Early Life Determinants of Infant Bone Health

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Dedication

This is dedicated to my husband, Ben, and my son Finlay, who provided the perfect balance of encouragement to complete this thesis and distraction to keep me sane.

Declaration

No part of this thesis has been submitted in support of an application for another degree or qualification of this or any other University.

All the studies performed as part of this thesis were approved by the local research ethics committee and informed consent was given by all subjects and/or their parents.

‘In my beginning is my end…….in my end is my beginning.’

T.S Eliot

East Coker

N^{o} 2 of ‘Four Quartets’

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Table of Contents **Page N°**

List of Figures **Page N^{o}**

List of Tables **Page N^{o}**

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McDevitt H, Tomlinson C, White MP, Ahmed SF. Quantitative Ultrasound Assessment of Bone in Preterm and Term Neonates. Arch Dis Child 2005; 90:F341-F342.

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McDevitt H, White MP, Ahmed SF

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Abbreviations

AGA	appropriate for gestational age
ANCOVA	analysis of covariance
BMC	bone mineral content
BMD	bone mineral density
BMI	body mass index
BTT	bone transmission time
BUA	broadband ultrasound attenuation
(CGA)	corrected gestational age
CLD	chronic lung disease
CRIB	clinical risk index for babies
CV	coefficient of variation
DXA	dual energy x-ray absorptiometry
ELBW	extremely low birthweight
GA	gestational age
ICTP	serum cross-linked carboxy-terminal telopeptide of type 1 collagen
IU	international units
IUGR	intrauterine growth retardation
LGA	large for gestational age
mmol/l	millimoles per litre
m/s	metres per second
MRI	magnetic resonance imaging
NS	not significant
OP	osteopenia of prematurity
PCA	post conceptual age

PICP	serum type one collagen c terminal peptide
PTH	parathyroid hormone
PYD	urinary pyridoline cross links of collagen
pQCT	peripheral quantitative computed tomography
QCT	quantitative computed tomography
QUS	quantitative ultrasound
SD	standard deviation
SDS	standard deviation score
SGA	small for gestational age
SIMD	Scottish index of multiple deprivation
SPA	single photon absorptiometry
SOS	speed of sound
TPN	total parenteral nutrition
VLBW	very low birthweight
wks	weeks

Summary

This thesis consists of a series of studies utilising quantitative ultrasound (Sunlight Omnisense 7000P) to assess bone health of infants. Preterm infants are at risk of osteopenia of prematurity (OP) which can result in fractures in the short term and may have an impact on growth in infancy and childhood. OP has a multifactorial aetiology including factors such as poor mineral intake and immobility. There is an increasing number of ex-preterm survivors therefore morbidity becomes more important. There is also increasing evidence from epidemiological studies that growth in infancy can have an effect on adult diseases such as osteoporosis.

The first study was a cross-sectional study of bone quantitative ultrasound measurements in 110 term and preterm infants shortly after birth. Speed of sound (SOS) was measured at the tibial and the radius. This validated the technique showing reproducible measurements with low inter and intra-observer error, and also showed no benefit to measuring multiple sites. Preterm infants were found to have a significantly lower SOS than term infants. There was a positive correlation between tibial SOS and gestation, with birthweight being a less significant factor than gestation.

The second study followed 18 preterm infants longitudinally from birth to hospital discharge or term corrected gestational age (CGA). SOS fell significantly with time in all infants. The most preterm infants had the greatest fall in SOS. SOS at the end of the study period was negatively associated with peak serum alkaline phosphatase and severity of illness score. SOS was significantly lower in the infants who required total parenteral nutrition for longer than 3 weeks. These results show that the neonatal course has a significant impact on SOS trajectory.

When preterm infants were followed up in the out-patient clinic over the first two years of life the SOS measurements taken as the next part of this study showed a catch up phenomenon. In the majority of infants, but not all infants, SOS moved into the normal range by 6 months CGA. In the subgroup of infants followed longitudinally those with the lowest SOS at hospital discharge/term corrected age had the greatest increase in SOS over time.

An interventional study of passive exercise was performed to explore its role in influencing the bone health of preterm infants. Thirty one infants born at less than 33 weeks gestation were randomised to receive range of motion flexion and extension exercises once daily for 5 days each week starting 'early'(n=15) or 'late' (when on 100kcal/kg/day enteral feeds, n=16) and continuing until term corrected gestational age (CGA) or discharge from hospital. Tibial SOS declined significantly from birth to end of physical activity in both 'early' and 'late' groups, and this was similar to the decrease seen in a group of historical controls from the earlier longitudinal study. Weight gain and head growth did not show a significant difference between groups or between study infants and controls. No infant was reported to have sustained a fracture, and length of hospital stay was not significantly different between groups. There was no significant increase in sepsis rate, retinopathy of prematurity or chronic lung disease in study infants but numbers were small. On longer term follow-up the intervention was not associated with any adverse effects.

To investigate the possibility that the maternal environment plays an important role in influencing infants' bone health we also studied SOS changes in 188 pregnant women and their offspring. Most women had SOS in the normal range antenatally, and there was no significant change in SOS across pregnancy in the group as a whole. There was a significant negative correlation with SOS SDS and BMI in early pregnancy. Women who smoked

cigarettes had lower SOS throughout pregnancy and so did their infants. Serum bone biochemistry was measured in the women antenatally and after delivery, and umbilical cord blood was also taken where possible. Vitamin D deficiency was found to be common at the end of pregnancy. Women of Asian origin had significantly lower vitamin D levels at all stages of pregnancy. There was no significant relationship between maternal and infant SOS, or between maternal vitamin D status and infant SOS.

The work of this thesis establishes quantitative ultrasound as a useful technique in the assessment of infant bone health. It is a radiation free tool which provides precise and reproducible measurements in both term and preterm infants. In agreement with a small number of other studies we found that preterm infants have a lower speed of sound at birth compared to term infants; speed of sound increases with increasing gestation while in utero. By including infants who were both appropriately grown and small for gestational age we found maturity to be a more important factor in bone strength than birthweight. Despite the apparent self limiting nature of osteopenia of prematurity an intervention to improve neonatal bone health is still desirable, to prevent fractures. Our results do not substantiate conclusions from previous studies that physical activity alone can improve neonatal bone health. .Findings are however limited by the small sample size. Further studies are needed which investigate alternative exercise regimens, taking into account mineral and nutrient supply. Vitamin D deficiency, smoking and obesity may adversely affect bone health of women and their offspring. In the west of Scotland vitamin D deficiency is common in pregnancy: women of south asian origin are at particularly high risk, and should be supplemented with Vitamin D.

Chapter 1 Hypotheses and Aims of the Thesis

The present studies were designed to investigate early life determinants of infant bone health. Using a number of techniques including quantitative ultrasound, the studies assessed bone health in term and preterm infants, and pregnant mothers.

1. Hypothesis: Quantitative ultrasound may be a useful adjunct for assessing bone health in neonates, including the sick preterm infant.

   Aim: Assess the feasibility and reliability of the technique of quantitative ultrasound in neonates.

2. Hypothesis: The neonatal course has an effect on bone development which can be assessed by quantitative ultrasound.

   Aim: Evaluate bone health of preterm infants at birth and during infancy, including use of quantitative ultrasound.

3. Hypothesis: The maternal environment, postnatal environment and genetic factors will all have a role in infants bone health.

   Aim: Investigate the determinants of maternal bone health during pregnancy, and the link between these and infant bone health.

4. Hypothesis: Skeletal growth is driven by functional requirements therefore the intervention of passive exercise in preterm neonates will improve their bone health.

   Aim: Assess passive exercise as an intervention for improving bone health in preterm infants.

Chapter 2 Introduction

Section 1 Neonatal bone health

Optimal bone health is dependent on achieving adequate bone mass, mineral content and geometry and these are influenced by a number of factors that can be broadly divided into three categories: genetics, maternal environment and the postnatal environment. There is increasing evidence to support the role of these factors in determining adult bone mass (1). Premature restriction of the *in utero* process of bone mass accretion and a greater *ex utero* need for bone nutrients predisposes the preterm infant to adverse bone health. Current methods of assessing bone health in the neonate have a low specificity and sensitivity and with an increasing survival rate of very low birthweight preterm infants (2) there is a need to explore novel, reliable, non-invasive methods for assessing bone health in this group of patients. However, a thorough understanding of these issues requires some knowledge of bone development, especially in the context of the preterm neonate.

Bone biology and development

The embryonic primordiae of the appendicular skeleton are the limb buds, which are mesodermal structures covered by ectoderm. The first visible outline of the embryonic limb follows a condensation of mesenchymal cells which subsequently differentiate into cartilage cells, the chondrocytes. These cells secrete a matrix and thus produce cartilaginous models of the future bones. Surrounding this cartilage is the perichondrium, the outer layer of which becomes a connective tissue sheath while the inner cells remain pluripotential (Fig.1). This cartilage rudiment grows by interstitial and appositional growth, and a vascular system develops to invade the perichondrium. A collar of bone is then laid down around the mid-shaft of the bone. This ossification is a result of the inner perichondrial cells differentiating into bone forming cells, the osteoblasts and by this time the three regions of the developing long

bone, diaphysis, epiphysis and metaphysis are evident. At the same time, increasing vascular development at the site where the cartilage cells and matrix have begun to disintegrate allows osteoblasts to invade the centre of the shaft to form a primary ossification centre. Trabecular bone is then deposited on cartilaginous remnants. In humans, primary ossification centres in the femur and humerus are visible by week 6 of gestation (3).

The epiphysis contains the growth plates and is responsible for the transverse and spherical growth of the ends of the bone, the shaping of the articular surfaces, and the longitudinal growth of the metaphysis and the diaphysis. The cells within the growth plate (chondrocytes) go through a sequential process of cell proliferation, synthesis of extra cellular matrix, cellular hypertrophy, mineralization of the matrix, localized vascular invasion, and apoptosis (4). The growth plate replenishes itself through the germinal zone and is continually replaced with bone at the physeal-metaphyseal junction. Not only is the hypertrophic chondrocyte crucial for longitudinal growth, it also prepares the matrix for calcification and vascularisation and directs the mineralisation of the adjacent matrix and attracts vessels by producing vascular growth factors.

The fetus has a high rate of mineral accretion, with high serum phosphate, calcium, calcitonin and a low PTH and low circulating levels of the active vitamin D metabolites. Approximately 99% of body calcium and 80% of phosphorus is in the skeleton at birth, at least 80% of this mineral deposition occurs between 25 weeks gestation and term (5).

At the same time as longitudinal growth, there is radial growth of the diaphysis and the metaphysis caused by direct apposition of cortical bone by periosteum-derived osteoblasts. This is coordinated closely with resorption of bone by osteoclasts on the inner cortical endosteal (medullary) surfaces and lateral metaphyseal surfaces to maintain the relative proportions of the marrow cavity to the cortices and the overall shape of the bone as it grows. The metaphyseal cortical bone is formed by the coalescence of peripheral endochondral

trabecular bone from the physis with intramembranous bone from the inner osteogenic layer of the periosteum. This dynamic process is under the influence of a number of intrinsic and extrinsic factors that include growth factors, sex steroids and external biomechanical stressors.

Detailed studies of fetal long bones show from 21-41 weeks gestation show that the bone geometry changes over the gestational period such that the medullary diameter increases at a greater rate than the outer cortical diameter growth rate thus resulting in a wider diaphyseal width with a greater medullary cavity and a relatively narrower cortex (6). Although, the velocity of medullary diameter growth was similar (0.05 mm/week) in three long bones (tibia, femur, humerus) studied, the velocity of outer cortical diameter growth was greater in the tibia (0.16 mm/week) than in the femur (0.14 mm/week) or humerus (0.13 mm/week). The diaphyseal growth rate decreased in the second half of the period studied in all three bones. This process of endocortical resorption and expansion of the marrow cavity, which in densitometric terms corresponds to a decrease in volumetric bone mineral density becomes even more pronounced after birth and, in densitometric terms, results in an effective reduction in bone density (7). Some factors such as estrogen promote endocortical bone formation and it is possible that premature birth may hasten the process of endocortical resorption. The change in geometry of the neonatal bone may be regarded as an adaptation process and defects in this process may predispose the infant to adverse bone health.

Overall, skeletal development does not depend solely on bone mineral accretion but also bone mass accrual which may be influenced by an imbalance between bone formation and resorption (8) as well as mechanical stimulation which is higher in utero with the muscular resistance of the uterine wall, compared to movements against little resistance after birth, putting smaller loads on the skeleton. Furthermore, the hormonal situation is also different postnatally, with the abrupt cessation of the placental supply of oestrogen and other hormones.

Neonatal Nutrition and Growth

Early nutrition is important as it has a short term effect on the disease course i.e. the baby's progress within the neonatal unit and it has an impact on long term health and development, particularly long term neurodevelopment. Achieving good nutrition and growth is particularly challenging in preterm infants. Infants born very prematurely, before 32 weeks gestation are likely to need total parenteral nutrition (TPN), at least in the initial period after birth. Calcium and phosphate are included in TPN at much smaller amounts than would be available to the baby from the placenta during the third trimester. This is because their solubility product is readily excreted and they precipitate out, their solubility depends on the acidity of the solution, and thus also on the protein of the solution so maximising protein can help to increase the amount of calcium and phosphate that can be given. This poor availability of calcium and phosphate, along with other factors can lead to osteopenia of prematurity. Once enteral feeds are established, human milk alone does not meet the nutritional requirements of a preterm infant and requires supplementation (9); however there is evidence for EBM reducing morbidity and mortality compared to using formula alone (10;11)

Multinutrient fortifiers or using a combination of EBM with preterm formula can be used to increase the protein, energy, calcium and phosphate intake. No randomized trials have been conducted on preterm infants breastfed exclusively post discharge from hospital, however there is some evidence that lower weight gain, lower bone mineral content along with lower serum phosphate and higher serum alkaline phosphatase occurs compared to those preterm infants fed formula (12;13). Further research is needed to determine if breast milk should be fortified after the preterm infant is discharged.

There is a trend towards earlier discharge home for preterm infants, as follow up in the community by home liaison sisters is possible in many areas, and more attention has been focused on the nutritional needs of preterm infants post discharge. Post discharge formulas

with increased protein and minerals compared to term formulae therefore evolved. There are a few studies which demonstrate advantages in weight gain and length in preterm infants fed post discharge formula although a recent Cochrane review did not find strong evidence that feeding using post discharge formula conferred benefit in growth or development of preterm infants up to 18 months post term (14) This was limited due to inter trial differences in presented outcomes, however a meta-analysis from the 2 trials (15;16) which performed follow up at 18 months of age show an increased length at 18 months but not weight or head circumference, and no difference between groups on formal developmental testing. This review did not cover indices of bone health.

As calcium accretion and bone mineralization is greatest during the first year of life then lack of early bone mass accumulation may have later consequences in childhood growth, accumulation of peak bone mass and subsequent risk of osteoporosis. Calcium is not only important for bone growth but also for blood clotting, intracellular metabolism, muscle conduction, nerve conduction and cardiac function. The placenta actively transports calcium to the fetus, driven by a magnesium adenosine triphosphatase-dependent calcium pump. This enables calcium transfer to the fetus of up to 150mg/kg of fetal weight each day in the third trimester. Preterm infants miss out on this calcium surge during the third trimester and are born with lower calcium stores and therefore require more dietary calcium postnatally. The recommended intake of calcium for preterm infants is 120-230mg/kg/day compared to 60-80 mg/kg/day for a term infant (17). Calcium intestinal absorption is determined by the amount of ingested calcium and the solubility of the calcium salt. Calcium absorption requires calcium to be ionized. In term infants studies have shown the calcium absorption rate approximates calcium retention or bone accumulation, in preterm infants calcium retention can be 48% of intake (18;19).There does appear to be a linear relationship between calcium retention and intake in preterm infants (19;20). Also, the calcium to phosphate ratio appears to be an

important determinant of calcium absorption and retention. In human milk there is calcium: phosphate ratio of 2:1 by mass. In preterm formula, a ratio of calcium: phosphate of 1.7 has previously been reported to be adequate for bone mineralisation of preterm infants, whereas a ratio of 1.4 at the same calcium concentration was not (21). Preterm formula has a ratio of approximately 1.9; this can be lower in term formulae.

In term infants at birth stores of vitamin D are usually adequate unless there is significant maternal deficiency. As skin synthesis of vitamin D requires exposure to UV B light, and most preterm infants are not exposed to sunlight initially the majority of vitamin D comes from maternal and dietary sources. Levels of 25 hydroxyvitamin D change with gestational age and thus are lower in preterm than in term infants. The 2^{nd} hydroxylation of vitamin D by the kidney occurs normally in preterm infants (22). Vitamin D is present in only very small amounts in breast milk (0.01mcg/100ml) and in larger amounts in preterm formula (1.2-2.4 mcg/100ml.) Glucocorticoid use and the use of theophylline can affect levels and function of 1, 25 dihydroxyvitamin D. It is recommended that vitamin D be supplemented at a dose of 200iu/ day in preterm infants until 6 months old (17). Dietary fats and fat soluble vitamins require adequate bile secretion for absorption. Deficient bile secretion in infants with chronic liver or biliary disease can lead to low vitamin D absorption, vitamin D deficiency, and low calcium absorption.

Osteopenia of Prematurity

At birth, preterm infants have low skeletal mineral stores as a consequence of delivery prior to completion of the third trimester when mineral accretion would be exponentially increasing. These infants are then often dependent on total parenteral nutrition (TPN) which has a low mineral content. The supply of both calcium and phosphate is low, but a critical factor leading to osteopenia of prematurity (OP) is lack of phosphate (23). OP ranges from mild osteopenia

to overt rickets with fractures. In infants with rickets frontal bossing, swelling of costochondral joints, ankles and wrists and decreased linear growth can occur. Even in milder cases disturbed mineral metabolism is followed by reduced bone mineralisation leading to abnormal bone remodeling and reduced linear growth. Preterm infants are frequently ill and immobile; this lack of mechanical stimulation may lead to less new bone formation and reduced osteoid. In OP, there is a combination of reduced osteoid and reduced mineral, and the relative deficit of these two factors may vary from one case to another. Drugs frequently used in the neonatal unit, such as steroids and loop diuretics can also alter bone mineralization (24;25).Dexamethasone, methylxanthines and frusemide have all been shown to have an effect on calcium regulation. Methylxanthines and frusemide increase renal calcium excretion, with secondary hyperparathyroidism and bone disease having been demonstrated in infants receiving long term frusemide therapy. (26;27) Use of frusemide has been consistently reported as a risk factor for fractures secondary to OP (28-31).

Studies in the 1980's found the incidence of radiological rickets and osteopenia in very low birthweight (VLBW) and extremely low birthweight (ELBW) infants to be 32% (30) and 54% (32) respectively. Despite improved nutrition and the regular use of oral phosphate supplements, fractures still occur, typically between 10 weeks and 6 months postnatal age. The true incidence of fractures is unknown as screening for fractures with skeletal surveys is not routinely undertaken. Most fractures, especially of the rib, are discovered incidentally when x-rays are performed for other reasons. Koo et al (30) prospectively studied 78 VLBW infants born between 23 and 26 weeks gestation and by day 88 found fractures plus rickets in 12 out of the 78, and fractures without rickets in a further 7 infants. Amir et al reported a lower fracture incidence of 2.1% in premature infants, occurring from 3 to 23 weeks. There have been many changes in neonatal feeding since these studies were done, so these figures may be less relevant to modern practice. More recently, two retrospective studies reported that 7% to

10% of VLBW infants may still be at risk of fractures (29;31) Risk factors for fracture identified by these studies included prolonged intravenous nutrition, cholestatic jaundice, bronchopulmonary dysplasia, longer duration of oxygen therapy, and prolonged use of frusemide. Dahlenburg et al (33) found an increased rate of fracture in preterm infants up to 2 years old, but by age 5 years this association was no longer significant.

In the short-term, OP might impair respiratory status, respiratory distress secondary to rachitic changes has been described (34) Bone softening and rib fractures may contribute to chest wall instability, respiratory distress and difficulty weaning ventilatory support (35). OP has been postulated to be a factor in the development of myopia of prematurity related to altered bone growth of the skull (36). OP seems to be a transient disease, 'catch up' in bone mineralisation has been described using SPA and DXA (37-43) however linear growth and bone strength may still be affected in the longer term. Studies have shown that former preterm infants tend to be shorter and lighter than their term counterparts (44;45) and high neonatal alkaline phosphatase has been significantly associated with short stature at 18 months and 10 years of age (45;46). Total body BMC and cortical area of the distal tibia have been reported to be lower in ex preterm preschool children, even after adjusting for current body weight (47). Lower bone mineral content and density has also been found in school age children born preterm (44;48) Ex preterm children have been shown to have increased urinary calcium excretion at age 8 yrs associated with a reduction in height and hip bone mineral density (49). A recent study of young adults born prematurely showed that height and lumbar spine BMD at age 20 years were lower compared to UK population reference values (50). The effect of premature birth (with modern treatment of prematurity) on accumulation of peak bone mass and subsequent risk of osteoporosis merits future evaluation.

Biomechanical Factors and Infant Bone Health

Stress on bone exerted by muscle is necessary for bone formation and growth. The mechanostat theory of a functional model of bone development suggests that bone cell action is coordinated by the mechanical requirements of the bone; and thus can be modified by influencing muscle force or longitudinal bone growth (51). Several studies have described a positive effect of physical activity on bone mass in children, adolescents and adults (52;53) Immobilisation has been demonstrated to result in low bone mineral density (54). Spontaneous movement has been related to changes in bone speed of sound (SOS) in three infants with reduced unilateral movements secondary to a contralateral brain insult (55). Tibial SOS was reduced in the legs with reduced spontaneous movements compared to the unaffected side. In controls with normal symmetrical movements tibial SOS was similar in both sides.

Preterm infants are hypotonic and have reduced muscle bulk, particularly if they are small for gestational age. Sedation or paralysis to optimise mechanical ventilation reduces movement, and any spontaneous movements which do occur don't meet any resistance. This is a marked contrast to in utero movements with strong resistance from the muscular uterine wall. Infants who are very unwell during the immediate neonatal period also have reduced spontaneous movement. In addition minimal handling of preterm infants is becoming routine in neonatal units, therefore there are many factors which mean preterm infants lack muscle stimulation and subsequently may contribute to osteopenia of prematurity. A few studies have performed physical activity interventions in neonates. Moyer-Mileur (56) enrolled preterm infants aged 2 to 4 weeks old to a physical activity programme consisting of range of motion exercises with gentle compression, extension and flexion of both upper and lower limbs. Each movement was done 5 times at each joint five times each week, and the infants receiving this had at the end of the study period (when they had reached a weight of 2kg) a higher mean radial BMC measured by SPA compared to controls. The same physical activity programme was repeated

in 2000 (57) and the infants then assessed by DXA, infants in the exercise group had larger forearm bone area, but there were no differences in forearm BMC or BMD when compared to the control group. This study group had DXA and anthropometry followed up at 12 months corrected age and there were no differences detected between the intervention and control groups. Litmanovitz et al (58) used the same exercise protocol designed by Moyer-Mileur but started the physical activity when the infants were immediately cardiovascularly stable and continued for only 4 weeks. Over this 4 weeks there was an attenuation in the decrease in bone speed of sound measured by QUS in the infants receiving physical activity. The effect of the same physical activity on bone turnover markers was examined by Moyer-Mileur (50) and Nemet et al (59) Both studies found an increase in bone formation markers; Moyer-Mileur found an increase in serum type one collagen c terminal peptide (PICP) in the exercise group compared to controls, both PICP and bone specific alkaline phosphatase were higher in the infants receiving physical activity in Nemet's study. Moyer-Mileur found no difference between groups in urinary pyridoline cross links of collagen (PYD), a marker of bone resorption; however Nemet describes a reduction in another marker of bone resorption, ICTP, in the exercised infants. Aly et al used a combination of massage and a physical activity protocol in preterm infants established on full enteral feeds and this resulted in significantly higher serum PICP on hospital discharge compared to control infants (60). These studies using a physical activity programme were done when infants were on enteral feeds and receiving the recommended dietary intakes of minerals. If physical activity is given when mineral intake is limited it may not confer any benefit, and it is unknown whether it may even have a negative effect on bone mineralisation. A longitudinal study of physical activity in term infants found physical activity in the infants with low calcium intake resulted in lower bone mineral content than the control infants (61).

So, the optimum timing, frequency and duration of exercise remain unanswered questions. There are currently no studies which have reported on both the short and longer term effects of passive exercise (e.g. on neurodevelopment) in preterm infants. None of the studies on physical activity have reported on the incidence of fractures.

Section 2 Assessment of neonatal bone health

Current assessment of neonatal bone health

The diagnosis of osteopenia of prematurity remains difficult, and there is a wide variation in practice reflecting the lack of evidence base. Serum biochemistry may be normal or abnormal in the presence of bone disease and serum calcium and urinary phosphate excretion correlate poorly with bone mineralisation (62-65). Despite this, clinical practice commonly includes monitoring of serum biochemistry, primarily phosphate and alkaline phosphatase with low serum phosphate and high alkaline phosphatase suggestive of osteopenia of prematurity. Plain x-rays in the absence of fractures are poor at diagnosing bone disease due to the subjectiveness of the interpretation and the fact that changes are not seen until there is a significant reduction in mineralization (66).Most fractures are detected as incidental finding on x-rays taken for other reasons

Other Means of Assessing Bone Health

Dual energy X-ray absorptiometry (DXA), quantitative CT (QCT) and quantitative ultrasound are other available techniques to assess bone health. They currently function as research tools only for newborns and are not used in the routine investigation of osteopenia of prematurity.

Dual energy X-ray absorptiometry (DXA)

DXA uses X-ray beams of 2 different energies which are projected on to bone and the absorption of each beam measured. Bone mineral content, bone area and average bone mineral density are calculated. Measurements can be made of both the axial and appendicular skeleton. DXA is considered the gold standard for diagnosis of osteoporosis in adults, with a T-score (standard deviation score) at any site of equal or less than 2.5 indicative of osteoporosis. DXA provides a 2D measurement of a 3D skeleton therefore BMD is influenced by bone size, and in the paediatric population size correction is required using height or weight. DXA results must be compared with normal data derived from healthy children of comparable age and maturity to calculate a standard deviation score. Although DXA is increasingly used to assess bone mineral status in newborn infants, the size and immobility of the scanner, the length of time to perform the scan and use of ionising radiation make it unsuitable for routine use in the setting of the fragile VLBW infant. In addition, the data obtained from DXA can be difficult to interpret in newborns due to both movement artifact (67) and variations in technique (68).

Quantitative computed tomography (QCT)

QCT obtains cross-sectional images of bone and muscle using lower doses of radiation than standard computed tomography. It measures true volumetric bone density, bone and muscle geometry and can distinguish between cortical and trabecular bone. Images are most commonly obtained of the forearm (radius and ulna) and midshaft of the tibia. It is currently not possible to measure newborn infants using QCT as the equipment is not validated for such small limbs. Both axial QCT and peripheral quantitative computed tomography (pQCT) have similar limitations to DXA when considering their use on a neonatal unit.

Quantitative ultrasound

Quantitative ultrasound (QUS) was first developed in 1984, as a non-ionising, portable and low cost method of assessing bone health. It measures attenuation of an ultrasound beam as it passes through a specified region of interest. Most commonly the broadband ultrasound attenuation (BUA, dB/MHz) or the speed of sound (SOS/VOS, m/s) are measured: some devices also measure bone transmission time (BTT). These measurements are thought not only to be related to the mineral density of the bone but also to reflect parameters of bone quality and strength (69;70).

The majority of current ultrasound scanners are designed to transmit the ultrasound wave through the bone, a receiver measuring the attenuated wave at the other side. QUS may only be applied to the peripheral skeleton and sites for measurement are the calcaneus, phalanges, patella and tibia. The most commonly measured site is the calcaneus; it is rich in metabolically active trabecular bone, weight bearing and has little surrounding soft tissue, making it ideal for ultrasound measurements. The application of calcaneal ultrasound in children is difficult due to lack of a dedicated paediatric transducer. The DBM Sonic BP (IGEA, Italy), originally designed to measure adult phalanges, can be used to measure the phalanges or humerus of children and infants. It uses two probes, mounted coaxial on two separate branches of a calliper. The device measures the distance between the probes and the time elapsing between emission and reception to calculate the speed of sound. However, a more recently developed device (Sunlight Omnisense 2000P) is based upon just one probe being used, the ultrasonic wave travelling along the cortical bone and the reflected wave being measured, this technique is called ultrasound critical angle reflectometry. These newer devices, which measure at sites such as the phalanges, radius and tibia are more suitable for measurement in paediatrics and some devices have been specifically designed for children and neonates with age and gestational specific reference data.

From a technical point of view QUS has many advantages: it does not involve ionising radiation and it is easily accessible and transportable. There are now new devices with a dedicated hardware design and a well defined quality assurance/quality control procedure, the measurements are relatively fast, and the overall cost is low.

QUS is reported to measure both qualitative bone properties, such as bone mineralization, and quantitative properties such as cortical thickness, elasticity and micro architecture (71-74). It is not yet possible to define more precisely what QUS measures. QUS measuring 'bone strength' is commonly used as a description, because of the association between higher QUS values and reduced fracture risk. In adults, QUS does not diagnose osteoporosis, but does (using calcaneal transverse transmission) predict clinical fractures independent of BMD (75;76). Although predictive of fractures in adults, QUS measurements in children and infants do not predict fractures. A recent study in adult women found tibial SOS correlated with site matched QCT and DXA (77). Similarly, in children tibial ultrasonography has been found to correlate with bone mineral assessment by DXA (78). The impact of skeletal properties on QUS variables assessed by measuring the proximal phalanges using QUS (transverse transmission), QCT and MRI, found SOS was affected most by cortical area, cortical bone density and cortical porosity (79). In vitro studies of both newborns (gestational age 20-41 weeks) (80) and adults have shown forearm QUS correlates significantly with bone strength (81).

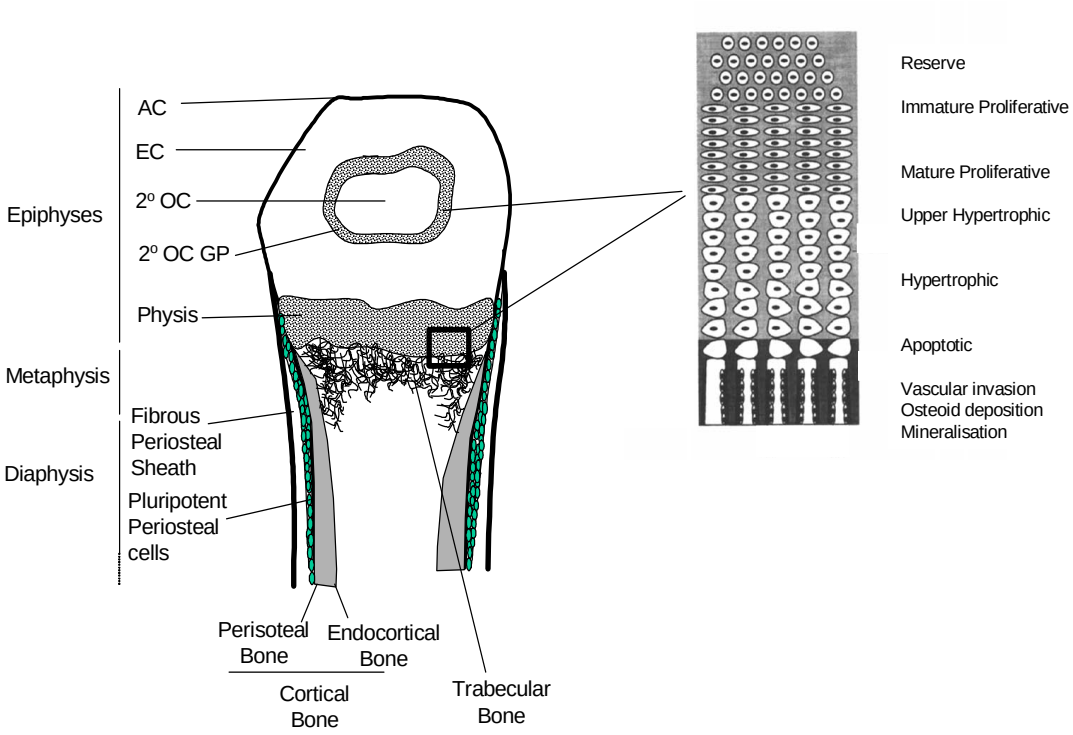
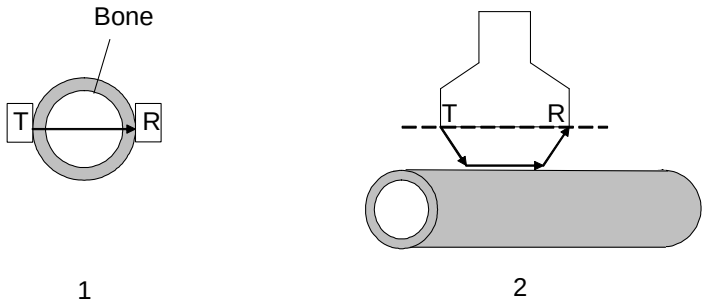


Figure 2.1 Schematic representation of the developing long bone. AC= Articular cartilage; EC= epiphyseal cartilage; 2^{o} OC= secondary ossification centre; 2^{o} OC GP = secondary ossification centre growth plate. Insert shows the cellular development of chondrocytes within the growth plate of the developing bone.

Figure 2.2 Principle of quantitative ultrasound in 1.DBM Sonic and 2.Sunlight Omnisense.



1 2

The arrows show the direction of ultrasound waves. T is the transmitter, R is the receiver.

Section 3 Maternal bone health and pregnancy

Plasma calcium is maintained by a complex mechanism involving fluxes of calcium between the extracellular fluid and the kidney, bone and gut. Dietary calcium is absorbed form the small intestine, and this is mediated by 1,25 dihydroxyvitamin D. Calcium absorbed by the gut is then filtered by the kidney and the majority is reabsorbed in the proximal renal tubules, mainly under the control of PTH, which maximises tubular reabsorption of calcium. Usually bone mineral absorption equals skeletal mineral resorption i.e. calcium flow to and from bone should be neutral. Calcium release from bone is mediated by PTH in response to a low plasma calcium, and 1,25 dihydroxyvitamin D enhances this flux of calcium from bone when PTH increases.

Mineralisation of the fetal skeleton places an increased demand on normal calcium homeostasis of the mother. Maternal intestinal calcium absorption increases early in gestation to double the normal pre –pregnancy state, largely mediated by increased 1,25 dihydroxyvitamin D which increases the concentration of calbindin$_{9k}$-D in the gut which binds calcium(82). In pregnancy the ionised calcium level remains steady, with a fall in total serum calcium and albumin. Serum phosphate levels are usually maintained in the normal range. The increase in vitamin D is independent of PTH, and is due to pregnancy induced renal changes, with a small contribution from the placenta and fetal kidneys (83). Renal hydroxylation is upregulated by factors such as PTH-related protein, oestradiol, prolactin and placental lactogen (84).

Studies of bone biopsies during pregnancy (85) and also studies looking at bone turnover markers suggest that bone resorption increases during pregnancy, and that bone formation also increases after an initial decrease. Changes in the maternal skeleton during pregnancy have no consistent pattern, studies using DXA have reported an increase in total body BMC (86) and also a decrease in BMD (87). Quantitative ultrasound has advantages for use in pregnant

women and the newborn population as it is radiation free and portable. Recent studies using calcaneal quantitative ultrasound in the pregnant mother point to bone loss that is dependent on maternal lifestyle, fat stores and seasonality of early pregnancy (88). Two studies published in 2004 measured amplitude dependent speed of sound (AD-SOS) at the hand phalanges; Pluskiewicz et al (89) prospectively studied 48 pregnant women and found a decrease in 46% of study participants however there was no correlation between fetal growth or newborn size with changes in maternal AD-SOS. Tranquilli et al (90) found a similar significant reduction in AD-SOS in 50 women measured longitudinally across pregnancy. To date, radial SOS, measured by axial transmission, to assess changes in bone health during pregnancy has not been studied. The effects of pregnancy therefore seem to have a wide inter-individual variation. Demographic and lifestyle factors are likely to exert some influence on these skeletal changes.

The pregnancy induced changes seems to provide the calcium needs for the fetus with rarely any long term effects on the maternal skeleton. Osteoporosis of pregnancy is rare, women can present with fragility fractures with or without low bone mineral density. The condition usually involves the spine or hip, and resolves spontaneously a few months after the end of pregnancy. It may be that some of these cases are women who had pre-existing low BMD, in others fractures can result from increased bone resorption secondary to pregnancy or calcium or vitamin D deficiency.

Section 4 Interaction between maternal and infant bone health

Maternal effects on the skeleton of her offspring can be mediated by both genes and the *in utero* environment. The intrauterine environment has not only an immediate effect on neonatal bone health but also there is increasing evidence that this effect persists into infancy and childhood and can extend into adulthood. The rapid rate of mineral gain during intrauterine and

early postnatal life coupled with skeletal cell differentiation and replication is postulated to offer the possibility of unique interactions between the genome and the early environment which can enable a type of skeletal phenotypic or developmental plasticity (91) . This is a phenomenon by which one genotype can give rise to a range of different morphologies in response to different prevailing environmental conditions during development. This occurs during a critical time window and is then irreversible. The effect of these early developmental effects persisting into adulthood is known as programming (92). Three studies have described birthweight and postnatal growth as predictors of adult bone mass and skeletal size (93-95) and short birth length with slow childhood growth has been shown to predict adult hip fracture (96). Maternal vitamin D status and nutrient intake has been described to have an effect on height, BMC, bone area and areal BMD in prepubertal children (97-100)

Section 5 Vitamin D

Vitamin D is vitally important for growth and maintenance of healthy bone. It is produced in the skin following exposure to sunlight, and in addition a small amount is produced from the diet. Vitamin D undergoes hydroxylation in the liver to 25 hydroxyvitamin D which is then further hydroxylated in the kidney to 1,25 dihydroxyvitamin D which is the active metabolite. This active metabolite acts on the gut to stimulate calcium and phosphate absorption. It acts to maintain calcium homeostasis, when dietary calcium is low calcium stores are mobilised from bone via PTH. Vitamin D status is usually assessed by measurement of 25(OH)D – which has two types, 25 hydroxyvitamin D2 and 25 hydroxyvitamin D3. Measurement of both together gives the best indicator of vitamin D status. Vitamin D2 is provided by some dietary sources and multivitamins, and is less potent than Vitamin D3. Vitamin D3 is the naturally occurring form in humans, is formed by action of ultraviolet light on vitamin D precursors in skin and is also present in some nutrients.

Vitamin D deficiency classically presents with rickets in childhood and osteomalacia in adulthood. Vitamin D deficiency is becoming increasingly reported (101-106). It is common in non caucasian individuals residing in countries with higher latitudes, and pregnant women and their children seem to be at particularly high risk. There are three factors which influence infant vitamin D status: vitamin D status at birth, vitamin D intake and exposure to sunlight. Exclusively breastfed infants of both caucasian and non caucasian origin are at an increased risk. A woman's vitamin D status during pregnancy correlates with her child's vitamin D status at birth, and babies born to mothers deficient in vitamin D are born with low stores. A recent study of 50 mother-infant pairs showed that mothers deficient in vitamin D had babies deficient in vitamin D, and that these infants had, relative to birthweight, a lower whole body and femur bone mineral content measured by DXA (107). A well recognized cause of neonatal hypocalcaemia is maternal vitamin D deficiency. Clinical presentations include seizures and cardiomyopathy. Vitamin D deficiency in infants can have acute and long term sequelae which should be wholly preventable. Vitamin D has effects on immune function and muscle function as well as its effect on bone, as there are vitamin D receptors in lymphocytes, skin, brain, heart, stomach, pancreas and gonads.

In one interventional study supplementing pregnant British Asian mothers with vitamin D resulted in a trend towards increased birthweight of offspring, with also higher weight and length at 1 year old (108). One recent study in the UK randomised pregnant women to receive vitamin D as a single oral dose of 200,000iu, a daily supplement of 800iu or no supplementation (109). The single or daily dosing both improved vitamin D levels significantly but only led to a small percentage of mothers and babies being vitamin D sufficient. Therefore further research is required to determine the optimal timing and dosing of vitamin D in pregnancy. Supplementation of infants who are exclusively breastfed is currently recommended by the UK government. There is currently one surveillance programme in the

UK to monitor the occurrence of rickets. This was recently started in Scotland and is co-ordinated by the Scottish Paediatric Society (ScotPSU.) A positive effect persisting to adolescence was described by Zamora et al whose Vitamin D supplementation of Swiss infants resulted in higher prepubertal bone mass (110).

Chapter 3 Quantitative ultrasound assessment of neonatal bone health at birth – cross-sectional study of term and preterm infants

Introduction

Preliminary studies suggest that the measurement of speed of sound (SOS) by quantitative ultrasound may be a useful adjunct for assessing bone health in infants (111;112). However, its methodology needs further exploration, especially in the sick, preterm infant. The current cross-sectional study was performed to assess the feasibility and reliability of the technique in this setting and to assess the relationship of SOS to the gestation and size of the infant.

Subjects and Methods

Study Population

All babies born during the period December, 2002 – January, 2004, at three maternity units in Glasgow, were eligible for recruitment. Following LREC approval and informed written consent from parents, speed of sound was measured soon after birth, at a median age of 3 days (quartiles, 2, 5) in 110 infants (male, 60) with a median gestational age (GA) of 36 wks (range, 24, 41) and median birthweight of 2565g (range, 680, 4600) (Table 3.1). The cohort, which included 5 sets of twins (4 of the same sex) was, arbitrarily, divided into three groups by gestation at birth, A (>37 wks), B (32-36 wks) and C (<32 wks).They were all clinically stable at the time of the scan (this included stable while ventilated for the extremely preterm infants) and without congenital malformations.

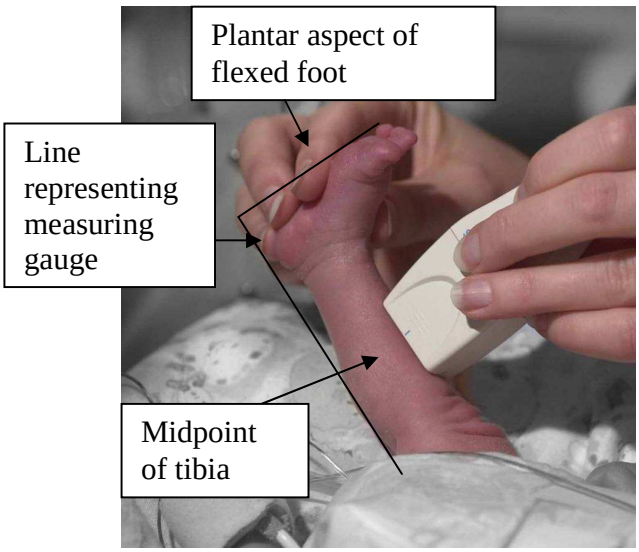
QUS Measurement

SOS was measured using the Sunlight Omnisense 7000P^{TM} scanner (Sunlight Medical, Israel). It is comprised of a main unit and a small hand held ultrasound probe. The Omnisense^{TM} generates pulsed acoustic waves, at a centre frequency of 1.25MHz (bandwidth 0.7 to 1.8). The probe contains two pairs of transducers; one acts as a transmitter and the other acts as a receiver. When ultrasound waves are incident on a subject such as bone, the waves are reflected, refracted or transmitted, depending on the angle of incidence. The refracted wave that propagates along the length of the bone can be measured. The time needed for the first detectable signal above noise to arrive at the receiving transducer is recorded. Because the transmitting and receiving transducers are at a fixed length, the length of the ultrasound pathway can be determined and, hence, the velocity can be calculated. A measurement consists of three scan cycles, each generating a representative SOS value. An internal algorithm checks the three SOS values for consistency and if the device detects any significant inconsistency, it instructs the user to obtain further measurements. An acoustic gel is used to couple the probe to the skin.

SOS was measured at the radius and tibia. The site of measurement on the radius was determined by identifying the midpoint between the tip of the middle finger and the dorsal aspect of the flexed elbow (distal third of the radius) and the site of measurement on the tibia was determined by identifying the midpoint between the plantar aspect of the flexed foot and the dorsal aspect of the flexed knee (mid shaft of the tibia) (see diagram). The probe was aligned along and parallel to the bone and moved in a semi-arc over the circumference of the site of measurement until a reliable estimate of the SOS is measured. Each SOS measurement cycle took about 30 seconds and the result was expressed in metres per second (m/s), and displayed together with a Z score value (units of standard deviations relative to the age-

matched population reference values) based on a reference range for term and preterm infants included with the software (113).

Determining site of measurement of tibial SOS



Validation studies were performed to assess (1) intraobserver variation – multiple measurements performed at one site (tibia) by the same observer in 15 infants, (2) interobserver variation – repeat measurements on two sites (left and right tibiae) in 6 infants, (3) variation between different sites in same infant – duplicate scans at each tibia and radius in 20 infants, (4) effect of temperature and humidity on SOS – performed on adult subjects by placing arm in incubator and varying temperature and humidity.

Statistical Analysis

Using XL STAT V7.0 (Addinsoft, France) and Microsoft Excel 2000 (Microsoft Corp, USA), precision of the measurements was determined by calculating the coefficient of variation and differences between groups were compared using the Mann-Whitney U test. Analysis of

covariance was performed to assess any associations between variables. Due to the small size of the study twins were treated as independent variables.

Results

Intra-observer and Inter-observer variation

The mean (1SD) intra-observer coefficient of variation (CV) for three repeat measurements at the tibia in 25 infants with a median GA of 37 wks (r, 33 - 41) was 1.2% (0.8%.) Each infant was measured and then the mark for that site of measurement was removed, and the infant repositioned between subsequent measurements. The technique precision error as calculated from the root-mean-square average of the CV was 1.4% (114). The mean interobserver CV for measurements performed by two observers at the tibia in 6 infants with a median GA of 26 wks (r, 24 - 32) was 1.2% (0.7%).

Inter-site variation

In 20 infants with a median GA of 37 wks (r, 26 - 41) measurements were performed at both tibiae and radii. The mean CV (1SD) for measurements at all 4 sites was 2.4% (1.2%), left and right radius was 2.1% (1.4%), right radius and right tibia was 2.3% (1.8%), left radius and left tibia was 1.8% (1.2%), and left tibia and right tibia was 1.7% (1.8%.)

Influence of Temperature and Humidity

Radial SOS in 15 adults was measured at ambient temperature, 35^0C, and 35^0C with 95% humidity. The SOS did not change with increasing temperature and humidity. Mean CV (1SD) for all measurements was 2.0% (1.1%), measurements at room temperature and 35 degrees was 1.7% (1.1%), room temperature and 35 degrees with 95% humidity was 2.1% (1.8%), and 35 degrees and 35 degrees plus 95% humidity was 1.7% (1.7%.)

Gestation and Birth Weight

Median tibial SOS was 3079m/s (q, 3010, 3142,) in Grp A and significantly higher than in Grp B who had a median SOS of 2994m/s (q, 2917, 3043) or Grp C with a median SOS of 2911m/s(q, 2816, 2982) ($p<0.001$) (Fig.3.1). There was no significant correlation between the birthweight and SOS in the infants in Grp A (Fig.3.2.) Analysis of covariance revealed that 40% of the variability of tibial SOS was explained by gestation, birthweight and gender ($p<0.001$) and gestation had the greatest impact, followed by birthweight, and then gender.

Influence of Size

In Grp A and B, there were no significant differences between the tibial SOS for the SGA and AGA infants. However, in Grp C, tibial SOS was greater in the two SGA than the AGA infants (SOS values 3011 and 3056) and median SOS 2909m/s (q, 2790, 2997) respectively ($p<0.05$). In the 5 sets of twins, tibial SOS tended to be higher in the lighter twin (Fig.3.2). There were no significant differences between the LGA and AGA infants.

Discussion

Not only does this study reinforce the finding of previous studies that quantitative ultrasound assessment of SOS can be performed successfully and precisely in infants from 24 weeks gestation through to term (112;113), it also shows that, at this age, the assessment is not site-specific, and measurements at one tibial site are sufficiently representative of the SOS at the other sites in the neonate. The validation studies also confirmed that the changes in temperature and humidity that are often encountered in an intensive care neonatal unit do not seem to alter the reproducibility of the measurements performed.

Unlike most previous studies, the infants in the current study were measured very shortly after birth, eliminating the confounding effect of the associated co morbidities that are often

encountered in the preterm infant (111;112). The close correlation of tibial SOS with gestational age rather than birth weight, agrees with other recent reports where measurements were performed within the first 4 days, suggesting that maturity may be a more important factor in bone health than birth weight (113;115). The relative lack of an association between birthweight and speed of sound was reinforced, firstly, by the findings in the SGA infants who did not have a lower tibial SOS than gestation-matched AGA infants, and secondly by the twin-studies where the tibial SOS was similar, and even slightly higher in the growth retarded twin.

A weakness of this study was that twins were included as independent variables. In the general population of preterm infants twins are over represented and in our small sample size the pragmatic approach of including both twins was taken. It would have been better to recruit only one of each twin pair. However, for clarity, we have presented raw data on graphs clearly identifying twins, rather than condensing data into groups.

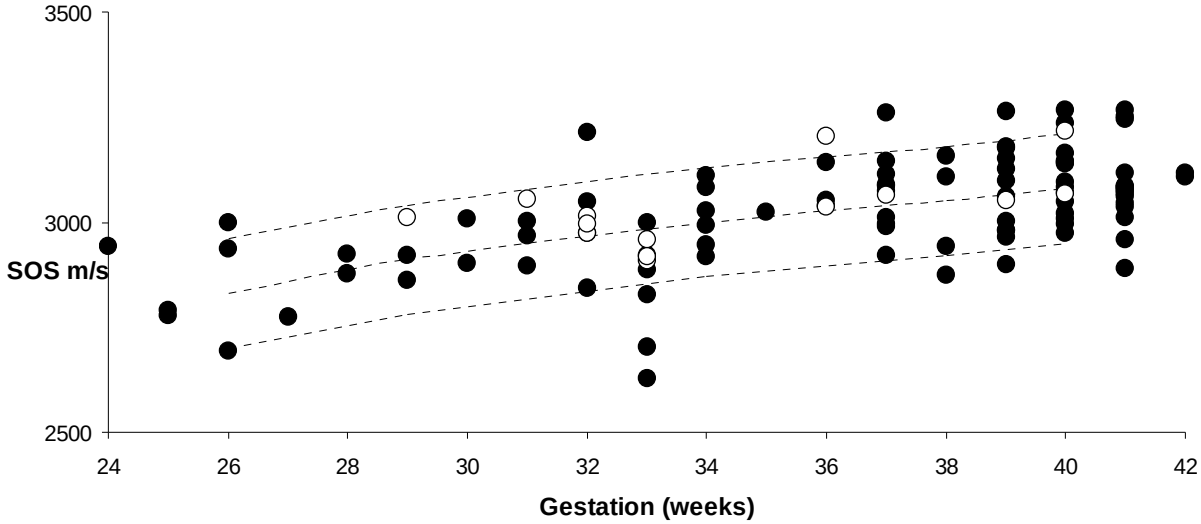
The future application of quantitative ultrasound in assessing the bone health of infants deserves further exploration and the data in this report shall prove beneficial in designing longitudinal studies.

Table 3.1

	>37 wks	32- 36 wks	<32 wks
Number of infants	62	28	20
Median gestation (wks) (25th,75th centiles)	40 (38, 41)	33 (32, 34)	28 (26, 30)
Male:Female	37:25	14:14	9:11
Median birth wt (g) (25th, 75th centiles)	3490 (3075, 3788)	1890 (1590, 2310)	1080 (920, 1280)
SGA[1]	4	9	2
AGA[2]	49	17	17
LGA[3]	9	2	1
Caucasian	57	28	18
Asian	4	0	1
Mixed race	1	0	1
History of PROM	1	1	1
Antenatal steroids	0	10	8
Twins	0	8	2
Oligohydramnios	1	3	0
SVD	34	10	10
Caesarian Section	16	13	8
Forceps	11	3	1
Vaginal breech	1	2	1
Breast	27	8	1
Formula	23	8	1
Breast and formula	12	9	3
TPN (+/- enteral feeds)	0	11	19

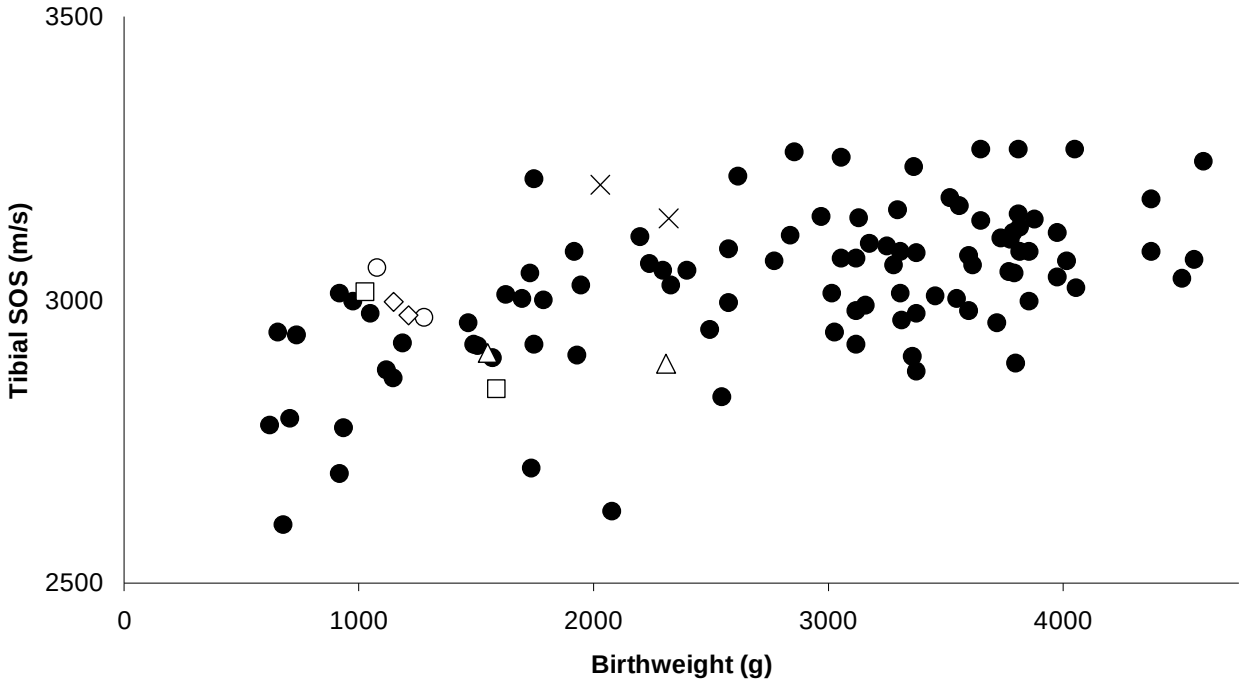
Table 3.1 Details of infants undergoing SOS measurement.
The cohort is divided into 3 groups according to gestation, those born at >37 weeks, those born between 32 and 36 weeks, and those born at <32 weeks. [1] SGA - small for gestational age, on or below the 9th centile for weight. [2] AGA - appropriate for gestational age, between 10th and 90th centile for weight. [3] LGA - large for gestational age, above 90th centile for weight. [4] PROM - prolonged rupture of membranes. [5] SVD - spontaneous vertex delivery.

Figure 3.1



The closed circles represent speed of sound (SOS) values in infants appropriate for gestational age (AGA), birthweight between the 10th and 90th centile for weight. The open circles represent SOS values in small for gestational age infants (SGA), birthweight on or below the 9th centile for weight.

Fig 3.2



The closed circles represent speed of sound (SOS) values in singleton infants. The other symbols represent SOS values in twin pairs.
Note that 4 twin pairs were of discordant growth at birth (one twin small for gestational age (SGA), on or below the 9th centile for birthweight and the other twin appropriately grown for gestational age (AGA), between the 10th and 90th centiles for birthweight).

Chapter 4 Quantitative ultrasound assessment of neonatal bone health from birth to 2 years in preterm infants

Section 1 Longitudinal evaluation of bone health as assessed by QUS in preterm infants from birth to term CGA

Introduction

Preliminary studies by our group suggest that the technique of quantitative ultrasound is a feasible and accurate method of assessing changes in bone health in preterm infants. We hypothesize that the neonatal course has an effect on bone development. In this study we performed serial measurements in a cohort of VLBW infants from birth to discharge and investigated the relationship between traditional markers of OP, markers of clinical illness and SOS.

Patients and Methods

Study Population

Between December 2002 and January 2004, infants who were less than 32 weeks gestation and less than 1500g birth weight were recruited from three neonatal units in Glasgow, UK. The study was approved by the Local Research Ethics Committee at all three maternity hospitals. Twenty five eligible infants with a median gestation of 27 weeks (range 24-31 weeks) and median birthweight of 980g (range 625-1500g) were recruited into the study following informed consent from their parents. Twenty three infants had an initial tibial ultrasound scan in the first week of life (one infant was too unwell, and in one case the scanner was out of order.) Eighteen of the twenty three infants had at least five serial scans until a median gestational age of 36 weeks (range 35, 37) (three infants died and two were discharged very early.) Routine clinical and anthropometric data, including details of nutrition, respiratory

complications and serum biochemistry were collected in these infants. Weekly measurements were performed until term corrected age or until the infant was discharged home. Details of the eighteen infants followed longitudinally are presented in Table 4.1. No infant sustained a clinically evident fracture during the study period.

The CRIB (Clinical Risk Index for Babies) score is a validated tool for assessing initial neonatal risk and severity of illness of the preterm infant with higher scores being associated with increasing mortality and morbidity (116). It is based on birthweight, gestational age, minimum and maximum oxygen requirement and base excess in the first 12 hours of life, and presence of congenital abnormalities. More recently, temperature at admission has been added to this to provide a new score(117).

Speed of Sound Measurement

SOS was measured at the tibia using the Sunlight Omnisense 7000P^{TM} scanner (Sunlight Medical, Israel) as described in the previous chapter. Ultrasound measurement was not performed if the infant was felt to be too unstable or if access to the tibia was difficult, for example, due to intravenous cannulation. Measurements were made by one of two operators.

Statistical Analysis

The data were expressed as medians and percentiles which were compared using the Mann-Whitney U test. Spearman rank correlations were used to compare any association between variables and analysis of covariance (ANCOVA) was performed to establish the level of associations between multiple measured variables. Statistical analysis was performed with XL STAT V7.0 (Addinsoft, France) and Microsoft Excel 2000 (Microsoft Corp, Redmond, WA, USA).

Results

Initial scan

In the 23 infants who had an initial scan, the median age at first scan was 4 days (range 1, 7.) The median SOS was 2923m/s (2672, 3107). There was a strong correlation between SOS and gestation (r, 0.8, $p<0.005$.) Conversion of the SOS data to SDS was performed to separately evaluate the association of SOS to birth weight and gestation. Median SDS z score was 0 (–1.3, 1.3.) The correlation of SOS SDS to birthweight was lower (r, 0.4, NS) compared to that for absolute SOS to birthweight (r, 0.6, $p<0.05$) indicating only a small effect of birthweight independent of gestation.

Serial Scans

A fall in SOS was noticed in all eighteen infants who had serial scans (Fig.4.1). The median fall in SOS from first to last scan was 95m/s (28, 289). The median SOS initially and at the end of the study was 2923m/s (2672, 3107) and 2802m/s (2502, 2991) respectively $p<0.05$.

The median Z score at the start and end of the study was 0 (–1.3, 0.55), and –2.15 (-0.45, -4.5). Expressed as a Z score, this fall was greater in the 24 - 27 weeks gestation cohort with a median reduction of 2.2 SDS (1.6–4.0) compared to a median reduction of 1.3 SDS (0.85-2.2) in the 28-32 weeks cohort ($p<0.05$).

Alkaline phosphatase

Peak serum total alkaline phosphatase (ALP) was reached by all infants at a median corrected age of 33 weeks gestation, and measured 741 iu/l (251, 2199.) In five infants serum ALP was within the normal reference range throughout the study period. Peak serum ALP was negatively correlated with tibial SOS at the end of the study (r, 0.6, $p<0.05$ (Fig 4.2)

Three infants in the study had a combination of high alkaline phosphatase (859, 1094, 2199 u/l) and low serum phosphate (<1.2mmol/l) suggestive of OP. These infants had the lowest Z scores (-4.5, -3.6, -4) and the lowest absolute speed of sound of any infant in the study (2502, 2508, 2532 m/s). A further six infants also had Z score <-2.0, four out of these six had a raised serum ALP with a normal phosphate, and two had both a normal serum ALP and phosphate. (Table 4.2)

Severity of illness

Of the infants who had serial scans the median CRIB and CRIB II scores were 2 (0-9) and 10 (5-14) respectively. There was a negative correlation between SOS at the end of the study and CRIB and CRIB II scores (r, 0.6 and 0.6 respectively, $p<0.01$) (Fig 4.3.)

There was no significant difference in SOS at the end of the study in infants with or without chronic lung disease. Two infants (both born at 25 weeks gestation) developed necrotising enterocolitis, these were two of the three infants with the lowest absolute SOS and biochemical evidence of OP.

Nutrition

All infants initially required infusion of total parenteral nutrition (TPN.) The median number of weeks to full enteral feeds was 3 (1, 17.) Out of the 18 infants, 11 required TPN for more than 3 weeks. The median tibial SOS at the end of the study for these 11 infants and the remaining 7 infants were 2746m/s (2502-2866) and 2842m/s (2778-2991) respectively ($p<0.05$, Fig.4.4.)

Growth

During the period of serial scans the median average weekly weight gain was 116g (range 100 – 191.7g.) There was no correlation between average weekly weight gain and either gestation (r, 0.07) or fall in SOS SDS (r, 0.27.)

Analysis of covariance

Gestation was the most significant variable in relation to SOS at 35-37 weeks when ANCOVA was performed in 3 combinations using gestation, gender, CRIB score, birthweight, alkaline phosphatase, age to full feeds, and chronic lung disease. (Table 4.3)

Discussion

In this study we have looked prospectively at changes in tibial SOS in 18 very low birth weight infants. The initial SOS for all infants was in the expected range, suggesting that all the infants had undergone skeletal development until the point of preterm birth. However, on longitudinal follow up the SOS fell rather than increasing as would be expected based on our cross-sectional reference data (118). The fall in SOS occurred in all infants but was greatest in those infants born earlier than 26 weeks gestation. Infants who were the most significantly unwell and predicted to have a greater risk of morbidity, as assessed by the CRIB and CRIB II scores, had the lower SOS scores at 35-37 weeks PCA. This emphasizes that even with advances in neonatal nutritional care, which has primarily concentrated on improving bone mineral accretion, the *exutero* environment remains a poor substitute for the *in utero* development. Both mineral deficiency causing decreased bone synthesis or lack of mechanical stimulation causing increased bone resorption could contribute to abnormal skeletal development in our preterm infants.

SOS fell despite adequate nutrition resulting in sustained weight gain. This might reflect an exacerbation of relative mineral deficiency by the demands of growth. Interestingly the presence of chronic lung disease did not have a significant effect on the fall of SOS.

Nemet et al (111) assessed tibial SOS in preterm infants at a median post-natal age of 4 weeks (range 1-18 weeks) and found the SOS correlated with gestational age, with preterm infants at term corrected age having a significantly lower SOS than term control infants. There was a significant inverse correlation of SOS with serum alkaline phosphatase in the preterm group. We also found an inverse correlation between tibial SOS at the end of the study and peak alkaline phosphatase. The three infants with the lowest SOS values had low serum phosphate as well as high alkaline phosphatase. However, all the infants in our study had a fall in SOS, even those with a serum ALP in the normal range. If a reduction in SOS indicates bone disease this would question the role of alkaline phosphatase as a screening test for OP. It should also be borne in mind that serum ALP is the sum of three isoforms (liver, intestine and bone) and some of the changes may reflect disorder of liver function. However, the bone isoform contributes to the largest proportion of ALP in infants and children and changes in that isoform generally mirror those in total serum ALP (108). Bone specific ALP in the neonate has not been found to improve sensitivity for OP (62).

It is well established that fractures do still occur in VLBW babies. Amir et al reported fractures in 1.2% of preterm infants between day 24-day 160 (28), Dabezies found a fracture rate of 10.5% diagnosed at a mean age of 50 days (29). The fall in SOS noted in our patients at 35-37 weeks might suggest an abnormality of bone strength which would correlate with an increased risk period for fractures.

Passive exercises might be of benefit in increasing bone strength. Two separate studies have demonstrated a positive effect using range of motion exercise interventions. The same exercise protocol consisting of daily flexion against passive resistance at the wrist, elbow, shoulder, knee and hip resulted in increased bone mineral density (determined by single-photon absorptiometry and DXA)(57) and an attenuation of the decrease in SOS postnatally in preterm infants (58) compared to controls. Moyeur-Mileur (57) also found an increase in body weight gain, forearm bone length, bone area and fat free mass in the exercise group.

In conclusion, very low birth weight infants show a fall in postnatal tibial SOS and by term the SOS is well below that of infants born at matched gestation suggesting there may have been an arrest in bone development. Gestation at birth is the most important influential factor, and the fall in SOS was greatest in the 24-27 week gestation cohort. The association of SOS with serum ALP, phosphate, as well as markers of illness severity suggests that routine measurement of SOS may have a place in non-invasive monitoring of bone health of the preterm infant.

Section 2 Changes in quantitative ultrasound in infants born at less than 32 weeks gestation over the first 2 years of life

Introduction

Studies by our group and others show reduced SOS as measured by QUS during the immediate neonatal period (112;115;119) however there is a scarcity of data on SOS changes following hospital discharge. Fractures are reported to occur most often around term CGA (which frequently coincides with discharge from the neonatal unit) and rarely after 6 months CGA (120). Most preterm infants who are small for their CGA at hospital discharge attain an appropriate weight and length (compared to term infants of the same post menstrual age) over the first year of life through a period of catch up growth. It is our hypothesis that tibial SOS increases over the first two years of life and that the rate of increase would be determined by the infant's neonatal course. Therefore the aim of this study was to assess bone health, including assessment of SOS from term to 2 years post term corrected age in infants born prematurely.

Materials and Methods

Study Population

Infants born at less than 32 weeks gestation were recruited from a neonatal follow up clinic in the Queen Mother's Hospital, a tertiary centre in Glasgow, between February 2004 and April 2005. The study was approved by the local ethics committee, and informed written consent was obtained from parents prior to their child being included in the study. Infants were eligible for inclusion if born before 32 completed weeks of gestation, and exclusion criteria were the presence of congenital abnormalities, as well as any congenital skeletal deformity. Details of the child's neonatal history and current feeding were recorded from the case notes, and weight

and total body length were measured at the clinic visit using Avery baby scales (accurate to 10g) and Holtain supine length measuring table (accurate to 200mm) respectively. Weight and length were converted to standard deviation scores (SDS) which are units of standard deviations relative to the mean for an age and sex matched population reference values. Corrected gestational age (CGA) was calculated for all infants, based on an age of 0 days at the 40 week post menstrual date. The number of days each infant received total parenteral nutrition (during their neonatal inpatient admission) was recorded from the case notes. All infants except one received oral phosphate supplementation once established on enteral feeds on the neonatal unit. All infants except one received vitamin D 400 IU daily once established on enteral feeds until 1 year corrected age. Six infants received diuretics (4 had furosemide and spironolactone, 1 had chorthiazide and spironolactone and 1 had spironolactone only) and one infant received postnatal dexamethasone. Chronic lung disease was defined as a requirement for oxygen at 36 weeks corrected gestational age (CGA.) The CRIB (Clinical Risk Index for Babies) score was used as a tool for assessing severity of illness of the preterm infant (116). Thirty nine infants were recruited, generating cross-sectional data. One infant was excluded from further analysis as the case notes could not be obtained. Fifteen of these 39 infants had serial measurements of SOS, generating longitudinal data. Eight of the fifteen had measurements at term CGA and early infancy (early group) and seven had serial measurements performed in later infancy (late group.) Details of these infants are outlined in Table 4.4.

Speed of Sound Measurement

SOS was measured by two operators at the tibia using the Sunlight Omnisense 7000P^{TM} scanner (Sunlight Medical, Israel) as described previously. The result was expressed in metres per second (m/s), and displayed together with a Z score based on a cross-sectional reference

range for term and preterm infants provided by the manufacturers (113;121). Two different probes were used, the CS and CM probe, and it should be noted that the manufacturer's reference range is slightly different for each probe. For consistency, all measurements until term were made using the CS probe, all measurements made post term corrected age were made using the CM probe. SOS SDS rather than absolute SOS values was used in analysis of longitudinal change, and also for correlation with variables such as serum biochemistry. This was to minimise the potentially confounding effect of the two different probes.

Serum Biochemistry

Peak total serum alkaline phosphatase (ALP, IU/l) measured, between 35-37 weeks corrected gestational age (CGA) was recorded. Lowest serum phosphate (PO_4, mmol/l) measured between 35-37 weeks CGA was also recorded. A conjugated hyperbilirubinaemia was recorded as present if the conjugated bilirubin was >10% of the total measured bilirubin during the neonatal inpatient stay (mmol/l).

Statistical Analysis

The data were expressed as medians and ranges. Qualitative variables were compared using the Mann-Whitney U Test. Pearson's correlation coefficients were used to compare any association between quantitative variables. P values <0.05 were taken as significant. Statistical analysis was performed with Minitab v.12.21 (Minitab Inc.)

Results

Cross-sectional Data

Thirty-nine infants were divided into 3 subgroups by age (CGA) at SOS measurement: 0-6 months, 6-12 months and >12 months. The characteristics of these infants and the relationships between qualitative and quantitative variables are reported in Table 4.4 and Table 4.5.

In the group as a whole there was a strong positive correlation between SOS and corrected gestational age, r=0.8, p<0.005 (Figure 4.5.) The youngest infants, 0-6 months CGA demonstrated a significant negative correlation between SOS SDS and TPN duration, (r -0.7, p<0.005) as well as a significantly lower SOS SDS for the 7 of 15 infants who required TPN for more than 14 days versus those who had TPN for less than 14 days, median SOS SDS -1.6 and -0.6 respectively, p<0.05. In this subgroup there was also a significant positive correlation between SOS SDS and neonatal serum phosphate (r 0.6, p<0.05). These correlations were not significant in the older children, or in the study group as a whole. In the infants age 0-6 months CGA CRIB score was negatively correlated with SOS SDS, r -0.6 but this was not statistically significant. There were 18 infants with CLD in our study group, and there was no significant difference in SOS SDS for those with or without CLD. Age at independent walking which was available in 17 patients was reported as a median of 17 months CGA (range, 10.5, 20.) There was no relationship between age at walking and SOS SDS, r=0.014, NS. Type of feeding was available in 30 infants, 6 received post discharge formula which was calcium and phosphate enriched, median SOS SDS for these 6 infants was -0.6 (range, -2.5, 2.1) compared to median SOS SDS of 0.13 (range, -3.55, 2.3) for the 24 infants discharged breastfeeding or on standard formula (ns.) The infants who received post discharge formulae were likely to be smaller and sicker.

Early Longitudinal Data

Seven infants (5 female) had at least two SOS measurements. Tables 4.6 shows the characteristics of these infants. All 7 had SOS measured around term CGA (36-40 weeks) and subsequently at a median age of 5 weeks CGA (range, 5 - 55) (Figure 4.5, absolute SOS values illustrated) Five of the 7 infants had a third measurement at a median age of 45 weeks (range, 15-80) which was a median of 27 weeks after 2nd measurement (range, 10-76.) In 6 of the 7 infants SOS SDS showed an increase between measurements. In 1 infant, although absolute SOS increased, SOS SDS continued to decrease post term until 6 weeks CGA then began to increase. This infant had the longest duration of TPN amongst the study cohort (120 days.) SOS SDS was low at term CGA, with a median of -2.2 (range, -3.6 to -0.5) and increased to a median of -1.5 (range, -4.1 to 0.8) by 2nd measurement, and median of 1.0 (range, -0.7 to 2.6) by third measurement. Median SOS SDS significantly increased from -2.2 (range, -3.6 to -0.5) to 0 (range, -1.7, 1.8) ($p<0.005$) between first (term corrected age) and final measurements (median age of 0.7 years, range, 0.1, 1.1.)

The median change in SOS between first and last measurements was 443m/s (range, 144-640) with a median change of 10.9 m/s per week (range, 5.4 – 15.1.) The babies with the lowest SOS at term had the greatest increase in SOS over time (r 0.9, $p=0.008$.) Figure 4.6. There was no correlation between change in weight SDS or length SDS and change in SOS SDS, r -0.3 and -0.9, NS.

Late Longitudinal Data

Eight infants had SOS measured twice over the age range 11 weeks to 2 years CGA. The characteristics of this group are shown in Table 4.6. Median age at first measurement was 33 weeks CGA (range, 11-88) and at second measurement was 65 weeks (range, 18-103.) Median SOS SDS was 0.55 (range, -1.75 to 2.3) and 1.2 (range, -0.9 to 2.5) at first and second measurements (NS.) The median time between measurements was 17.5 weeks (range, 7-71)

and the median change in SOS SDS was 0.2 (range, -0.15 – 2.5.) The median change in SOS per week was 7.2 m/s (range, 1.2-8.45.)

Median weight SDS was -0.99 (range, - 2.56 to 0.96) and did not significantly change over time. Median length SDS was -0.15 (range, -1.33 – 2.11) and -0.64 (range, -1.87, 1.13) at first and second measurements (NS.) There was no correlation between change in length and change in SOS SDS score, r= -0.01 NS.

The characteristics of infants followed longitudinally did not differ significantly from the infants who were only included in the cross sectional results.

Discussion

Our cross-sectional data show that in most, but not all infants SOS was within the manufacturer's reference range which is based on children born at term. This is interesting as published studies (119;122;123) show SOS to plateau or decrease in preterm infants during the period from birth to discharge. Therefore, there has been catch up in SOS, which parallels the catch-up in BMC measured by SPA and DXA reported in some studies (37-43).

One of the critical aetiological factors in osteopenia of prematurity is inadequate phosphate. It is therefore not surprising that there was a significant correlation between serum phosphate and SOS SDS score in the infants age 0-6 months. Indeed, Kurl et al (124) reported hypophosphataemia at 6 weeks of age to be associated with a 7.8 fold risk of having low BMC later in infancy. Backstrom also showed low serum phosphate at 3 weeks to be negatively associated with a change in BMAD between 3- 6 months of age in ex preterm infants (62). Our previous data showed a negative relationship between duration of TPN and SOS SDS at term (119), and TPN duration also had a significant effect on SOS SDS in study infants aged 0-6 months. CRIB score, conjugated hyperbilirubinaemia, elevated ALP and IUGR were not significant factors. This may be because the number of infants was too small, however

Backstrom et al (62) also found there to be no significant difference in forearm BMAD (measured by DXA) at 6 months CGA in preterm infants with complications in the neonatal period as compared to the non-complicated group, whereas at 3 months CGA there had been a difference in BMAD between the two groups. Only one infant older than 6 months CGA had a low SOS SDS score (-2.5 at 20 months CGA.) This infant was IUGR at birth, growing between 2nd and 10th centiles at time of measurement, and did not have a low serum phosphate, raised alkaline phosphatase or conjugated hyperbilirubinaemia in the neonatal period. She did not have chronic lung disease, however her mother was a heavy smoker during pregnancy.

The significant effect of neonatal factors in infants at 0-6 months which then disappears in the older infants, coupled with the rapid increase in SOS from term to 6 months in the early longitudinal group points towards an early window when catch-up occurs. This pattern has similarities with SGA infants who have an early period of catch up growth (125). However, we found no significant effect of weight or length gain on SOS SDS. According to the manufacturer's reference range (based on cross-sectional measurements), in-utero SOS increases steadily from a mean of 2850m/s to 3100m/s between 26 and 40 weeks gestation, this equates to an increase in SOS of 15.7m/s per week. The infants in our early longitudinal group gained 10.9m/s per week post term. This raises the possibility of an internal biostat which is switched off in preterm infants after birth, and which when it restarts works closer to the higher in-utero rate of accretion until catch up is achieved. This may explain why the greatest gains over the first 6 months were seen in those with the lowest speed of sound at term. A similar trend in bone mineral accretion with a rapid phase of increase starting at 40 weeks PCA has been previously described (38;39).

A significant weakness in this study is the use of two different probes, each with a different reference range. Although the larger probe (CM) is set up by the manufacturers to be used at term corrected age (immediately following use of the CS probe designed for preterm infants) the reference ranges do not merge perfectly. This raises the question of minor errors in the manufacturer's reference ranges and indicates that a study comparing both probes in babies aged between 36 to 42 weeks CGA is needed. Because the reference range for the CM probe is slightly lower, the duration of time to 'catch up' may actually be longer than suggested by our data. Following hospital discharge increased handling and movement may be important in determining the timing and extent of recovery in the SOS. Passive exercise has been shown to attenuate the decrease in SOS from birth in a small group of preterm infants (58). Lower physical activity levels, with a concomitant decrease in bone loading have been suggested as a potential cause of long term bone deficiency in infants born prematurely (47;126). It is possible that those infants who are slow to catch up do have an increased risk of fractures during the period of time that SOS is low. Our finding of SOS reaching the normal range in the majority of ex preterm by 6 months would fit in with the observation of Koo who did not observe fractures or radiological rickets in ex VLBW babies after 26 weeks postnatal age(120).

Although the relative size of the population sample restricts the power of this study, our observation that the window of recovery in bone SOS maybe restricted to the first 6 months following discharge is novel. Greater numbers of study infants are needed to confirm this finding as the numbers are too small for definite conclusions to be made. Although after discharge, feeding with enriched formula may confer additional benefit in bone mineralisation or growth compared to standard term formula (127), future studies should, nevertheless, study carefully any link between feeding regimens and recovery in bone health.

In summary, we have observed a period of catch up in bone SOS in preterm infants that may be limited to the first 6 months following hospital discharge. Interventions that are aimed at improving bone health in these infants need to consider this period of spontaneous improvement in bone health.

Table 4.1

No of infants	18
Male: Female	7:11
Gestation (weeks)	27 (24,32)
Birthweight (g)	957 (625, 1500)
SGA[1] AGA[2] LGA[3]	4 14 0
Initial SOS (m/s)	2923 (2672, 3107)
SOS at 35-37 wks PCA[4] (m/s)	2802 (2502, 2991)
CRIB score	2 (0,9)
CRIB 2 score	10 (5,14)
Peak alkaline phosphatase (iu/l)	741 (251,2199)
Age at full enteral feeds (days)	24 (6,120)
No of infants ventilated	13
No of infants with chronic lung disease[5]	9
Twin pregnancy	5
No of infants with radiologically proven NEC[6]	2

Table 4.1 – Characteristics of infants who had serial ultrasound scans.
All values are medians (ranges) 1- SGA, small for gestational age, birthweight below 10^{th} centile for gestation on a standard UK growth chart. 2 - AGA, appropriate for gestational age, birthweight $>10^{th}$ and $<90^{th}$ centiles for gestation on a standard UK growth chart. 3 - LGA, large for gestational age, birthweight above the 90^{th} centile for gestation on a standard UK growth chart. 4 – PCA, postconceptional age, number of weeks postconception. 5 – CLD, chronic lung disease, a requirement for supplemental oxygen at 36 weeks PCA. 6 – NEC, radiologically proven necrotising enterocolitis.

Table 4.2

	High ALP[1]		Normal ALP[1]	
	SOS> 2SDS[2]	SOS<2SDS[3]	SOS>2SDS[2]	SOS<2SDS[3]
Low phosphate[4]	0	3	0	0
Normal phosphate[4]	4	4	4	2

Table 4.2 – Serum alkaline phosphatase (ALP) and serum phosphate in seventeen study infants (one infant had no serum phosphate or alkaline phosphatase measured) categorised according to speed of sound (SOS) SDS.
1 - ALP was defined as high and normal by a level above or below 420iu/l, respectively. 2 – SOS>2 SDS, speed of sound measurement within 2 standard deviation scores of the mean for an age and sex matched population. 3 – SOS<2 SDS, speed of sound measurement more than 2 standard deviation scores below the mean for an age and sex matched population. 4 – phosphate was defined as low or normal defined on serum phosphate level below or above 1.2 mmol/l, respectively.

Table 4.3

	Variables	Goodness of fit coefficient, R	Type 1 SS Pr>F
ANCOVA 1	Gestation	0.8	0.001
	Birth weight		0.742
	CRIB[1]		0.057
	Sex		0.654
ANCOVA 2	Gestation	0.8	0.000
	Serum ALP[2]		0.116
	Age at full feeds		0.465
	Sex		0.261
ANCOVA 3	Gestation	0.8	0.001
	CRIB		0.116
	Age at full feeds		0.465
	CLD[3]		0.261

Table 4.3 – Analysis of covariance of variables in relation to speed of sound at the end of the study.
1 – CRIB, clinical risk index for babies. 2 – serum ALP, serum total alkaline phosphatase. 3 – CLD, chronic lung disease, a requirement for supplemental oxygen at 36 weeks post conceptual age.

Table 4.4

Quantitative Variables	0-6 months (n=15)		6-12 months (n=10)		12+ months (n=13)		Study group (n=38)	
	Median (range)	R value[a] (p value)	Median (range)	Rvalue (p value)	Median (range)	R value (p value)	Median (range)	R value (p value)
Gestation (wks) [b]	27 (26,31)		27.5 (24, 31)		31 (27, 31)		28.5 (24, 32)	
Age at scan (wks post term corrected) [c]	11 (2, 26)		35.5 (28, 52)		73 (54,104)		34 (2, 104)	
SOS (m/s) [d]	2942 (2609, 3064)		3269 (3009, 3413)		3327 (3110, 3495)		3203 (2609, 3495)	
SOS SDS[e]	-1.5 (-4, 1)		1.6 (-0.6,2.4)		0.2 (-2.5, 3.5)		-0.6 (-4, 3.5)	
Birthweight (g) [f]	1010 (625, 1810)	-0.1 (ns)	1138 (740, 2250)	0.2 (ns)	980 (845, 1600)	0.4 (ns)	1090 (625, 1430)	
Weight SDS[e]	-1.5 (-2.6,2.1)	0.3 (ns)	-0.9 (-1.9,1.1)	0.5 (ns)	-0.5 (-2.7, 1)	-0.2 (ns)	-1 (-2.6,2.1)	-0.1 (ns)
Length SDS[e]	-0.6 (-3.3,2.1)	0.1 (ns)	0.3 (-1.9,2.6)	0.1 (ns)	-0.4 (-4.2, 1.5)	0.0 (ns)	-0.3 (-4.2,2.6)	-0.2 (ns)
CRIB score[g]	2 (1, 9)	-0.6 (ns)	1 (1, 7)	-0.2 (ns)	2 (0, 10)	-0.1 (ns)	2 (1, 10)	-0.2 (ns)
Phosphate (mmol/l) [h]	1.4 (0.9, 1.8)	0.6 (<0.05)	1.2 (0.8, 1.8)	-0.1 (ns)	1.5 (0.8,2.3)	0.2 (ns)	1.4 (0.8, 2.4)	0.1 (ns)
TPN duration (days) [i]	14 (5, 120)	-0.7 (<0.005)	10 (4, 95)	-0.1 (ns)	10 (5, 42)	0.2 (ns)	13 (5, 120)	-0.2 (ns)
ALP (u/l) [j]	376 (163, 1094)	-0.5 (ns)	678 (175, 1287)	0.2 (ns)	424 (143, 648)	-0.4 (ns)	426 (143, 1287)	0 (ns)

Table 4.4 Characteristics of Study Infants: Quantitative Variables

[a]R, Pearson's correlation coefficient for correlation of variable and SOS SDS. P value significant if <0.05, ns=not significant.[b] Wks, weeks.[c] post term corrected age in weeks.
[d] SOS, speed of sound in m/s, metres per second.[e]SDS, standard deviation score.
[f] g, grams.[g]CRIB score, clinical risk index for babies score (21)[h] Phosphate, lowest serum phosphate recorded in the neonatal period, measured in mmol/l, millimoles per litre.
[i] TPN, total parenteral nutrition.[j] ALP, peak serum total alkaline phosphatase recorded in the neonatal period.

Table 4.5

Qualitative Variables	0-6months CGA (n=15)		6-12months CGA (n=10)		12+months CGA (n=13)		Study Group (n=38)	
	n	p value [a]	n	p value	n	p value	n	p value
IUGR [b]	2	ns	1		6	ns	9	ns
Chronic lung disease [c]	10	ns	5	ns	3	ns	18	ns
Hyperbilirubinaemia [d]	4	ns	1		1		6	ns
Antenatal Steroids	14		10		13		37	
TPN duration > 2 weeks [e]	7	<0.05	3	ns	4	ns	16	ns
ALP>1000u/l [f]	1		3	ns	0		4	ns

Table 4.5 Characteristics of Study Infants: Qualitative Variables

[a] p values for a significant effect of the variable on speed of sound standard deviation score (SOS SDS.) P value significant if <0.05, ns=not significant.[b] IUGR, intrauterine growth retardation, below 2nd percentile for weight on a standard UK growth chart.

[c] Chronic lung disease, oxygen requirement at 36 weeks corrected gestational age.

[d] Hyperbilirubinaemia, presence of a conjugated hyperbilirubinaemia, with conjugated bilirubin>10% of total serum bilirubin.[e] TPN, total parenteral nutrition.

[f] ALP, peak serum total alkaline phosphatase recorded in the neonatal period.

Table 4.6

	Early Group	***n=7***	***Late Group***	***n=8***
	Median	(Range)	Median	(Range)
Gestation (wks)[a]	26	(25, 31)	27.5	(24, 31)
Birthweight (g)[b]	870	(625, 1080)	1013	(740, 1460)
CRIB score[c]	4	(1, 9)	1	(1, 10)
Phosphate (mmol/l)[d]	1.4	(0.9, 1.6)	1.1	(0.8, 1.6)
TPN duration (days)[e]	16	(6, 120)	11	(5, 95)
ALP (u/l)[f]	445	(163, 1096)	552	(175, 1287)
Chronic lung disease (N^{o} infants)[g]	7		5	
Antenatal steroids (N^{o} infants)	6		8	
Female (N^{o} infants)	5		5	

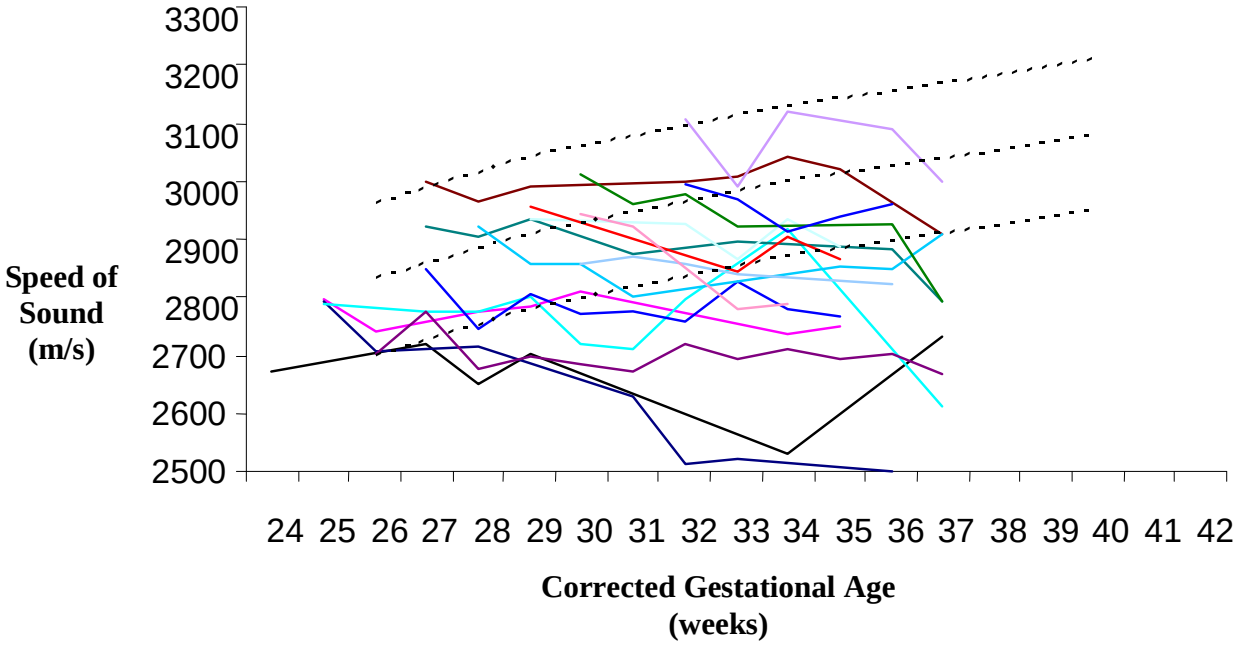
Table 4.6 Characteristics of Study Infants Followed Longitudinally

[a] Wks, weeks.[b] g, grams.[c]CRIB score, clinical risk index for babies score (21)

[d] Phosphate, lowest serum phosphate recorded in the neonatal period, measured in mmol/l, millimoles per litre.[e] TPN, total parenteral nutrition.[f] ALP, peak total serum alkaline phosphatase recorded in the neonatal period, measured in u/l, units per litre.

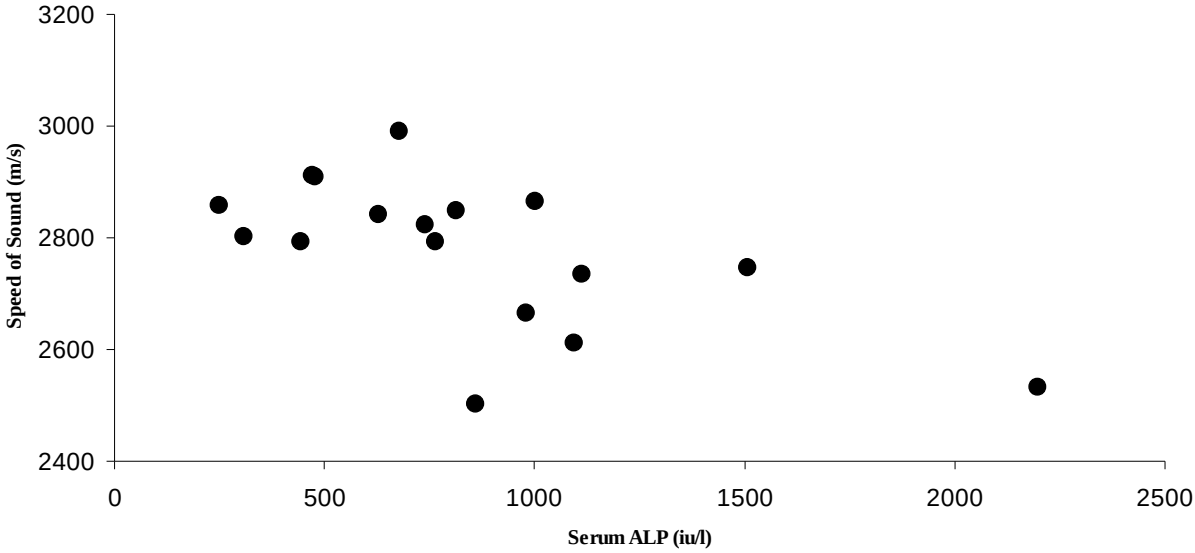
[g] Chronic lung disease, oxygen requirement at 36 weeks corrected gestational age.

Figure 4.1



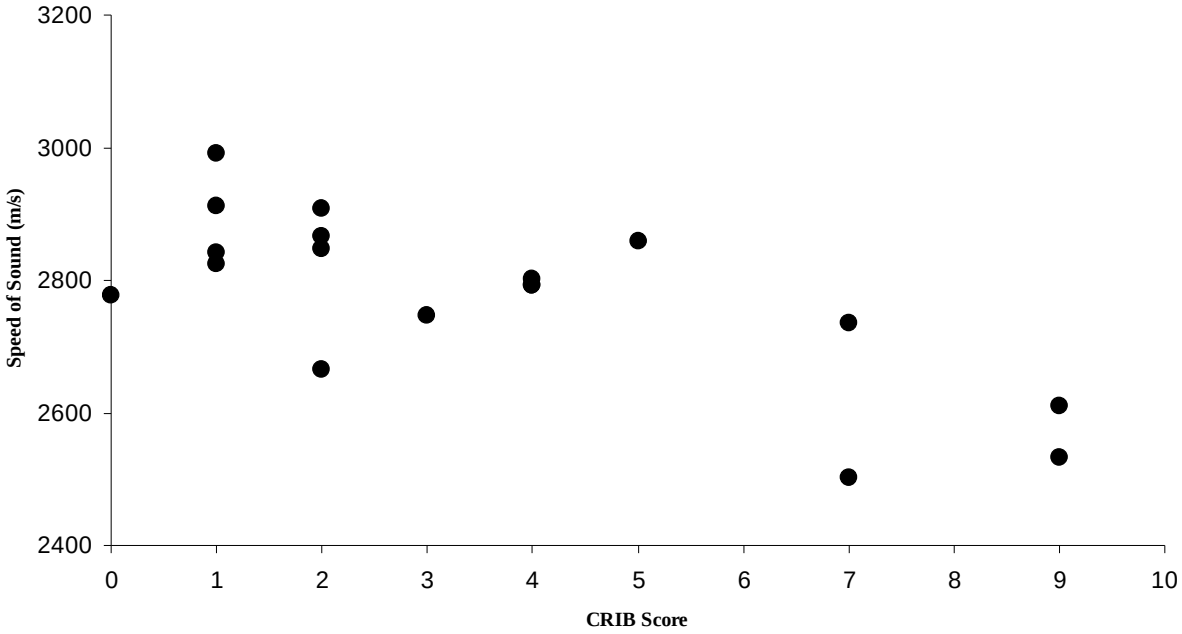
Each line represents one patient's speed of sound (SOS) measurements, measured with the CS probe. Birth gestation is by completed number of weeks at delivery. The dotted lines represent the manufacturers reference range, mean +/- 1 SD.

Figure 4.2



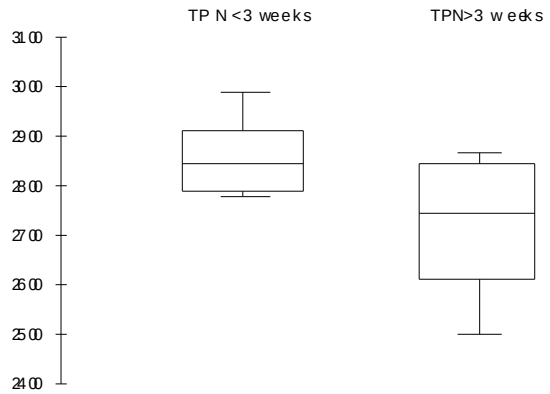
The relationship between serum total alkaline phosphatase (ALP, IU/L) and speed of sound (SOS) at the end of the study period. The closed circles represent tibial SOS in metres per second. $R=0.6$ $p<0.05$

Figure 4.3



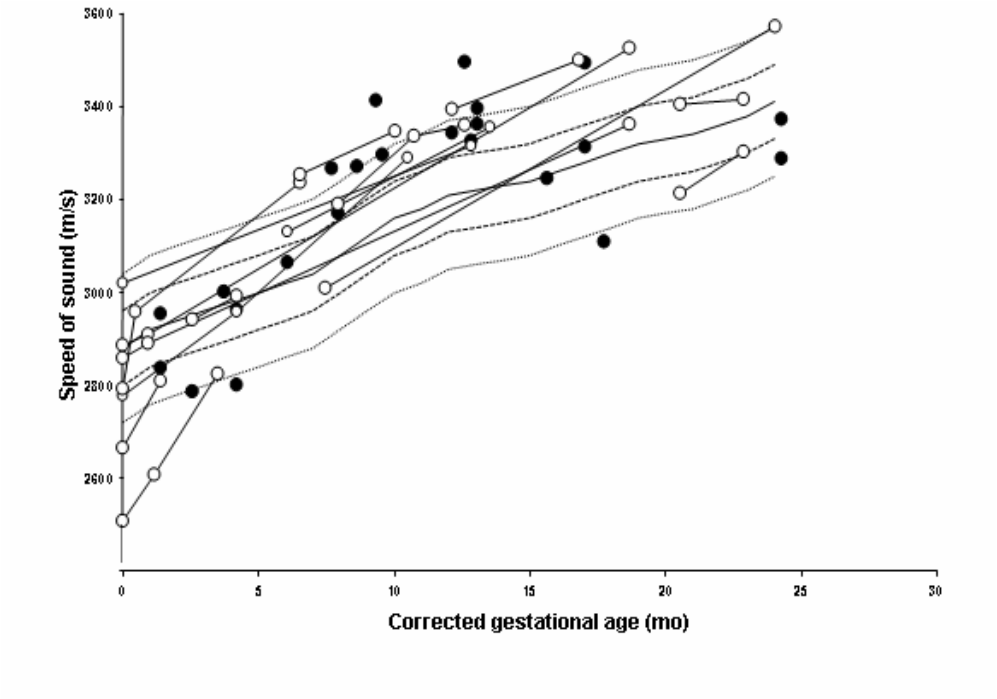
The relationship between CRIB score (116) and tibial speed of sound (SOS) at the end of the study. The closed circles represent tibial SOS in metres per second.

Figure 4.4



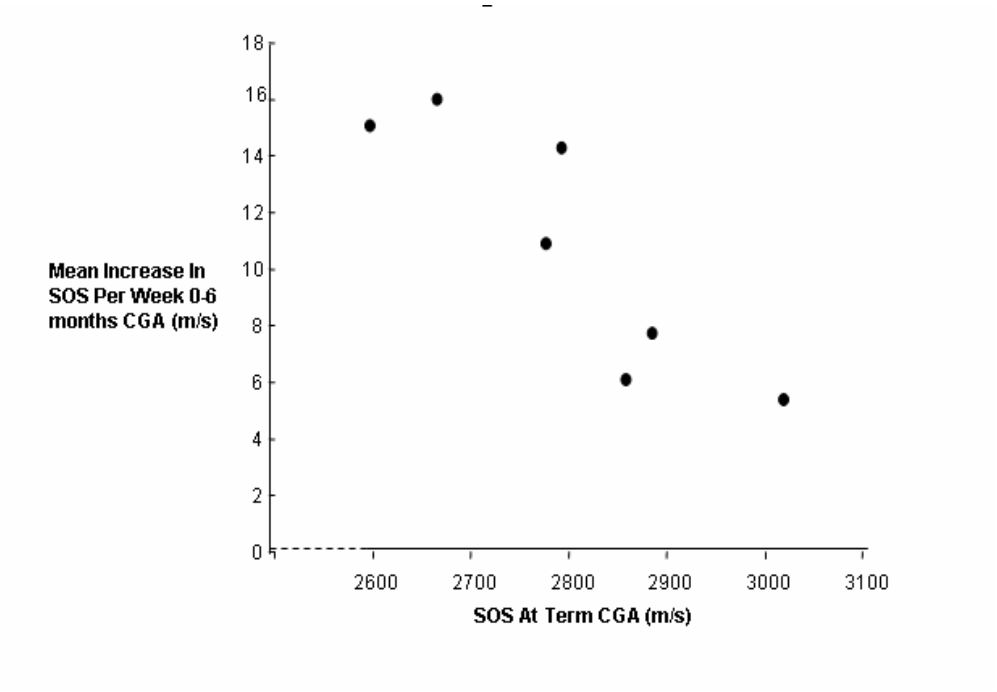
Box plot showing the relationship between duration of total parenteral nutrition (TPN) and tibial speed of sound (SOS) at the end of the study. SOS (metres per second) is on the y axis. The black line represents the median values, the whiskers represent the range.

Figure 4.5



The relationship between corrected gestational age and speed of sound. The closed circles represent each patient's speed of sound in metres per second in patients measured only once. The open circles represent each speed of sound value in patients who had serial measurements, the first and subsequent values are joined by a solid line. These measurements were made using the CM probe. The solid line is the mean for age according to the manufacturers' reference range, the dotted lines are +/- one and two standard deviations from the mean.

Figure 4.6



Early group changes in speed of sound over time showing greatest percentage change in infants with the lowest speed of sound at term. The closed circles represent each patient's increase in speed of sound in metres per second.

Chpt 5 Maternal factors and infant bone health at birth

Introduction

Maternal effects on the skeleton of her offspring can be mediated by both genes and the in utero environment. Maternal factors that have an effect on placental function and hormonal factors influencing growth are likely to be important for fetal bone development. A study of 50 mother-infant pairs showed that mothers deficient in vitamin D had babies deficient in vitamin D, and that these infants had, relative to birthweight, a lower whole body and femur bone mineral content measured by DXA shortly after birth (107). Besides the short term effect on neonatal bone health, there is increasing evidence that the effect of the intrauterine environment may persist into infancy and childhood and perhaps even into adulthood. A relationship between maternal vitamin D levels in late pregnancy and the offspring's childhood BMC at 9 years old has recently been described (99). A genetic influence on peak bone mass has been demonstrated however, current genetic markers can explain only a small proportion of the variation in individual bone mass or fracture risk (128) therefore it is likely that early environment – genome interactions are influential in determining skeletal growth. A possible interaction in the human growth hormone gene, with weight at one year and rate of bone loss has also been recently reported (129).

There is no consistent pattern to changes in bone mineral content in pregnancy, with both bone loss and bone gain reported in studies using DXA or SPA. Quantitative ultrasound has advantages for use in pregnant women and the newborn population as it is radiation free and portable. Recent studies using calcaneal quantitative ultrasound in the pregnant mother point to bone loss that is dependent on maternal lifestyle, fat stores and seasonality of early pregnancy (88). Two studies published in 2004 measured amplitude dependent speed of sound (AD-SOS) at the hand phalanges and found a decrease across pregnancy, with no correlation

between fetal growth or newborn size with changes in maternal AD-SOS.(89)To date, radial SOS, measured by axial transmission, to assess changes in bone health during pregnancy has not been studied. Furthermore, there are increasing reports of maternal vitamin D deficiency during pregnancy and it is unclear whether the extent of vitamin D deficiency changes during pregnancy and whether there is a relationship between changes in maternal vitamin D status or bone health and that of the offspring.

Therefore, the following clinical study was designed to assess bone QUS status of pregnant women, to investigate the factors which influence maternal bone changes in pregnancy and the interaction between maternal bone status and that of her offspring. We also aimed to assess vitamin D status of mothers and infants and explore the link between vitamin D status and bone QUS measurements.

Materials and Methods

Study population

Pregnant women attending for their first visit to the antenatal clinic of the Queen Mother's Hospital between May 2006 and January 2007 were initially approached at the booking hospital appointment and informed consent was obtained in 188 women for a bone health study at booking and delivery, a further 22 women consented postnatally. Some of the women who consented antenatally subsequently miscarried or delivered still born infants. Information was collected on milk intake, recalled birthweight, history of fracture, age, confirmed gestation at booking, postcode, parity, time since last pregnancy, medical history, current medication, multivitamin intake and cigarette and alcohol intake. Mothers who had been smoking within the last 6 months were categorised as smokers. These details of the study participants are outlined in Table 5.1. The presence of gestational diabetes and hypertension, as well as medication during pregnancy was prospectively recorded from the case notes.

Season at early pregnancy was divided into spring and summer versus autumn and winter. A deprivation score was calculated based on postcodes which were allocated a data zone score from the 2006 Scottish Index of Multiple Deprivation (130). The Scottish Index of Multiple Deprivation (SIMD) identifies small area concentrations of multiple deprivation across Scotland using indicators in 7 domains: current income, employment, health, education skills and training, geographic access to services, housing and crime. The data zones scores are attributed population weighted deciles of 1= least deprived to 10= most deprived. There is also a record of which data zones contain the most deprived 15% of the population. For the newborn infants, anthropometric data and details on feeding were recorded. Infants had tibial length and circumference measured. Tibial length was measured from the top of the knee to the bottom of the heel with a calliper and at the midpoint of this measurement tibial circumference was measured in centimetres using a standard paper measuring tape.

SOS Measurement of Mothers

Out of a total of 210 women recruited into the study, 167 had a radial speed of sound (SOS) measurement at booking, and 113 out of these 167 also had a SOS following delivery at a mean age of 2 days (SD 1 day.) In addition 12 women had a single postnatal measurement, having missed the antenatal QUS. All SOS measurements were performed by a single operator using the Sunlight Omnisense 7000P^{TM} scanner (Sunlight Medical, Israel) as described in chapter 3. This is the same method used by Weiss et al, and the result generated was converted to a standard deviation score (age and sex matched) using their published database (131).

SOS Measurement of Infants

Infants of all mothers participating in the study were eligible for a single tibial SOS measurement shortly after birth. One hundred and twenty five term infants (53 male) from 125

mothers had QUS measurements at a mean of 2 days old (SD 1 day.) Forty three infants were born and discharged when the QUS operator was unavailable. Thirty one preterm infants from 22 mothers had QUS measurements in the first week of life. Median gestational age was 30 weeks (10^{th}, 90^{th} percentiles 27, 33) and median birthweight was 1540g (1060, 1966). SOS was measured at the tibia using the Sunlight Omnisense 7000P^{TM} scanner (Sunlight Medical, Israel) as described in chapter 3. Measurements were made by one single operator.

Bone biochemistry

A sample of blood was only collected from those study participants who were having other samples collected for clinical reasons at the booking clinic or around the time of delivery. An aliquot of this sample was analysed immediately for serum calcium, phosphate, albumin and alkaline phosphatase. The remaining serum was then frozen at -80^{o}C and stored for analysis of 25-hydroxy vitamin D (25VitD) and PTH. 25-hydroxy vitamin D levels were measured by tandem mass spectrometry after solid phase extraction as described by Knox et al (132). Both intra and inter- assay precisions are <10% over the assay range for both 25-hydroxyvitamin D3 and D2. Assay sensitivity for D3 was 5nmol/L and for D2 7 nmol/L

Serum vitamin D3 level of <25nmol/l was considered vitamin D deficient, between 25 and 55 nmol/l was insufficient, and >55 nmol/l was considered sufficient. Intact parathyroid hormone was analysed by solid phase two site chemiluminescent enzyme-labelled immunometric assay using the Immulite 2000^{TM} (Siemens Medical Solutions, 5210 Pacific Concourse Drive, Los Angeles, CA 90045-6900). Sensitivity was 0.3 pmol/l and CV at 5.8 was 8.3% and at 33.8 was 9%. Sufficient samples were available to measure serum calcium, phosphate, albumin and alkaline phosphatase in 151 and 49 women antenatally and at delivery, respectively; for 25VitD, the respective figures were 140 and 42 women; for PTH, the respective figures were

122 and 33. Umbilical cord calcium, phosphate, albumin, alkaline phosphatase and vitamin D was obtained in 45 infants, and was sufficient for PTH in 28 infants. Blood was taken from the umbilical vein immediately after delivery of the placenta and was stored and analysed in an identical manner to the serum samples.

The study had approval from the hospital research ethics committee and all participants gave written informed consent.

Statistical Analysis

Data analysis was carried out using Minitab Release 14.1 statistical software (Minitab Inc.) and data were described as medians and 10^{th} and 90^{th} centiles. Correlation (Pearson's) methods were used to explore the determinants of maternal SOS, infant SOS and the relationship between these. Wilcoxon Signed Ranks tests were used to test for differences in SOS SDS at the start and end of pregnancy. Mann Whitney tests, comparing medians, were used to determine differences between groups. Fisher's exact test was used to compare unpaired binary data. Significant factors in univariate analyses were entered into multiple regression analysis. Significance was taken as $p<0.05$. A sample size of 147 provided 95% power to detect a 0.3 SD change.

Results

Maternal Vitamin D Status

Vitamin D2 levels were undetectable (<7.5 nmol/l) in all women, including those who reported the use of multivitamins. At booking, median serum vitamin D3 (n, 140.) was 66nmol/l (24, 120) and median PTH (n, 122) was 1.8nmol/l (0.9, 4.6). Based on these Vitamin D3 levels, 59% of mothers were vitamin D sufficient, 29% were insufficient, 11% were deficient at booking. At delivery, median serum vitamin D3 (n, 42) was 26nmol/l (7, 63) and

median PTH (n, 33) was 3.8nmol/l (1.4, 7.6). Based on these Vitamin D3 levels, 19% of mothers were sufficient, 31% insufficient, 50% deficient. There was a significant difference between Vitamin D3 levels at booking and delivery ($p<0.001$) (figure 5.1). PTH was significantly higher at delivery ($p<0.01$). Median PTH: vitamin D3 ratio was 0.027 (0.009, 0.13) antenatally, and increased significantly by delivery 0.13 (0.03, 0.84) ($p<0.005$.) Serum calcium and alkaline phosphatase levels were within the normal reference range in all study participants at all time points. Serum phosphate levels were low at booking in 2 women and at delivery in 4 women and ranged between 0.75 and 2.82 mmol/l.

Infant Vitamin D Status & Its Link To Maternal Status

Out of 110 infants, cord samples were available in 45 infants for analysis. Median umbilical cord vitamin D3 was 23nmol/l (7, 51). Umbilical cord vitamin D levels were sufficient in 9% of infants, insufficient in 44%, and deficient in 47%. Although, there was no correlation between maternal vitamin D at booking and umbilical cord vitamin D (r,0.1, NS), there was a strong positive correlation between maternal vitamin D at delivery and umbilical cord vitamin D (r,0.7, $p<0.001$) (fig 5.2). PTH was <1.0 on 42 out of 45 cord blood samples. The remaining 3 PTH levels were 1.7, 3 and 3.2, the maternal samples for these 3 infants were insufficient in 2 samples, but the 1 result available was 25.6, the highest PTH of any woman at delivery.

Parity, Deprivation, Smoking Status and BMI and maternal/ infant Vitamin D

Women residing in an area of deprivation did not have significantly lower vitamin D antenatally, postnatally or in umbilical cord, medians 69nmol/l (31, 101), 14nmol/l (8, 21), and 25nmol/l (0, 40) respectively, compared to medians of 61nmol/l (23, 123), 31nmol/l (7, 64), and 23nmol/l (3, 55) when women were not resident in the most deprived areas, ns. Women who smoked cigarettes had slightly lower vitamin D antenatally, postnatally and in

umbilical cord samples, median 58 nmol/l (30, 94), 19 nmol/l (14, 32), 15 nmol/l (5, 26) respectively, compared to non smokers, 68 nmol/l (24, 123), 27 nmol/l (7, 64) and 31 nmol/l (7, 54), however this did not reach statistical significance. Obesity only had a significant effect on antenatal vitamin D, a lower median of 46nmol/l (25, 89) compared to 70nmol/l (25, 127) in non obese women ($p<0.005$.). Higher PTH antenatally was also found in obese women, median 2.7 (1.6, 7.8) compared to 1.6 (0.9, 4.2) in women with a normal BMI ($p<0.005$), but not in smokers, median 1.9 (2.7, 17), compared to non smokers, median 1.8 (1.4, 7.0) (NS). Obesity had no significant effect on PTH: vitamin D3 ratio, median 0.02 in women with BMI<30, and 0.07 in obese women (NS). Also the PTH: vitamin D3 ratio was not significantly different between smokers and non smokers, median 0.113 and 0.14 respectively, (NS).

Race, Head Coverings, Seasonality and maternal/ infant Vitamin D levels

At booking, median vitamin D levels in non-caucasian (n, 18) and caucasian women (n, 122) were 23nmol/l (7, 36) and 70nmol/l (32, 123), respectively ($p=0.0000$) (fig 5.1). South-asian women with head coverings (n, 6) had the lowest vitamin D levels at booking with a median value of 18.5nmol/l (7, 30) and significantly lower than the other women (n, 134), $p=0.0003$ (figure 5.1). Median vitamin D at delivery was lower at 10nmol/l (8, 16) in the non-caucasian women (n, 3) compared to 27nmol/l (7, 63) in caucasian mothers (n, 39) but this did not reach significance ($p=0.09$) (fig 5.1). Median umbilical vitamin D was 25nmol/l (0, 53) in caucasian infants. Only 2 infants of south-asian origin had cord vitamin D measured and they were both undetectable. A significant association with season and vitamin D levels was only evident for maternal vitamin D levels at booking where the median vitamin D level, in mothers booking in spring and summer median was 70nmol/l (30, 127) and in autumn and winter was 57nmol/l (14, 98) ($p=0.02$). This association was not evident for maternal vitamin D at delivery or

umbilical cord vitamin D. At booking, median serum PTH was 3.3 (1.5, 10) and 1.7 (0.9, 3.7) in non-caucasian and caucasian women, respectively (p=0.001), and higher in those women who kept their skin covered (n, 5) at median 3.3 (2.4, 7.7) compared to 1.8 (0.9, 4.5) in non covered women (n, 117), p=0.01. PTH: vitamin D3 ratio was significantly higher at booking in non caucasian women, median 0.3 (0, 0.7) than in caucasian women, median 0.03 (0, 0.1) (p=0.003). The ratio was similar for covered and non covered women (NS).

Multivitamin Supplementation

Out of the 167 women, 19 (11%) were taking vitamin supplements at booking. However, these women as well as the rest of the cohort had undetectable vitamin D2 levels at booking and delivery. These women did, however, have a higher vitamin D3 levels at booking with a median value of 82nmol/l (67, 154) compared to those not on multivitamins with a median of 59 nmol/l (23, 120) (p= 0.01). There was no difference between the vitamin D levels of the two groups at delivery and neither was there a difference between the umbilical cord vitamin D levels of the offspring of the two groups of mothers.

Maternal SOS

One hundred and sixty seven women, median age 32 yrs (22, 38) had a median antenatal SOS measurement of 4147 m/s (3993, 4360) which converted to a median SOS SDS of 0.0 (-1.4, 2.3) In 10 women, the SOS SDS was less than -2.0, and in 17 was greater than 2.0. There was a strong significant correlation with age, r=0.4 p<0.001, therefore all associations between variables were tested using SOS SDS rather than SOS values, to correct for age. Median postnatal SOS in the mothers who had both an antenatal and postnatal measurement (n=111), was 4134m/s (3986, 4317) and median SOS SDS was 0.0 (-1.7, 1.6) in the immediate postnatal period. Median change in SOS SDS was 0 (-2.4, 1.6) across pregnancy (NS)

Women who had a decrease in SOS SDS (n=56) had a significantly higher median SOS SDS antenatally compared to those who had an increase in SOS SDS over pregnancy (n=57), 0.9 and -0.4 respectively, $p<0.0001$.

Infant SOS & Its Link To Maternal SOS

Median gestation of the infants was 40 weeks (38, 41) and the median birthweight was 3450g (2867, 4122), length was 51cm (46, 55) and occipitofrontal circumference (OFC) was 35cm (33, 36.6.) The median tibial SOS SDS at birth was -0.4 (-1.3, 0.6). Two infants had a SOS which was below -2 SDS and no infant had a SOS SDS of greater than 2. There was a significant negative correlation between infant SOS SDS and birthweight, r,-0.3, $p<0.005$, but there was no correlation with length or OFC. Tibial length and circumference was recorded in 96 infants, tibial circumference divided by tibial length had a median value of 0.86 (0.78, 0.94) so tibial circumference was most frequently 86% of tibial length. We used the tibial circumference:length ratio as a proxy for the amount of soft tissue present in the lower leg; the higher the ratio, the greater amount of soft tissue relative to leg length. There was a significant negative correlation between SOS SDS and tibial circumference:length, $r= -0.2$, $p<0.05$. Neither maternal SOS SDS (antenatal and postnatal) nor change in maternal SOS SDS during pregnancy correlated significantly with the SOS SDS of her offspring. Although the median birthweight, head circumference and SOS SDS of the babies born to mothers who had an increase in SOS SDS was similar to that of babies born to mothers who showed a decrease, the median length was greater at 52cm (47.5, 56) compared to 50cm (46, 53) ($p<0.005$).

Parity, Deprivation, Smoking Status and BMI and maternal/ infant SOS

Out of the 167 mothers, 27 (16%) lived within the 15% most deprived areas in Scotland as categorised by the Scottish Index of Multiple Deprivation (130). Median SOS SDS of women

in this band was 0.3 (-1.2, 2.4) compared to 0.0 (-1.9, 2.3) for the rest of the cohort (NS). In addition, change in SOS SDS during pregnancy, infant SOS SDS as well as all measures of infant anthropometry were similar in the two groups._Out of the 167 mothers, 77 (46%) women were primigravid. At booking, median SOS SDS was -0.15(-2.0, 2.4 0 in these women compared to 0.3(-1.3, 2.3) in parous women (ns). In addition, change in SOS SDS during pregnancy, infant SOS SDS as well as all measures of infant anthropometry were similar in the two groups. The median SOS SDS in the 29 women who were smokers was -0.6 (-2.6, 2.2) compared to 0.3 (-1.2, 2.4) in the 92 non-smokers, $p<0.005$. Postnatal SOS SDS was also lower in smokers at -0.6 (-1.9, 1.1) compared to non smokers at 0.1 (-1.5, 1.7) (NS). The median SOS SDS in infants of smokers was -0.75 (-1.8, 0.5), compared to -0.3 (-1.3, 0.6) in infants of non smokers ($p<0.05$) (Figure 5.3) There were no significant differences in birthweight, length or OFC between infants of smoking or non smoking women. Median maternal BMI at booking was 25 (20, 32) and there was a significant negative correlation with SOS SDS (r,-0.2, $p<0.01$) (Figure 5.4), There was no significant relationship between BMI at booking and change in SOS SDS during pregnancy, or any measure of infant anthropometry.

Race, Head Coverings, Seasonality and maternal/ infant SOS

In caucasian and non-caucasian women, median SOS SDS at booking was similar at 0 (-1.4, 2.3) and 0.3 (-1.4, 1.7) respectively. Median change in SOS SDS from booking to post-natal measurement pregnancy was 0 (-2.5, 1.8) in caucasian and -0.2 (-2.3, 1.2) in non caucasian women (NS). The median SOS SDS of a subgroup of 5 women of South-Asian origin who wore head coverings for religious reasons was 1.5(0.3, 4.4) and not significantly different from the rest of the cohort. However, median change in SOS SDS in this group was -1.8 (-3.6, -0.8) and this was significantly different from the median change in SOS SDS in the rest of the cohort of 0.05 (-2.4, 1.8) ($p=0.000$) (Figure 5.5). Median SOS SDS at booking in spring and

summer and in autumn and winter was 0.3 (-1.4, 2.6) and -0.1 (-1.4, 1.2) respectively (ns). However, the mothers who booked in spring and summer showed a median change in SOS SDS of -0.3 (-2.5, 1.3) whereas the remainder showed an increase of 0.35 (-1.7, 2.1) (p=0.02). Season at booking had no significant association with infant SOS SDS or anthropometry. When smoking status, BMI and maternal covering was entered into a multiple linear regression model for effect on antenatal SOS SDS all 3 factors remained significant and explained 15% of the value. When the same factors were entered for effect on change in SOS SDS only maternal skin covering remained a significant factor (p=0.03).

Vitamin D and SOS

Women who were deficient in vitamin D at booking had a median SOS SDS of -0.1 (-1.1, 1.5) and 0.5 (-1.7, 1.1) at booking and delivery, respectively and this was similar to the mothers who were not vitamin D deficient who had a median SOS SDS of 0.05 (-1.4, 2.3) and -0.15 (-1.7, 1.6) at booking and delivery, respectively (NS). The median SOS SDS of those mothers who were vitamin D deficient at delivery was 0.5 (-1.2, 1.7) and similar to the rest of the cohort at -0.05 (-2.7, 0.9) (NS). Median SOS SDS in the infant was 0.0 (-0.9, 0.4) when their mothers were vitamin D deficient at booking and -0.4 (-1.3, 0.6) when mothers were not vitamin D deficient at booking (NS). Median SOS SDS was -0.6 (-1.6, 0.2) in those infants whose mothers were deficient at delivery, and -0.8 (-1.3, 0.6) in those whose mothers were not deficient (NS). There was no relationship between maternal vitamin D or umbilical cord vitamin D and infant SOS or infant size. In addition, change in SOS SDS during pregnancy, infant SOS SDS as well as all measures of infant anthropometry were similar in the two groups. There was no correlation between antenatal PTH and maternal or infant SOS SDS. However, there was a negative correlation between maternal PTH at booking and infant birth weight r,-0.2, p=0.04, and birth length r,-0.34, p=0.002 (figure 5.6 and 5.7). At delivery,

maternal PTH had no significant correlation with SOS SDS or infant anthropometry. The PTH: vitamin D3 ratio did not correlate with antenatal SOS SDS, postnatal SOS SDS or change in SOS SDS.

Discussion

SOS is a promising measure of bone health in pregnant women. In our cohort, median SOS SDS in early pregnancy was 0, which suggests that our study participants, women of child bearing age, had normal bone health at the start of the study. Our data would support current evidence that bone is not usually lost during pregnancy and that the maternal skeleton is protected while adaptive responses allow for adequate mineralisation of the fetal skeleton (133) This would seem to be the case in nutritionally adequate and vitamin D sufficient women.

Vitamin D deficiency in early pregnancy was almost exclusively found in women of south asian origin, the lowest values in those who were covered. PTH was also significantly higher in these women. During the pregnancy, those south asian mothers who kept their skin covered had a reduction in SOS that was 17 times greater than the decrease in the caucasian and asian mothers with more exposed skin, but interestingly, their infants had normal SOS at birth. By the end of pregnancy, vitamin D deficiency was present in both caucasian and non-caucasian mothers. It was interesting to note that none of the mothers, even those who were taking supplements had detectable Vitamin D2 suggesting that current methods of supplementation were inadequate. Caucasian women taking multivitamin supplementation had a slightly higher serum vitamin D3 level in early pregnancy and it is possible that this may reflect lifestyle. There was a trend towards a correlation between maternal vitamin D at the end of pregnancy

and SOS SDS, so it may be that there is a lag between becoming vitamin D deficient and the bone changes associated with it to be seen on QUS.

In our cohort the mothers who had a decrease in SOS SDS during pregnancy started off with a higher SOS SDS antenatally. This was a similar finding to Sowers et al (134) who attributed it to a greater bone mass giving more cancellous bone surfaces available for turnover. This may indeed be the case as Shahtaheri et al (135) demonstrated fluctuations in cancellous bone transilial biopsy specimens during pregnancy. In early pregnancy the quantity of cancellous bone was lower than in controls, primarily due to a decline in trabecular thickness, which was entirely restored by term by addition of new trabeculae.

Pregnancy is a high oestrogen state that should, through inhibition of osteoclasts recruitment and activity, maintain bone mass. The observation of a direct association between the change in maternal SOS SDS and the length of the offspring is intriguing. Women with an increase in SOS SDS during pregnancy had larger babies whilst women with a reduction in SOS SDS had smaller babies. It, therefore, seems that the positive effect on the maternal skeleton is mirrored in the birth size.

Lifestyle factors which negatively affected bone SOS SDS were cigarette smoking and obesity. The association between smoking and bone health was particularly clear at booking, the lower impact on postnatal bone health and vitamin D status may have been due to a reduction in the amount of cigarettes smoked while pregnant as well as other contributory factors playing a more important contributory role on bone health during pregnancy. The increased likelihood of vitamin D deficiency in obese women has been described before (136) and our studies confirm the existence of this association in pregnant women too. Whilst both

smoking and obesity can be associated with social deprivation, deprivation scores by themselves did not show any association with SOS, infant size or vitamin D status.

In our unselected group of preterm infants median SOS SDS was -0.4, rather than the expected 0. It may be that there was a systematic error in the measurement of SOS in these infants. However this would be unlikely as the operator was the same as in our previous cross-sectional study of term infants, also measured shortly after birth, and absolute values of SOS were then found to be comparable to the manufacturer's reference range.

In conclusion, vitamin D deficiency, smoking and obesity may adversely affect the bone health of women. Vitamin D deficiency is common in pregnancy and becomes more pronounced during pregnancy, and south asian women are at particularly high risk. Whilst the bone health of the fetus, as assessed by QUS seems to be protected, the vitamin D deficient neonate is likely to be at risk of other sequelae of early vitamin D deficiency. Women living in Scotland should be supplemented with adequate amounts of vitamin D. There is a need to study longer-term functional outcomes of vitamin D deficiency in pregnant women and their offspring.

Table 5.1

		All Study Participants n=210		***Antenatal Group n=167***		***Postnatal Group n=34***		***No QUS n=9***	
		median	10th,90th	median	10th,90th	median	10th,90th	median	10th,90th
Age		31	21, 38	32	22, 38	30	20, 38	31	26, 38
BMI[1]		24	20, 32	25	20, 32	23	20, 32	24	21, 27
SIMD decile[2]		5	1,10	5	1, 10	6	1, 10	2	1, 9
15% most Deprived[3]	yes	40		27		11		2	
	no	170		140		23		7	
Race	caucasian	188		146		34		8	
	asian	16		15		0		1	
	other	6		6		0		0	
Smoking	yes	45		29		13		3	
	no	162		138		18		6	
Alcohol	none	64		53		0		4	
	pre-pregnancy	9		106		26		5	
	during pregnancy	137		8		1		0	
Parity	primigravid	102		77		22		3	
	parous	108		90		12		6	
Season early pregnancy	spring	36		29		7		0	
	summer	71		63		6		2	
	autumn	64		45		12		7	
	winter	39		30		9		0	
Vit D suppl	yes	20		19		1		0	
	no	190		148		33		9	
Milk intake	<0.5 pint/day			80		7		0	
	>0.5 pint/day			60		8		0	
	unsure			27		19		9	
Childhood fracture	yes			34		1		0	
	no			130		27		0	
	unsure			3		6		9	

Table 5.1 Characteristics of Study Participants
1- BMI, body mass index, 2- SIMD decile, Scottish Index Multiple Deprivation decile score (124), 3- 15% most deprived, in the 15% most deprived area of Scottish population according to the SIMD (124).

Table 5.2

Lifestyle Factors/Demographics	*N*	*Median SOS SDS*	*10^{th} ,90^{th} centiles*	*P value*
Cigarette Smoking				
Y	29	-0.50	-2.26, 0.72	**<0.005**
N	138	0.20	-1.24, 2.31	
BMI >30				
Y	30	-0.10	-2.1, 1.94	0.13
N	133	0.10	-1.29, 2.4	
Multivitamins				
Y	19	-0.20	-1.0, 3.60	0.99
N	148	0.10	-1.40, 1.96	
Resident In Deprived Area				
Y	27	0.30	-1.14, 1.94	0.42
N	139	0.00	-1.41, 2.05	
Parous				
Y	90	-0.05	-1.21, 1.99	0.65
N	77	0.10	-1.78, 2.16	
Season of early pregnancy				
Autumn/winter	76	-0.1	-1.41, 1.24	0.12
Spring/summer	91	0.3	-1.35, 2.58	
Childhood fracture				
Y	34	-0.40	-1.35, 1.87	0.25
N	130	0.15	-1.40, 2.29	
Low milk intake				
Y	80	0.30	-1.21, 2.08	0.15
N	60	-0.10	-1.78, 2.11	
Race				
Non caucasian	21	0.30	-1.36, 1.69	0.97
Caucasian	146	0.00	-1.41, 2.32	
Covered skin				
Y	8	0.95	-1.49, 3.11	0.38
N	159	0.00	-1.41, 2.08	

Table 5.2 Demographics and Lifestyle factors And SOS SDS in Early Pregnancy
All values are medians

Table 5.3

Lifestyle Factors/Demographics	*N*	*Booking SOS SDS*	*centiles*	*p value*	*Delivery SOS SDS*	*centiles*	*p value*	*SOS SDS Change*	*centiles*	*p value*
Cigarette Smoking										
Y	21	-0.6	-2.6,2.2	**0.001**	-0.6	-1.9,1.1	0.06	0.3	-1.1,1.8	0.16
N	92	0.3	-1.2,2.4		0.1	-1.5,1.7		-0.05	-2.4,1.7	
BMI >30										
Y	22	0.1	-2.0,2.0	0.3	-0.35	-1.8,1.6	0.31	0.25	-2.5,1.5	0.79
N	87		-0.5,2.5		0.10	-1.4,1.6		0.00	-2.4,1.9	
Multivitamins										
Y	13	-0.2	-0.9,2.1	0.45	-0.2	-1.1,1.7	0.92	0	-2.2,2.5	0.62
N	100	0.2	-1.8,2.3		0.05	-1.7,1.6		0	-2.5,1.6	
Resident In Deprived Area										
Y	22	0.35	-1.2,2.4	0.69	-0.35	-1.1,1.9	1.00	-0.05	-3.3,1.6	0.81
N	91	0.0	-1.9,2.3		0.1	-1.6,1.5		0.0	-2.3,1.8	
Parous										
Y	57	0.3	-1.3,2.3	0.08	0.50	-1.6,1.9	0.10	0.1	-2.9,1.8	0.83
N	56	-0.15	-2.0,2.4		-0.2	-1.5,0.9		-0.1	-2.3,1.7	
Season at Booking										
Autumn/winter	48	-1.5	-1.7,2.0	0.06	0.1	-1.2,1.5	0.36	0.35	-1.7,2.1	**0.02**
Spring/summer	65	0.4	-1.6,2.6		-0.2	-1.7,1.6		-0.3	-2.5,1.3	
Childhood fracture										
Y	27	0	-1.3,2.3	0.88	0	-2.1,1.8	0.88	0.00	-2.9,1.5	0.89
N	83	0.1	-1.7,2.4		0.1	-1.5,1.5		-0.1	-2.4,1.8	
Low milk intake										
Y	60	0.3	-1.1,2.5	0.18	0.2	-1.4,1.5	0.88	-0.1	-2.4,1.5	0.31
N	34	-0.05	-2.1,2.2		0.1	-1.7,1.6		0.4	-2.4,1.9	
Race										
Non caucasian	13	0.4	-1.0,1.9	0.61	0.8	-1.5,1.6	0.44	-0.2	-2.3,1.2	0.57
Caucasian	100	0.05	-1.8,2.4		0.00	-1.5,1.6		0.00	-2.5,1.8	
Covered skin										
Y	5	1.5	0..3,4..3	0.05	0.8	-1.8,1.2	0.75	-1.8	-3.6,-0.8	**0.000**
N	108	0	-1.8,2.3		0	-1.6,1.6		0.05	-2.4,1.8	

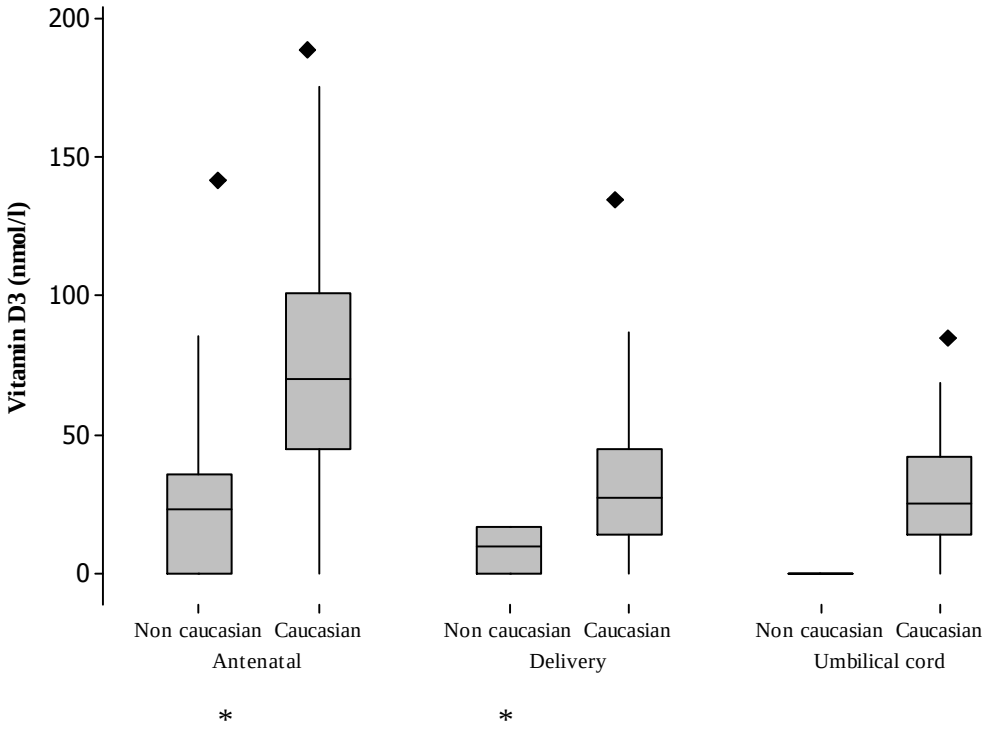
Table 5.3 Demographics and Lifestyle factors And Change In SOS SDS Across Pregnancy
All values are medians

Table 5.4

Lifestyle Factors/Demographics	*N*	*SOS SDS*	*centiles*	*p value*	*N*	*Birth weight*	*centiles*	*p value*	*N*	*Length*	*centiles*	*p value*	*N*	*OFC*	*centiles*	*p value*
Cigarette Smoking																
Y	20	-0.75	-1.8,0.5	**0.04**	27	3500	2955,4381	0.75	18	50.5	47,55.7	0.71	27	35	32.4,36	0.78
N	90	-0.3	-1.3,0.6		126	3435	2910,4160		97	51	47.54.7		117	35	33,36.6	
BMI >30																
Y	21	-0.3	-1.6,0.4	0.93	28	3610	2982,4408	0.65	18	51	47.7,56.6	1.0	25	35	33.5,37	0.50
N	86	-0.4	-1.3,0.5		122	3435	2888,4145		94	51	46.9,55		116	35	33,36	
Multivitamins																
Y	13	-0.5	-1.3,0.7	0.70	17	3490	2872,4348	0.33	13	52	48.9,56	0.50	14	35.5	35,37	**0.04**
N	97	-0.4	-1.1,0.1		136	3448	2917,4049		102	51	46.7,54.3		130	34.7	33,36	
Resident In Deprived Area																
Y	21	-0.4	-1.1,0.1	0.90	26	3268	2920,3810	0.07	15	53	46.3,54.4	0.56	24	35	32.7,35.7	0.93
N	89	-0.4	-1.4.0.7		127	3495	2910,4194		100	51	47,55		120	35	33,36.5	
Parous																
Y	54	-0.3	-1.3,0.5	0.33	84	3515	2930,4135	0.14	64	51	47,56.4	0.84	78	34.9	33.4,36.6	0.47
N	56	-0.55	-1.4,0.8		69	3370	2884,4176		51	51	47.2,53		66	35	32.8,36	
Season of early pregnancy																
Autumn/winter	47	-0.3	-1.1,0.9	0.37	66	3435	2610,4305	0.31	46	51	47.2,55.6	0.67	62	34.8	32.5,36	0.59
Spring/summer	63	-0.4	-1.5,0.5		87	3470	3011,4058		69	51	47,054.8		82	35	33,37	
Childhood fracture																
Y	26	-0.5	-1.2,0.4	0.39	32	3500	2957,4082	0.76	22	50.8	47,52.5	0.22	29	35	32.8,36	0.60
N	82	-0.4	-1.4,0.8		119	3450	2911,4179		91	51	47,56		113	35	33,36.5	
Low milk intake																
Y	60	-0.4	-1.3,0.9	0.99	75	3440	2814,4133	0.99	56	51	47.5,54	0.57	69	35	32.5,36.4	0.63
N	32	-0.25	-1.3,0.4		55	3445	2920,4180		40	51	46.1,56.6		53	34.5	33,36.6	
Race																
Non caucasian	13	-0.1	-0.7,0.4	0.15	19	3125	2734,3593	**0.007**	16	50.5	46,53	0.2	19	34	33,35.5	**0.03**
Caucasian	97	-0.4	-1.4,0.7		134	3495	2961,4183		99	51	47,55.4		125	35	33,36.5	
Covered skin																
Y	5	0.0	-1.3,0.3	0.67	8	3128	3002,3327	0.09	8	49.5	45.8,51.9	0.26	8	34	33,34.6	0.09
N	105	-0.4	-1.3,0.7		145	3480	2908,4175		107	51	47,55		136	35	33,36.5	

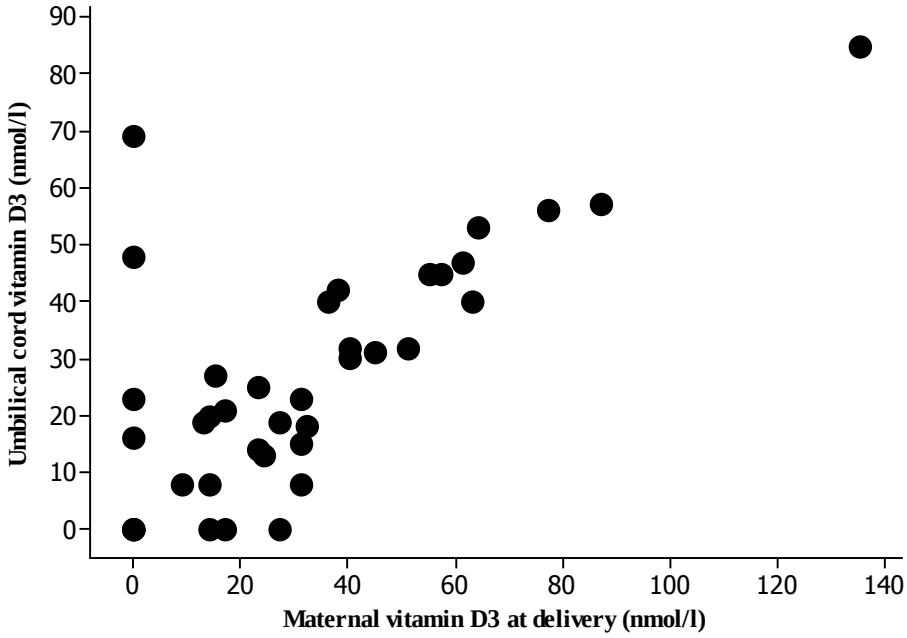
Table 5.4 Demographics and Maternal Lifestyle Factors And Offspring SOS SDS /Growth
All values are medians

Figure 5.1



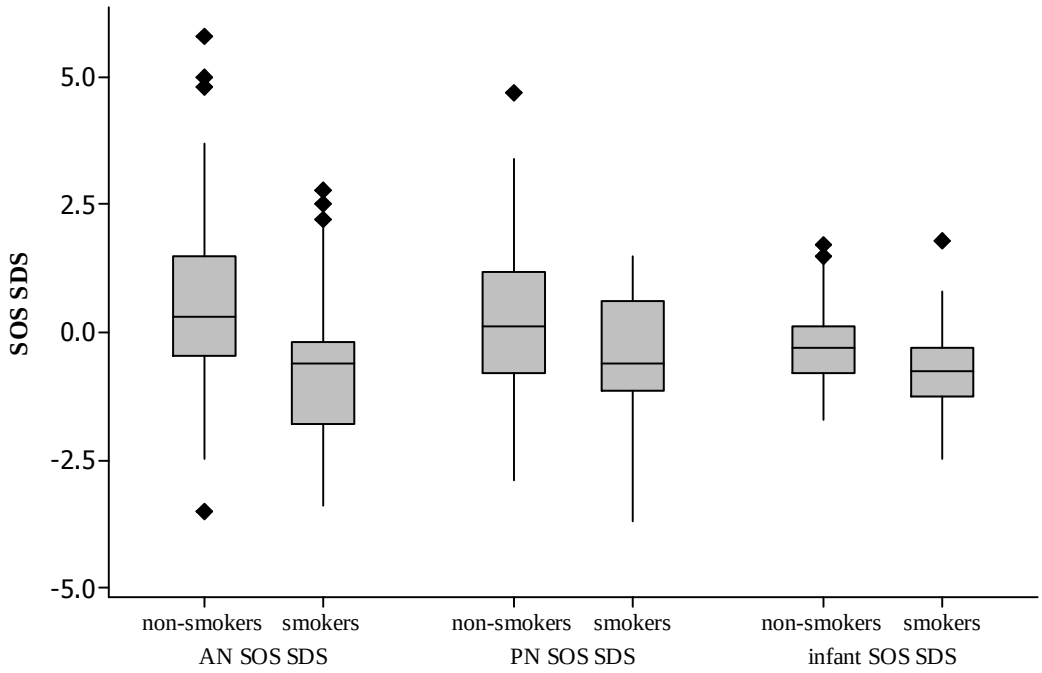
Boxplot showing vitamin D3 levels across pregnancy in caucasian and non caucasian women, and in their offspring. The boxes represent the interquartile range, with the median marked as a straight line, and outliers are marked as diamonds. The asterisks show a statistical difference at $p<0.05$

Figure 5.2



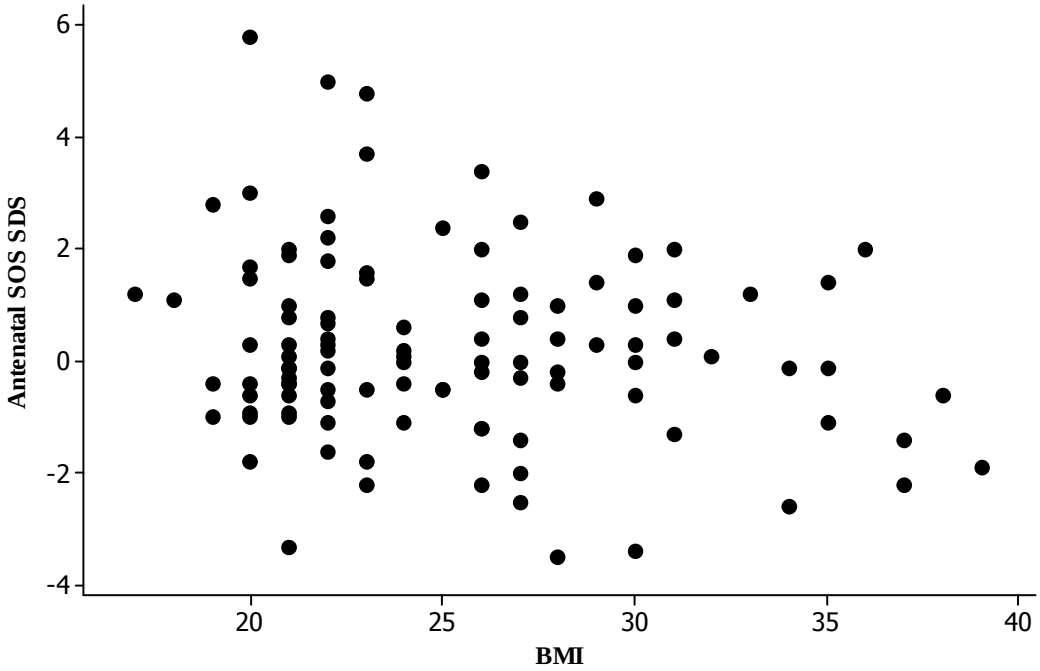
The closed circles represent vitamin D3 levels in mother-infant pairs. R=0.7, p<0.001

Figure 5.3



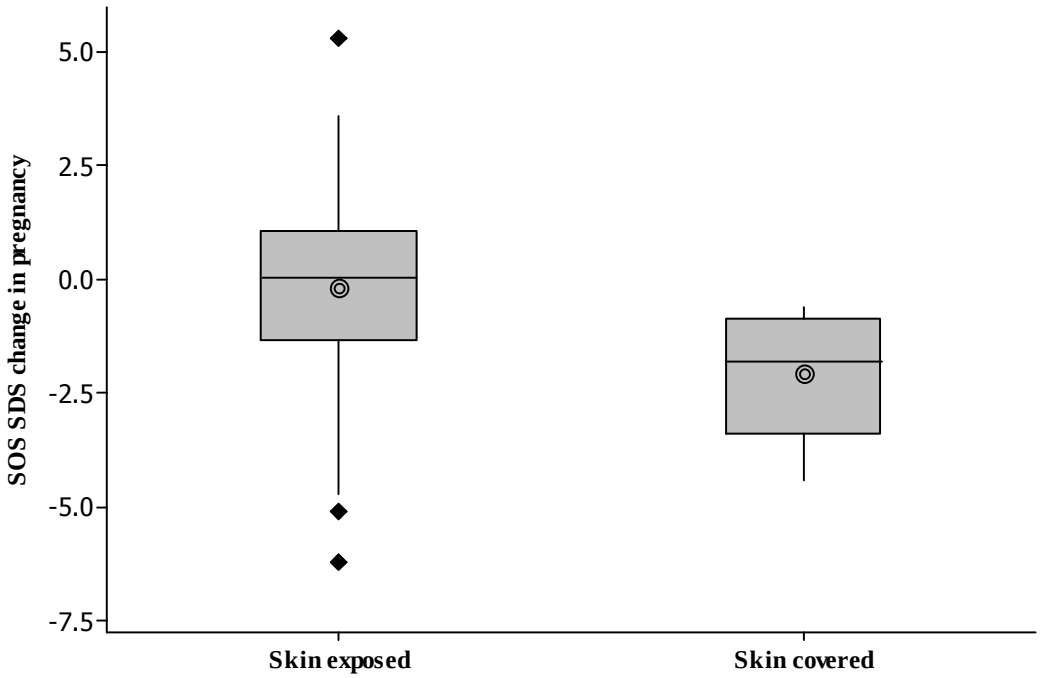
This box plot shows antenatal speed of sound standard deviation score (AN SOS SDS), postnatal speed of sound standard deviation score (PN SOS SDS) and infant speed of sound SDS (infant SOS SDS) in smoking and non-smoking mothers. The boxes represent the interquartile range with the median marked as a straight line, and the outliers marked as diamonds.

Figure 5.4



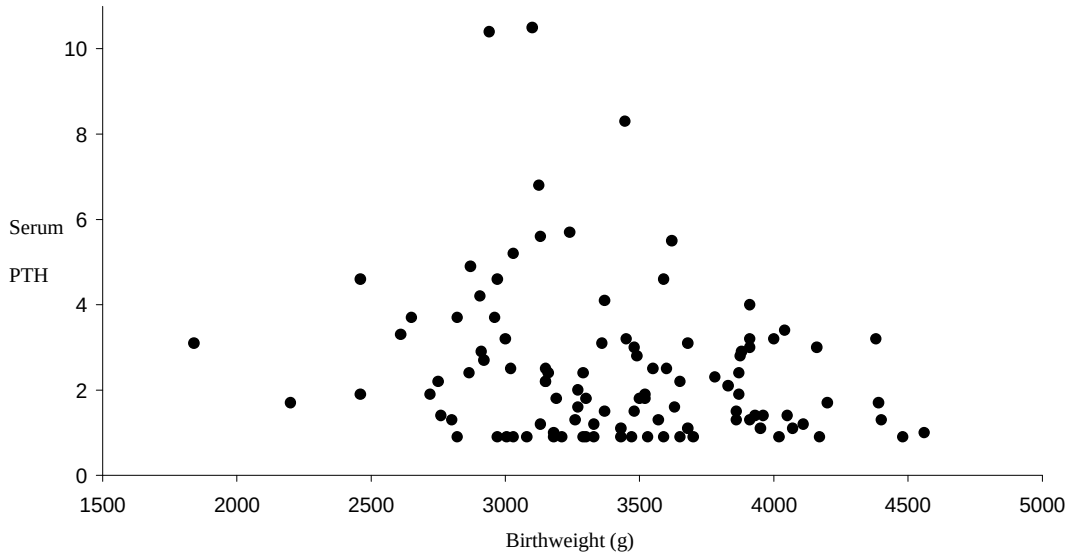
The closed circles represent each patient's antenatal SOS SDS, speed of sound standard deviation score, plotted against their BMI, body mass index.

Figure 5.5



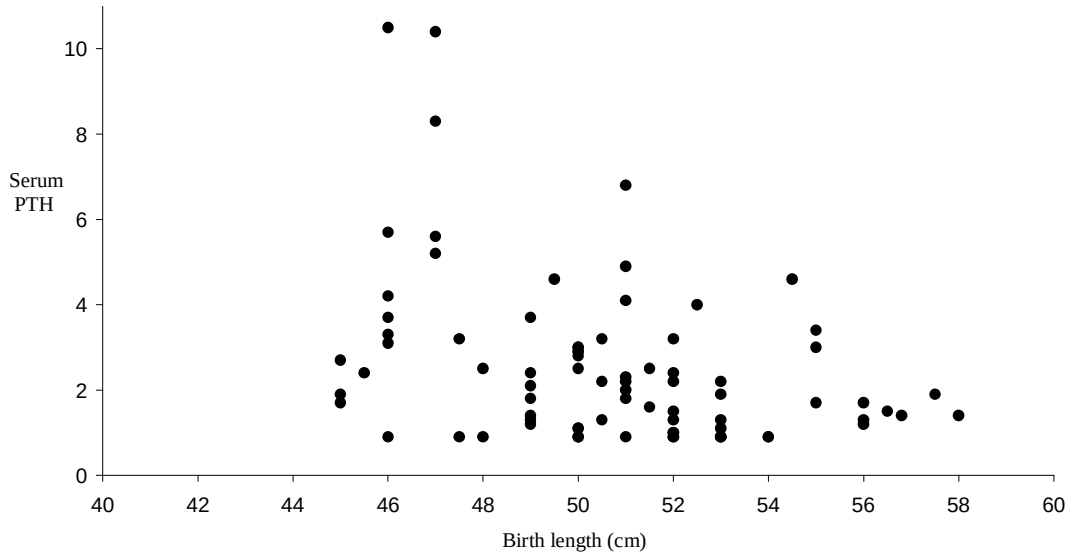
This box plot shows the change in speed of sound standard deviation score (SOS SDS) across pregnancy, in women who kept their skin covered and women who did not. The median is represented by a horizontal line, the boxes extend to the interquartile range and the diamond shapes are the outliers.

Figure 5.6



The closed circles represent the birthweight of offspring plotted by maternal antenatal serum parathyroid hormone (PTH).

Figure 5.7



The closed circles represent birth length of offspring plotted against maternal antenatal serum parathyroid hormone (PTH)

Chapter 6 Passive exercise as an intervention for improving bone health in preterm infants

Introduction

Skeletal growth is driven by functional requirements. Both spontaneous movement and exercise has been related to changes in bone speed of sound (SOS) (55;58). The mechanostat theory of a functional model of bone development suggests that bone cell action is coordinated by the mechanical requirements of the bone; and thus can be modified by influencing muscle force or longitudinal bone growth (51). Minimal handling of preterm infants is becoming routine in neonatal units, and may contribute to the suboptimal bone mineralisation of these infants by lack of muscle stimulation. Moyer-Mileur (56) enrolled preterm infants aged 2 to 4 weeks old to a physical activity programme consisting of range of motion exercises with gentle compression, extension and flexion of both upper and lower limbs. Each movement was done 5 times at each joint five times each week, and the infants receiving this had at the end of the study period (when they had reached a weight of 2kg) a higher mean radial BMC measured by SPA compared to controls. The same physical activity programme was repeated in 2000 (57)and the infants then assessed by DXA, infants in the exercise group had larger forearm bone area, but there were no differences in forearm BMC or BMD when compared to the control group. This study group had DXA and anthropometry followed up at 12 months corrected age and there were no differences detected between the intervention and control groups. Litmanovitz et al (58) used the same exercise protocol designed by Moyer-Mileur but started the physical activity when the infants were immediately cardiovascularly stable and continued for only 4 weeks. Over this 4 weeks there was an attenuation in the decrease in bone speed of sound measured by QUS in the infants receiving physical activity. So, the optimum timing, frequency and duration of exercise remain unanswered questions. There are currently

no studies which have reported on both the short and longer term effects of passive exercise (e.g. on neurodevelopment) in preterm infants. The hypothesis that the intervention of passive exercise in preterm neonates will safely improve their bone health will be evaluated in this clinical study.

Materials and Methods

Study Population

Following approval from the Local Research Ethics Committee, 31 preterm infants were recruited (13 female) were recruited during the period May 2006 to February 2007 from the neonatal unit in the Queen Mother's Hospital, Glasgow. One infant was transferred back to his district general hospital at 3 weeks old and was therefore excluded from further analysis. The study group included 7 sets of twins (4 same sex.) Median gestational age was 30 weeks (10^{th}, 90^{th} percentiles 27, 33) and median birthweight was 1540g (1060, 1966). There were no significant differences in gestation or size at birth between infants in the early exercise group and those in the late exercise group (Table 6.1). Routine clinical and anthropometric data, including details of respiratory complications and serum biochemistry were collected. Median duration of total parenteral nutrition (TPN) for the whole study group was 6 days (1.9, 16). As 3 of the infants were discharged at 3 weeks old, data on nutritional intake over the first 3 weeks of life was analysed, with average daily calcium, phosphate, and calorie intake calculated over this period. Once discharged from hospital the infants were followed up in the out patient clinic, and data on growth, feeding, fracture, presence of skeletal deformities and neurodevelopmental progress was recorded.

A group of previously studied (ref- chapter 4 in thesis) were used as historical controls. Each study infant was paired with 2 different gestation matched historical controls, (therefore some

of the historical controls were used more than once) to compare SOS SDS and serum alkaline phosphatase only. There had been no significant changes in newborn care in the neonatal unit in the time between studies, eg the same TPN preparations were used across both study periods.

SOS Measurement of Infants

SOS was measured at the tibia using the Sunlight Omnisense 7000P^{TM} scanner (Sunlight Medical, Israel) as described in chapter 3. Weekly measurements were made by one single operator (HM) in the neonatal unit unless the infant was too unstable to tolerate the procedure, or if access to the tibia was difficult, e.g. because of intravenous cannulation. Measurements were also performed at each out-patient clinic visit following hospital discharge.

Passive exercise protocol

Range of motion passive exercises of upper and lower limbs were performed as previously described by Moyer-Mileur (57). Flexion and extension movements with gentle compression at the ankle, knee, hip, wrist, elbow and shoulder were done five times at each joint five times each week. Infants were randomised by computer to either 'early' or 'late' exercise protocol. A random number was generated by computer for each sequential patient, odd random numbers were allocated to 'early' exercise, and even random numbers were allocated to 'late.' Early exercise was started as soon as the infant was cardiovascularly stable. Late exercise commenced once the infant was on 100kcal/kg/day of milk feeds. No stratification was made for twins due to the small numbers in the study. Both exercise protocols involved 5 minutes of exercise 5 times a week until hospital discharge. Exercises were performed by HM in all infants. There were 14 infants in the early group (1 excluded infant was randomised to early group) and 16 in the late group. The infants in the early group started the range of motion

protocol at a median of day 5 (3, 7), and the late group day 11 (6, 17), and in both groups continued until discharge. Table 6.1 shows the characteristics of infants in each group.

Statistical Analysis

The data were summarised as medians and 10^{th} and 90^{th} percentiles. Wilcoxon Signed Ranks test was used to test for differences in SOS SDS at the start and end of the study period. Mann Whitney U tests were used to determine differences between groups. Fisher's exact test was used to compare unpaired binary data. Pearson's correlation method was used to analyse the associations between SOS SDS and other continuous variables. Data analysis was performed with Minitab Release 14.1 statistical software (Minitab Inc.).

Results

Initial SOS SDS

In the 'early' group median SOS at the enrolment to the study was 2986 m/s (10^{th}, 90^{th} centiles 2864, 3098) and SOS SDS was 0.4 (-0.8, 1.3.) This was similar to the 'late' group, with median SOS 2982m/s (2874, 3086) and SOS SDS 0.1 (-0.44, 1.3) ns.

SOS SDS at end of the study

Median age at discharge was 42 days (24, 69) in the early group and 46 days (23, 110) in the late group. There was a significant fall in SOS SDS from study enrolment to hospital discharge in both groups; median change was -1.1 (-2.94, -0.44) in the 'early' group and -0.8 (-3.8, -0.25) in the 'late' group (both p=0.001)(Figure 6.1). Median SOS SDS at discharge was not significantly different between groups -0.6 (-2.2, -0.1) and –1.05 (-1.95, -0.3) in the early and late groups respectively (ns). In both groups there were significant correlations between

SOS SDS at discharge and both gestation at birth and also birthweight r=0.57, p=0.001, and r=0.6, p=0.000 respectively, figure 6.2.

Inpatient Nutrition and Growth

Median duration of TPN was 7 days (3, 8) in the early group, and 5 days (1, 16) in the late group, which was not significantly different. Over the first 3 weeks of life average daily intakes of calcium, phosphate and calories did not differ significantly between groups; 1.94 mmol/kg/day (1.31, 3), 1.55 mmol/kg/day (1.15, 2.57), 112 kcal/kg/day (94, 118) in the early group and 2.02 mmol/kg/day (1.31, 4.2), 1.47mmol/kg/day (1.12, 3.12), 104 kcal/kg/day (86, 129) in the late group (ns). Weight at 3 weeks of age were similar between groups, 1703g (1263, 2235), and 1650g (1160, 2441) in the early and late groups respectively (ns.) Weight gain over the first 3 weeks were also comparable, 240g (73, 425) in the early group and 115g (30, 327) in the late group (ns) In the early group SOS SDS at 3 weeks was -0.6 (-1.1, 0.18), which was a median change of -0.8 (-1.46, -0.1) from the start of the study, compared to -0.5 (-1.4, 0.3) and -0.65 (-1.25, -0.2) in the late group, ns. There was no significant correlation between SOS SDS at 3 weeks and daily calcium, phosphate or calorie intake over that period. However there was a significant positive correlation between SOS SDS change to discharge and both calcium and calorie intake over the first 3 weeks, r=0.54, p=0.018, and r=0.57, p=0.011 respectively (figures 6.3 and 6.4). The correlation between SOS SDS until discharge and phosphate intake over the first 3 weeks came close to statistical significance, r=0.42, p=0.07 (figure 6.3). There was no significant correlation between average daily weight gain until discharge and SOS SDS.

Serum Biochemistry

Median serum alkaline phosphatase (ALP) at discharge was not significantly different between groups, 327 iu/l (294, 509) and 278 iu/l (186, 496) in the early and late groups respectively. There was also no significant difference between groups in the lowest serum phosphate or calcium during the study period, 1.7mmol/l (1.05, 1.87) and 1.97mmol/l (1.85, 2.1) in the early group compared to 1.59mmol/l (0.98, 1.96) and 1.93mmol/l (1.67, 2.26) in the late group. The reference range in our laboratory for serum phosphate is 1.5 -2.6 mmol/l, and calcium is 2.2 – 2.7 mmol/l. There was a significant negative correlation between change in SOS SDS in both groups and serum ALP at discharge, r=-0.7, p=0.000 (figure 6.5). There was also a positive correlation in all infants between SOS SDS until discharge and both lowest serum phosphate and also serum calcium, r=0.48, p=0.008 and r=0.55, p=0.002, respectively (figure 6.6) Three infants in the early group and 4 infants in the late group had a combination of high serum ALP with a concurrently low serum phosphate, suggestive of OP. These infants were significantly more preterm (median gestation 29 weeks compared to 32 weeks), lighter at birth (median birthweight 1290g compared to 1700g) and had a greater decrease in SOS SDS (median -1.7 compared to -0.8) than the infants without that combination of biochemical changes.

Complications of Prematurity

There were no significant differences in duration of respiratory support between the different exercise groups, in the early group median days of ventilation were 0 (0,2) and CPAP were 1 (0, 4) compared to 1 (0,13) and 1.5 (0, 19) (ns). The number of episodes of proven sepsis were also similar, 0 (0, 0.7) and 0 (0, 1.5) in the early and late groups respectively. The rate of intraventricular haemorrhage (IVH) and periventricular leukomalacia (PVL) were also not significantly different between groups; 2 infants in the early group had an IVH (1 unilateral

grade 1, 1 bilateral grade 3) compared to 5 in the late group (3 unilateral grade 1, 1 bilateral grade 3, 1 bilateral grade 3/4), and 1 infant from each group developed PVL. Two infants from the early group developed chronic lung disease, compared to 3 infants from the late group, No infant from either group developed retinopathy of prematurity. One infant from the early group and 2 infants from the late group had a persistent ductus arteriosus. No infant developed a conjugated hyperbilirubinaemia and there were no fractures noted during the neonatal period.

Post discharge growth, bone status and neurodevelopment.

Twenty six out of the thirty one infants were followed up in the out patient clinic at a median age of 12 months (4, 24.) There was no difference in weight, length or OFC, corrected for age, between infants who received the early exercise protocol or the late exercise protocol. SOS SDS was 0.1 (-1.3, 1.7) in the infants from the early group, and 0.5 (-0.6, 1.0) in the late group, ns. One infant from the early group developed post haemorrhagic hydrocephalus, two infants from the late group developed a unilateral hemiplegia, one following PVL, the other secondary to a middle cerebral artery infarct which was likely to have happened antenatally. Two infants from the late group still needed home oxygen at 9 months CGA, one of these infants also had sensorineural deafness. No infant was found to have a fracture or any skeletal deformity. Fourteen infants were seen as out patients when they had all reached a corrected gestational age of 2 years; no infant had a neurodevelopmental abnormality, all having reached motor and communication milestones appropriate for their age. (The 2 infants with hemiplegia and deafness were not seen at 2 years.)

Comparison with historical control group

Median initial SOS SDS was 0 (-0.4, 1.1) and 0.1(-0.4, 1.1) in the controls for the early and late group respectively, this was not significantly different from the early and late exercise infants. Median change in SOS SDS was -1.4 (-2.2, -0.85) in the early controls, compared to -1.1 in the early exercise infants (ns). In the late controls median change in SOS SDS was -1.58 (-2.2, -0.85) which was a larger decrease over time compared to the late exercise group, (median -0.8); the difference was almost statistically significant at p=0.06. Median serum ALP at discharge was significantly higher in both control groups, 472IU/l (315, 678) compared to the early exercise infants (p=0.04) and late exercise infants (p=0.0008.)

Discussion

There was no difference in bone SOS in infants who received a physical activity intervention starting once immediately cardiovascularly stable, or starting when established on 100kcal/day enteral feeds, and neither intervention conferred any benefit compared to a group of historical controls.

The same exercise protocol which improved bone mineralisation in the Moyer-Mileur studies(56;57) did not have any impact on bone SOS SDS in our study infants. As SOS should increase with gestational age, the attenuation in absolute SOS from the same exercise intervention as shown by Litmanovitz et al (58), would still result in a decrease in SOS SDS, however this would be of a smaller magnitude than in our study group. It is likely that our infants did not respond to the physical activity intervention because of suboptimal mineral intake. Daily calcium and phosphate was marginally below that recommended for very low birthweight infants (17;137), and was substantially lower than the daily intake of infants in the Moyer-Mileur study. Infants in both the former studies received fortified EBM, whereas our

infants did not. Calorie intake was similar in our infants and the infants in the aforementioned studies, and similarly exercise had no significant effect on weight gain.

The correlation between SOS SDS at discharge and early mineral and calorie intake reinforces the importance of striving for the best possible nutrition in preterm infants. As observed in our earlier studies (119) the most preterm infants had the largest negative change in SOS SDS from birth to discharge, and they are the group who pose the greatest challenge in provision of good nutrition. Trying to improve bone health by challenging immobility using a physical exercise programme appears less effective in the presence of suboptimal intake of calcium and phosphate. However, there was an effect on serum alkaline phosphate at the end of the study, interestingly ALP was lower in infants who received physical activity compared to the group of historical controls, perhaps reflecting a positive effect of exercise on bone turnover, with reduced bone resorption at hospital discharge. This may be important in the risk of subsequent fracture and long term growth.

This is the first study to look at complications and medium term outcomes of a physical activity programme. A recent Cochrane review on the effects of exercise in preterm infants (138) concluded that the current data were inadequate to assess the possibility that exercise may be harmful. There were no fractures or skeletal deformities found on follow up of our study group. Growth and bone strength assessed by QUS were neither negatively affected nor improved by having either of the physical activity protocols during the neonatal period. Our study has many limitations, particularly the small numbers involved, and the large proportion of twins and greater numbers would be needed to establish the safety of passive exercises in this population.

Our data raises the possibility that nutritional intake may modify the effect of physical activity. There was no difference in outcomes between exercise that started early or late. The optimal physical activity programme for preterm infants remains undetermined and there is a need to explore the interaction between nutritional status and exercise. Future studies including comparison between intervention groups and contemporaneous controls are required.

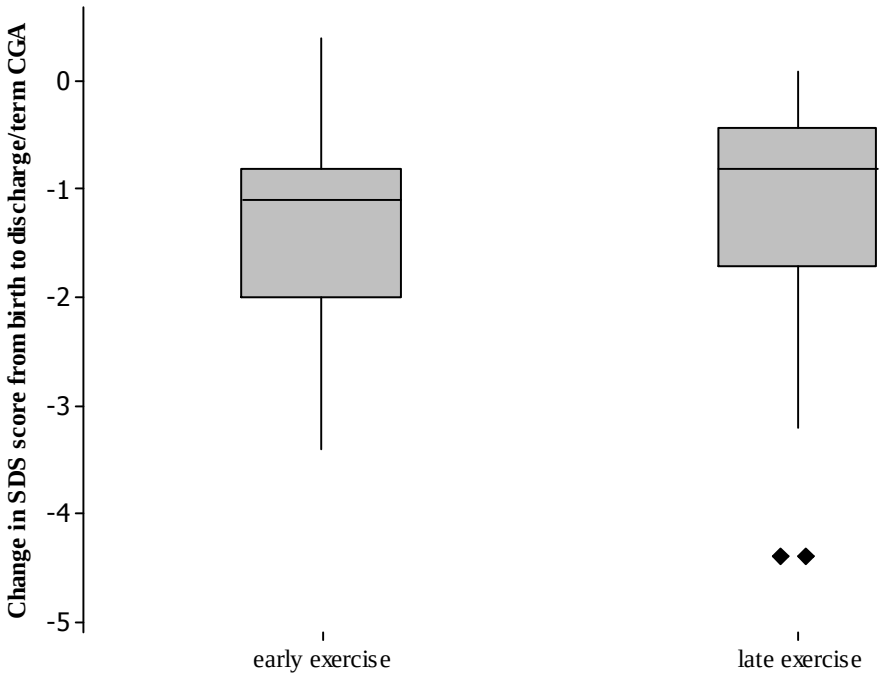
Table 6.1

	Early Group		**Late Group**		
	Median	10^{th}, 90^{th} centiles	Median	10^{th}, 90^{th} centiles	P value
Gestation	30	27.4, 33	31.5	27.5, 33	0.89
Birthweight	1525	1126, 2002	1560	985, 2255	0.77
Weight discharge	2510	2042, 3220	2525	2058, 3215	0.84
OFC[1] birth	29.5	26.5, 30.5	30	26.8, 32.3	0.24
OFC discharge	32.6	32.1, 34.7	34.7	33.3, 36.8	0.06
Initial SOS	2986	2864, 3098	2982	2874, 3086	
SOS discharge	2932	2785, 3038	2900	2826, 2992	
Initial SOS SDS	0.4	-0.8, 1.3	0.1	-0.55, 1.3	0.43
SOS SDS discharge	-0.6	-2.2, -0.1	-1.0	-1.95, -0.3	0.68
Days of exercise	24	14, 38	21	10, 50	0.90
Age at discharge	42	24, 69	45	22.5, 110	0.79
Days ventilation	0	0, 2	1	0, 13	0.25
Days CPAP[2]	1	0, 4	1.5	0, 19	0.53
Days TPN[3]	6.5	3.3, 8	4.5	0.5, 6	0.50
Incidents[4]	0.1	0, 1.4	0.1	0, 0.7	0.81
Sepsis episodes	0	0, 0.7	0	0, 1.5	0.31
Serum PO_4[5]	1.7	1.05, 1.87	1.59	0.98. 1.96	0.69
Serum calcium	1.97	1.85, 2.1	1.93	1.67, 2.23	0.60
Serum ALP[6]	327	239, 509	278	186, 496	0.42
N^o infants CLD[7]	2		3		
N^o infants IVH[8]	2		5		
N^o infants PVL[9]	1		1		
N^o infants ROP[10]	0		0		

Table 6.1 Characteristics of Early and Late Groups

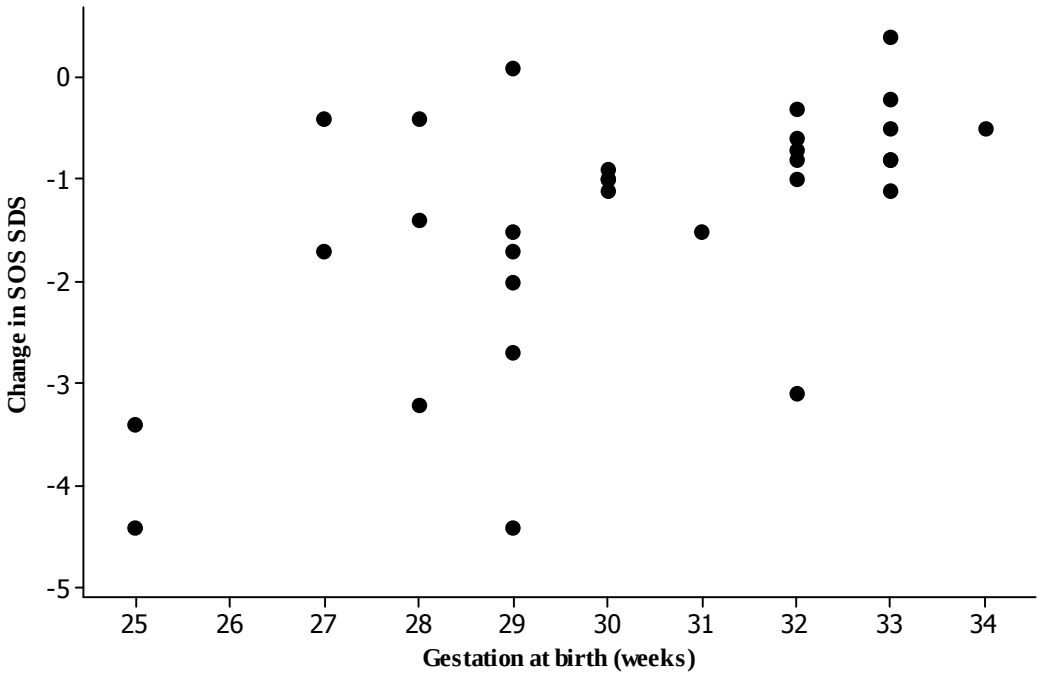
1- OFC, occipitofrontal circumference , 2- CPAP, continuous positive airways pressure, 3- TPN, total parenteral nutrition, 4- Incidents, number of episodes of apnoea, bradycardia or desaturation, 5- PO_4, serum phosphate, 6- ALP, serum total alkaline phosphatase, 7- CLD, chronic lung disease, oxygen dependent after 36 weeks corrected gestational age, 8- IVH, intraventricular haemorrhage, 9- PVL, periventricular leukomalacia, 10- ROP, retinopathy of prematurity.

Figure 6.1



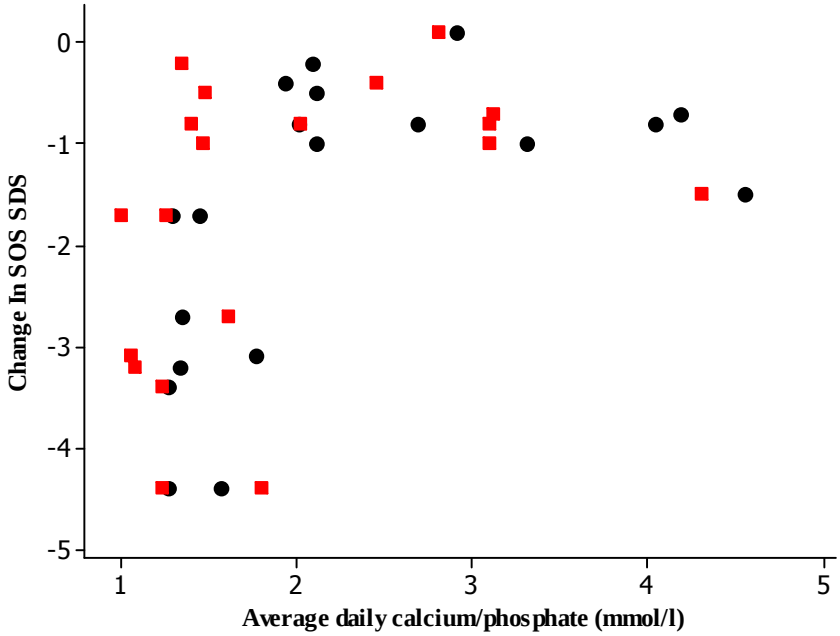
The boxplot shows change in SOS SDS from start to end of study period. The straight line represents the median, and the whiskers show the interquartile range. The diamond shape represents an outlier.

Figure 6.2



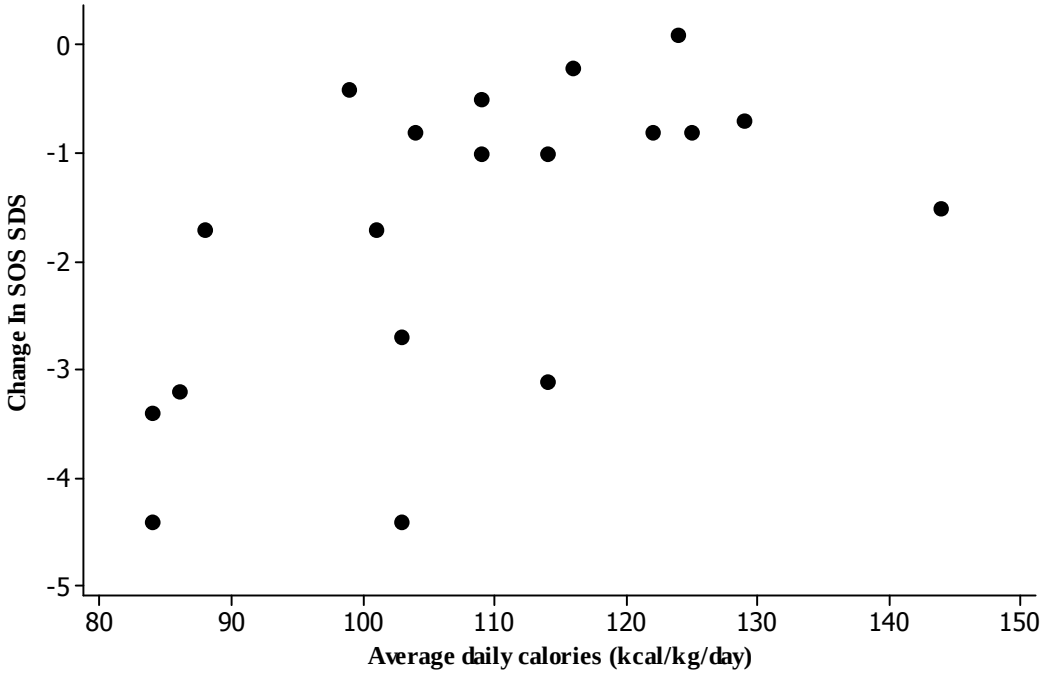
The closed circles represent each infants change in SOS SDS from start to the end of the study period.

Figure 6.3



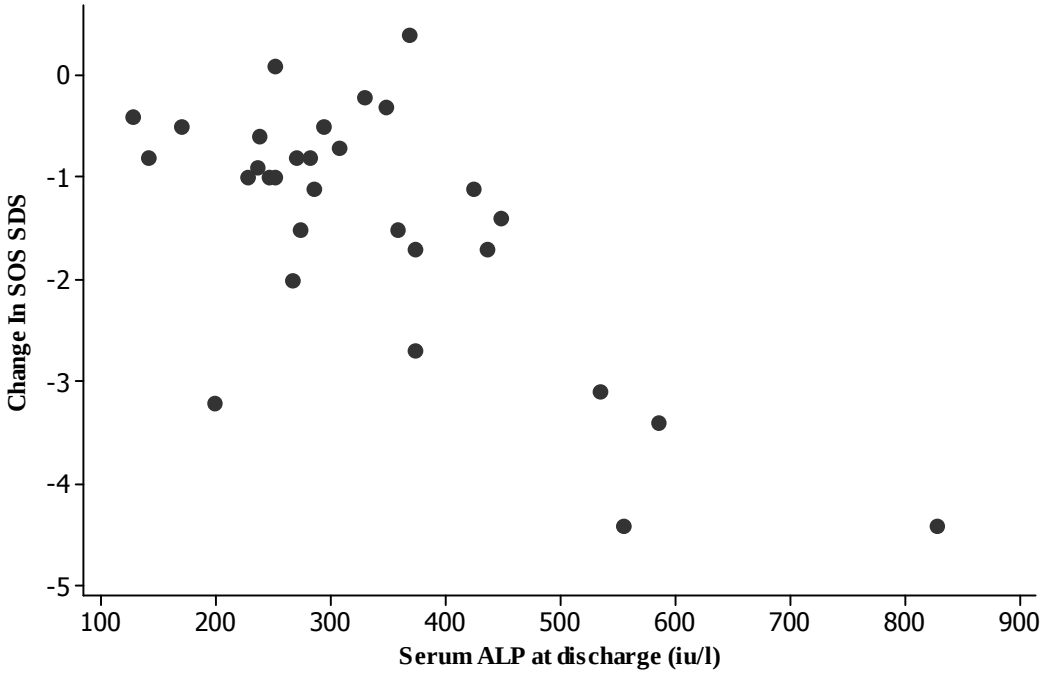
The black circles represent values for each patient's change in SOS SDS until hospital discharge plotted by average daily calcium intake (mmol/kg/day) over the first 3 weeks of life. The black squares represent values for each patient's change in SOS SDS until hospital discharge plotted by average daily phosphate intake over the first 3 weeks of life.

Figure 6.4



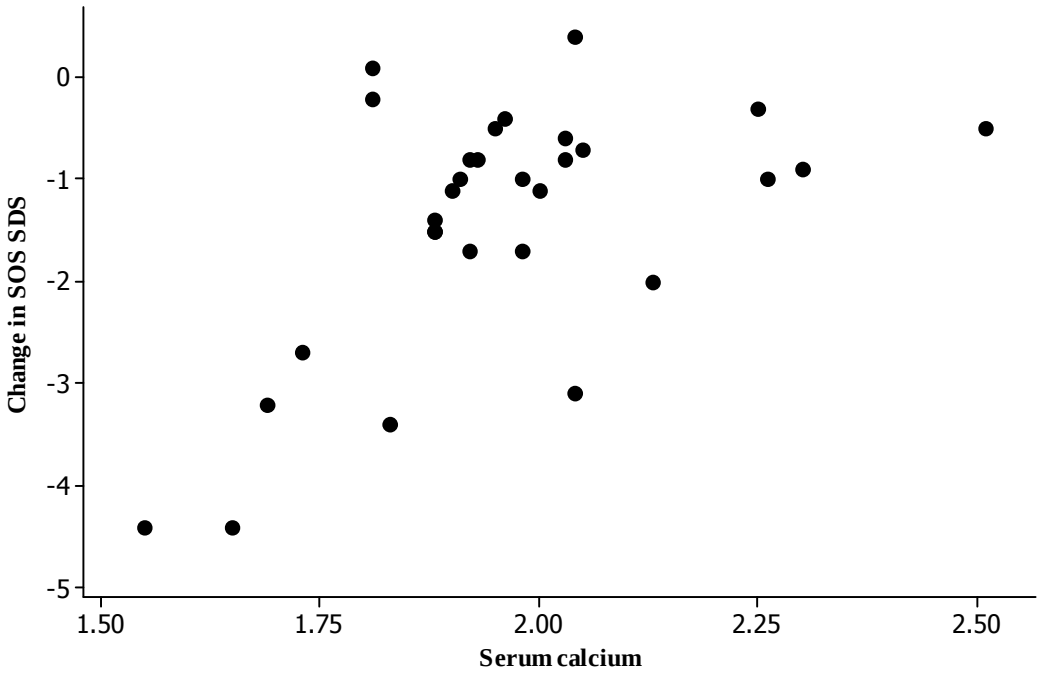
The closed circles represent each patient's change in SOS SDS from start of the study until hospital discharge plotted by their average calorie intake (kg/kg/day) over the first 3 weeks of life.

Figure 6.5



The closed circles represent each patient's change in SOS SDS until hospital discharge plotted with their serum alkaline phosphatase (ALP, iu/l) at hospital discharge.

Figure 6.6



The closed circles represent each patient's change in SOS SDS until hospital discharge plotted against their lowest serum calcium during the neonatal period (mmol/l.)

Chapter 7 Conclusions and Future Directions

Conclusion

The work of this thesis establishes quantitative ultrasound as a useful technique in the assessment of infant bone health. It is a radiation free tool which provides precise and reproducible measurements in both term and preterm infants. The pattern of speed of sound changes in a neonatal population was assessed, and then used to assess the effect of a clinical intervention.

In agreement with a small number of other studies we found that preterm infants have a lower speed of sound at birth compared to term infants; speed of sound increases with increasing gestation while in utero. By including infants who were both appropriately grown and small for gestational age we found maturity to be a more important factor in bone strength than birthweight. In infants born before 32 weeks speed of sound decreases from birth to term corrected gestational age, this decline was largest in the sickest, most preterm infants but occurred in all study infants. The low SOS close to term CGA coincides with the peak time for fractures secondary to osteopenia of prematurity. From term to 2 years CGA tibial speed of sound in preterm infants increases rapidly, exhibiting a catch up phenomenon which, in the majority, produced SOS values in the normal range by 6 months CGA. This trajectory of SOS is similar to bone changes demonstrated in preterm infants using SPA and DXA(37-43), and reflects the common clinical course of neonatal bone disease due to osteopenia of prematurity.

Despite the apparent self limiting nature of osteopenia of prematurity an intervention to improve neonatal bone health is still desirable; to prevent fractures which occur in up to 10% of cases (29;31) and to prevent long term effects on growth (45-47;50). Passive exercise has been used successfully in a few studies (56-58) to improve bone health in the short term in preterm infants, however those studies lacked information on benefits and harm in the longer term. Optimal duration and timing of exercise remains unclear; our study of an ‘early’ versus

'late' physical activity programme was designed to explore the benefit versus harm of extra handling of preterm infants at the earliest opportunity (when cardiovascularly stable) compared to the same passive exercises when the infants were older but had a better mineral supply. In both groups there was a significant fall in SOS from birth to hospital discharge, of a similar magnitude to gestation matched historical controls. There was no significant difference between the exercise groups in SOS to discharge, serum bone biochemistry and growth. It is likely that suboptimal mineral intake in our study infants affected the response to the physical activity programme. The changes in SOS SDS correlated strongly with early nutritional intake of phosphate, calcium and kilocalories. Our results challenge conclusions from previous studies that physical activity alone can improve neonatal bone health. No significant adverse effects occurred secondary to physical activity during the neonatal period or in the first year of life. There were two main drawbacks to the study, the small numbers of infants recruited, and the limitation of the technique of quantitative ultrasound. Recruitment to the cross-sectional and interventional studies of preterm infants was limited due to smaller than expected numbers of preterm infants being delivered at the recruiting centre. It is possible that the small sample size of our study may have underestimated either the benefits of exercise or associated complications. Larger studies are needed to define better the role of passive exercise in the neonatal period. QUS is reported to measure both qualitative bone properties, such as bone mineralization, and quantitative properties such as cortical thickness, elasticity and micro architecture (71-74) but exact correlations are not clear at present. In adults, QUS predicts clinical fractures independent of BMD (75;76), but this has not yet been demonstrated in children and infants. Therefore there is currently no role for quantitative ultrasound in routine clinical practice in neonatology.

In the west of Scotland vitamin D deficiency is common in pregnancy, and women of south asian origin are at particularly high risk. SOS was normal in most women at the start of

pregnancy, even those with risk factors for adverse bone health. It is likely that osteomalacia is not seen immediately upon vitamin D becoming deficient, and therefore changes in SOS may take some time to evolve and may not be seen at booking or at delivery depending on the duration and severity of vitamin D deficiency**.** Term fetuses appear relatively protected and at birth have normal bone strength. There may be short term consequences relating to calcium metabolism in the vitamin D deficient neonate. Preterm and term infants may be susceptible to the effect of programming; therefore there are potentially longer term effects on the cardiovascular system, risk of cancer and bone disease. Vitamin D supplementation should be given to pregnant women, particularly those at high risk.

Future Directions

'Imagination is more important than knowledge. For while knowledge defines all we currently know and understand, imagination points to all we might yet discover and create' (Albert Einstein)

There are unanswered questions in the current literature which stem from my project

- What is the relationship between QUS measurements and risk of fracture in preterm infants?

  There is a need to define the true incidence of fracture in preterm infants via a prospective study. A multicentre study which actively screened for fractures at discharge would be desirable.

- What is the optimal physical activity programme for infants born prematurely?

  Larger randomised controlled studies of physical activity in neonates are needed to determine an optimal exercise regimen, which must be considered alongside mineral and nutrient supply. Follow up for at least 2 years would be desirable. The role of genetic influences such as the vitamin D receptor merits further investigation within the infant population and could be done easily on umbilical cord blood.

- How many infants and children will present with clinical consequences of vitamin D deficiency in Scotland, and what are the longer term outcomes of women and children with vitamin D deficiency?

  Future studies should focus on functional outcomes of vitamin D deficiency; this was outwith the scope of this thesis. A national surveillance programme of infants and children presenting with vitamin D deficiency with long follow up would be needed to provide accurate information. As there is the potential for vitamin D deficiency to

affect many systems longitudinal evaluation of bone health, growth, cardiovascular system and occurrence of malignancy would be necessary.

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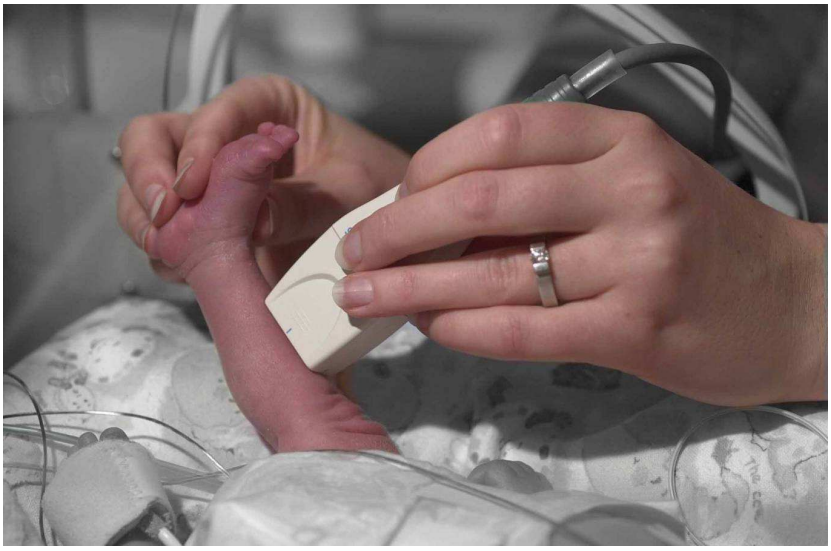
Photograph 1

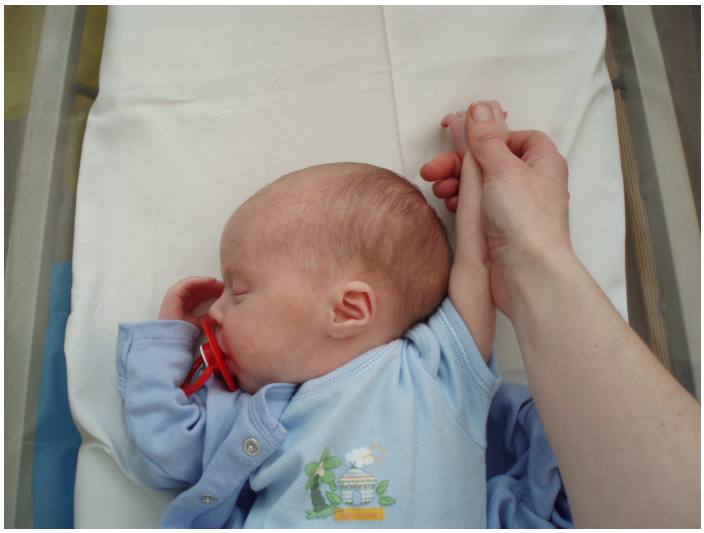
Photograph 2



Photographs 1 and 2 show a tibial quantitative ultrasound scan being done on a preterm infant.

Photograph 3

Photograph 4



Photographs 3 and 4 show preterm infants receiving passive exercises.

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