate to the responsibilities, composition, function, operations and records of an independent Ethics Committee/Independent Review Board. To this end it undertakes to adhere as far as is consistent with its Constitution, to the relevant clauses of the ICH Harmonised Tripartite Guideline for Good Clinical Practice, adopted by the Commission of the European Union on 17 January 1997.

Yours sincerely

Mrs Clair Wright
Research Ethics Manager