

Assessment of mineralised bone mass and mineral supplementation in the newborn infant

Adele Harrison

MB ChB, MRCP, FRCPC

Doctor of Medicine
University of Edinburgh
2006



Declaration

This thesis has been composed in its entirety by me for the purposes of submission for the degree of Doctor of Medicine, University of Edinburgh. The work undertaken is my own, unless (where acknowledged) it has been undertaken with colleagues. The work described has not been submitted for any other degree, diploma or professional qualification.

Signed

Adele Harrison

MB ChB, MRCP, FRCPC

Acknowledgements

With grateful thanks to:

Philip Holland, John Truscott and John Puntis for their continuous help and support,

Trish McKinney, Roger Parslow, Richard Feltblower, and Liz Pryde of the Paediatric Epidemiology Group for assistance with the database,

Mike Collins, Donald Purvis, and Maria Utting for all their work on the biochemical assays,

Lisa Walder and Darren Greenwood for statistical advice,

Bryan Gill and Malcolm Levene for their cooperation in the Peter Congdon Neonatal Unit,

the paediatric outpatient nursing staff led by Sandra Garbutt, for help in patient follow-up,

the nursing and medical staff of the Peter Congdon Neonatal Unit, the delivery, transitional care and postnatal wards for their assistance in patient recruitment and sample collections,

Mike Whitfield, Brian Lupton, Horacio Osiovič, Philippe Chessex of the Children and Women's Health Centre of British Columbia for giving me the time and encouragement to finish writing, and to

Barb Jones for clerical assistance.

Special thanks to the parents and infants, without whom the work would not be possible.

Thanks also to *SPARKS* and Leeds General Infirmary Special Trustees for the financial support.

Abstract

This work consists of original research in aspects of fetal and neonatal mineralised bone mass, as measured by a unique portable single photon absorptiometer. The aim of the study is to explore potential materno-fetal mechanisms implicated in the determination of mineralised bone mass at birth, and to examine the effect of individualised calcium and phosphorus supplementation on mineralised bone mass in preterm infants.

Methods – Bone mineral content (BMC), area normalised bone mineral density (BMD) and radial width (RW) were measured within 5 days of birth using single photon absorptiometry (SPA) of mid-radius in a cohort of 99 infants. Venous cord blood was collected for parathyroid hormone (PTH) assay. The relationship between antenatal factors and mineralised bone mass was explored using multiple regression.

In a randomised controlled trial 12 infants received individualised mineral supplementation based on plasma concentrations and urinary excretion, while 13 control infants received standard calcium and phosphorus supplements according to European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) guidelines.

Results – BMC, BMD and RW were significantly linearly related to birthweight. In each model, all maternal factors and PTH concentrations were excluded at 0.1 level, leaving only birthweight in the final models for BMC, BMD and RW. High PTH

concentrations were found only in low birthweight infants. Categorising PTH into <11 (group 1), 11-55 (group 2) and >55ng/L (group 3), there is a significant difference in birthweight between group 1 and 2 ($p<0.05$) and group 1 and 3 ($p<0.05$).

Compared to controls, individualised mineral supplementation failed to enhance radial BMC, BMD or RW. BMC increased from a mean of 7.2mg at birth to 9.2mg at 37 weeks post-conceptual age (PCA) ($p<0.05$), RW increased from 0.43 to 0.57cm ($p<0.001$), with no change in BMD. There was a significant difference in the preterm infants from weight-adjusted values of BMC of 13.7mg ($p<0.001$) and BMD of 105.5mg/cm² ($p<0.001$) at 37 weeks PCA but no significant difference in RW adjusted for weight.

Conclusion – BMC, BMD and RW are directly related to birthweight in infants of any viable gestational age. This study failed to demonstrate a relationship between materno-fetal factors, cord PTH concentrations, and mineralised bone mass at birth.

Individualised mineral supplementation of preterm infants did not improve mineralisation over standard supplementation regimens. Preterm infants have a reduced mineralised bone mass at term compared to expected weight-adjusted values.

Content

<i>Declaration</i>	<i>ii</i>
<i>Acknowledgements</i>	<i>iii</i>
<i>Abstract</i>	<i>iv</i>
<i>Content</i>	<i>vi</i>
Figures	x
Tables	xii
<i>List of Abbreviations</i>	<i>xiii</i>
<i>1 Bone Mineralisation in the Newborn Infant</i>	<i>1</i>
1.1 Bone formation and growth	2
1.1.1 Intramembraneous ossification.....	2
1.1.2 Intracartilaginous ossification.....	3
1.2 Metabolic Bone Disease	4
1.2.1 Mineral Accretion.....	5
1.2.2 The environment <i>in-utero</i>	5
1.2.3 The post-natal environment.....	6
1.2.4 Incidence of Metabolic Bone Disease.....	6
1.3 Mineral Metabolism	8
1.3.1 Calcium Metabolism.....	8
1.3.1i Gastrointestinal absorption of calcium.....	9
1.3.1ii Renal excretion of calcium.....	10
1.3.2 Phosphorus metabolism.....	12
1.3.2i Gastrointestinal absorption of phosphorus.....	12
1.3.2ii Renal excretion of phosphorus.....	13
1.3.3 The relationship between calcium and phosphorus.....	16
1.3.4 Calcium and phosphorus requirements.....	17
1.3.4i Parenteral feeding.....	18
1.4 Complications in the Preterm Infant	19
1.4.1 Renal function.....	19
1.4.1i Development of renal function.....	19
1.4.1ii Diuretics.....	20
1.4.2 Bronchopulmonary dysplasia.....	22
1.4.2i Corticosteroids.....	22
1.5 Control of bone mineralisation	24
1.5.1 Parathyroid hormone.....	24
1.5.2 Calcitonin.....	26
1.5.3 Vitamin D.....	27
1.5.3i Vitamin D and the fetus.....	29
1.5.3ii Vitamin D requirement of the preterm infant.....	29
1.5.3iii Genetic influence of vitamin D receptor genotype.....	32
1.5.4 Parathyroid hormone related protein.....	32
1.6 Assessment of bone mineralisation	35
1.6.1 Biochemical markers.....	35
1.6.1i Alkaline Phosphatase.....	35
1.6.1ii Osteocalcin.....	36
1.6.1iii Collagen assays.....	37
1.6.1iv Hydroxyproline.....	38

1.6.1v Pyridinium Crosslinks	38
1.6.2 Imaging Techniques.....	39
1.6.2i Single Photon Absorptiometry	39
1.6.2ii Dual Photon Absorptiometry	42
1.6.2iii Quantitative Computed Tomography.....	42
1.6.2iv Dual energy x-ray absorptiometry.....	43
1.6.2v Broad band ultrasound attenuation.....	44
1.7 Bone mineralisation in the newborn infant	45
1.7.1 Biochemical markers of mineralisation	47
1.7.2 Supplementation	48
1.7.3 Follow up.....	50
1.7.4 More than just bones.....	51
1.8 Fetal origins of adult disease.....	53
1.9 Physical activity and mineralisation.....	55
1.10 Effects of prematurity on cellular control	56
1.10.1 Cytokines	56
1.10.2 Sex hormones	57
1.10.3 Growth hormones	58
1.10.4 Preterm delivery	59
2 Methodology.....	61
2.1 Cohort study.....	61
2.1.1 Introduction	61
2.1.1i Aim:	61
2.1.1ii Hypothesis:	62
2.1.2 Patients and Methods.....	63
2.1.3 Parathyroid hormone	65
2.1.4 PTHrP	66
2.1.5 1,25 dihydroxycholecalciferol	67
2.1.6 Single photon absorptiometry.....	68
2.1.6i Bone mineral density.....	69
2.1.6ii Bone mineral content	71
2.1.7 Statistics.....	73
2.2 Randomised controlled trial of individualised calcium and phosphorus supplementation	75
2.2.1 Introduction	75
2.2.1i Aim:	76
2.2.1ii Hypothesis:	76
2.2.2 Patients and Methods.....	77
2.2.2i Setting	77
2.2.2ii Population	77
2.2.2iii Consent	77
2.2.2iv Randomisation.....	77
2.2.2v Primary Outcome	78
2.2.2vi Secondary Outcomes.....	78
2.2.2vii Confounding variables	78
2.2.2viii Data sampling	78
2.2.2ix Study design.....	79
2.2.2x Data Collection.....	81
2.2.2xi Blinding.....	81
2.2.2xii Laboratory Methods	81
2.2.2xiii Ethical approval	81
2.2.3 Statistical Methods	82

3 Results of Cohort study	83
3.1 Single photon absorptiometer	83
3.1.1 Coefficient of variation.....	83
3.1.2 Loss of images.....	83
3.2 Patient characteristics	85
3.3 Linear Regression	90
3.4 Parathyroid hormone	97
3.4.1 Parathyroid hormone and pregnancy-induced hypertension.....	103
3.4.2 Parathyroid hormone and season of birth.....	107
3.5 PTHrP	108
3.6 1,25-dihydroxyvitamin D	109
4 Results of randomised controlled trial	111
4.1 Characteristics of trial and control groups	114
4.1.1 Parenteral and enteral intake.....	114
4.1.2 Mineralised bone mass.....	118
4.3 Group characteristics	119
4.3.1 Weight gain.....	119
4.3.2 Ventilation.....	119
4.3.3 Medication.....	120
4.4 Morbidity and Mortality	122
4.5 Alkaline Phosphatase	123
4.6 Post hoc analysis	125
4.6.1 Birthweight group analysis.....	126
4.7 Follow-up	128
5 Discussion	129
5.1 Cohort study	129
5.1.1 What does this study add?.....	129
5.1.2 Critique of the Methodology.....	135
5.1.2i Population.....	135
5.1.2ii Consent.....	135
5.1.2iii Measured variables.....	136
5.1.2iv Data Sampling.....	136
5.1.2v Study Design.....	137
5.1.3 Limitations of single photon absorptiometry.....	139
5.1.4 Effect on future research.....	141
5.2 Randomised controlled trial	143
5.2.1 Mineralised bone mass at discharge.....	143
5.2.2 Follow-up.....	144
5.2.3 Individualised supplementation.....	145
5.2.4 Alkaline Phosphatase.....	145
5.2.5 Cortical Width.....	146
5.2.6 Critique of the Methodology.....	151
5.2.6i Protocol.....	151
5.2.6ii Assignment.....	153
5.2.6iii Masking.....	153
5.2.6iv Participant Flow and Follow-up.....	154
5.2.6v Analysis.....	154

5.2.7 What does this study add?	155
5.2.8 Effect on future research.....	160
5.3 Implications of reduced mineralisation	161
5.4 Conclusion	162
6 Appendix.....	163
Appendix A: Phosphorus and calcium content of preterm formula and breast milk	163
Appendix B: Individual variation in bone mineral content and density.....	165
Appendix C: Structured form used for obstetric and neonatal note extraction	167
Appendix D: Parent Information Sheets	188
Study 1.....	188
Study 2.....	189
Appendix E: Assessment of calcium and phosphorus solubility.....	190
Appendix F: Abidec.....	192
7 Presentations/Publications	193
8 References	194

Figures

<i>Figure 1: Hormonal control of hypocalcaemia (Mawer & Berry, 1995)</i>	9
<i>Figure 2: Calcium excretion index</i>	11
<i>Figure 3: Percentage tubular reabsorption of phosphorus</i>	15
<i>Figure 4: Image of forearm bones; cursor positioned for BMD measurement</i>	70
<i>Figure 5: Image of forearm bones; alignment for BMD measurement</i>	70
<i>Figure 6: Image of forearm bones; cursor positioned for BMC measurement</i>	72
<i>Figure 7: Image of forearm bones; alignment for BMC measurement</i>	72
<i>Figure 8: Bone images of infants recruited</i>	86
<i>Figure 9: Parathyroid analysis of recruited infants</i>	87
<i>Figure 10: Bone mineral content and birthweight</i>	91
<i>Figure 11: Bone mineral density and birthweight</i>	92
<i>Figure 12: Radial width and birthweight</i>	93
<i>Figure 13: Birthweight and administration of antenatal steroids</i>	96
<i>Figure 14: PTH and birthweight</i>	98
<i>Figure 15: Parathyroid hormone and birthweight</i>	100
<i>Figure 16: Parathyroid hormone and gestation</i>	100
<i>Figure 17: Parathyroid hormone and birthweight centile</i>	101
<i>Figure 18: Parathyroid hormone and bone mineral content</i>	102
<i>Figure 19: Parathyroid hormone and bone mineral density</i>	102
<i>Figure 20: Pregnancy-induced hypertension and birthweight</i>	104
<i>Figure 21 : Birthweight and pregnancy-induced hypertension (all infants)</i>	105
<i>Figure 22: Birthweight and pregnancy-induced hypertension (PTH measured)</i>	106
<i>Figure 23: PTH and month of birth</i>	107
<i>Figure 24: PTHrP concentrations and bone mineral content</i>	108
<i>Figure 25: PTH and PTHrP concentrations</i>	109
<i>Figure 26: 1,25-dihydroxycholecalciferol and birthweight</i>	110
<i>Figure 27: PTH and 1,25-dihydroxycholecalciferol</i>	110
<i>Figure 28: Tubular resorption of phosphorus in trial and control groups</i>	117
<i>Figure 29: Calcium excretion index in trial and control groups</i>	117
<i>Figure 30: Alkaline phosphatase concentrations in trial and control groups</i>	124
<i>Figure 31: SPA image of 25 week gestation infant at birth</i>	147

<i>Figure 32: SPA image of 25 week gestation infant at term</i>	147
<i>Figure 33: Determination of cortical width</i>	148
<i>Figure 34: Relationship between change in estimated volumetric bone density and change in radial width</i>	149
<i>Figure 35: Individual bone mineral content changes throughout the study period</i>	165
<i>Figure 36: Individual bone mineral density changes throughout the study period</i>	166

Tables

<i>Table 1: Effect of independent variables on BMC, BMD and RW.....</i>	<i>89</i>
<i>Table 2: Regression coefficients for mineralised bone mass and birthweight.....</i>	<i>90</i>
<i>Table 3 :General linear model for BMC.....</i>	<i>94</i>
<i>Table 4: General linear model for RW</i>	<i>94</i>
<i>Table 5 :General linear model for BMD.....</i>	<i>95</i>
<i>Table 6: PTH concentrations and pregnancy-induced hypertension.....</i>	<i>103</i>
<i>Table 7 : Details of infants entered into study.....</i>	<i>113</i>
<i>Table 8: Details of trial and control groups.....</i>	<i>114</i>
<i>Table 9: Daily parenteral intake in trial and control groups by week of age.....</i>	<i>116</i>
<i>Table 10: Daily enteral intake in trial and control groups by week of age</i>	<i>116</i>
<i>Table 11: Details of trial and control groups at discharge</i>	<i>118</i>
<i>Table 12: Ventilatory support in trial and control infants.....</i>	<i>120</i>
<i>Table 13: Drug administration to trial and control infants</i>	<i>121</i>
<i>Table 14: Mineralised bone mass changes in recruited infants compared to cohort data</i>	<i>125</i>
<i>Table 15: Mineralised bone mass changes in recruited infants by birthweight... </i>	<i>127</i>
<i>Table 16: Follow-up of trial and control groups.....</i>	<i>128</i>

List of Abbreviations

AGA	appropriate for gestational age
AP	alkaline phosphatase
BMC	bone mineral content
BMD	bone mineral density
BMP	bone morphogenic protein
BPD	bronchopulmonary dysplasia
ICTP	c-terminal telopeptide of type I collagen
Ca _E	calcium excretion index
cAMP	cyclic adenosine monophosphate
CCD	charge couple device
CPAP	continuous positive airway pressure
CV	coefficient of variation
DBP	vitamin D binding protein
DHCC	dihydroxycholecalciferol (vitamin D)
DPA	dual photon absorptiometry
Dpd	deoxypyridine
DXA	dual energy Xray absorptiometry
EDF	end-diastolic doppler flow
ELBW	extremely low birthweight
ESPGHAN	European Society of Paediatric Gastroenterology, Hepatology and Nutrition
GFR	glomerular filtration rate
GH	growth hormone
HCC	hydroxycholecalciferol
hGH-V	human growth hormone variant (placental growth hormone)
HMM	humoral hypercalcaemia of malignancy
hPL	human placental lactogen
Ihh	Indian hedgehog
IL	interleukin
IV	intravenous
LBW	low birth weight
MBD	metabolic bone disease
MBM	mineralised bone mass
OC	osteocalcin
PICP	c-terminal propeptide of type I procollagen
PCA	post-conceptional age
PMA	post-menstrual age
PN	parenteral nutrition
PIH	pregnancy-induced hypertension
PTH	parathyroid hormone
PTHrP	parathyroid hormone-related protein
Pyd	pyridinium
RIA	radioimmunoassay
ROI	region of interest
RW	radial width

SEM	standard error of the mean
SD	standard deviation
SGA	small for gestational age
SOL	spontaneous onset of labour
SPA	single photon absorptiometry
TBBC	total body bone content
TGF	transforming growth factor
TmP/GFR	maximum tubular resorption of phosphorus
TNF	tumour necrosis factor
TRP	percentage tubular resorption of phosphorus
VDR	vitamin D receptor

1 Bone Mineralisation in the Newborn Infant

Increasing numbers of preterm infants are surviving the acute complications of extreme prematurity (Stevenson et al, 1998), such that the focus of neonatal care is moving towards reducing morbidity associated with their survival (Jobe, 2001). Recent studies on the impact of fetal and infant nutrition on diseases in adult life (Barker et al, 1990) suggest that the nutritional status of the preterm infant during the first weeks of life (Ehrenkrantz et al, 1999) may be relevant not only to the neonatal period but may have long term consequences (Lucas, 1991).

This thesis examines the impact of prenatal factors and the hormonal environment which may influence mineralised bone mass in the newborn infant, and the effect of early mineral supplementation on bone mineral content in the preterm infant.

1.1 Bone formation and growth

The musculoskeletal system develops from mesoderm and neural crest cells. Mesodermal cells initially condense to form models of the bones before differentiating into fibroblasts, chondroblasts and osteoblasts. Bone structures develop within mesenchyme either by intramembraneous ossification or by transforming into cartilage models and ossifying by endochondral ossification (Moore & Persaud 1993).

Cartilage first appears in the embryo in the 5th week. The mesenchyme condenses and cells proliferate to form a matrix in which collagenous or elastic fibres are deposited. Mesodermal cells differentiate within the matrix and secrete collagenous fibrils and the ground substance of the matrix.

1.1.1 Intramembraneous ossification

The mesenchyme condenses and becomes highly vascular. Some cells differentiate into osteoblasts and begin to deposit matrix or osteoid tissue into which calcium phosphate is deposited. Bone osteoblasts are trapped within the matrix and become osteocytes. Spicules of bone become organised and coalesce into lamellae. Concentric lamellae develop around blood vessels forming Haversian systems. Some osteoblasts remain at the periphery of the developing bone and continue to lay down layers, forming plates of compact bone on the surfaces. Between the surface

plates the intervening bone remains spiculated, accentuated by the resorbing action of osteoclasts. In the interstices of this spongy bone, the mesenchyme differentiates into bone marrow. Continuous remodelling by the action of osteoclasts and osteoblasts during fetal and postnatal life remodels the bone.

1.1.2 Intracartilaginous ossification

Primary ossification centres appear in the diaphysis of a long bone in the 8th week of embryonic life. The cartilage cells hypertrophy, the matrix calcifies and the cells die. Concurrently a thin layer of bone is deposited under the perichondrium surrounding the diaphysis and the perichondrium becomes periosteum. Invasion of vascular connective tissue from the periosteum breaks up the cartilage. Invading cells differentiate into haemopoietic cells of the marrow or into osteoblasts that deposit bone matrix on the spicules of calcified cartilage. This process continues towards the epiphyses. The spicules of bone are remodelled by the action of osteoclasts and osteoblasts. Lengthening of bone occurs at the diaphyseal-epiphyseal junction. Growth in diameter results from the deposition of bone at the periosteum and absorption on the medullary surface.

1.2 Metabolic Bone Disease

The term “metabolic bone disease” (MBD) applies to a spectrum of hypomineralisation in the preterm newborn, ranging from mild undermineralisation or “osteopenia” to radiological evidence of rickets (Brooke & Lucas, 1985). Rickets is characterised by the accumulation of unmineralised osteoid interrupting mineralisation of the growth plate (Greer, 1994), similar to osteomalacia which occurs in non-growing bone. Fractures may occur in osteopenic bones, with or without the radiological appearances of rickets.

Histologically MBD is characterised by reduced matrix formation and decreased osteoblastic activity (Greer, 1994). Diagnostic radiological findings include fraying, widening and irregularity of the metaphyses; subperiosteal new bone formation; and fractures of long bones and ribs. This characteristically occurs at 2-3 months age, usually representing 38 to 42 weeks post-conceptual age (PCA) (Campbell & Fleischman, 1988).

1.2.1 Mineral Accretion

For many years it has been recognised that premature infants have high requirements for calcium and phosphorus and that human milk is an inadequate source of minerals (Benjamin et al, 1943). This view was supported by von Sydow who also suggested that vitamin D supplementation was essential to enhance calcium absorption in preterm infants and so reduce the incidence of rickets (von Sydow, 1946). Despite this early literature the use of mineral supplementation for human milk fed preterm infants has taken many years to become established.

1.2.2 The environment *in-utero*

Calcium and phosphorus are actively transported across the placenta, with 70% of mineral accretion occurring during the third trimester of pregnancy (Givens & Macy, 1933). Calcium and phosphorus accretion rates peak at 34 to 36 weeks post-menstrual age (PMA) at 3.0 mmol/kg/day (120 mg/kg/day) and 2.4 mmol/kg/day (75 mg/kg/day) respectively (Ziegler et al, 1976).

At term the appropriately grown infant has a total body calcium content of 30 gm and phosphorus of 16 gm (Ziegler et al, 1976). This may be reduced if placental mineral transport has been compromised, for example in pregnancy-induced hypertension or from other causes of placental insufficiency (Khattab & Forfar, 1971).

1.2.3 The post-natal environment

Postnatally mineral accretion in the preterm infant is largely dependant on the tolerance of enteral and parenteral feeds, with dietary mineral deficiency being primarily responsible for metabolic bone disease.

In utero bone mineral accretion rates of phosphorus and calcium can be achieved in preterm infants supplemented with energy, protein and minerals, leading to improved BMC at 32 weeks PCA (Schanler & Abrams, 1995). Some consider that achieving 2/3 of the *in utero* accretion rate is a more appropriate goal (Ziegler, 1985; Bentur et al, 1987).

1.2.4 Incidence of Metabolic Bone Disease

Prior to the use of mineral supplemented human milk or preterm formula milk, most infants born before 30 weeks PCA demonstrated some degree of hypomineralisation, which persisted into early infancy (Greer, 1994). The incidence of radiographic rickets in newborns of birthweight <1500g has been reported to be 20-32% (Callenbach et al, 1981; Evans et al, 1989), being more prevalent in black infants and those who have sustained greater weight loss in the early neonatal period, and increasing to up to 50-60% in infants fed unsupplemented human milk (McIntosh et al, 1982).

In the extremely low birth weight infant (birth weight <1000g) dependent upon parenteral nutrition in the first weeks of life, the incidence of MBD has been reported to be as high as 80% (Lindroth et al, 1986). However, since the widespread introduction of human milk supplementation and mineral-enhanced preterm formula biochemical rickets has diminished (Warner et al, 1998), although osteopenia continues to occur in up to 30% of infants (Koo et al, 1988). X-ray diagnosis of MBD is no longer routinely performed, and as such there are no recent reports of the incidence of radiological MBD.

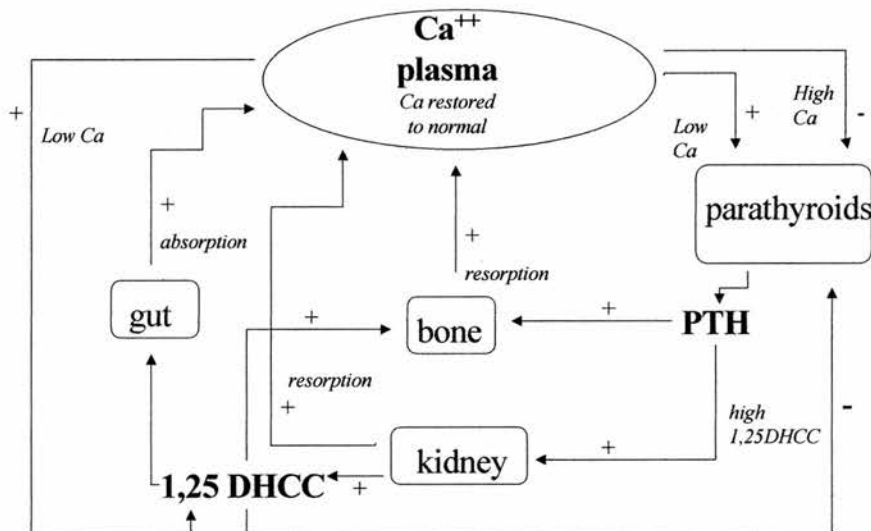
1.3 Mineral Metabolism

1.3.1 Calcium Metabolism

99% of total body calcium is contained within the skeleton; the remainder is distributed within the intravascular, interstitial and intracellular spaces. Total serum calcium is tightly maintained within 2 and 2.8 mmol/L (8-11 mg/dl), with 50% in ionised form. Serum calcium concentration is regulated mainly by the rate of gastrointestinal absorption, bone metabolism and resorption, with glomerular filtration and renal tubular absorption playing a minimal role.

Fluctuations in ionised calcium concentrations are controlled by parathyroid hormone (PTH), calcitonin and 1,25-dihydroxycholecalciferol (1,25-DHCC) (Figure 1). Other factors which affect calcium homeostasis are oxygenation, acid-base state, and interactions with hormones, e.g. thyroid hormone, growth hormone, adrenal steroids, oestrogens, insulin and glucagon (Campbell and Fleischman, 1988).

Figure 1: Hormonal control of hypocalcaemia (Mawer & Berry, 1995)



1.3.1i Gastrointestinal absorption of calcium

Calcium is absorbed in the duodenum by both active and passive transport, with the rate-limiting factor being the calcium supply. Calcium absorption and retention by the preterm infant are difficult to measure. Measurements of net absorption do not take account of calcium loss from bone, and so may underestimate dietary absorption in high bone turnover states. However, absorption appears to be influenced by gestational age, PCA, endogenous (intestinal) loss of calcium, and the quantity and quality of fat in the diet (Shaw, 1976; Lucas et al, 1997). The percentage of calcium absorbed by both human and formula milk fed infants is increased by vitamin D supplementation, although calcium in human milk may have

a higher bioavailability (70% absorption) than in formula milk (50% absorption) (Senterre & Salle, 1982). Other studies suggest that the preterm infant may achieve percentage absorption of calcium as high as 80% of intake irrespective of the dietary source (Ehrenkranz et al, 1985).

In the very low birthweight infant gastrointestinal absorption and retention of calcium appears to be related directly to calcium intake as long as an adequate intake of vitamin D (Day et al, 1975; Salle et al, 1986) and phosphorus (Moya & Doménech, 1982; Giles et al, 1987) are maintained. The ratio of calcium to phosphorus therefore appears to be less important than absolute amounts. An inadequate phosphorus intake results in increased urinary losses of calcium (Senterre et al, 1983). Increases in calcium supplementation may result in increased faecal calcium losses, although net retention improves. As calcium combines with intestinal fat to form insoluble long chain saturated fatty acids concurrent increases in faecal loss of fat may also occur (Katz & Hamilton, 1974; Salle et al, 1986).

1.3.1ii Renal excretion of calcium

In blood 35 to 40% of total calcium is bound to plasma proteins and the remainder is filtered across the glomerulus. Calcium is actively resorbed in the distal tubule resulting in only 0.5 to 1% of filtered calcium appearing in the urine (Massry et al, 1973). Calcium excretion is, therefore, related to the glomerular filtration rate (GFR).

The calcium excretion index (Ca_E) allows for variation in renal function and is calculated by multiplying the ratio of urinary calcium to creatinine by the plasma creatinine concentration (Nordin, 1976) (Figure 2). As creatinine excretion is related to lean body mass, this provides a reference. Normal fasting calcium excretion in adults is 0.05 to 0.15 mg/100ml GFR (Nordin et al, 1967). The term infant has comparable calcium excretion, although the preterm infant may exhibit much larger calcium losses (Brion et al, 1994).

Figure 2: Calcium excretion index
(mg/100 ml glomerular filtration rate)

$$Ca_E = \frac{\text{Urinary calcium (mmol/L)} \times \text{Plasma creatinine } (\mu\text{mol/L})}{\text{Urinary creatinine (mmol/L)} \times 250}$$

1.3.2 Phosphorus metabolism

Inorganic phosphorus is involved in nearly all metabolic processes, with 85% of total body phosphorus being contained in bone (Campbell & Fleischman, 1988). Plasma phosphorus is almost entirely inorganic and therefore rapidly diffuses through the extracellular space, and is quickly incorporated into nucleotides, phospholipids and proteins. Phosphorus is essential for soft tissue growth and metabolism by the body. In low phosphorus intake states, phosphorus is withdrawn from the skeleton; calcium cannot be utilised for bone metabolism and so is lost in the urine (Brooke & Lucas, 1985; Bentur et al, 1987).

1.3.2i Gastrointestinal absorption of phosphorus

Serum inorganic phosphorus concentrations vary widely between 0.8 and 2.5 mmol/l (2.5 and 8.0 mg/dL) depending on age, intake and growth rate. A narrow range is unnecessary and intestinal absorption is efficient (70-90%).

Phosphorus is absorbed primarily from the jejunum by active and passive transport, and absorption rates are high reaching up to 95% in the preterm infant fed on human milk (Senterre, 1983; Rowe, 1984). However, absorption from soy-based products is reduced due to the binding of phosphate by phytates.

1.3.2ii Renal excretion of phosphorus

The renal tubule is the major regulator of extracellular phosphorus, involving PTH and 1,25-DHCC. PTH inhibits tubular resorption of phosphorus in the proximal tubule through activation of adenylcyclase and elevation of cyclic adenosine monophosphate (cAMP), promoting urinary excretion of phosphorus. At least 90% of plasma phosphorus is filtered at the glomerulus with negligible phosphorus secretion occurring at the nephron (Massry et al, 1973).

The renal phosphorus threshold or maximum tubular reabsorption of phosphorus (TmP/GFR) can be assessed clinically by simultaneous measurement of phosphorus loading and GFR, or in adults by the nomogram described by Walton and Bijvoet (Walton & Bijvoet, 1975).

Brodehl (Brodehl et al, 1982) demonstrated that infants have higher plasma phosphate and higher urinary phosphate excretion than children. The net tubular reabsorption of phosphorus is lower in infants due to reduced GFR, but the fractional tubular reabsorption is elevated in association with higher plasma phosphorus concentration. It has been suggested that growth hormone may act on the nephron to reduce renal excretion of phosphorus resulting in an elevated plasma phosphorus concentration (Massry et al, 1973).

Furthermore, in infants and children the TRP during the fasting state is already maximal and phosphorus loading is therefore unnecessary (Brodehl et al, 1988).

They suggest that the nomogram of Walton and Bijvoet is correct in predicting T_{m_p}/GFR when the clearance of phosphate/GFR ratio is above 0.2. However, below 0.2 the nomogram overestimates the directly measured value. For clinical assessment of renal phosphate clearance in infants and children they recommend the formula:

$$\text{plasma phosphorus} - \frac{\text{urinary phosphorus} \times \text{plasma creatinine}}{\text{urinary creatinine}}$$

Expressed as a percentage of plasma phosphorus this gives the percentage of tubular reabsorption of phosphorus (TRP), which corrects for variation in renal function and cancels out errors in urine collection (Figure 3) (Massry et al, 1973). Adult reference values range from 78 to 94% (Kyle et al, 1958; Chambers et al, 1956). Preterm infants have a higher fractional excretion of phosphorus at birth than term infants. This falls from 20% to 3% in the first week, and remains low over the first 3 months of life (Karlén et al, 1985).

The maximal TRP seen in preterm infants after the first week of life suggests relative phosphorus deficiency (Senterre et al, 1983). High TRP persists in human milk fed infants (Schanler et al, 1985). As the concentration of plasma phosphorus falls, the urinary phosphorus diminishes to almost disappear from the urine (Massry et al, 1973). When supplemental phosphorus is given a dramatic reduction in TRP is seen, with between 45 and 100% urinary excretion being documented in mineral-supplemented preterm infants (Carey et al, 1985).

Figure 3: Percentage tubular reabsorption of phosphorus

$$\%TRP = 1 - \frac{\text{Urine phosphate}}{\text{Urine creatinine}} \times \frac{\text{Plasma creatinine}}{\text{Plasma phosphate}} \times 100$$

All units mmol/L. (N.B. Plasma creatinine is usually measured in $\mu\text{mol/L}$.)

1.3.3 The relationship between calcium and phosphorus

Hypercalciuria with hypercalcaemia is seen in the preterm infant as a result of hypophosphataemia, with a reduction in renal calcium excretion in very low birthweight infants given supplemental phosphorus (Senterre et al, 1983; Lyon et al, 1984; Carey et al, 1985; Chessex et al, 1985; Holland et al, 1990). Low serum phosphorus concentrations stimulates the hydroxylation of 25-hydroxycholecalciferol to 1,25-dihydroxycholecalciferol (1,25-DHCC). The action of 1,25-DHCC on bone raises both plasma phosphorus and calcium concentrations by the action on bone and kidneys (See section 1.5.3).

With increased availability of plasma phosphorus without calcium supplementation, the deposition of both phosphorus and calcium in bone results in reduced circulating calcium concentrations. However, animal studies suggest that the effect on reduced renal calcium excretion may be independent of the reduced plasma calcium concentration and of PTH (Coburn et al, 1971; Lau et al, 1982).

Improved calcium absorption resulting from vitamin D supplementation results in increased urinary losses of calcium unless phosphorus intake is adequate (Senterre et al, 1983) as calcium alone cannot be deposited into bone. Supplementation with phosphorus alone leads to high urinary phosphorus losses (Carey et al, 1985) and hypocalcaemia (Rowe et al, 1979; Kovar et al, 1983), as additional calcium is unavailable for bone mineral accretion.

1.3.4 Calcium and phosphorus requirements

In low birth weight infants the estimated requirements of calcium and phosphorus based on intestinal loss, urinary excretion and the rate of tissue increments at birth are 3.0 mmol/kg/day of calcium and 2.4 mmol/kg/day of phosphorus (Ziegler et al, 1976).

Due to the low phosphorus content of human milk breast-fed infants become primarily deficient in phosphorus. Low birth weight infants fed human milk demonstrate high TRP (99.7%) compared with formula fed infants (82%), indicative of phosphorus depletion (Rowe et al, 1984). The early introduction of phosphorus supplementation has been shown to reduce the incidence of rickets in LBW infants (Holland et al, 1990).

Very low birth weight infants require both calcium and phosphorus supplementation. Individualised supplementation of calcium and phosphorus based on absolute urinary mineral excretion has been shown to improve bone mineralisation in the preterm infant (Pohlandt, 1994a).

The European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) recommendations for very low birth weight formula fed infants are currently 1.8-3.5 mmol/100 kcal calcium and 1.6-2.9 mmol/100 kcal phosphorus. Human milk fed infants, at risk of phosphorus deficiency, require up to 4.2 mmol/100 kcal phosphorus in order to maintain the plasma concentration of

phosphorus above 1.5 mmol/L and avoid excessive urinary calcium loss. Calcium and phosphorus should be provided in a ratio of 1.4-2:1 mg/mg (1.1-1.6:1 mmol/mmol) (Bremer et al, 1987).

1.3.4i Parenteral feeding

The amount of phosphorus and calcium which can be made available in parenteral nutrition (PN) is limited by poor solubility of the mineral salts, although this can be improved by the use of organic phosphate salts (Hanning et al, 1989).

Conventionally calcium gluconate or calcium chloride is administered with mono or dibasic potassium phosphate. The use of glycerophosphate salts permits increased concentrations of calcium and phosphorus even in fluid restricted infants.

Increasing the phosphorus and calcium content of PN by 35% to 1.8 mmol/kg/day calcium and 2.5 mmol/kg/day phosphorus results in improved rate of increase in BMC over the first 8 weeks of life (Prestridge et al, 1993). The aluminium content of PN may have implications for mineralisation in the preterm infant (Koo et al, 1989).

1.4 Complications in the Preterm Infant

1.4.1 Renal function

1.4.1i Development of renal function

The mature kidney has one million nephrons. In the normal mature kidney two thirds of the filtered sodium chloride and water are resorbed in the proximal tubule along with glucose, bicarbonate, phosphorus and amino acids. Nephrons begin to form during the 8th week of life and begin to function at the end of the first trimester. All nephrons are formed by 34 weeks post-conceptual age, but they remain immature in function (Bailie, 1993). Glomerular filtration rate (GFR) in both the preterm and term infant is reduced in comparison to adult GFR even when corrected for surface area (Corey & Spitzer, 1992).

Between 28 and 40 weeks PCA GFR increases 4 fold to 40 ml/1.73m², and reaches adult levels of 100ml/1.73m² by 2 years of age. The fetus has a very low renal blood flow and following birth this rises rapidly, again reaching adult levels by 2 years of age. Likewise tubular function is immature, with the newborn kidney unable to excrete a high solute load and having poor resorptive capacity.

Phosphorus is filtered at the glomerulus with probably no active secretion (Massry et al, 1973). Reduced clearance resulting from diminished GFR is offset by a raised plasma concentration, which allows total excretion to remain constant (Brodehl et al, 1982). Plasma calcium concentration is determined largely by non-renal factors, so that the absolute urine calcium output is related to the GFR. Expressing calcium

excretion per 100ml of glomerular filtrate allows comparison of excretion irrespective of body mass and abolishes the effects of reduced renal function.

1.4.1ii Diuretics

Many preterm infants are treated with diuretics for chronic lung disease and persistent ductus arteriosus. Frusemide is calciuric, inhibiting electrolyte absorption from the ascending loop of Henle (Duarte, 1968). Human adult studies suggest that frusemide increases urinary phosphorus excretion (Puschett & Goldberg, 1968). In preterm infants a single dose of frusemide may result in persisting urinary excretion of both calcium and phosphorus (Morgan & Evans, 1986).

In newborn infants the prolonged use of frusemide in conjunction with high urinary calcium produces an the incidence of nephrocalcinosis of 2.5% (Hufnagle et al, 1982; Ezzedeen et al, 1988). Although radiological features resolve, an association between renal calcification in the neonatal period and functional renal abnormalities at 1 to 2 years (including reduced creatinine clearance and reduced ability to excrete hydrogen ions) has been demonstrated (Downing et al, 1992).

Thiazide diuretics reduce the excretion of calcium and increase the excretion of phosphorus in adults (Brickman et al, 1972). However, in premature infants the administration of thiazides may result in hypercalciuria (Atkinson et al, 1988).

Thiazides act on the distal tubule to increase calcium absorption and decrease the resorption of sodium. A large sodium load may override this mechanism, leading to

natriuresis and calcium wasting, such that sodium supplementation may contribute to urinary bone mineral losses and hence promote the development of metabolic bone disease (Campfield et al, 1997).

1.4.2 Bronchopulmonary dysplasia

In bronchopulmonary dysplasia (BPD), the combination of fluid restriction, reduced caloric and nutrient intake, delay in enteral feeding, and diuretics may have a profound effect on mineral availability. Despite this, a follow up study of preterm infants failed to demonstrate a significant difference in weight, length or BMC (measured by radial SPA) in infants with BPD compared to preterm controls without BPD (Greer & McCormick, 1987). However BMC comparable to term infants at birth was not attained by any of the study infants until at least 6 months post partum.

1.4.2i Corticosteroids

Deleterious effects of elevated cortisol levels on bone have long been recognised (Cushing, 1932). Glucocorticoid-induced bone disease is characterized by decreased bone formation (Dempster, 1989) and cell death of isolated segments of bone by apoptosis (Weinstein et al, 1998). The mechanism is poorly elucidated but appears to involve a reduction in bone formation and bone mineral density with impaired osteoblastogenesis and osteoclastogenesis in bone marrow, and enhanced apoptosis of osteoblasts and osteocytes. In particular, the programmed cell death of osteocytes may explain the continued evolution of bone disease after steroid administration is discontinued (Weinstein et al, 2000).

The long term administration of corticosteroids in adults result in increased urinary excretion of calcium due to reduced tubular resorption (Laake, 1960). Animal

studies show that glucocorticoid administration results in lower circulating calcidiol and calcitriol concentrations, and reduced intestinal absorption of calcium. Piglets receiving dexamethasone exhibit reduced linear growth and lower total body BMD. This occurs as a result of a direct effect on osteoclast and osteoblast activity, and the changes in the endocrine regulation of calcium metabolism (Weiler et al, 1995).

In a cohort of preterm infants given postnatal dexamethasone for prevention of chronic lung disease, trial infants developed early elevation in PTH concentration in association with increased phosphorus excretion and reduced calcium excretion. Radiographic assessment of bone growth was the same in both groups (Lin et al, 1998). Short course dexamethasone therapy administered to premature infants for chronic lung disease results in an initial period of reduced linear growth velocity. Catch-up growth is achieved by 30 days after the treatment is discontinued (Gibson et al, 1993).

1.5 Control of bone mineralisation

1.5.1 Parathyroid hormone

Parathyroid hormone (PTH) primarily maintains the extracellular fluid calcium concentration. It acts directly on bone and kidney and indirectly on intestine through the synthesis of 1,25 DHCC (Bentur et al, 1987). There is evidence of an action on a variety of bone cells, including osteoblasts, osteocytes and osteoclasts. Mature osteoclasts do not appear to have PTH receptors, and it is believed that osteoblasts signal to osteoclasts via mediators such as prostaglandins and interleukins (Gillham et al, 1997).

Within a few minutes of the exposure of bone to PTH a release of calcium and phosphorus from osteoclasts and osteocytes occurs. Subsequently RNA and protein synthesis proceeds within osteoclasts, with release of collagenase and other lysosomal enzymes into the bone matrix. Continuous exposure to PTH results in erosion of calcified bone and release of calcium and phosphorus into the circulation (Gillham et al, 1997) The effects of PTH on osteoblasts are variable and include cell proliferation, matrix protein synthesis and secretion, enzyme synthesis and release of growth factors and cytokines (Potts & Jüppner, 1998).

Intermittent administration of PTH leads to an increase in bone mass, and has been used in the treatment of osteoporosis (Lindsay et al, 1997). Animal models confirm an increase in osteoblast number and bone formation (Dobnig & Turner, 1997), initially thought to be attributed to activation of bone lining cells into osteoblasts

(Dobnig & Turner, 1995). More recently, the increase in bone mass on exposure to PTH has been attributed to reduced apoptosis of mature osteoblasts (Jilka et al, 1999).

In the kidney PTH inhibits the absorption of calcium and phosphorus in the proximal tubule, with increased delivery to the distal tubule. In the distal nephron increased quantities of both sodium and calcium are resorbed with calcium absorption further enhanced by PTH. There is minimal phosphorus absorption in the distal tubule, resulting in phosphaturia (Massry et al, 1973).

PTH is an 84 amino acid which is rapidly synthesised and almost immediately undergoes intracellular degradation. Interpretation of early research on maternal, fetal and neonatal concentrations is complicated by the inability of assays to detect the intact molecule. Inactive fragments and intact molecules are stored and released together. Previous work using radioimmunoassay (RIA) for either the carbon or nitrogen moiety may overestimate the total PTH, or fail to detect an increase in active PTH if the intact active molecules are released with a reduced number of detectable fragments (Bishop, 1989).

The evidence regarding placental transfer of PTH is conflicting. Animal data suggests that intact maternal PTH does not cross placenta (Erenberg et al, 1978). Human studies indicate that placental transfer and degradation of maternal PTH occurs (Balabanova et al, 1986). In the same study, the finding of a higher concentration of PTH in the umbilical artery than umbilical vein suggests that the

fetus is able to produce PTH. Umbilical cord PTH may also be influenced by gestation, mode of delivery and morbidity, such that high PTH concentrations are associated with prematurity, delivery by caesarean section and poor neonatal outcome (Bruchi et al, 1984).

1.5.2 Calcitonin

Calcitonin is a peptide hormone secreted by the parafollicular cells of the thyroid gland. It acts in many ways as an antagonist to PTH, being hypocalcaemic and hypophosphataemic. It reduces bone resorption and increases renal calcium clearance. Synthesis and secretion is regulated by extracellular calcium concentrations and by gastrointestinal hormones, e.g. gastrin (Mundy, 1995). It appears to have little physiological effect in adults and is thought to exert effects only in high bone turnover states (Bentur et al, 1987).

Calcitonin concentration in cord blood is elevated above adult levels (Venkataraman et al, 1985), and may be implicated in the pathogenesis of early neonatal hypocalcaemia (Venkataraman et al, 1987). Concentrations are further elevated in the preterm infant, being inversely related to gestational age (Hillman et al, 1977, David et al, 1981; Seki et al, 1994a). The effect of calcium infusion on calcitonin has been examined in infants developing hypocalcaemia during exchange transfusion (Dincsoy et al, 1982). Calcitonin concentration was seen to rise in response to intravenous calcium administration, reaching significantly higher

concentrations in preterm infants, suggesting a protective effect of calcitonin on bone resorption.

Elevated calcitonin concentrations seen in preterm low birthweight infants are not found in term small-for-gestational age infants (Romagnoli et al, 1987). Mean serum calcium concentrations are lower in preterm infants, with higher calcitonin concentrations in the first week of life. In preterm infants, but not SGA infants, calcitonin levels were further increased in the hypocalcaemic infants.

Similarly, in infants of well-controlled diabetic mothers, calcitonin levels are low at birth and rise over the first 5 days of life (Salle et al, 1982a), although concentrations do not differ from those seen in term control infants (Cruikshank et al, 1983; Mimouni et al, 1990). The authors suggest that calcitonin levels do not appear to be related to the hypocalcaemia seen in infants of diabetic mothers.

1.5.3 Vitamin D

Vitamin D (cholecalciferol) is acquired both through metabolism from provitamin D₃ in the skin, and through the ingestion and absorption of vitamin D of plant (vitamin D₂) or animal (vitamin D₃) origin. In skin provitamin D₃ is converted to previtamin D₃ by the action of ultraviolet radiation, and subsequently isomerises to vitamin D₃. Vitamin D₂ and vitamin D₃ are bound to vitamin D-binding protein (DBP) and are transported to the liver. In the liver, vitamin D is hydroxylated to

produce 25-hydroxycholecalciferol (25-HCC), which is bound and transported to the kidney, where it is hydroxylated again to form 1,25-DHCC (Tsang et al, 1981). 25-HCC is thought to reflect overall vitamin D status, as it is the most abundant circulating form.

Low calcium, low phosphate and high parathyroid hormone levels facilitate the synthesis of 1,25-DHCC in the kidney. Persistent hypophosphataemia stimulates 1,25-DHCC synthesis, enhancing renal tubular absorption of phosphorus and intestinal absorption of both calcium and phosphorus. In the presence of calcium and phosphorus sufficiency when the PTH level is low 24,25-DHCC is synthesised by the kidney. This has a weak effect on mineral mobilisation from bone and increases intestinal absorption of calcium.

1,25-DHCC increases serum calcium and phosphorus concentrations by enhancing intestinal absorption of calcium and phosphorus and increasing the renal tubular absorption of phosphorus and calcium. In mineral sufficiency, it promotes calcium and phosphorus deposition in the epiphyseal-growth plate cartilage and in newly formed bone. However, with PTH it regulates the release of calcium from bone and in the event of mineral unavailability 1,25-DHCC may cause bone resorption, with increased serum and urinary calcium and urinary hydroxyproline excretion (Maierhofen et al, 1983).

Vitamin D is available in a variety of pharmacological preparations. Vitamin D₃ (cholecalciferol) requires hydroxylation by the kidney; alfalcidol (1 α -

hydroxycholecalciferol) and calcitriol (1,25-dihydroxycholecalciferol) are active forms of the vitamin and do not require further metabolism.

1.5.3i Vitamin D and the fetus

Several studies have demonstrated a correlation between maternal and umbilical cord 25-HCC concentrations, confirming that this metabolite crosses the human placenta (Delvin et al, 1982; Hollis & Pittard, 1984; Zeghoud et al, 1997). The relationship between umbilical cord and maternal 24,25-DHCC concentrations is less clear, with some studies suggesting a correlation (Delvin et al, 1982; Hollis & Pittard, 1984), and others refuting this (Hillman et al, 1978).

The fetal supply of 1,25-DHCC is predominantly synthesised by the feto-placental unit (Delvin et al, 1982). Renal 1α -hydroxylase appears to be functional in the human fetus immediately after birth (Glorieux et al, 1981). 1α -hydroxylation is the rate limiting step, the activity of which is enhanced by a low plasma calcium (via PTH) and low plasma phosphorus level.

1.5.3ii Vitamin D requirement of the preterm infant

Early studies indicated that in the very preterm infant plasma concentrations of 25-HCC may fall following delivery (Hillman & Haddad, 1975), and there is consensus

agreement that preterm infants require supplemental vitamin D. However the suggested dose ranges from 400 to 1200 IU. Early studies involved infants on low mineral supplementation and results were often based on the finding of radiological rickets rather than more precise measures of MBD.

In a large cohort, 50% of formula fed premature infants developed low 25-HCC concentrations despite supplements of 400 IU/day vitamin D₂; radiographic evidence of hypomineralisation correlated with concurrent dietary mineral insufficiency (Hillman et al, 1985a). In the absence of mineral supplementation a reduction in radiographic hypomineralisation can be seen with vitamin D₂ supplementation of 800 IU/day (Hillman et al, 1985b). Other studies in premature infants have failed to demonstrate improvements in plasma 25-HCC concentrations or rickets on doses of vitamin D supplementation exceeding 400-500 IU/day (Robinson et al, 1981; Markestad et al, 1983a; Evans et al, 1989). The administration of 25-HCC appears to be no more effective than cholecalciferol (Hillman, 1985b).

Although it has been shown that 1 α -hydroxylation is taking place in the preterm infant (Glorieux et al, 1981), infants receiving daily vitamin D supplements who later developed rickets have been shown to have lower serum 1,25-DHCC concentrations than those infants who did not develop rickets (Seino et al, 1981). The administration of 1,25-DHCC has been shown to elevate plasma concentrations of 1,25-DHCC, but this also results in a high fractional excretion of hydroxyproline due to the direct mobilisation of calcium from bone (Salle et al, 1982b). Currently it

is suggested that 1,25-DHCC is given only to infants with a defect in 1α -hydroxylation, and that calciferol is the analogue of choice.

Some authors feel that there is little evidence that vitamin D deficiency is implicated in MBD (McIntosh et al, 1982). Despite high doses (2000 IU/day) of vitamin D their human milk-fed infants had a high incidence of demineralisation. 1,25-DHCC has been found to be elevated in association with osteopenia of prematurity on dietary supplementation of 500 IU/day vitamin D. In conjunction with low plasma phosphorus concentration this suggests phosphorus deficiency as the primary pathology (Markestad et al, 1983b). In a case report plasma 1,25-DHCC concentrations reduced following phosphorus supplementation (Rowe et al, 1979), suggesting that adequate mineral supply was more important than high dose vitamin D supplementation. Currently it is accepted that adequate mineral supplementation negates the need for high vitamin D supplementation, although infants receiving prolonged parenteral nutrition are at increased risk of metabolic bone disease and may require higher doses of vitamin D when enteral feeds are commenced (Campbell & Fleischman, 1988). However, the current ESPGHAN guidelines recommend a daily intake of 1000 IU vitamin D in enterally fed premature infants (Bremer et al, 1987).

1.5.3iii Genetic influence of vitamin D receptor genotype

There are conflicting data regarding the influence of the vitamin D receptor (VDR) gene on bone mineralisation (Jorgensen et al, 1996). Twin studies have suggested an important link between the VDR gene and BMD (Spector et al, 1995), with the bb allele conferring higher BMC than the BB allele (Morrison et al, 1994).

However intrapair differences in bone mineralisation increase with age (Smith et al, 1973) and it has been suggested that environmental factors influence a genetic predisposition (Pocock et al, 1987).

1.5.4 Parathyroid hormone related protein

Parathyroid hormone-related protein (PTHrP) is a polypeptide that exerts biological properties similar to PTH, acting via the PTH receptor. It consists of a variable length protein species of 139, 141, and 173 amino acids, with 8 of the 13 N-terminal sequence being shared with PTH (Moseley et al, 1987). Pharmacological studies indicate that the action of PTHrP in animals mimics that of PTH excess. PTHrP is responsible for the hypophosphataemia, hypercalcaemia and increased urinary cyclic AMP seen in humoral hypercalcaemia of malignancy (HHM) (Martin & Moseley, 1990).

PTHrP has also been shown to exhibit paracrine functions as a developmental regulatory molecule. These functions include the regulation of endochondral ossification, involving a negative feedback loop involving the paracrine factor, Indian hedgehog (Ihh) (Lanske et al, 1996; Vortkamp et al, 1996). Ihh is produced by prehypertrophic chondrocytes, which in turn increases the production of PTHrP. This binds to the PTH/PTHrP receptor on prehypertrophic chondrocytes, inhibiting their differentiation and hence further production of Ihh, in a negative feedback loop. As the committed cells progress, they cease to produce Ihh, attenuating the negative feedback loop and allowing the differentiation of prehypertrophic chondrocytes. By this mechanism, the actions of PTHrP and Ihh inhibit chondrocyte differentiation and promote cell proliferation and linear growth. Evidence for the importance of functioning PTHrP receptors in man has been demonstrated by both delayed endochondral ossification caused by active PTHrP receptors in Jansen's metaphyseal dysplasia (Schipani et al, 1995), and advanced ossification due to nonfunctional receptors in Blomstrand chondrodysplasia (Jobert et al, 1998).

Low plasma concentrations of PTHrP are normally present in the adult, but both placenta and fetal parathyroid glands show evidence of PTHrP production. PTHrP stimulates calcium transport across the sheep placenta (Rodda et al, 1988). It is present in large amounts in the placenta early in gestation, suggesting that this may be the initial source of PTHrP, with fetal parathyroid glands playing a part later in gestation (Martin & Moseley, 1990). Maternal milk of several mammalian species, including humans, has been shown to contain high concentrations of PTHrP, at concentrations more than 100 times those seen in HHM (Budayr et al, 1989).

Concentrations in infant formula milk vary from undetectable to 1/3 of those in human milk, and it is absent from soy-formula.

PTHrP concentrations have been shown to be higher in umbilical cord blood than in non-pregnant and pregnant serum samples (Hillman et al, 1990; Thiebault et al, 1993). Umbilical arterial concentrations are elevated above umbilical venous concentrations, suggesting synthesis of PTHrP by the fetus (Seki et al, 1994b).

Other researchers have failed to demonstrate this materno-fetal gradient and suggest factors other than PTH or PTHrP may contribute to placental calcium transport (Khosla et al, 1990; Papantoniou et al, 1996).

1.6 Assessment of bone mineralisation

Accurate determination of bone mineralisation is difficult to obtain in the clinical setting. Severe demineralisation occurs long before changes of craniotables and rachitic rosary are apparent by clinical examination. Similarly hypomineralisation is not detectable by radiography until 30-40% bone loss has occurred (Ardran, 1951).

The x-ray classification of MBD describes degrees of severity from grades I to III as follows: I - loss of dense white line at metaphyses, increased submetaphyseal lucency and thinning of the bone cortex (osteopenia), II - grade I plus irregularity and fraying of metaphyses, with splaying and cupping (overt rickets), III - grade II plus fractures (Koo et al, 1982).

Biochemical markers of bone turnover have been used to estimate bone mineral status with limited success. Bone imaging techniques provide a more reliable assessment of bone mineralisation, but are often not available for clinical use.

1.6.1 Biochemical markers

1.6.1i Alkaline Phosphatase

Alkaline phosphatase (AP) is an enzyme located on the membrane of the osteoblast-derived “matrix vesicles”, and in parent osteoblasts is close to the mineralisation front. Membrane phosphatase is a phosphotransferase which transfers phosphate residues into matrix vesicles where, together with calcium ions, crystallisation is

initiated. As mineralisation proceeds, vesicles and osteoblasts are disrupted and alkaline phosphatase leaks into the circulation. When substrate deficiency is present an increase in alkaline phosphatase production may occur, such that an increase in plasma activity is detected despite a reduction in mineralisation (Lucas et al, 1989).

Most circulating alkaline phosphatase is derived from bone and liver, but bone forming osteoblasts have high amounts of alkaline phosphatase. In infants 90% of serum AP activity is derived from bone (Pittard et al, 1992), and in the absence of liver disease plasma AP activity correlates with bone formation. Measurement of plasma AP concentration has poor sensitivity and specificity in relation to radiographic bone changes of osteopenia (Walters et al, 1986; Evans et al, 1989; Pittard et al, 1992). Although levels up to five times the normal adult range may be seen in preterm infants (Kovar et al, 1982), with MBD as diagnosed by radiography and clinical pathology at 7.5 times the adult reference range (Kovar et al, 1982), some infants have evidence of rickets without a rise in AP (McIntosh et al, 1984).

1.6.1ii Osteocalcin

Osteocalcin (OC) or gamma carboxyglutamic acid-containing (Gla) protein (BGP) comprises 20% of the non-collagen protein in bone. It is synthesised by osteoblasts, and provides a marker of remodelling activity (Mundy, 1995). γ -carboxylation of osteocalcin is vitamin K dependent. Serum OC concentrations fall with age, and are higher in breast-fed than formula fed infants (Lichtenstein et al, 1987). OC is

removed from the circulation by renal clearance and serum concentrations in both adults and infants are significantly altered by impaired renal function (Delmas et al, 1983; Charles et al, 1985). A correlation has not been found between OC and MBD or AP in the preterm infant (Pittard et al, 1992).

1.6.1iii Collagen assays

There are at least 13 genetically distinct types of collagen molecules in mammalian connective tissue. Bone is composed primarily of type I collagen which constitutes 90% of bone matrix. It is a complex molecule consisting of 2 pro- α 1(I) and 1 pro- α 2(I) polypeptide chains. Crosslinking between molecules occurs at both the N-terminal and C-terminal ends by specific N-terminal and C-terminal peptidases. The molecules are organised in a characteristic staggered component with gaps between the end of one molecule and the beginning of the next, the “hole zones”, where mineralisation occurs.

Collagen molecules are packed end to end within collagen fibrils. Interactions occur between collagen and extracellular macromolecules, e.g. fibronectin, osteonectin and proteoglycans. Once alignment of collagen molecules and related macromolecules occurs mineralisation can take place (Mundy, 1995).

C-terminal propeptide of type I procollagen (PICP) quantitatively reflects type I collagen synthesis and is produced by proliferating osteoblasts. It is principally a

marker of bone formation and has been shown to correlate with changes in BMC in preterm infants (Crofton et al, 1999).

The cross-linked C terminal telopeptide of type I collagen (ICTP) is a marker of type I collagen breakdown. Serum concentrations are high at birth and subsequently fall (Crofton et al, 1999). This marker has not been shown to correlate with changes in BMC.

1.6.1iv Hydroxyproline

Hydroxyproline is found almost exclusively in collagen, and its presence in urine is seen as a marker of collagen degradation. In preterm infants urinary excretion of hydroxyproline is increased although there is no correlation between urinary concentrations in infants with and without rickets or fractures. The high excretion rates may be related to immature renal function (Koo et al, 1990).

1.6.1v Pyridinium Crosslinks

Pyridinoline (Pyd) and deoxypyridinoline (Dpd) can be measured in the urine as markers of collagen degradation, with Dpd being more bone specific. Both have been found to correlate positively with birthweight (Crofton et al, 1999), and may

rise after birth (Naylor et al, 1999). More data is required to assess the use of pyridinium crosslinks as a surrogate for bone imaging techniques.

1.6.2 Imaging Techniques

1.6.2i Single Photon Absorptiometry

Single photon absorptiometry (SPA) was first described by Cameron and Sorensen in 1963 (Cameron & Sorenson, 1963). An iodine-125 source is used to produce a beam of collimated photons that pass to a photomultiplier detector. The bone attenuates the photon beam and as BMD is inversely related to the logarithm of the bone compared to the soft tissue, BMD may be calculated. It assumes that the site of measurement is a cylinder of constant width and that bone is surrounded by a water-equivalent material such as muscle. Fat attenuates the beam less than water. As the depth of the bone is not measured the measurement is expressed as a result of photon attenuation per unit area, an areal density.

It has accuracy and precision of 2-5%, a radiation dose of 5mRem and a scan time of 10-15 minutes (Shaw & Bishop, 1995). It is limited by being useful in only measuring bones in the peripheral skeleton, most commonly the radius. The scan may be performed at the mid-radius (97% cortical bone), distal radius (80% cortical bone) or ultradistal radius (30% cortical bone, 70% trabecular bone). Cortical bones have a low turnover. Trabecular bone is therefore more sensitive to mineral changes. There are no significant differences between BMD of the midradius and

distal radius by SPA, but a difference between either of these and lumbar spine as measured by dual photon absorptiometry (DPA) in adult subjects (Seeman et al, 1982). Conventional methods of measuring the distal 1/3 radius may be a problem in longitudinal studies as the bone lengthens with time giving a different location of measurement (Greer et al, 1982)

A portable single photon absorptiometer, specifically designed for use in preterm infants has been developed (Truscott 1996). The neonatal bone mineral device uses a charge couple device (CCD) to acquire a 2-dimensional bone image. Photons are produced at 27.5keV by the decay of ^{125}I point source, and a cone beam is passed through the infant's forearm. Photons are differentially absorbed by bone of varying mineral content resulting in a beam of differing intensities, inversely proportional to the bone mineral, falling on the detector. A bone mineral equivalent image can be calculated on a pixel by pixel basis. In order to compensate for the different radiation path lengths produced by the cone beam a water bolus image is obtained and subtracted from the image of the infant arm. The bone mass per unit area (M_B) is given as:

$$M_B = [\rho_B / (\mu_B \rho_B - \mu_S \rho_S)] \ln I_0 / I$$

where μ_B and μ_S are the mass attenuation coefficients for hydroxyapatite (3.1704 cm^2/g) and water (0.48016 cm^2/g) and are ρ_B and ρ_S are the density values for hydroxyapatite (3.225 g/cm^3) and water (1.0 g/cm^3). I is the photon intensity of the

image pixel and I_0 the photon intensity in the same pixel through the water bolus alone.

BMC in grams of hydroxyapatite is calculated by integration of the bone image within a region of interest (ROI) and an equivalent area in the non-bone background is subtracted. The area normalised BMD is then calculated by dividing the BMC by the area of the BMD.

The Xray sensitive video camera coupled to the CCD imager provides input to an integrated image processor and frame grabber allowing real time image capture, with an image region of 40mm by 30mm. Data is stored as a 16-bit image file and archived to hard disk drive.

The ^{125}I source is held in a shielded container with simple shutter. The source, forearm holder and camera are encased in perspex, and are coupled to the video output, camera power, and control signals mounted on a small trolley. The instrument measures 52cm by 20cm permitting use within a neonatal incubator.

The infant forearm is placed between 2 perspex sheets, the sheet furthest from the detector being adjustable to allow for the width of the arm. The distance between the positioned sheets is read from a 1 mm gauge. Following image acquisition the infant arm is removed and a background image is taken of a water bolus of identical width, using a water-filled condom.

The system has been shown to produce forearm BMD ranging from 43 to 115 mg/cm² in babies aged 23-41 weeks post-conceptual age. Linearity has been demonstrated using aluminium foils. The absorbed radiation dose to skin is 6uSv.

1.6.2ii Dual Photon Absorptiometry

Dual photon absorptiometry (DPA) uses an isotope with two energies, usually gadolinium (¹⁵³Gd) with photopeaks at 44 and 100keV. Bone attenuates the low energy photons more than the high energy photons and so summation of the relative attenuation of the two energies may be used to estimate BMD of the spine, hip and total skeleton. A single scan takes 15-20 minutes and has a radiation dose of 5mRem. DPA has an accuracy of 4-10% and precision of 2-4%, although the precision is reduced as the source decays (Shaw & Bishop, 1995).

1.6.2iii Quantitative Computed Tomography

Quantitative computed tomography is the only technique that measures true bone density. It relies on the principle that mineralised tissues absorb ionising radiation to a greater extent than soft tissue. It can separately measure trabecular and cortical bone within the spine and is not influenced by vertebral size. Scan takes 10-20 minutes, accuracy of 10-15%, precision 2-4%. It provides a significant radiation

dose of 100mRem, limiting use in children and longitudinal studies (Shaw & Bishop, 1995).

1.6.2iv Dual energy x-ray absorptiometry

In dual energy x-ray absorptiometry (DXA) a beam of collimated x-rays are transmitted from a source to a detector located above the subject, producing a measure of bone mineral density by correcting the bone mineral content for the projected bone area. The beam is of higher intensity than DPA and may be more highly collimated allowing for improved spatial resolution and increased scan speed (Eastell & Wahner, 1990). The first generation machines took up to 8 minutes to obtain an image, but this has been reduced to 45 seconds on fan beam machines. Accuracy and precision is 1-2% and the radiation dose is small at 3-5mRem. DXA also provides estimates of total lean body and fat mass in all but small infants. Different machines use different methods of generating the dual-energy source and this may result in differing estimates of BMD (Eastell & Wahner, 1990).

SPA, DPA and DXA measure area and do not allow for differences in bone thickness. Therefore BMD is overestimated in large bones and underestimated in small bones. An attempt to correct for this has been described, using bone mineral apparent density which is adjusted for bone volume (Carter et al, 1992).

1.6.2v Broad band ultrasound attenuation

Broad band ultrasound attenuation of the calcaneum is correlated with total body bone mineral density. This method uses no ionising radiation but is less sensitive than DXA. It provides information about bone architecture, and may be a useful adjunct to radiological techniques of bone density measurement (Mughal et al, 1996).

1.7 Bone mineralisation in the newborn infant

BMC of the forearm correlates with weight rather than gestation, such that low birthweight infants have reduced BMC for gestation (Minton et al, 1979; Ryan et al, 1988; Pohlandt & Mathers, 1989). Similarly, total body bone content (TBBC) as measured by DPA and DXA is related to birthweight, being reduced in the small for gestational age (SGA) infant (Petersen et al, 1989; Koo et al, 1996; Lapillonne et al, 1997). The appropriate for gestational age (AGA) preterm infant however has a lower TBBC than the SGA term infant of the same weight.

Extremely low birthweight infants fail to achieve any increment in radial BMC between birth and 40 weeks PCA, such that at 39 weeks PCA BMC in premature born infants is 50% of control infants born at term. These infants also fail to exhibit weight gain and increase in crown-heel length, suggesting that osteopenia is a result of both reduced growth and reduced density of mineralised bone mass (Horsman et al, 1989a). However by 65 to 100 weeks PCA there is no difference in BMC between the premature and term infants, although the preterm group remain significantly lighter with reduced crown-heel length (Horsman et al, 1989b).

Using whole body DXA, preterm infants have been shown to have reduced TBBC at term as compared to infants born at full-term, irrespective of dietary supplementation (Lapillonne et al, 1994; Wauben et al, 1998). By 6 months of age (3 months post-term) Lapillonne's infants had a TBBC similar to full-term infants at birth. However, there is a lack of consensus as to whether TBBC as measured by

DXA should be interpreted in relation to weight-matched or age-matched infants (Lapillonne & Salle, 1999). In low birthweight infants lumbar spine BMD is more closely correlated with birthweight than whole body BMD (Ichiba et al, 2001). The authors found no correlation between lumbar spine BMD and total body BMD at 40 weeks PCA and suggested that in infants less than 4kg lumbar spine BMD is more suitable than TBBC for serial evaluation of changes in mineralised bone mass, due to the rapid metabolic turnover of cancellous bone, and the large contribution of the mineralised bone mass of the skull in low birthweight infants

1.7.1 Biochemical markers of mineralisation

North American summer-born infants have been shown to have lower radial BMC than winter-born term infants, in association with elevated osteocalcin and 1,25-DHCC concentrations (Namgung et al, 1994). Conversely, a study using DXA to assess total body bone mineral content demonstrated lower TBBC in Korean winter-born infants despite similar changes in 1,25-DHCC and cord calcium concentrations (Namgung et al, 1998), similar to findings in adults and older infants. The authors suggest that these differences may be related to vitamin D deficiency seen in the Korean mothers, in contrast to the 25-HCC sufficient American mothers.

SGA infants have reduced radial BMC proportional to birthweight as compared to AGA infants, in association with reduced 1,25-DHCC and OC concentrations (Namgung et al, 1993). In conjunction with mineralised bone mass, SGA infants have been shown to have an unexplained elevation of PTH concentration at birth compared to AGA controls (Minton et al, 1983). They suggest that this may be detrimental to bone growth, but found a subsequent reduction in serum concentration over the first 12 weeks of life in both AGA and SGA infants, by which time the BMC of SGA infants had reached the normal BMC of AGA infants at birth.

No differences in carboxyterminal propeptide of type 1 procollagen (PICP) or cross-linked carboxyterminal telopeptide of type 1 collagen (ICTP) have been shown in SGA and AGA infants suggesting that reduced BMC in SGA infants is not related to

altered fetal bone collagen synthesis or degradation, but to mineral supply (Namgung et al, 1996).

1.7.2 Supplementation

In infants born at term BMC is independent of type of milk feeds received in the first year (Chan et al, 1982). In contrast, breast fed preterm infants are demineralised at 1 year compared to formula milk-fed infants, although this has resolved by 2 years of age (Abrams et al, 1989; Schanler et al, 1992). Protein and mineral fortification of human milk results in improved bone mineralisation and rate of growth comparable to that achieved with preterm formula (Greer & McCormick, 1988). However, low birthweight infants fed with either fortified human milk or preterm formula for the first 3 months of life remain significantly demineralised compared to full-term newborns at birth (Lapillonne et al, 1994).

In a non-randomised study of 74 low birthweight infants (birthweight median 970g, range 430-1580g) those demonstrating urinary excretion of both calcium and phosphorus in more than 50% of collected urine samples (n=30) were shown to have improved humeral BMC, although there were no significant differences in the amount of calcium and phosphorus received by the two groups (Pohlandt, 1994a). The authors suggest that individualised mineral supplementation based on urinary calcium and phosphorus excretion may enhance bone mineralisation in the preterm infant.

The Cochrane Library contains 2 reviews pertaining to the assessment of mineral supplementation of human milk on bone mineral content (Kuschel & Harding, 1998; Kuschel & Harding, 2001). A review of multicomponent fortification of human milk (Kuschel & Harding, 1998) evaluates the impact of commercially-manufactured human milk fortifiers against human milk feeding alone. A statistically significant improvement in bone mineral content is concluded, although the effect is produced from one larger study, with several smaller studies failing to demonstrate a difference. In an attempt to isolate the effects of calcium and phosphorus from multi-nutrient supplementation of human milk in the preterm population (Kuschel & Harding, 2001), the same authors conclude that there are no randomised controlled trials eligible for inclusion.

Several studies demonstrate that the low birthweight infant should continue to receive supplemented feeds after hospital discharge in order to enhance bone mineralisation. At 16 weeks post discharge infants receiving high calcium containing formula have increased radial BMC compared to breast-fed infants (Chan, 1993). Infants fed nutrient-enriched formula feeds after discharge showed improved radial BMC at 3 and 9 months post term after adjusting for body weight (Bishop et al, 1993).

Similarly Raupp demonstrated improved mineralisation at 3 months post-term in preterm infants receiving calcium and phosphorus enriched formula at hospital discharge (Raupp et al, 1997). In this cohort bone width increment remained greater in the supplemented group at 6 months, although no significant differences were

found in radial BMC at this time. Raupp's trial infants received more phosphorus and calcium, and the control group more calcium than the infants in Bishop's study.

1.7.3 Follow up

The effect of early nutrition on mineralisation in the preterm infant remains difficult to quantify, with studies providing conflicting conclusions. It has been suggested that mineralisation in preterm-born children aged 5 months to 15 years is not influenced by early nutrition, but related directly to birthweight and current weight (Rubinacci et al, 1993; Kurl et al, 1998). However, a study of the effect of early nutrition on bone mineralisation in preterm infants at 5 years of age suggests that maternal milk enhances radial BMC (Bishop et al, 1996).

In the newborn period the infants had all received maternal breast milk and had been randomly assigned top-up feeds of banked donor breast milk or preterm formula. In multiple linear regression analysis BMC was positively correlated with the amount of maternal milk received in the postnatal period. The authors suggest that the reduced mineral content of human milk may "programme" these infants to have "thrifty" bones such that when exposed to an increased mineral supply "over-mineralisation" occurs. They found no difference in mean BMC between the 2 groups, or between AGA and SGA infants at 5 years of age.

A larger follow-up study at 8 to 12 years of age of preterm infants randomised to the same feed regimens during the neonatal period, found that the differences in BMC seen at 5 years of age had disappeared. BMC and BMD as measured by both DXA and radial SPA were related only to body size, and not to early feed regimen. The preterm children were shorter and lighter, but when corrected for size BMC and BMD were not different from term controls. Growth retarded preterm infants were smaller still, but again BMC and BMD remained related to size (Fewtrell et al, 1999). The authors suggest that efforts to increase bone mass during childhood should be directed towards increasing body size.

A Finnish study found that preterm infants were within control reference ranges for height, weight and BMC at 6-7 years age. BMC was again related to current weight and bone area, but a correlation also exists with weight and height at one year of age, such that prematurely born children who were thinner at one year had higher BMC at 6-7 years (Kurl et al, 1998).

1.7.4 More than just bones

Early intervention to prevent the onset of metabolic bone disease in preterm infants may have wider implications than the prevention of rickets and fractures.

Dolicocephalic head flattening of preterm infants is influenced by mineral supplementation and BMC, as shown by a 27% contribution to head shape by BMC/body weight ratio in preterm infants at discharge (Pohlandt, 1994b).



Alterations in head shape may also have an impact on the incidence of myopia in the child born prematurely (Pohlandt, 1994c). As well as reduced BMC measured at term, dental enamel hypoplasia and hypomineralisation has been described in a cohort of preterm infants at 3 years PCA when compared to control infants born at term (Drummond et al, 1992). Osteopenia is also a contributing factor in respiratory distress (Glasgow & Thomas, 1977) and possibly chronic oxygen dependency.

Several studies have indicated a link between reduced linear growth and reduced mineralisation in preterm infants. Preterm infants fail to exhibit catch-up growth comparable to term peers in the first 3 years of life (Casey et al, 1990; Casey et al, 1991).

Biochemical evidence of rickets coexists with reduced short term linear growth. Infants with high peak AP in the neonatal period were significantly shorter at 18 months (Lucas et al, 1989). They also had significantly lower plasma phosphorus, urinary phosphorus and higher urinary calcium concentrations, suggesting phosphorus deficiency. These infants exhibited slower growth rate in the neonatal period. It has been suggested that although weight gain in preterm infants is related to energy intake, phosphorus supplementation may influence linear growth (Mize et al, 1995).

1.8 Fetal origins of adult disease

The concept of programming was described by Lucas (Lucas, 1991) as a stimulus or insult acting at a critical or sensitive point in development and resulting in a long term effect on the structure or function of the organism. Barker (Barker & Osmond, 1992) observed that areas of the UK which currently has high mortality rates from cardiovascular disease had high infant mortality 50 years previously. The Barker group began a series of geographical studies exploring this relationship, and a wealth of epidemiological research has followed, supporting the hypothesis that poor nutrition and health in the female population results in increased death rates from cardiovascular disease in their offspring. The most striking relationship was found between low birth weight and high systolic blood pressure in adult life (Barker et al, 1990), but relationships between newborn anthropometric characteristics and non-insulin dependent diabetes (Hales et al, 1991), cholesterol concentrations (Fall et al, 1992) and coagulation factors (Barker et al, 1992) have also been explored.

Other researchers have sought to develop Barker's theory by investigating twin pairs in an attempt to exclude maternal and genetic factors, and focusing on the birthweight differences of the infants (Dwyer et al, 1999; Poulter et al, 1999). However, findings have been inconclusive, confounded by small numbers of monozygotic pairs.

Although SGA infants appear to be at increased risk of cardiovascular disease in adult life, the mechanism remains unclear. Epidemiological studies have focused on birthweight, length, head circumference and placental weight, but largely exclude gestation as a variable, primarily as accurate information is not available (Wilson, 1999). More recent studies describe the SGA age infant without discriminating between the growth-restricted infant and constitutionally small infant, although some authors have controlled for target height (Leger et al, 1997).

Early nutrition appears to play a part in subsequent growth and cognitive function (Lucas et al, 1990; Sorenson et al, 1997), but larger twin studies are required to exclude genetic predisposition to adult disease in growth-restricted infants. The preterm infant provides a unique opportunity to study the programming effect of early nutrition as they are at a stage of rapid growth and maturation (Morley & Lucas, 1994).

1.9 Physical activity and mineralisation

During weight bearing and muscle contraction, minor deformations of bone occur.

These stresses are important in the normal development of bone. The *in utero* environment provides a weightlessness and physical containment in flexion in which the term infant develops muscle tone (Short et al, 1996). Movement in the sick preterm infant is frequently reduced by the use of analgesics, sedatives and paralysis. Even the active preterm infant is exposed to a gravitational environment without containment.

The importance of muscular activity on bone development is supported by the presence of hypomineralised long bones in newborns with fetal akinesia syndromes (Rodríguez et al, 1988). In severe cases cortical bone is replaced by cartilaginous tissue. However in all cases the long bones were thinner than expected; a phenomenon seen in rickets of prematurity.

In vitro studies in bones of fetal mice has demonstrated increased mineralisation in bones exposed to minimal physical loading comparable to that produced by muscle contraction (Van't Veen et al, 1995). A small randomised study in preterm infants suggests a beneficial effect of passive range-of-motion exercises on both BMC and weight gain (Moyer-Mileur et al, 1995).

1.10 Effects of prematurity on cellular control

Potential candidates in the control of mineralisation *in utero* include cytokines, human placental lactogen, human growth hormone-variant and oestrogen.

1.10.1 Cytokines

Cytokines are a diverse group of proteins which mediate autocrine or paracrine interactions in many tissues. Cytokines are implicated in the control of bone cell activity and abnormal control of cytokine production may be relevant in the pathogenesis of adult bone disease. It has been suggested that the effects of the various cytokines on bone may be dependent on the stage of development of the skeleton (MacDonald and Gowan, 1992). The role of cytokines in reduced mineralisation in the newborn infant has not been explored.

Insulin-like growth factors, transforming growth factor- β (TGF- β) and bone morphogenic proteins (BMPs) stimulate bone formation. Conversely, interleukin- $I\alpha$ and β (IL- $I\alpha$ and β), and tumour necrosis factors α and β (TNF α and β) are potent stimulators of bone resorption (Gowen et al, 1983; Pfeilschifter et al, 1989), with interleukin-6 (IL-6) exerting a permissive effect on bone resorption. Both groups of cytokines stimulate bone cell proliferation, but have opposing effects on mature osteoblast function, such that TGF- β , IGFs and BMPs stimulate osteoblast function, whereas IL-Is and TNFs lead to activation of bone resorption and paralysis of local

bone formation to allow unopposed osteoclast action (MacDonald and Gowan, 1992).

1.10.2 Sex hormones

In postmenopausal women oestrogen has a protective effect on the skeleton. This effect may be modulated by cytokines, particularly IL-6.

Oestrogen, testosterone and dihydrotestosterone have been shown to inhibit IL-6 production in human and animal models *in vitro* (Jilka et al, 1992; Girasole et al, 1992; Bellido et al, 1995). Oestrogen loss results in up-regulation of IL-6 production (Passeri et al, 1993). Following loss of sex steroids, the IL-6 knockout mouse fails to show the expected loss in bone mass (Manolagas, 1998).

In vitro studies in fetal rat metatarsal bones have demonstrated that oestrogen promotes chondrocyte proliferation with subsequent bone lengthening, whereas dihydrotestosterone has a greater effect on increasing bone width. A combination of oestrogen, dihydrotestosterone and progesterone together resulted in less bone growth but increased calcification (Chamoux et al, 1997).

Evidence for the importance of oestrogen in human fetal bone development is found in pathological oestrogen deficient states. Oestrogen deficiency due to defects in the aromatase gene and oestrogen resistance result in abnormalities of bone

development, including increased bone turnover, reduced bone mineral density and failure of epiphyseal fusion in both males and females (MacGillivray et al, 1998; Smith et al, 1994).

During pregnancy high concentrations of fetal, maternal and placentally derived oestradiol and progesterone are available to the fetus (Tulchinsky et al, 1972). This supply is disrupted in preterm delivery. A recent study in premature infants examined the effects of oestrogen and progesterone replacement on bone mineral accretion in extremely preterm infants. This randomised controlled trial suggests that postnatal hormone replacement may improve mineralisation in this population (Trotter et al, 1999).

1.10.3 Growth hormones

The growth hormone-prolactin-related hormones are classified into two categories, some having primarily lactogenic effects and others having mainly somatogenic effects. As a group they modulate linear bone growth mediated by the generation of local and hepatic IGF-1.

During the first 2 trimesters circulating GH is derived from the fetal pituitary gland. However in the last trimester this is replaced by placental growth hormone or human growth hormone variant (hGH-V), which is detectable in the maternal circulation between 21 and 26 weeks and increases to a peak at 36 weeks (Frankenne et al,

1987). hGH-V is not detectable in the fetal circulation. Maternal IGF-I concentrations are positively correlated with hGH-V rather than human placental lactogen (hPL) or pituitary GH (Caufriez et al, 1990). It has been suggested that hGH-V may influence fetal growth by interfering with the maternal metabolism of IGF-I or by modulating placental development (Mirlesse et al, 1993).

hPL is secreted by the placenta and is detectable in the fetal circulation. It has low somatotrophic action, but has a direct action on fetal tissues stimulating IGF-I production (Handwerger & Freemark, 2000). A role in bone metabolism is suggested by evidence that 1,25-DHCC stimulates the synthesis and release of hPL by the placenta (Stephanou et al, 1994).

GH receptors have been demonstrated in a variety of fetal tissues at 14-16 weeks gestation, but appear to be absent from fetal skeletal tissue and epiphyseal growth plate (Hill et al, 1992). The appearance of the GH receptor in late gestation may play a role in the transition from GH-independent fetal growth to GH-dependent postnatal growth (Handwerger & Freemark, 2000). Conversely the hPL receptor is expressed widely in the fetus from early in development. Studies of transgenic mice bearing deletions of the prolactin receptor gene showed delayed ossification and defects of bone formation persisting into adulthood (Clément-Lacroix et al, 1999).

1.10.4 Preterm delivery

At delivery the preterm infant loses the placental supply of hPL, hGH-V, and oestrogen. Control of IGF-1 by pituitary GH is insufficient in the extremely preterm

infant due in part to lack of receptors, and may have an immediate impact on growth. The reduction in hPL and oestrogen results in increased IL-6 production, producing a permissive effect on osteoclastic bone resorption. Postnatally, increases in vitamin D may influence mineralisation via the effect on oestrogen receptors.

Further studies in animal models will be helpful in clarifying the control of fetal mineralisation. The regulation of fetal growth changes at delivery (Gluckman, 1989). The preterm infant is not only deprived of the uteroplacental nutritional supply, but is exposed to a new hormonal environment for which it may not be prepared.

2 Methodology

2.1 Cohort study

2.1.1 Introduction

Osteoporosis is a major public health problem, with osteoporotic fractures being directly related to peak bone mass acquired through skeletal maturation and subsequent bone mineral losses related to disease states, age and/or menopause. The optimisation of peak bone mass has been targeted through dietary and exercise interventions during childhood, however the concept of nutritional programming suggests that the nutritional and/or hormonal environment in fetal and early neonatal life may also have long term implications.

Fetal nutrition is dependent on the integrity of the materno-placento-fetal unit. In placental insufficiency the fetus is at risk of mineral insufficiency and consequent reduced mineralised bone mass. It has been suggested that high ionised cord calcium concentrations in infants of diabetic mothers are responsible for suppressed parathyroid function (Tsang et al, 1975).

2.1.1i Aim:

To explore the relationship between mineralised bone mass at birth and potential fetal mechanisms implicated in the control of materno-fetal calcium transport across the gestational range of viability.

2.1.1ii Hypothesis:

In impaired transplacental calcium delivery, PTH production by the fetus is increased, such that the growth restricted infant will demonstrate elevated cord blood PTH concentrations, in association with reduced mineralised bone mass (MBM).

2.1.2 Patients and Methods

Caucasian infants born at the General Infirmary of Leeds between October 1997 and July 1999 were considered for inclusion in the study. Infants with suspected or antenatally diagnosed chromosomal abnormalities or skeletal anomalies were excluded. Recruitment was targeted at preterm infants and infants who were expected to be small for gestational age. Appropriately grown term infants were recruited on an opportunistic basis, with an attempt to include both AGA and SGA infants in each month of recruitment.

Up to 8 ml of umbilical venous cord blood was taken by a single researcher (AH) from the placental circulation following delivery of the placenta. The samples were decanted into 2ml lithium heparin bottles and centrifuged within 15 minutes (by AH). The plasma was then separated, frozen at -70°C , and stored for later analysis of PTH. Paired venous and arterial umbilical cord samples were taken on a cohort of babies.

Following informed written parental consent forearm bone images were taken by portable single photon absorptiometer within 5 days of birth, and stored for analysis at the completion of the study period.

A structured standard form designed for obstetric note abstraction (McKinney et al, 1997) was modified and completed from the maternal hospital and patient-held records where available (Appendix C). Information collected included the first

antenatal visit, illnesses in pregnancy, ultrasound scans, labour record, delivery and neonatal details. The diagnosis of pregnancy induced hypertension was accepted where designated by the obstetrician as a reason for admission or delivery. Placental abnormality was used in all cases where the description of the placenta deviated from the designation of normal. All information was entered into Microsoft Access for Windows database designed by the Paediatric Epidemiology Group at Leeds General Infirmary.

Ethical approval was granted by General Infirmary at Leeds Ethical Committee. Parental consent was obtained prior to bone measurements, but anonymised blood samples were analysed without consent on the approval of the Hospital Ethical Committee.

2.1.3 Parathyroid hormone

PTH was assayed using the Nichols Advantage Chemiluminescence Intact Parathyroid Hormone Immunoassay (Nichols Institute Diagnostics, USA), an automated method.

The assay detects intact PTH using two goat polyclonal antibodies. One of the antibodies is coupled to biotin (39-84 segment) and the second is labelled with acridinium ester for detection (N terminal 1-34 sequence); the 2 antibodies sandwiching the intact PTH molecule. Following initial incubation of the plasma sample and labelled antibodies, streptavidin coated magnetic particles are added. These bind the PTH-antibody complex via the high-affinity interaction between biotin and streptavidin. Aspiration of the reaction mixture and subsequent washing separates the bound from free labelled antibody.

Hydrogen peroxide and sodium hydroxide are added, oxidising the acridinium ester. This leads to emission of light which is quantified and expressed in relative light units by the integrated luminometer. The amount of labelled antibody is directly proportional to the concentration of intact PTH in the sample. The automated system calculates test results from the stored calibration curve.

Precision at 8 $\mu\text{g/ml}$ is 6.7% CV (expressed as 90% confidence level for variance). Interassay variation at 34 $\mu\text{g/ml}$ is 9%. The reference ranges for the assay are 11 to

55 ng/L, such that the lower limit of detectability of the assay is 10 ng/L, and >55 ng/L is regarded as a high concentration.

2.1.4 PTHrP

PTHrP assay was performed by Dr W Fraser, University of Liverpool (Fraser et al, 1993). Cord blood samples were collected into 2cc collection tubes containing protease-inhibitors, spun, separated and frozen within 30 minutes of collection. The samples were stored at -70°C until completion of the study when they were packaged on ice and sent to the University of Liverpool for analysis.

Since the original publication (Fraser et al, 1993), the assay has been refined to a detection limit of 0.3-0.5 pmol/L, with the upper limit of normal considered to be 1.8 pmol/L (Personal communication Dr W Fraser).

2.1.5 1,25 dihydroxycholecalciferol

1,25-DHCC was assayed using the IDS Gamma-B kit (Immunodiagnostic systems Ltd, UK) by immunoextraction followed by quantitation by ^{125}I radioimmunoassay. Serum samples are delipidated using a solution of dextran sulphate and magnesium chloride, and incubated for 3 hours with highly specific solid phase monoclonal anti-1,25-DHCC to extract 1,25-DHCC. The immunoextraction gel is then washed and the purified 1,25-DHCC eluted into glass assay tubes.

The samples are incubated overnight with highly specific 1,25-DHCC sheep anti-1,25-DHCC. ^{125}I -1,25-DHCC is added for a further 2 hours incubation. Separation of bound from free is achieved with short incubation with Sac-Cel (anti-sheep IgG), followed by centrifugation, decanting and counting. Bound radioactivity is inversely proportional to the concentration of 1,25-DHCC. Intraassay variation is 5-8%, sensitivity (mean minus 2sd of 10 replicates) of 5pmol/l and 100% specificity for 1,25 vitamin D₃ and 94% for 1,25 vitamin D₂.

2.1.6 Single photon absorptiometry

For the purposes of the clinical studies, BMC and area normalised BMD were measured using a portable single photon absorptiometer, specifically designed for use in preterm infants (Truscott 1996). This scanner produces a measurement of mineralised bone mass of the infant forearm in both the extremely-low birthweight population and the ventilated infant, permitting data collection in the first few days of life in these high risk populations, an advantage over DXA scanning.

Images were taken at the midpoint of the right radius wherever possible. This point was determined using the formula

$$d = 0.391 \times Du + 1.19$$

where d is the separation of the midpoint of the radius from the radial styloid process and Du is the distance between the distal styloid process and the proximal tip of the olecranon, measured in millimetres (James et al, 1986).

Du and d were measured using perspex calipers and the midpoint marked on clear clinical tape (Blenderm, 3M, USA).

The system has previously been shown to produce forearm BMD at the distal radius ranging from 43 to 115 mg/cm² in babies aged 23-41 weeks post-conceptual age (Truscott 1996). Linearity has been demonstrated using aluminium foils. The absorbed radiation dose to skin is 6uSv.

2.1.6i Bone mineral density

Area-normalised BMD of the radius is estimated from the 2-dimensional bone image by lining up the cursor along the axis of the radius, with the base of the T-bar at the fiduciary mark (Figure 4). This offsets the centre of the ROI 10mm from the fiduciary mark. The cursor is then rotated 90^0 , and the length of the ROI box adjusted to match the width of the radius. The edges of the radius are confirmed using the profile of bmc/pixel (Figure 5). Box width is maintained at 38 pixels (2mm). Both radial width and areal density within the ROI are obtained.

Figure 4: Image of forearm bones; cursor positioned for BMD measurement

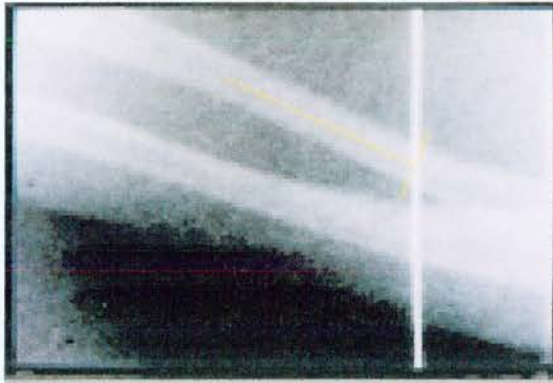
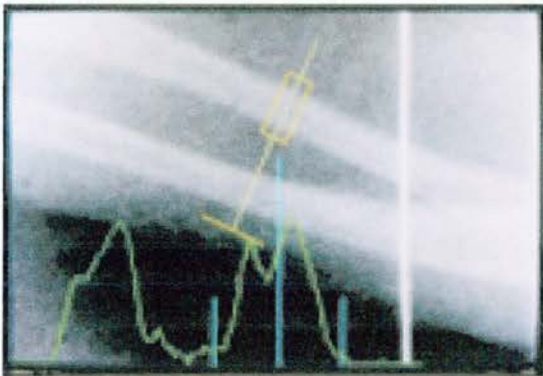


Figure 5: Image of forearm bones; alignment for BMD measurement



2.1.6ii Bone mineral content

BMC of a 1mm section of mid-radius and ulna is estimated at the mid-radial point.

The cursor is lined up along the axis of the 2 bones with the base of the T-bar at the fiduciary mark (Figure 6). This offsets the centre of the ROI 10 mm from the

fiduciary mark. The cursor is then rotated 90^0 , and the length of the ROI box

adjusted to extend beyond the radius and ulna (Figure 7). Box width is maintained

at 19 pixels (1mm). Bone mineral content of the 2 bones (and soft tissue) within this area is obtained.

Figure 6: Image of forearm bones; cursor positioned for BMC measurement

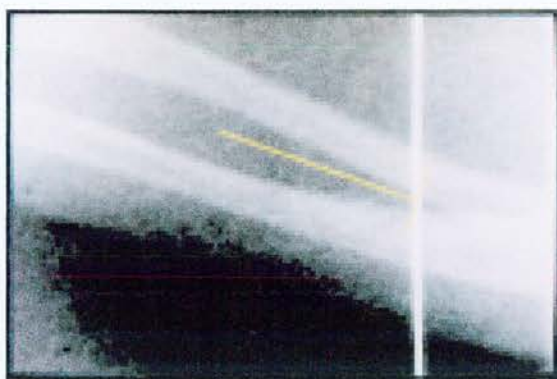
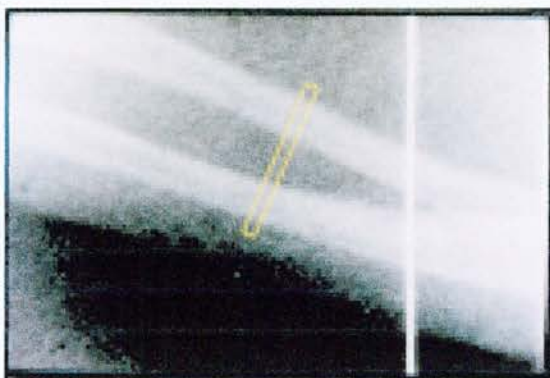


Figure 7: Image of forearm bones; alignment for BMC measurement



2.1.7 Statistics

As a pilot study, Dr P Holland measured PTH in cord blood of a small number of preterm infants. The mean PTH in SGA infants was 34 pg/ml (4 infants, gestation 27-31 weeks, PTH range 25-54 pg/ml). In AGA infants PTH was undetectable at <10pg/ml (4 infants, gestation 30-38 weeks). Because the AGA group of infants all had PTH levels below the limit of detectability (<10 pg/ml), an s.d. was estimated for the power calculation, following discussion with a statistician (Darren Greenwood). Given the range in the SGA group, 20 was considered to be a generous, but realistic figure.

To detect a difference in mean PTH concentrations between SGA and AGA infants, assuming a mean of 10 and 34 and a standard deviation of 20, a study with 90% power at a significance level of 5% would require a sample size of 15 in each group. However, in order to perform multiple regression analysis a larger sample size was targeted, with the aim to recruit 150 infants.

Independent t-tests were performed to determine the effect of each of independent variables thought to influence mineralised bone mass: sex of infant, maternal smoking, antenatal dexamethasone, caesarean section, spontaneous onset of labour, pregnancy induced hypertension, absent/reversed end diastolic flow on one or more doppler ultrasound scans, and placental abnormality, on each of the three dependent variables BMC, BMD and RW separately.

Pearson's correlation was performed on each of the continuous variables: birthweight, gestation, birth centile, maternal weight and height at booking, maternal systolic and diastolic blood pressure at booking, and the three dependent variables separately.

For each of the dependent variables a final model predictive equation was generated by linear regression analysis using backward elimination. Independent variables were weight, gestation, caesarean section, placental abnormality, PTH, spontaneous onset of labour, pregnancy induced hypertension and maternal smoking. BMC and BMD models used regression through the origin (no-intercept model).

Mann-Whitney and Kruskal-Wallis tests for non-parametric data were used for analysis of PTH concentrations in relation to BMC, BMD and RW.

Statistics were performed using Statistical Package for Social Sciences (SPSS) version 9.0 for Windows. SAS system was used for regression analysis when non-dichotomous categorical data was included (PTH).

2.2 Randomised controlled trial of individualised calcium and phosphorus supplementation

2.2.1 Introduction

Previous studies have demonstrated that bone mineralisation at birth is related to weight. Postnatally, the preterm infant fails to demonstrate *in utero* mineral accretion rates, despite the provision of comparable quantities of calcium and phosphorus either enterally, or parenterally.

Plasma calcium concentrations are maintained at the expense of calcium deposition within bones, such that monitoring plasma calcium concentrations alone is an unreliable measure of calcium requirements. In calcium deficiency states urinary excretion of calcium will be reduced, with high phosphorus losses due to hyperparathyroidism, leading to reduced plasma phosphorus concentrations.

2.2.1i Aim:

To examine the effect of individualised calcium and phosphorus supplementation on bone growth and mineralised bone mass of the radius in preterm infants, using the portable single photon absorptiometer.

2.2.1ii Hypothesis:

A personalised mineral supplementation regimen based on routine plasma and urine calcium and phosphorus concentrations promotes mineralised bone mass in the preterm infant, such that bone mineral content is increased at term by 20% in those receiving individualised mineral supplementation.

2.2.2 Patients and Methods

2.2.2i Setting

Peter Congdon Neonatal Unit between November 1997 and October 1999.

2.2.2ii Population

Infants below 32 completed weeks gestation and of birthweight less than 1800g, less than 96 hours of age, born to caucasian parents. Infants with chromosomal anomalies, skeletal abnormalities or primary gastrointestinal pathology were excluded. Infants in whom transfer to another unit prior to discharge home, and infants in whom survival was precarious in the first days of life were also excluded. Infants subsequently requiring surgical intervention were subsequently managed by the surgical team and were therefore removed from the study.

There was no process in place recording the total number of eligible infants for the study and the reasons for non-recruitment.

2.2.2iii Consent

Informed written parental consent was obtained by the researcher (AH).

2.2.2iv Randomisation

Infants were randomised using sealed opaque envelopes to the control or study groups. Randomisation was performed by table of random numbers, in advance of study commencement by AH, in blocks of four and six, stratified for gestation as less than 28 completed weeks and equal to or above 28 completed weeks. All

aspects of randomisation, including enrolment of patients, group assignment and storage of randomisation envelopes and study records was carried out by AH.

2.2.2v Primary Outcome

Bone mineral content of radius and ulna at 40 weeks PMA

2.2.2vi Secondary Outcomes

Bone mineral areal density and radial width at term; BMD, RW and BMC at 64 weeks PMA; alkaline phosphatase concentrations.

2.2.2vii Confounding variables

The following potentially confounding variables were included: parental and enteral intake, ventilation parameters, medications.

2.2.2viii Data sampling

Plasma and urinary calcium, phosphorus and creatinine concentrations were measured weekly along with plasma alkaline phosphatase by the department of biochemistry. Plasma samples were taken as part of routine clinical monitoring and were drawn by technicians as capillary samples or by medical staff from an indwelling arterial line when present.

Details were taken from the medical and nursing records on all respiratory support, drugs administered, weekly weight (measured on seca model 724 or 727), and any medical complications (Appendix C). Forearm bone images were obtained within the first week of life and 4 weekly thereafter until discharge from the neonatal unit.

A cohort of these infants had further measurements taken after 56 weeks PMA. Images were analysed primarily for radial and ulnar BMC, and also for RW and BMD.

Regular Xrays were not included as part of the study protocol.

2.2.2ix Study design

Infants randomised to the control arm received the neonatal unit protocol of mineral supplementation at the discretion of the neonatal unit medical staff, following guidelines (Bremer et al, 1987). Local recommendations suggested that intravenously fed infants were commenced on 0.7 mmol/kg/day each of both phosphorus and calcium added to parenteral nutrition; enterally fed infants received supplementation when plasma phosphorus concentration fell below 1.6 mmol/l and plasma calcium concentration below 2.2 mmol/l.

Infants randomised to the study group received mineral supplementation based on plasma concentrations of phosphorus and calcium, and urinary calcium excretion and renal tubular absorption of phosphorus. Phosphorus supplementation was adjusted weekly to maintain a plasma phosphorus concentration of >2.0 mmol/l and a tubular resorption of phosphorus \leq 95%. Calcium supplementation was adjusted weekly to maintain plasma calcium concentration within the range 2.2-2.6 mmol/l with a urinary calcium excretion > 0.3 mg/100 ml glomerular filtrate. All infants on full enteral feeds received 400 IU/day of vitamin D as abidec (Appendix F).

Calcium and phosphorus were added to parenteral feed solution as calcium gluconate and potassium glycerophosphate, to a maximum of 1.5 mmol/kg/day calcium and 2.0 mmol/kg/day phosphorus. Where appropriate, oral supplements were commenced at one week of age or when tolerating at least half the daily volume requirement by enteral route. Phosphorus was given as potassium phosphate 17.42% commencing 2 mmol/day in 2 divided doses, and increasing to 3 mmol/day in divided doses. Supplements were reduced by half to a minimum of 1mmol/day prior to discontinuation when the criteria for supplementation were no longer met. Calcium was prescribed as calcium sandoz (0.54 mmol/ml) commencing at 0.5 mmol/kg/day, increasing to a maximum of 1 mmol/kg/day, reducing to a minimum of 0.5 mmol/kg day prior to discontinuation. All supplements were prescribed on the patient's medication chart and administered by the nursing staff as boluses via nasogastric tube, with calcium and phosphorus supplements prescribed at different times of day and administered immediately prior to bolus feeds. All prescriptions were written by AH. Infants who were unable to tolerate enteral feeds for a period of 10 days or more received parenteral nutrition throughout that time.

Decisions regarding commencement of feeds and the type of feed were made by the neonatal unit staff, incorporating parental preference. All parenteral and enteral feeds were recorded until the infant was 40 weeks PMA, discharged from the neonatal unit or receiving primarily breast feeds.

2.2.2x Data Collection

All information was entered into Microsoft Access for Windows database designed by the Paediatric Epidemiology Group at the University of Leeds.

2.2.2xi Blinding

There was no blinding of study and control groups from the researcher (AH).

Extraction of BMC, BMD and RW from archived images was performed by JT who was blind to group allocation.

2.2.2xii Laboratory Methods

Plasma and urinary calcium and phosphorus concentrations were assayed using automated methods, by the department of clinical biochemistry. Calcium was carried out by o-cresolphthalein complexone method (Roche Ltd, Welwyn Garden City, Hertfordshire) and phosphorus by ammonium molybdate method (Roche). Plasma and urinary creatinine was assayed by Jaffe method (Roche) and alkaline phosphatase by p-nitrophenyl phosphate/DEA buffer method (Roche).

2.2.2xiii Ethical approval

Ethical approval was granted by General Infirmary at Leeds Ethical Committee.

2.2.3 Statistical Methods

Power calculations were based on previous studies of BMC in term and preterm infants (Horsman et al, 1989a). The mean BMC at 30 weeks gestation in preterm babies being 80 mg/cm and reaching 81 mg/cm (s.d. 15.9) at 38 weeks, compared with term infants of 187 mg/cm (s.d. 24.7) at birth. A study with 90% power at the 5% significance level would need to recruit 21 infants into each arm to show a 20% difference (1 s.d.) at term in BMC ($\mu_1=81$, $\mu_2=97$, $\sigma=15.9$). On statistical advice (DG) we aimed to recruit 48 infants.

Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) version 9.0 for Windows. Paired and non-paired t-tests were used for BMC, BMD and RW, plasma calcium and phosphorus concentrations. The non-parametric Mann-Whitney test was used for protein, carbohydrate, fat, calcium, phosphorus and vitamin D intake, TRP, and Ca_E . Fisher's Exact test and Chi-squared test were used for categorical data.

3 Results of Cohort study

3.1 Single photon absorptiometer

3.1.1 Coefficient of variation

Repeated measurements were taken on 20 images selected randomly, and the coefficient of variation (CV) calculated for BMC and BMD using 3 measurements (Bland M, 1997). CV = 3.4% (0.33mg) for BMC, 4.4% (4.0mg/cm²) for BMD and 2.9% (0.036 cm) for RW.

Reproducibility of BMC and BMD was assessed using measurements from 21 pairs of images taken following repositioning of the forearm within the cradle. CV of BMC is 10.9% (1.7 mg), BMD 11.5% (13.7 mg/cm²) and RW 14.3% (0.089 cm) (Bland M, 1997).

3.1.2 Loss of images

The edges of the bone were determined using the profile of bmc/pixel in an attempt to remove observer error. In small infants the profile produced distinct edges which were easy to identify. In larger infants the interosseous membrane and tendons produced an image incompletely distinct from bone, potentially leading to error in the measurement of BMD and RW. For each image 3 estimates of BMD were taken. The first measurement was taken from the best estimate, with 2 subsequent

measurements taken at 2 pixels wider and 2 pixels narrower than the best estimate.

The mean of these 3 measurements was taken as BMD.

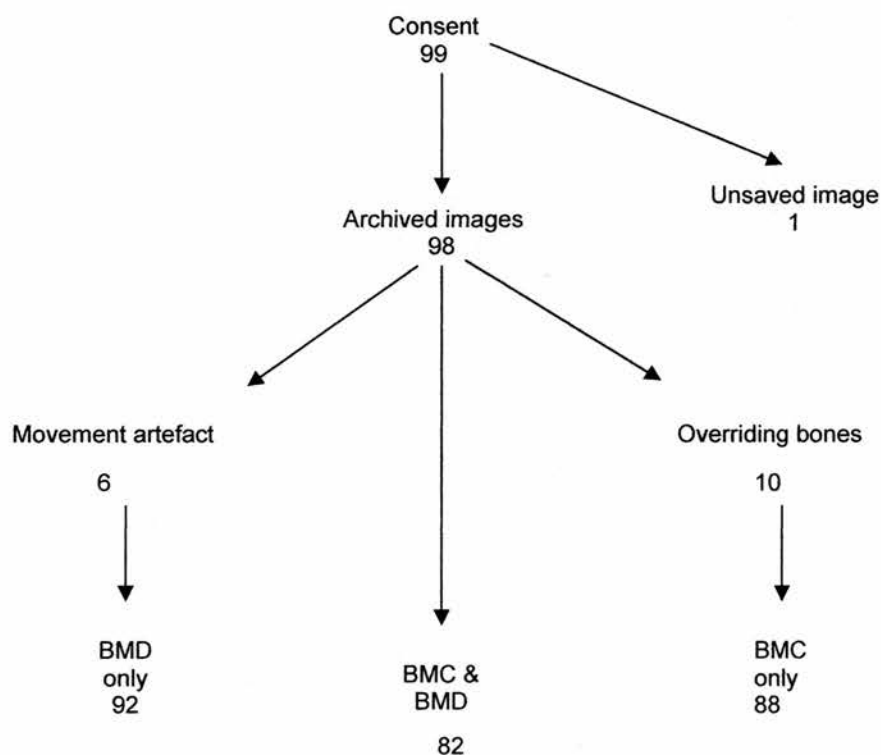
Some images were not suitable for interpretation due to movement artefact during imaging, and these were discarded for both BMC and BMD. In some small infants overlapping of the radius and ulna occurred at the midpoint, preventing interpretation of BMD measurements. In these infants only BMC of the combined radius and ulna were used.

3.2 Patient characteristics

Structured questionnaires were completed on 99 infants. All questionnaires were completed from maternal notes to avoid bias in recollection. However, some notes were incompletely available from referring hospitals, or had not been completed during pregnancy. Not all mothers had undergone detailed antenatal ultrasound scans providing information on umbilical or uterine artery blood flow. All infants were born to caucasian mothers.

Bone images were taken on all 99 infants and were available for BMC on 92 and BMD on 88. On one infant the image failed to save to the archive, and in 6 infants movement artefact causing indistinct edges to the radial image prevented estimation of BMC. Overriding of radius and ulna at the midpoint prevented BMD estimation of 10 images (Figure 8). Data on how many parents were approached for inclusion in the study and did not consent was not collected.

Figure 8: Bone images of infants recruited

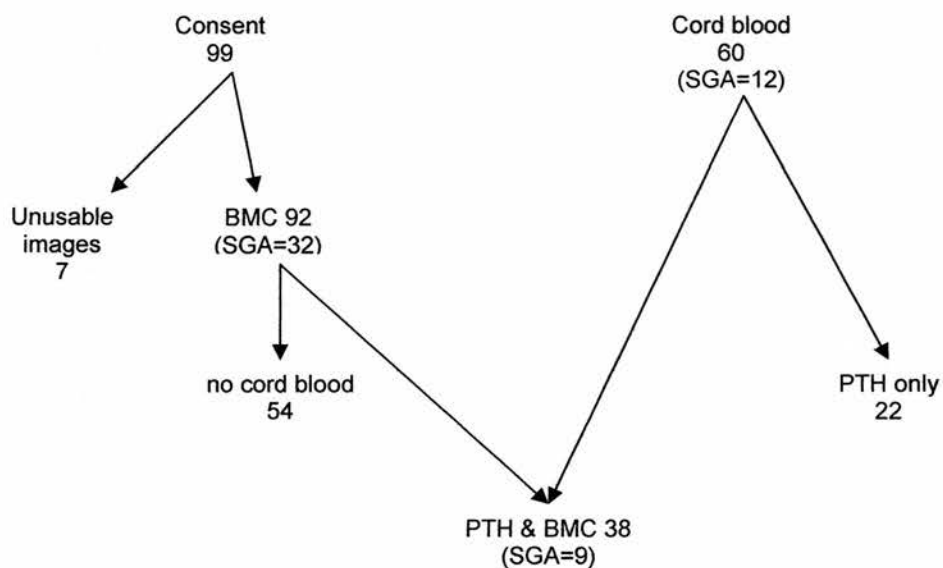


Gestational age as determined by maternal menstrual dating were within 2 weeks of gestational age by ultrasound scan before 15 weeks where available. Gestation ranged from 23 to 41 completed weeks, mean 33.4 weeks, standard deviation 4.6. Birthweight ranged from 0.540 to 4.975 kg, mean 1.935 kg (0.887). 59 infants were preterm (<37 weeks) and 71 were low birth weight (<2.5 kg). 32 infants were SGA (<10th centile; Freeman et al, 1995).

BMC ranged from 0.3 to 44.0 mg. BMD ranged from 9.8 to 183.0 mg/cm², and was measured only in infants with birthweight 0.540 to 4.520 kg. All infants with BMD measurements had RW estimated.

Samples were acquired for PTH 60 infants. In 38 of the infants parents consented for BMC measurement. A further 22, declined to have bone images taken or were discharged home within 6 hours of delivery such that images were unable to be obtained (Figure 9). Cord blood was drawn on an opportunistic basis by the single researcher (AH) due to the necessity to spin and separate the samples immediately. Where cord blood samples had been insufficient or were not obtained at delivery, parents were subsequently approached for BMC measurements, accounting for 54 infants on whom PTH was not measured.

Figure 9: Parathyroid analysis of recruited infants



BMC, BMD and RW were correlated with birthweight; BMC $r=0.847$, BMD $r=0.752$, RW $r=0.682$ (Figures 10,11,12); and gestation, BMC $r=0.706$, BMD $r=0.662$, RW $r=0.663$. $p<0.001$ for all correlations. Statistically significant

differences were found in BMC, BMD and RW between infants whose mothers had received antenatal dexamethasone and those who had not, $p < 0.001$ for all variables, with the administration of steroids being associated with reduced mineralised bone mass. Significant differences were also found in lower RW in those infants who had been delivered by caesarean section ($p = 0.021$), and those with placental abnormality ($p = 0.043$).

Absent or reversed end-diastolic doppler flow (EDF) was associated with a significant reduction in BMC ($p = 0.042$) and BMD ($p = 0.038$), but not RW ($p = 0.077$). No significant correlation was found between BMC, BMD or RW and maternal booking weight, height, systolic or diastolic blood pressures. Sex of infant, maternal smoking, diagnosis of pregnancy induced hypertension (PIH) or spontaneous onset of labour (SOL) was not related to BMC, BMD or RW (Table 1).

Table 1: Effect of independent variables on BMC, BMD and RW

	n	n			
	BMC	BMD	BMC*	BMD*	RW*
Sex of infant	92	88	0.320	0.335	0.873
Antenatal dexamethasone	59	53	<0.001	<0.001	<0.001
Caesarean section	73	68	0.073	0.176	0.021
Placental abnormality	70	65	0.170	0.424	0.043
Spontaneous onset of labour	74	69	0.249	0.263	0.592
Pregnancy induced hypertension	74	69	0.880	0.590	0.491
Absent/reversed end diastolic flow	74	69	0.042	0.038	0.077
Maternal smoking	61	59	0.134	0.405	0.832

*p-value, independent t-test

3.3 Linear Regression

Complete data sets including weight, gestation, caesarean section, placental abnormality, spontaneous onset of labour, pregnancy induced hypertension and maternal smoking were available on 72 infants. Addition of PTH to the model reduced the dataset to 38 infants (Figure 9). In each model, all maternal factors were excluded at 0.1 level, leaving only birthweight in the final models for BMC, BMD and RW, with beta coefficients as shown in Table 2. Regression through the origin produced good fit for BMC and BMD. Regression equations were derived using beta values, and for RW a constant:

$$\text{BMC (mg)} = 6.371 \times \text{birthweight (kg)}$$

$$\text{BMD (mg/cm}^2\text{)} = 48.788 \times \text{birthweight (kg)}$$

$$\text{RW (cm)} = 0.356 + 0.08 \times \text{birthweight (kg)}$$

Table 2: Regression coefficients for mineralised bone mass and birthweight

	n	Constant	Beta	95% CI	r ²	p value
BMC	92		6.371	5.975/6.767	0.918	p<0.001
BMD	88		48.788	45.83/51.746	0.925	p<0.001
RW	88	0.356	0.08	0.065/0.104	0.465	p<0.001

Figure 10: Bone mineral content and birthweight

BMC (mg) = 6.371 x birthweight (kg)

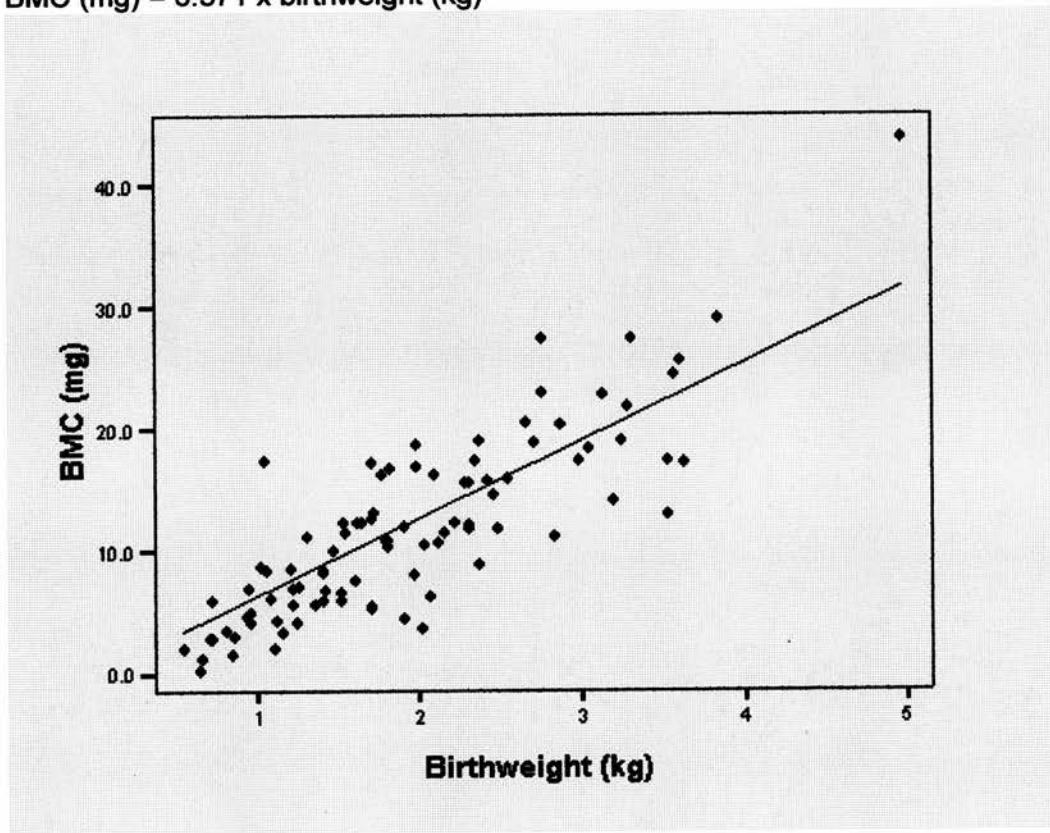


Figure 11: Bone mineral density and birthweight

$$\text{BMD (mg/cm}^2\text{)} = 48.788 \times \text{birthweight (kg)}$$

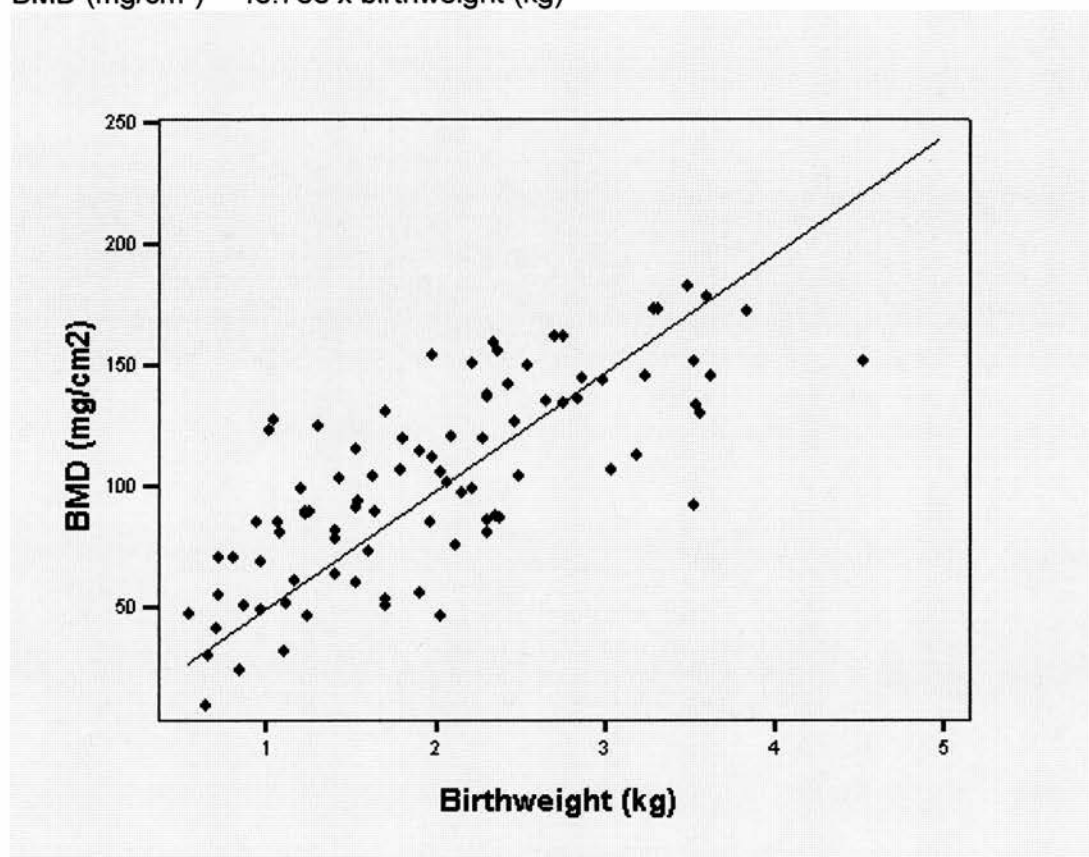
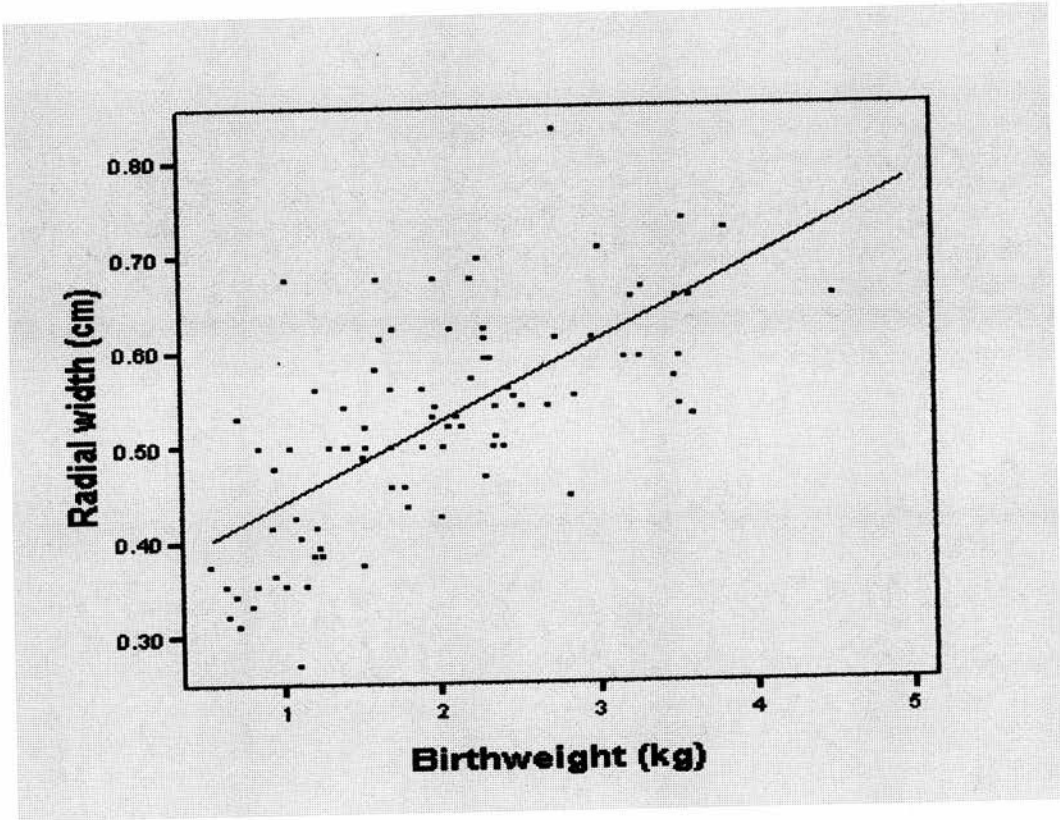


Figure 12: Radial width and birthweight

$$RW \text{ (cm)} = 0.356 + 0.08 \times \text{birthweight (kg)}$$



As birthweight had a massive effect on bone mass, it was subsequently excluded from the model to examine effects of the other variables. Using backward elimination, the general linear model for BMC excluded maternal smoking, PIH, absent or reversed EDF, sex of infant, delivery by caesarean section and spontaneous onset of labour, leaving antenatal dexamethasone and placental abnormality in the model (Table 3).

Table 3 :General linear model for BMC

Included variables			Excluded variables	
	beta	p		p
Dexamethasone	-5.45	0.001	Maternal smoking	0.467
Placental abnormality	-4.93	0.004	PIH	0.549
Constant	15.715	<0.001	Absent/reversed EDF	0.544
			Sex	0.429
			C-section	0.803
			Labour	0.434

Similarly for RW, antenatal dexamethasone and placental abnormality remained in the model, excluding maternal smoking, PIH, absent or reversed EDF, sex of infant, delivery by caesarean section and spontaneous onset of labour (Table 4).

Table 4: General linear model for RW

Included variables			Excluded variables	
	beta	p		p
Dexamethasone	-0.084	0.003	Maternal smoking	0.158
Placental abnormality	-0.087	0.006	PIH	0.096
Constant	0.578	<0.001	Absent/reversed EDF	0.994
			Sex	0.338
			C-section	0.726
			Labour	0.588

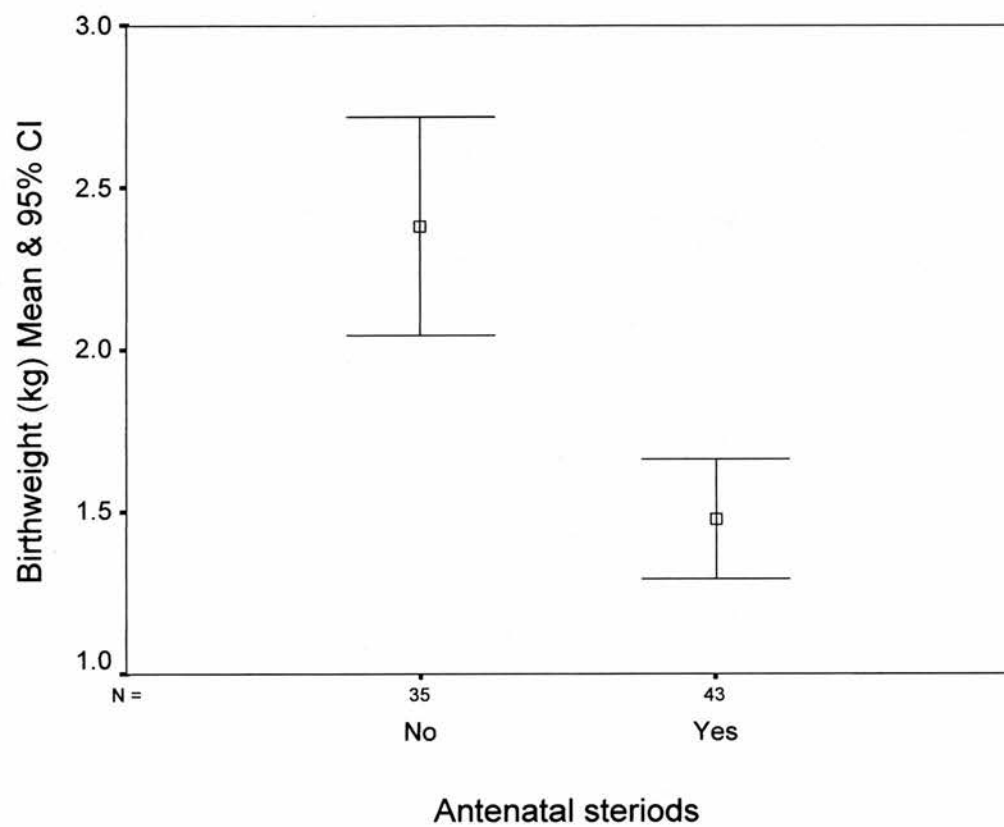
The model for BMD excluded only maternal smoking, PIH, absent/reversed EDF and delivery by caesarean section. Significant predictors were antenatal steroids, placental abnormality, spontaneous onset of labour and sex of infant (Table 5).

Table 5 :General linear model for BMD

Included variables			Excluded variables	
	beta	p		p
Dexamethasone	-45.72	<0.001	Maternal smoking	0.783
Placental abnormality	-21.54	0.030	PIH	0.805
Labour	-19.68	0.029	Absent/reversed EDF	0.529
Male sex	18.635	0.039	C-section	0.375
Constant	131.08	<0.001		

However, there was a significant difference in birthweight between those infants born to mothers who received antenatal dexamethasone and those who did not, $p < 0.001$ (Figure 13), suggesting that the effect of antenatal steroids was a birthweight phenomenon. There were no differences in birthweight between the groups when analysed by delivery after spontaneous onset of labour ($p = 0.10$), placental abnormality ($p = 0.126$), or sex of infant ($p = 0.652$).

Figure 13: Birthweight and administration of antenatal steroids



3.4 Parathyroid hormone

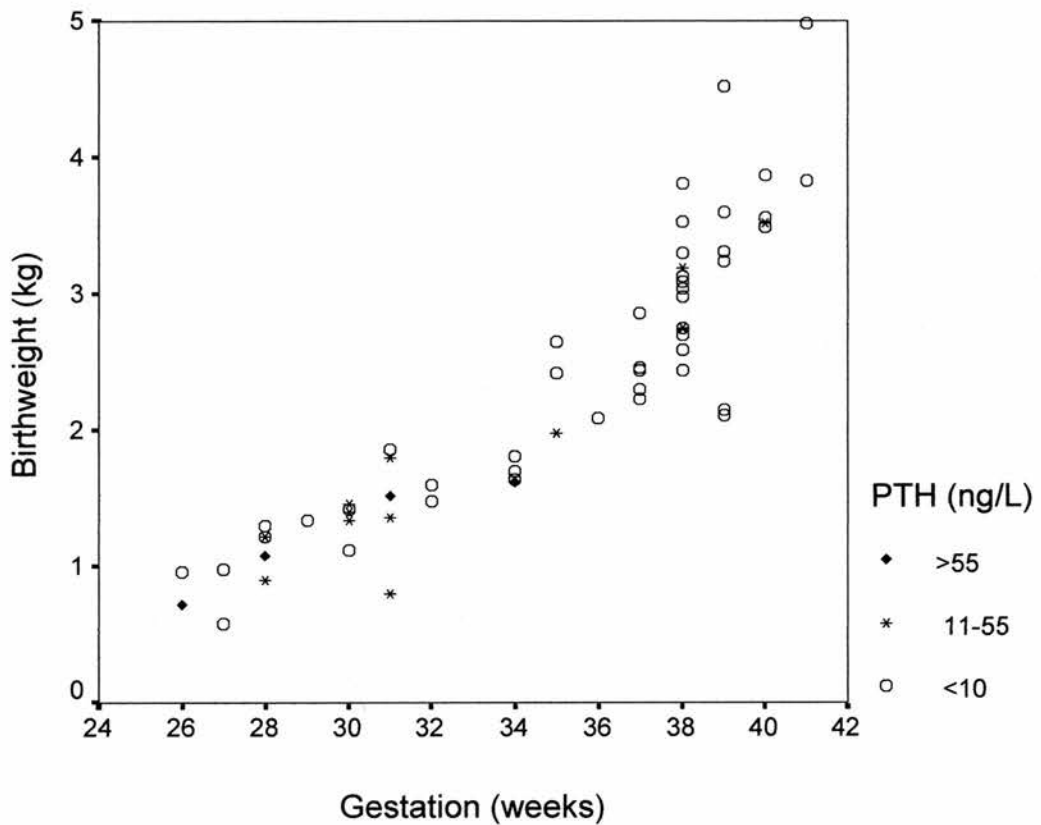
60 venous cord blood samples were obtained and analysed for PTH, on infants of birthweight 0.580 to 4.975 kg and gestation 26 to 41 weeks. All infants were caucasian.

Very high PTH concentrations were found only in low birthweight infants (Figure 14).

Paired venous and arterial samples were obtained in 10 of the larger term infants and run for PTH only. In all arterial samples, PTH was below the detection limit of 10ng/L, as were the corresponding venous samples. Duplicate samples were not available on smaller infants due to the size of the umbilical cords.

As birthweight is related to BMC and BMD, high PTH concentrations were also seen in those infants with low radial BMD and low forearm BMC. Only 3 infants of gestational age 37 weeks and over had PTH >10 (11, 15,17 ng/L). Many of the term infants had concentrations below the lower limit of detectability (<10 ng/L), therefore the PTH data is treated as categorical, using the reference ranges to divide the categories into <10 ng/L, 11-55 ng/L, and >55 ng/L.

Figure 14: PTH and birthweight

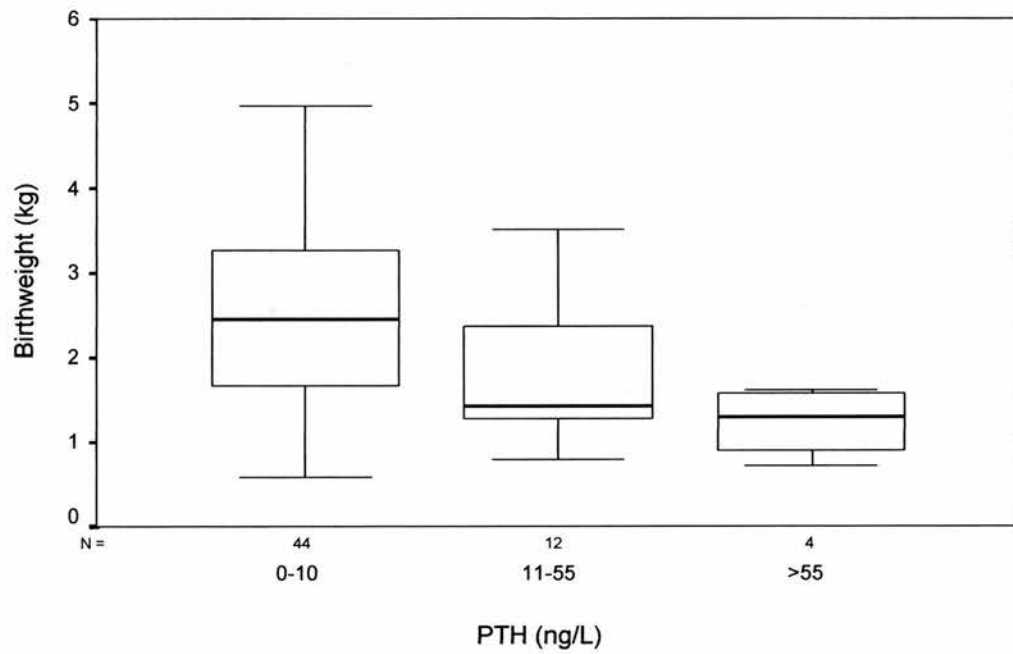


Categorising PTH into <11 (group 1, n=44), 11-55 (group 2, n=12) and >55 ng/L (group 3, n=4), there is a significant difference in birthweight between group 1 and 2 ($p=0.03$) and group 1 and 3 ($p=0.012$), with high PTH seen in low birthweight infants (Figure 15).

There is also a significant difference in gestation between groups 1 and 2 ($p=0.046$) and groups 1 and 3 ($p=0.015$), with high PTH in low gestation infants (Figure 16). However, there is no relationship between PTH concentration and birthweight centile (Figure 17).

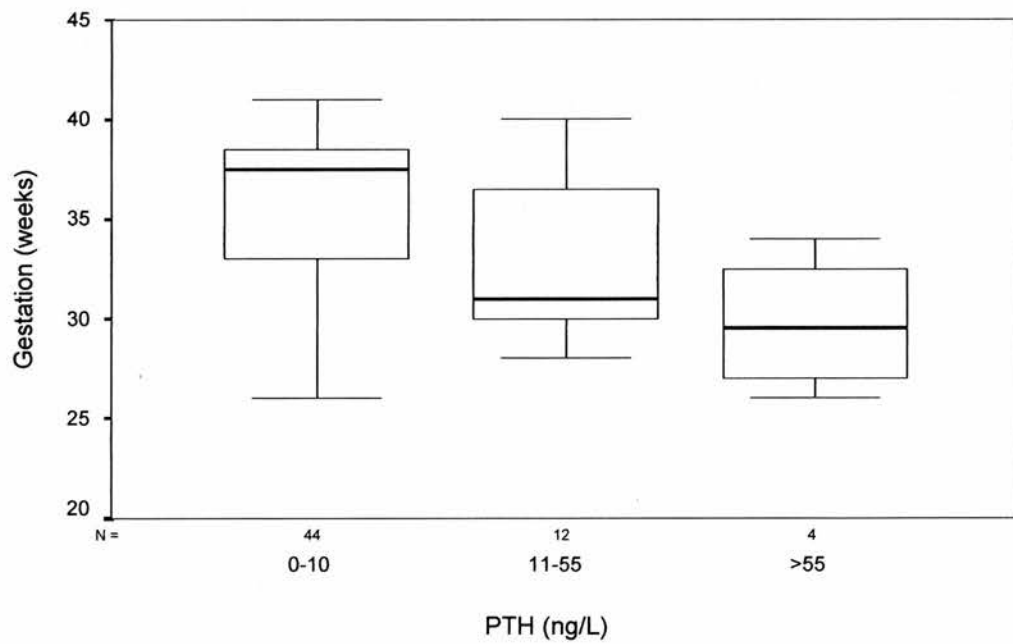
BMC and BMD are linearly related to birthweight, however the difference in BMC (Figure 18) and BMD (Figure 19) was only significant between groups 1 and 3: BMC $p=0.017$, BMD $p=0.020$.

Figure 15: Parathyroid hormone and birthweight



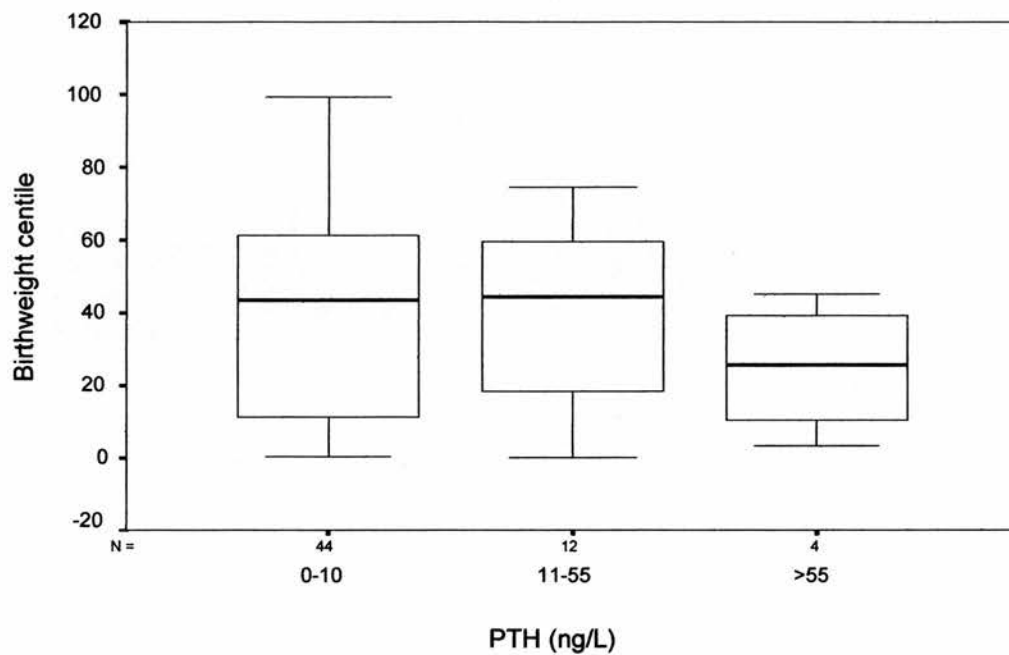
Bar=median, box=interquartile range, whiskers=range

Figure 16: Parathyroid hormone and gestation



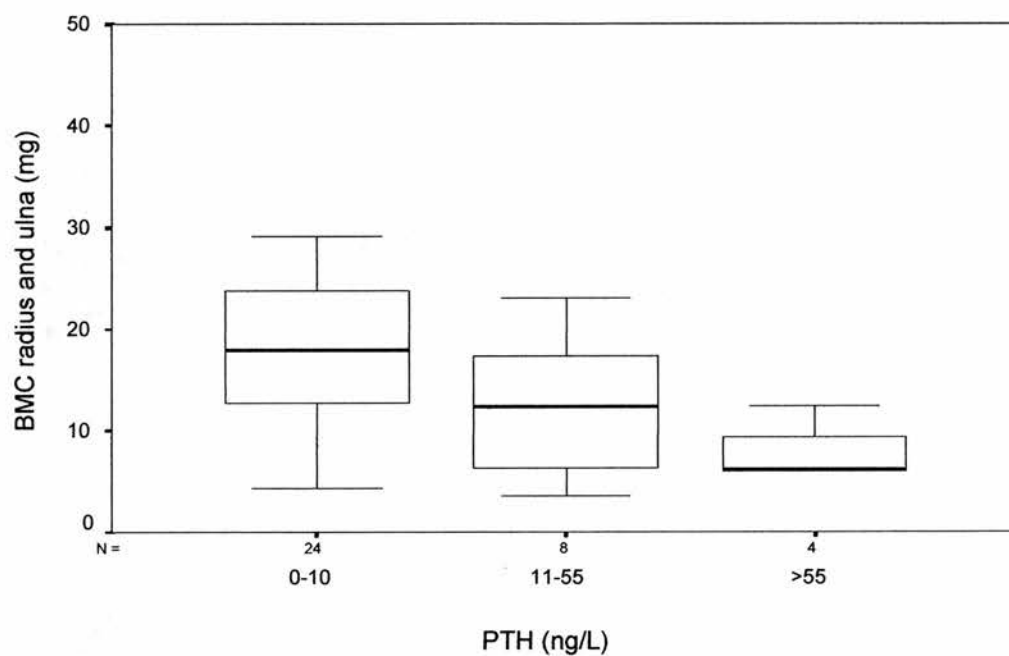
Bar=median, box=interquartile range, whiskers=range

Figure 17: Parathyroid hormone and birthweight centile



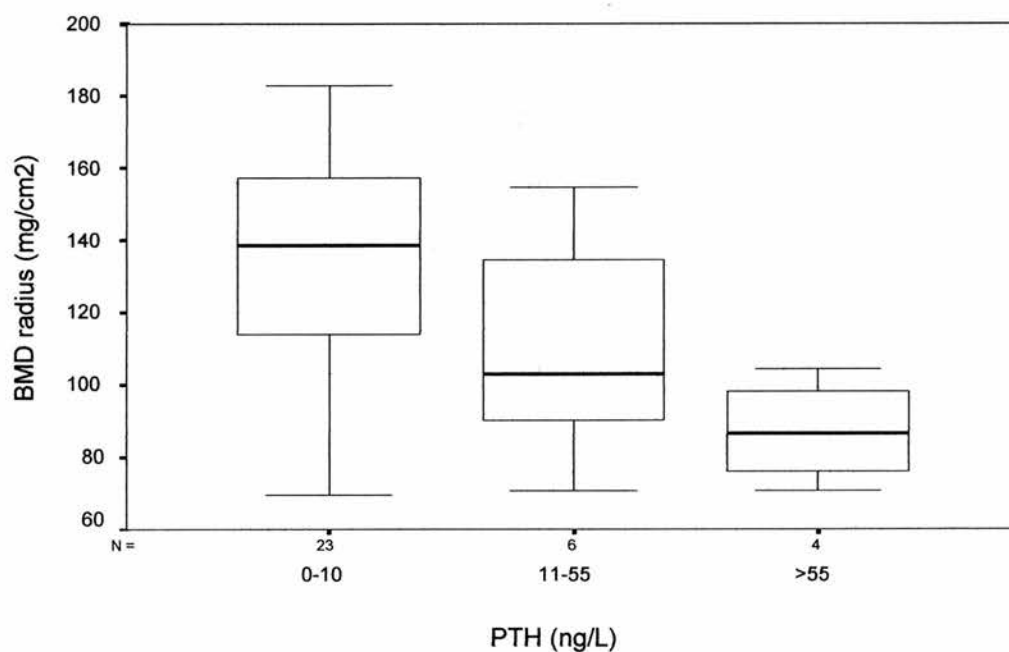
Bar=median, box=interquartile range, whiskers=range

Figure 18: Parathyroid hormone and bone mineral content



Bar=median, box=interquartile range, whiskers=range

Figure 19: Parathyroid hormone and bone mineral density



Bar=median, box=interquartile range, whiskers=range

3.4.1 Parathyroid hormone and pregnancy-induced hypertension

A significantly increased incidence of PIH is seen in infants with elevated PTH concentrations, Fisher's Exact test $p=0.008$ (Table 6, Figure 20), although only 4% of infants were born to mothers diagnosed as having PIH. In all infants there was no statistical difference in birthweight between those born to mothers with and without a diagnosis of PIH ($n=99$, $p=0.194$; Figure 21). Similarly, there was no difference in birthweight between those born to mothers with and without a diagnosis of PIH in the subgroup of infants on whom PTH was analysed ($n=38$, $p=0.517$; Figure 22). However, the 95% confidence intervals are wider in those born to mother's with PIH reflecting the lower birthweight infants in that group.

Table 6: PTH concentrations and pregnancy-induced hypertension

PTH	PIH	
	no	yes
<10	26	0
10-55	5	3
>55	3	1
	34	4

Figure 20: Pregnancy-induced hypertension and birthweight

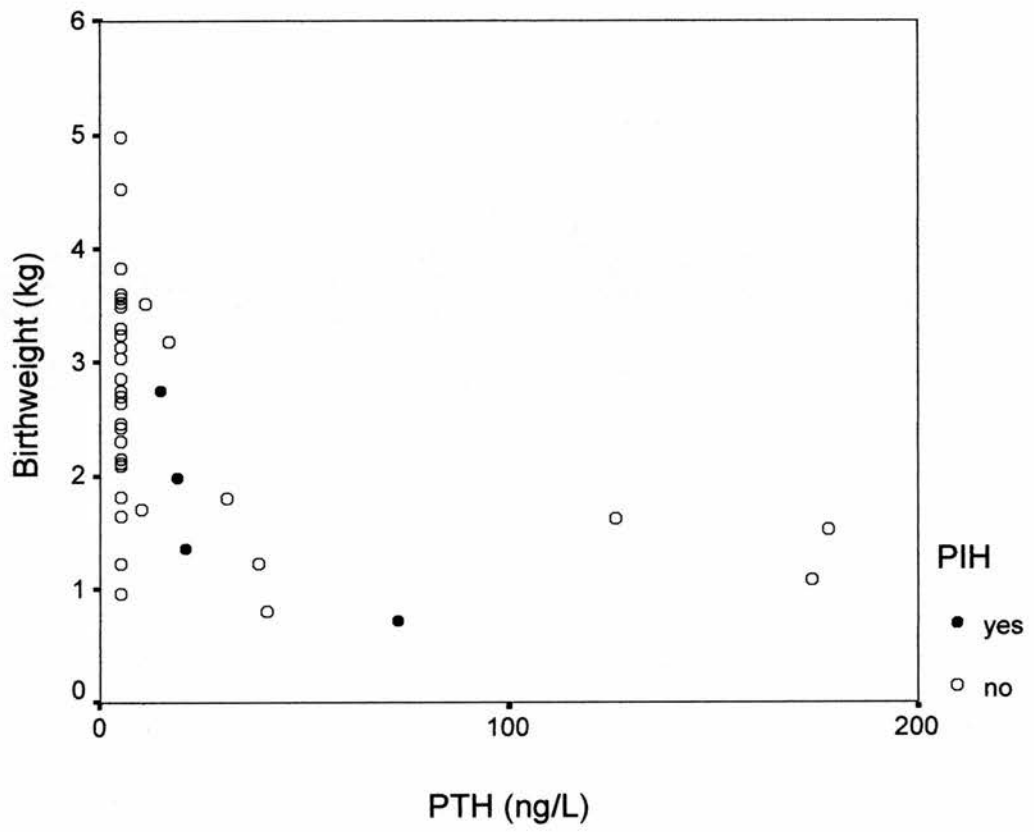


Figure 21 : Birthweight and pregnancy-induced hypertension (all infants)

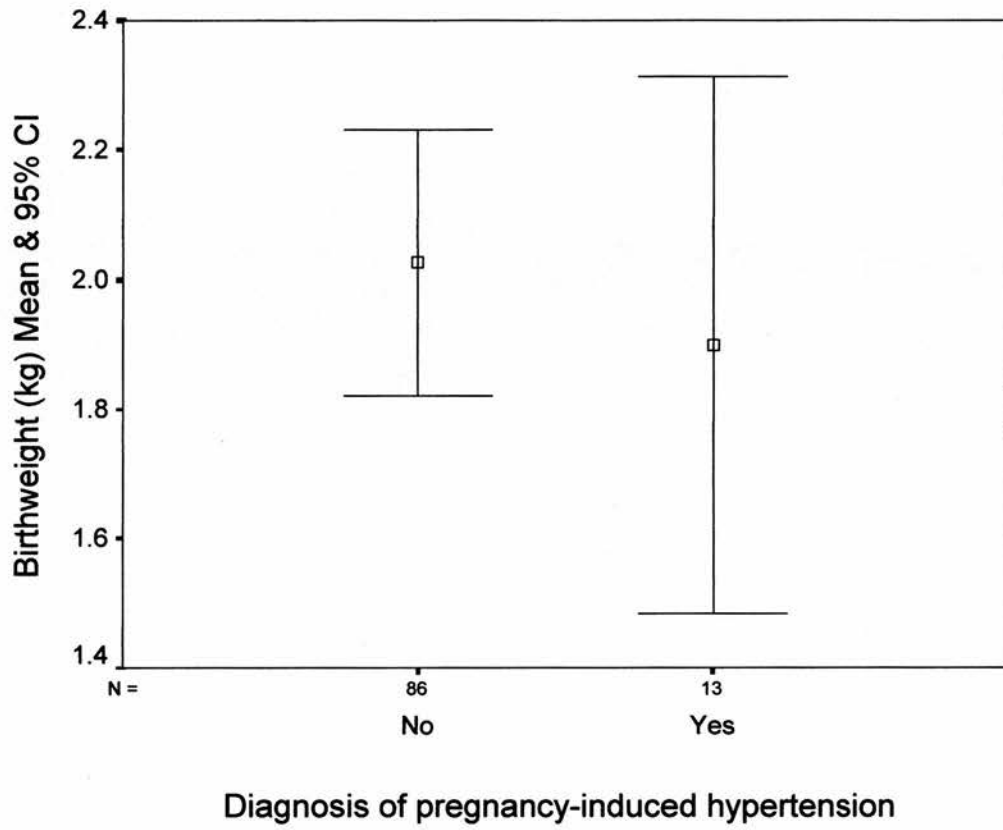
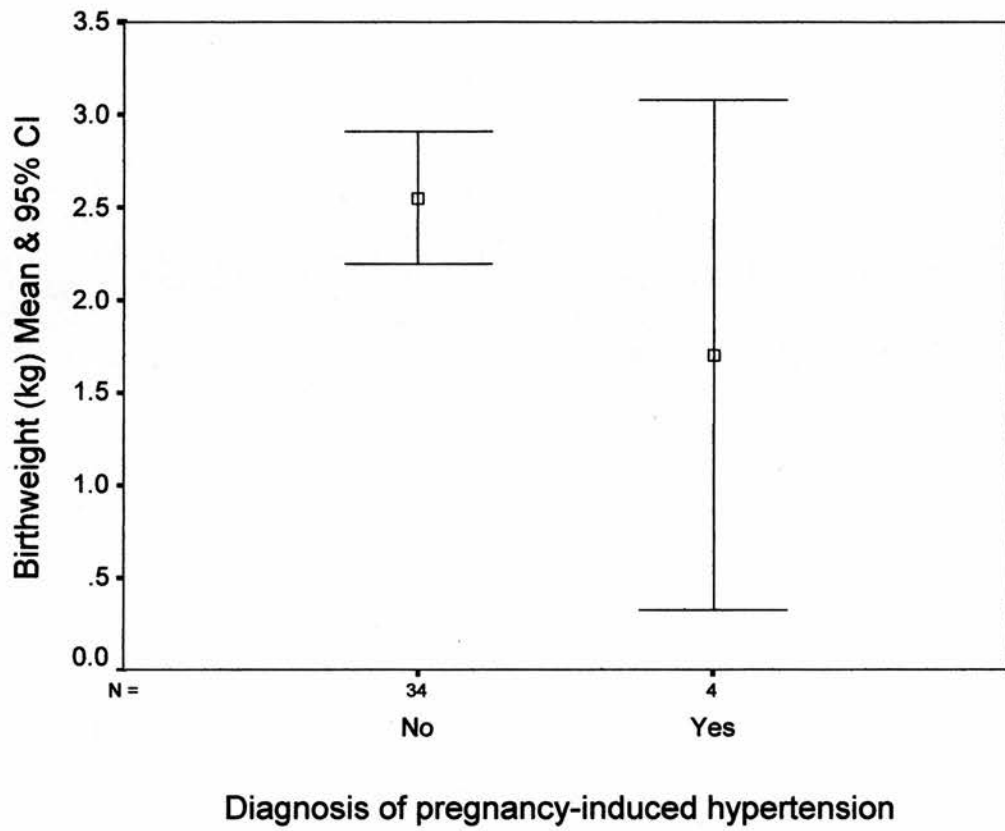


Figure 22: Birthweight and pregnancy-induced hypertension (PTH measured)

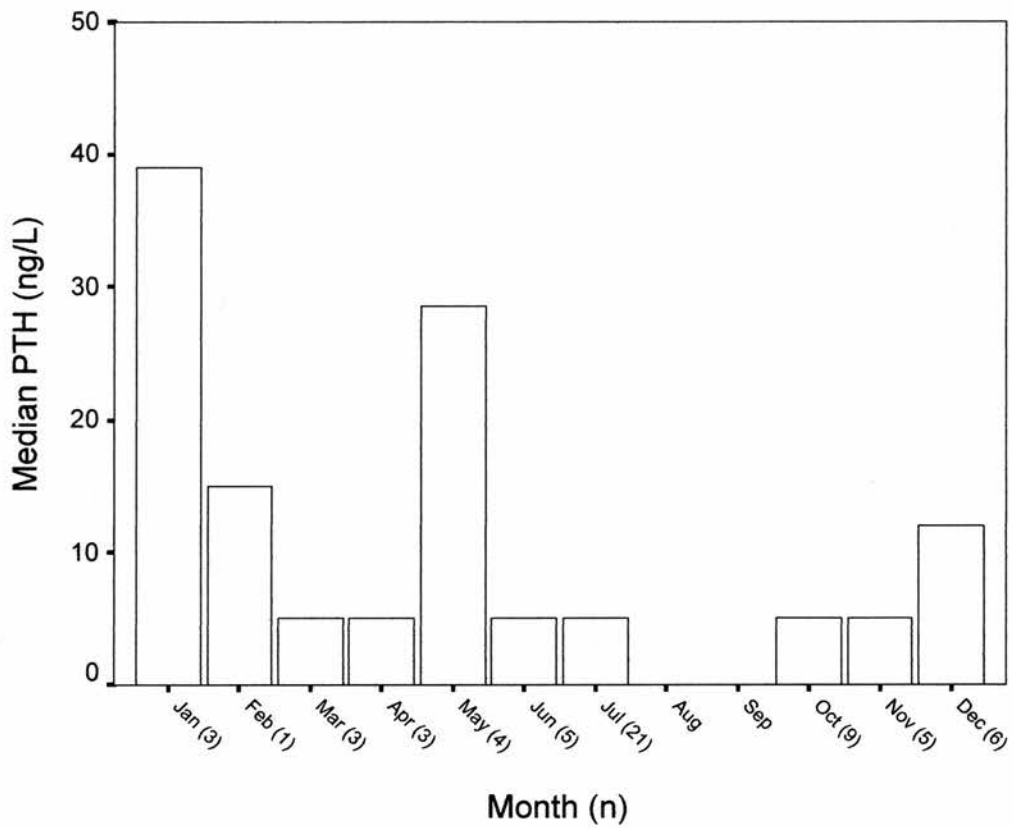


3.4.2 Parathyroid hormone and season of birth

PTH concentrations were analysed according to month of birth. Concentrations below the limit of detectability were assigned the value 5ng/L for data handling.

There was no significant difference between median PTH and season of birth, Kruskal-Wallis $p=0.132$ (Figure 23).

Figure 23: PTH and month of birth



3.5 PTHrP

Cord blood was assayed for PTHrP concentrations on 18 infants on whom excess venous blood was opportunistically obtained. Birthweight ranged from 0.720 to 4.975g and gestation 26 to 41 weeks. PTHrP concentrations ranged from 0.35 to 0.95 pmol/L, with Normal distribution, mean 0.55 pmol/L, s.d. 0.17.

PTHrP does not correlate with birthweight, birthweight centile, gestation. PTH concentrations, BMC or BMD. There was no relationship to antenatal steroid administration, pregnancy-induced hypertension, delivery by caesarean section, maternal smoking, absent end diastolic doppler flow or placental abnormality.

Figure 24: PTHrP concentrations and bone mineral content

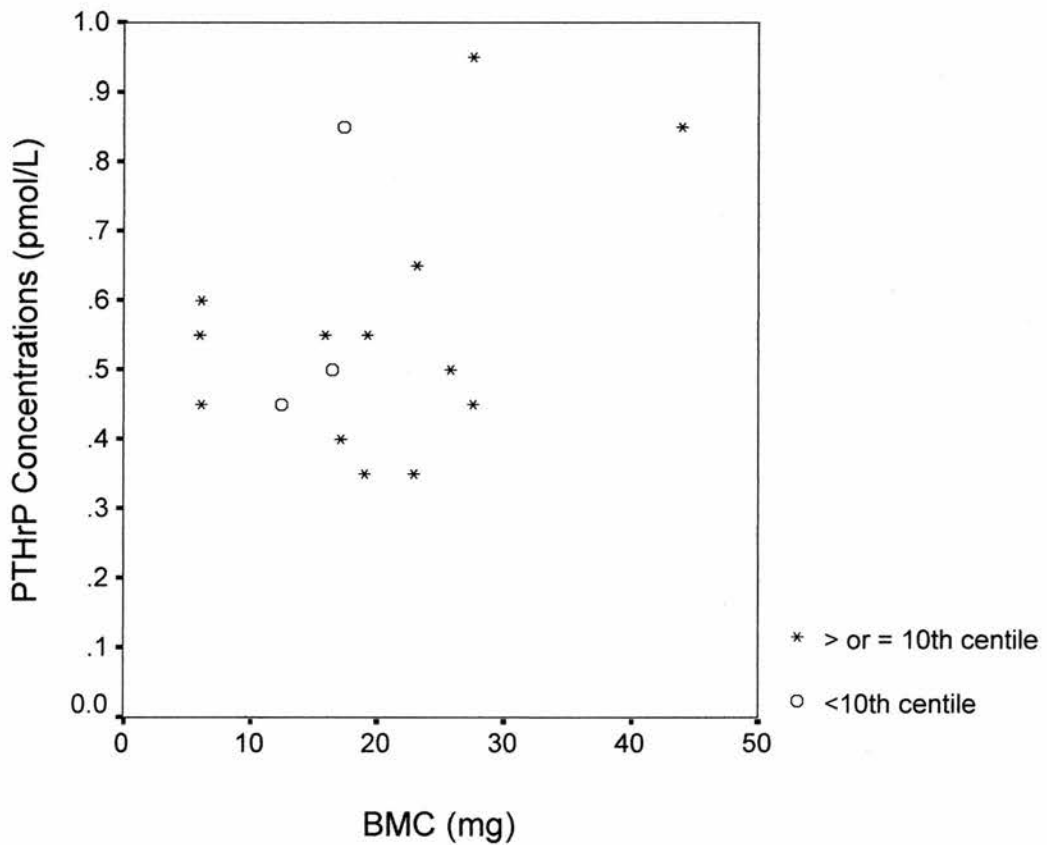
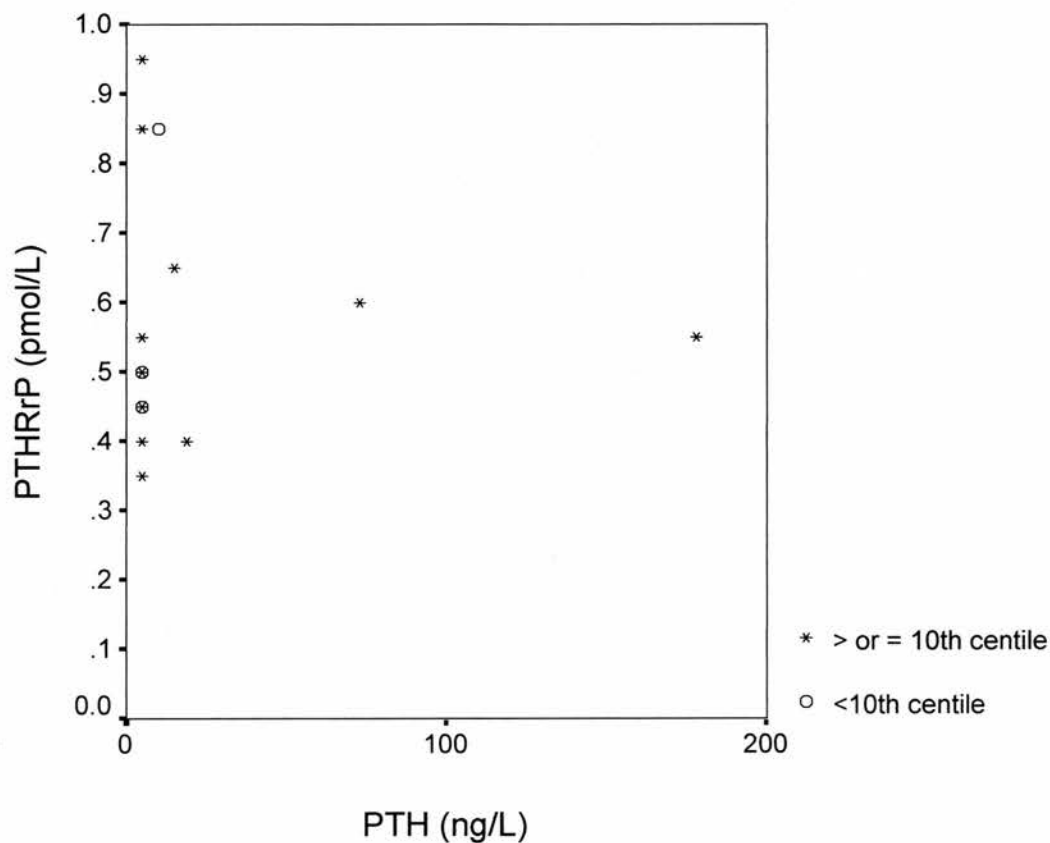


Figure 25: PTH and PTHrP concentrations



3.6 1,25-dihydroxyvitamin D

Cord blood was assayed for 1,25-DHCC concentrations on 48 infants ranging from birthweight 0.720 to 4.975g and gestation 26 to 41 weeks, with Normal distribution, mean 86.5 pmol/L, s.d. 27.09.

1,25-DHCC did not relate to birthweight (Figure 26), birthweight centile, gestation, BMC, BMD or PTH concentration. The infants with high PTH values had low 1,25-DHCC concentrations but this was not statistically significant (Figure 27).

Figure 26: 1,25-dihydroxycholecalciferol and birthweight

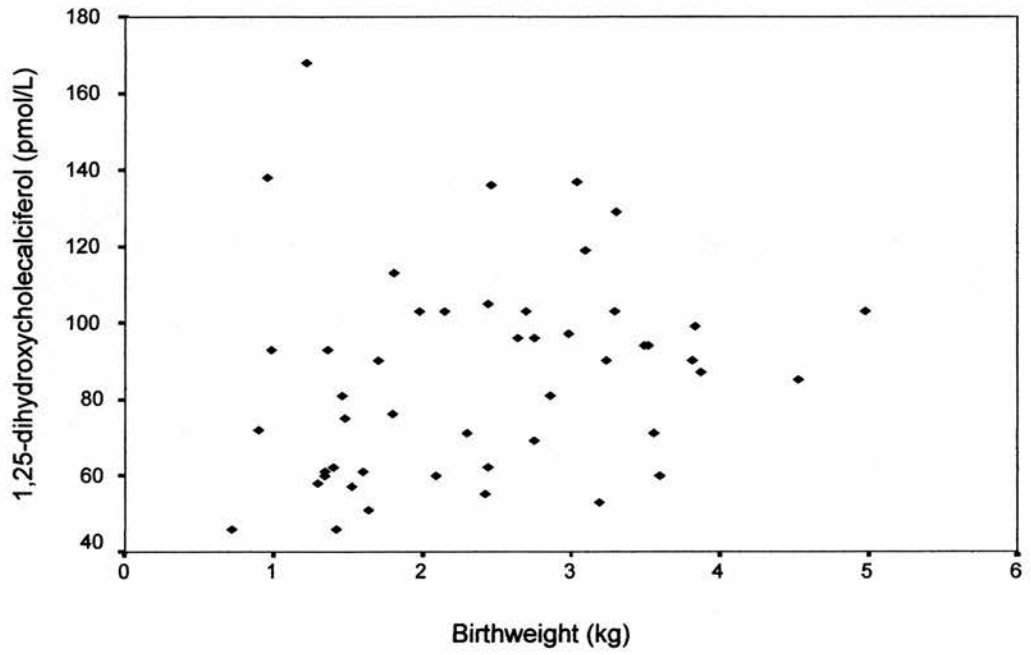
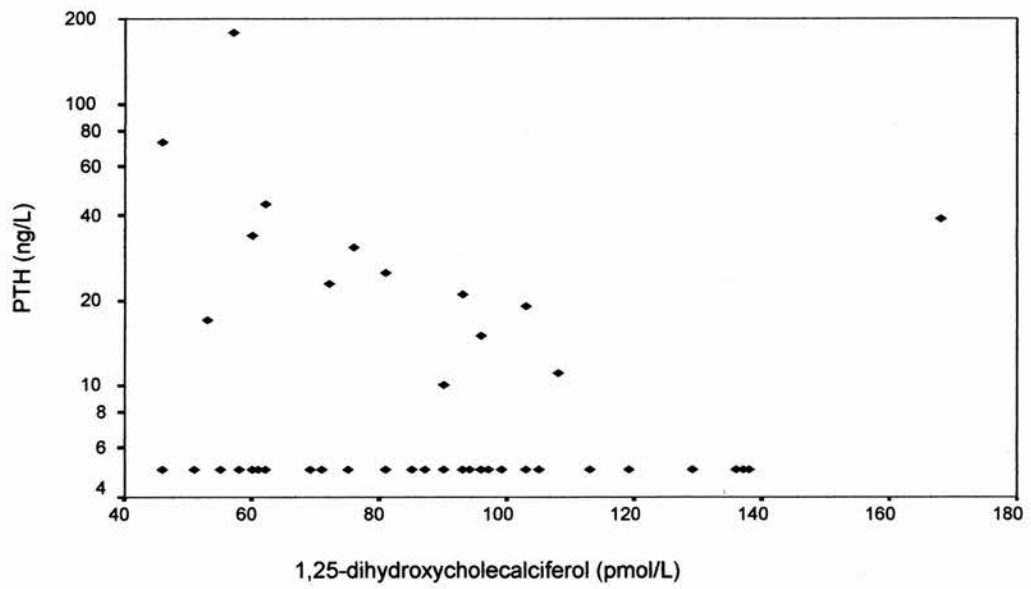


Figure 27: PTH and 1,25-dihydroxycholecalciferol



4 Results of randomised controlled trial

26 infants were recruited to the study between November 1997 and October 1999. Only parents of inborn infants with local addresses were approached for consent, excluding those who were likely to be transferred to other hospitals prior to discharge home. Permission from the attending medical team was sought on infants less than 25 weeks completed gestation who were unstable in the first days of life prior to approaching the parents, excluding some extremely low birth weight infants from the study (numbers not recorded). Some of these infants not approached for consent due to early complications of extreme prematurity subsequently died. Parents of two infants who remained eligible declined participation in the study.

On commencement of the study it was anticipated that infants would be recruited at a rate of 20 per year, allowing achievement of the target of 48 infants by March 2000, during which time the clinical researcher (AH) was available. During the time period of the study, the admission profile to the neonatal unit changed, reducing the number of preterm infants admitted to the tertiary referral centre in favour of infants with complex surgical and cardiac anomalies. In October 1999, radio-active iodine (I^{125}) manufacture ceased in the UK and a replacement source for the scanner could not be obtained, necessitating early termination of the study.

13 infants were randomised each to trial and control groups. One infant (trial group) was prematurely discharged from the neonatal unit and was excluded from the analysis. One infant (control group) developed necrotising enterocolitis with

perforation and was excluded from further data collection at 7 weeks of age (33 weeks PMA). 2 infants died (control group); one at 39 weeks PMA in the neonatal unit, and one at 40 weeks PMA following discharge from the neonatal unit. Overriding of radius and ulna at the midpoint prevented estimation of BMD and RW in 3 infants at birth (1 trial, 2 control) and 2 trial infants at term. In one trial infant the image was unusable due to movement artefact, and in one control infant at term the bone image was indistinct, preventing measurement of RW. In this infant BMC and BMD were designated zero.

Of the remaining 22 infants, 14 reached 64 weeks PCA within the study period, ending in November 1999. Bone measurements were made on 11 of these: 5 trial, 6 control. The remaining 3 infants were discharged from hospital follow-up prior to 64 weeks PCA (all trial group). 8 infants were recruited late in the study period such that the study ended before reaching 64 weeks PCA (4 trial, 4 control). 1 image (trial group) was lost from the archive and was not included in the analysis (Table 7).

Analysis was made on an intention-to-treat basis.

Table 7 : Details of infants entered into study

	Control	Trial	Total
Recruited	13	13	26
Excluded prior to entry		1 discharged from NICU	1
Entered	13	12	25
Excluded prior to term	1 excluded for NEC and bowel resection		1
Completed (term)	12	12	24
Excluded prior to 64 weeks PMA	2 died	3 discharged from follow-up, 1 lost to F/U	6
Recruited late, study ended	4	4	8
Completed (>64 weeks PMA)	6	4	10

4.1 Characteristics of trial and control groups

Trial and control groups were comparable at entry in terms of birthweight, gestation, BMC, BMD and RW (Table 8).

Table 8: Details of trial and control groups

	Trial (n=12)	Control (n=13)	p value
Birthweight (kg)	1.21 (0.11)	1.20(0.07)	0.94
Gestation (weeks)	28.9 (0.52)	28.4 (0.45).	0.60
Male/female	5/7	10/3	0.85
Initial BMC (mg)	7.4 (1.19) n=12	7.1 (1.36) n=13	0.54
Initial BMD (mg/cm ²)	80.2 (11.05) n=11	71.2 (9.25) n=11	0.73
Initial RW (cm)	0.42 (0.029) n=11	0.43 (0.030) n=11	0.85

Mean (sem)

n: number of infants included in analysis

4.1.1 Parenteral and enteral intake

Intravenous intake was analysed over the first 4 weeks of life. This included parenteral nutrition and IV dextrose/saline solution. There was no significant difference in carbohydrate, protein, fat, phosphorus or calcium received from IV fluids by the trial and control groups (Table 9). Some infants received no IV nutrition.

Enteral nutrition was analysed over the first 5 weeks of life. The trial group received more enteral phosphorus over the first 5 weeks ($p=0.037$) and had a significantly lower TRP ($p=0.002$) (Figure 28). Ca_E was not significantly different between the two groups (Figure 29). There was no difference in enteral carbohydrate, protein, lipid, calcium, vitamin D intake (Table 10). Without making assumptions about percentage of gastrointestinal absorption which may have differed due to varying absolute amounts of minerals, it was not possible to combine parenteral and enteral intake between the 2 groups.

Table 9: Daily parenteral intake in trial and control groups by week of age

	Week 1		Week 2		Week 3		Week 4	
	Trial n=12	Control n=13	Trial n=12	Control n=13	Trial n=12	Control n=13	Trial n=12	Control n=13
Calcium (mmol)	0.56 (0.06)	0.65 (0.06)	0.38 (0.12)	0.54 (0.09)	0.32 (0.11)	0.28 (0.10)	0.20 (0.09)	0.23 (0.09)
Phosphate (mmol)	0.35 (0.11)	0.59 (0.08)	0.49 (0.19)	0.57 (0.13)	0.38 (0.18)	0.24 (0.10)	0.25 (0.18)	0.19 (0.10)
Lipid (g)	0.70 (0.24)	0.88 (0.14)	1.04 (0.39)	1.36 (0.38)	0.83 (0.39)	0.63 (0.30)	0.36 (0.26)	0.59 (0.35)
Protein (g)	0.75 (0.23)	1.09 (0.15)	0.83 (0.29)	1.15 (0.27)	0.58 (0.26)	0.52 (0.22)	0.29 (0.20)	0.4 (0.25)
Carbohydrate (g)	9.3 (0.7)	10.7 (0.8)	5.5 (1.7)	8.8 (1.5)	4.8 (1.7)	4.7 (1.7)	3.4 (1.4)	4.7 (2.0)

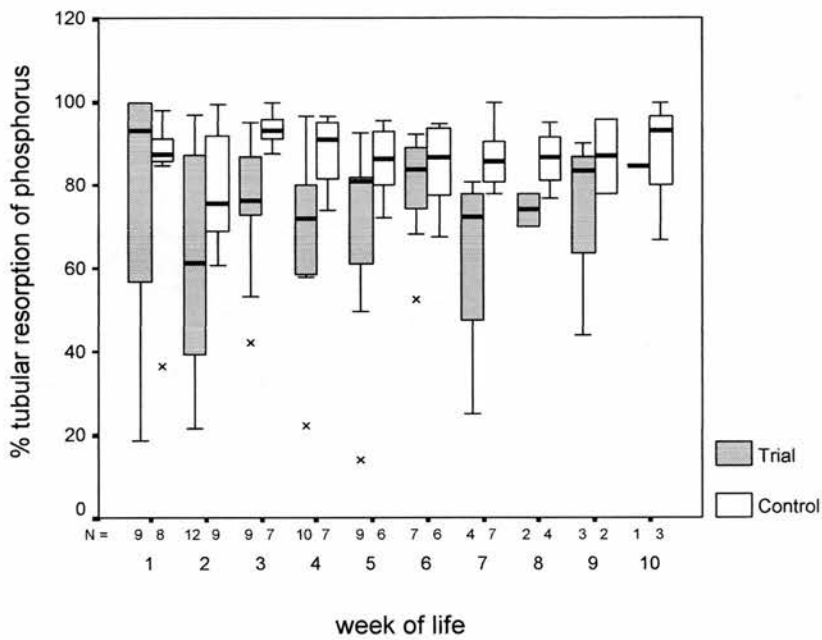
mean (sem)

Table 10: Daily enteral intake in trial and control groups by week of age

	Week 1		Week 2		Week 3		Week 4	
	Trial n=12	Control n=13	Trial n=12	Control n=13	Trial n=12	Control n=13	Trial n=12	Control n=13
Calcium (mmol)	0.26 (0.09)	0.26 (0.17)	1.63 (0.48)	1.27 (0.30)	2.27 (0.54)	2.20 (0.41)	2.98 (0.49)	2.48 (0.43)
Phosphate (mmol)	0.26 (0.08)	0.20 (0.13)	1.66 (0.44)	0.91 (0.21)	2.41 (0.47)	1.61 (0.30)	2.44 (0.39)	1.86 (0.33)
Lipid (g)	1.14 (0.32)	0.68 (0.31)	4.37 (0.85)	3.52 (0.66)	4.98 (0.80)	4.68 (0.65)	5.69 (0.52)	4.50 (0.77)
Protein (g)	0.54 (0.14)	0.32 (0.15)	1.99 (0.46)	1.75 (0.34)	2.68 (0.47)	2.53 (0.39)	3.03 (0.31)	2.82 (0.47)
Carbohydrate (g)	2.2 (0.6)	1.2 (0.6)	8.6 (1.8)	6.6 (1.3)	10.6 (2.0)	9.6 (1.5)	12.2 (1.3)	10.4 (1.7)
Vitamin D (IU)	18 (10)	12 (9)	307 (92)	135 (58)	485 (116)	370 (97)	552 (96)	513 (111)

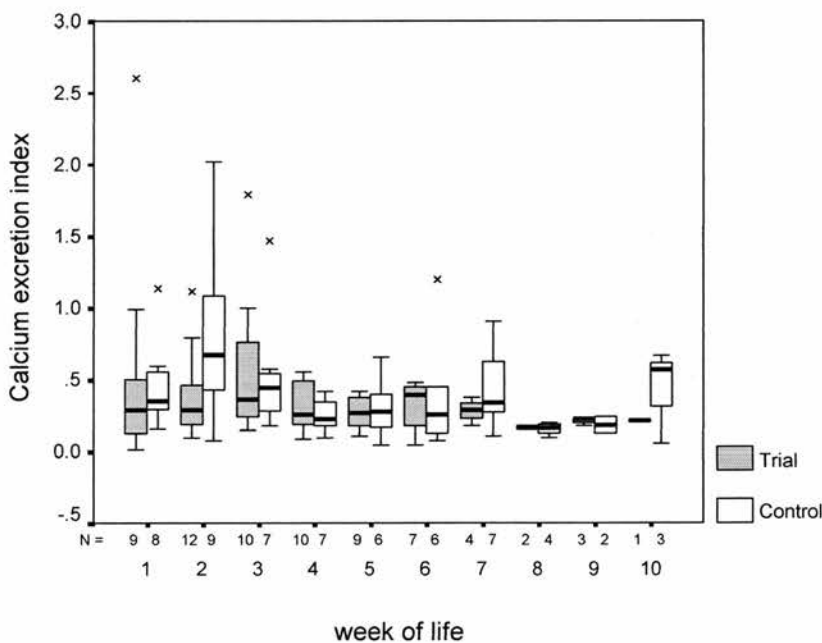
mean (sem)

Figure 28: Tubular resorption of phosphorus in trial and control groups



(Bar=median, box=interquartile range, whiskers=range, x=outliers greater than 1.5 times box length)

Figure 29: Calcium excretion index in trial and control groups



(Bar=median, box=interquartile range, whiskers=range, x=outliers greater than 1.5 times box length)

4.1.2 Mineralised bone mass

Analysis at discharge showed no significant difference between trial and control group in weight, gestation, BMC, BMD or RW at term, despite individualised supplementation regimens and significantly higher phosphorus intake with a fall in TRP in the trial group (Table 11).

With 12 infants in each group the study has 80% power to detect 25% difference and 90% power to detect 28% difference.

Table 11: Details of trial and control groups at discharge

	Trial (n=12)	Control (n=12)	p value
Weight (kg)	2.08(0.078) n=12	2.27 (0.085) n=12	0.11
Gestation (weeks)	36.2 (0.46) n=12	36.8 (0.44) n=12	0.33
Term BMC (mg)	8.3(1.064) n=11	10.0 (1.413) n=12	0.37
Term BMD (mg/cm ²)	62.1 (7.603) n=9	77.3 (9.789) n=12	0.26
Term RW (cm)	0.57 (0.018) n=9	0.57 (0.034) n=11	0.93

Mean (sem)

n: number of infants included in analysis

4.3 Group characteristics

Daily weight gain, number of babies ventilated, length of time on a ventilator, and the use of caffeine, dexamethasone, diuretics or antibiotics was the same in both groups.

4.3.1 Weight gain

There was no difference in mean daily weight gain between the 2 groups, with 16.9g (0.91) in the trial group and 17.3g (1.2) in the control group.

4.3.2 Ventilation

9 infants in the trial group and 11 in the control group were intubated and ventilated from birth. Trial infants received a median of 1 day intubation initially (range 0-5, n=12) with a total of 2 (0-53, n=12) days of intubation and nasal CPAP. Control infants received 2 (0-12, n=13) days of intubation, with a total of 6.5 (0-48, n=13) days of ventilatory support (Table 12).

4 infants in the trial group and 5 infants in the control group were discharged on home oxygen. The number of days until no longer requiring supplemental oxygen

in the remaining infants was 1 (0-40, n=8) in the trial group and 3 (0-64, n=8) in the control group.

Table 12: Ventilatory support in trial and control infants

	Intubation (days)	Intubation + CPAP (days)
Trial n=12	1 (0-5)	2 (0-53)
Control n=13	2 (0-12)	6.5 (0-48)

Median (range)

n: number of infants included in analysis

4.3.3 Medication

There was no significant difference in the use of caffeine, dexamethasone, ranitidine, frusemide, chlorothiazide and spironolactone (Table 13). The dose and length of time the drug was administered was not considered.

No significant differences were found in the use of benzyl penicillin, ampicillin, amoxicillin, flucloxacillin, gentamicin, aztreonam, vancomycin, meripenem, trimethoprim, metronidazole and acyclovir.

Table 13: Drug administration to trial and control infants

	Trial (n=12)	Control (n=13)
Caffeine	9	13
Dexamethasone	2	0
Ranitidine	3	1
Frusemide	3	7
Chlorothiazide	4	7
Spironolactone	4	7
Indomethacin	0	1
Diamorphine	3	7
Pancuronium	2	4
Dopamine	1	1
Insulin	1	0

n: number of infants included in analysis

4.4 Morbidity and Mortality

4 infants in the control group and none in the trial group developed necrotising enterocolitis; 3 Bell's stage 1, 1 Bell's stage 3 (Bell et al, 1978). One was excluded from the study after undergoing laparotomy and ileal resection. The remaining 3 remained within the study as nutrition was not discontinued for more than 10 days.

2 infants in the control group died; one of ventriculitis following shunt insertion for posthaemorrhagic hydrocephalus at 39 weeks PCA, and the other died at home following discharge at 40 weeks PCA, with a diagnosis of unexplained sudden infant death.

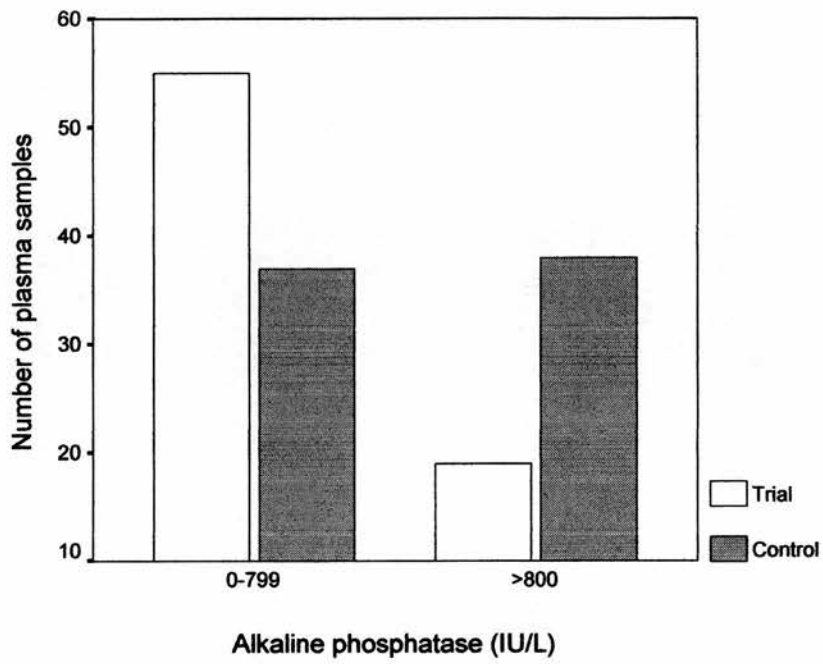
4.5 Alkaline Phosphatase

Maximum alkaline phosphatase was significantly different ($p=0.05$) between the 2 groups, trial 918 (s.d 81.5, $n=12$), control 1132 (s.d. 65, $n=13$).

An alkaline phosphatase concentration in excess of 800 IU/L is used in the clinical setting to indicate the upper limit of normal for the preterm population. Plasma concentrations exceeded this limit more frequently in the control group than trial group ($p<0.001$). As the concentrations measured in the first 2 weeks of life were unlikely to have been influenced by mineral supplementation, these were subsequently excluded, leaving a significance of $p=0.002$ (Figure 30).

There was no correlation between alkaline phosphatase and birthweight or gestation. BMC, BMD, and RW were measured every 4 weeks during the study period. There was no relationship between any of the bone mineral parameters and AP in trial or control groups.

Figure 30: Alkaline phosphatase concentrations in trial and control groups



4.6 Post hoc analysis

With 12 infants in each group the study has 80% power to detect 25% difference and 90% power to detect 28% difference between the 2 groups. As there was no difference between trial and control infants following intervention further analysis has been performed on the cohort of 25 infants.

Of the 3 parameters of mineralised bone mass, a significant increase was seen in BMC ($p=0.011$), and RW ($p<0.001$), with no increase in BMD in the first weeks of life, despite a significant increase in weight (Table 14). Using the regression equation derived from the cohort data, study infants had a significantly lower BMC and BMD but not RW at term compared to term-born infants of the same weight.

Table 14: Mineralised bone mass changes in recruited infants compared to cohort data

	Entry	Discharge	p-value (discharge v. entry)	Weight adjusted estimate [#]	p-value (estimate v. discharge)
Weight (kg)	1.21 (0.07) n=25	2.16 (0.06) n=24			
PCA (weeks)	28.6 (0.34) n=25	36.5 (0.32) n=24			
BMC (mg)	7.2 (0.89) n=25	9.2 (0.91) n=23	0.011	13.7 (12.9/14.6)	<0.001
BMD (mg/cm ²)	75.7 (7.10) n=22	70.8 (6.84) n=21	0.54	105.5 (99.1/111.9)	<0.001
RW (cm)	0.43 (0.02) n=22	0.57 (0.02) n=20	<0.001	0.53 (0.50/0.57)	0.044

Mean (sem)

[#]Based on regression equations derived in cohort study (Table 2) with weight of 2.16 kg (95% CI)
n: number of infants included in analysis

4.6.1 Birthweight group analysis

The data was further analysed according to birthweight, comparing extremely low birthweight infants (ELBW <1kg, n=7) and low birthweight infants (non-ELBW \geq 1kg, n=18). At birth there were significant differences in BMC ($p=0.006$), BMD ($p=0.004$) and RW ($p=0.028$) between the two groups, as expected due to difference in birthweight. At term, differences between the two groups remained in RW ($p=0.018$), with no difference in BMC or BMD. Higher birthweight infants had greater increase in RW, although both groups had a significant increase in RW from birth to discharge (ELBW $p=0.038$, non-ELBW $p=0.001$). There was no difference in weight between the 2 groups at term measurements but the ELBW infants were more mature ($p=0.034$), with a mean weight gain in both groups of 17g/day (Table 15).

The ELBW group had a significant increase in BMC ($p=0.041$), but this was not seen in the non-ELBW group ($p=0.09$).

This subgroup analysis suggests that RW increases without mineralised bone mass even in the higher birthweight preterm infants.

Table 15: Mineralised bone mass changes in recruited infants by birthweight

	Birth		Term	
	<1kg (n=7)	≥1kg (n=18)	<1kg (n=7)	≥1kg (n=18)
Birthweight (kg)	0.822(0.051) n=7	1.354(0.060) n=18	2.046(0.156) n=7	2.218(0.049) n=18
Gestation	27.0(0.5) n=7	29.2(0.3) n=18	37.4(0.7) n=7	36.0(0.3)** n=18
BMC (mg)	3.5(0.8) n=7	8.7(1.0)* n=18	6.6(1.4) n=7	10.3(1.0) n=15
BMD (mg/cm ²)	47.9(9.7) n=7	88.7(7.4)* n=15	64.7(8.9) n=6	73.3(8.5) n=15
RW (cm)	0.36(0.03) n=7	0.46(0.02)* n=15	0.50(0.04) n=6	0.60(0.02)** n=14

Mean (sem)

n: number of infants included in analysis

*p<0.05 between <1kg and ≥1kg at birth

**p<0.05 between <1kg and ≥1kg at term

4.7 Follow-up

BMD and RW was measured at 71.9 (sem 1.52) weeks PMA in 10 infants: 4 trial, 6 control. There was no difference between trial and control groups, with $p=0.95$ (Table 16).

This group of 10 infants has a BMD of 112.9 mg/cm^2 (sem 12.2) and RW 0.87 cm (sem 0.05). Using the cohort regression equations this corresponds to a newborn infant of birthweight 2.314 kg for BMD and 3.73 kg for RW. There was no difference between ELBW and non-ELBW groups (5 in each group).

Table 16: Follow-up of trial and control groups

	Trial	Control
n	4	6
BMD (mg/cm^2)	114.0 (23.6)	112.1 (14.9)
RW (cm)	0.86 (0.02)	0.88 (0.08)

Mean (sem)

n: number of infants included in analysis

5 Discussion

The portable single photon absorptiometer has permitted measurement of radial BMC, BMD and RW in extremely low birth weight infants during the first few days of life. This technique has confirmed that radial BMC, BMD and RW are linearly related to birthweight from 23 to 41 weeks gestation. BMC measurements are comparable to previous studies using SPA (Minton et al, 1979; Pohlandt & Mathers, 1989).

5.1 Cohort study

5.1.1 What does this study add?

The hypothesis stated that in impaired transplacental calcium delivery, PTH production by the fetus is increased, enhancing fetal calcium resorption from bone such that the growth restricted infant will demonstrate elevated cord blood PTH concentrations in association with reduced MBM. In order to explore this hypothesis, it was important to confirm that modifications to the portable single absorptiometer rendered it a suitable tool to use in extremely low birthweight infants.

This study demonstrated that the portable single photon absorptiometer permitted measurement of radial BMC, BMD and RW in extremely low birth weight infants during the first few days of life. It has confirmed that radial BMC, BMD and RW is

linearly related to birthweight from as early as 23 weeks PMA. The regression model demonstrates a strong relationship between BMC, BMD, RW and birthweight in infants across the gestational age range, but excludes refining factors included in the model. Several investigators have used experimental techniques to examine mineralisation in the preterm infant (Pohlandt & Mathers, 1989; Koo et al, 1996; Lapillonne et al, 1997), however none of these have been used in the extremely low birth weight infant immediately following birth. We found that birthweight was the single best predictor of radial BMC, BMD and RW, such that retrospective estimation of radial mineralised bone mass at birth can be inferred on any infant whose birth weight is known. However, this does not suggest a causal effect of birthweight on mineralised bone mass.

This study also confirms the SGA infant to have radial mineralised bone mass comparable to the AGA infant of the same birthweight, as shown by Pohlandt using mid-humeral SPA (Pohlandt & Mathers, 1989), and Lapillonne using whole body DXA (Lapillonne et al, 1997).

Secondly, it was necessary to describe the infant with potentially impaired transplacental function. Antenatal and maternal factors which were thought to be potentially associated with fetal mineralisation were chosen and used in a regression model against estimates of mineralisation in the newborn infant. Infants of mothers who had received antenatal dexamethasone had reduced BMC, BMD, and RW. However these infants were also likely to be of low gestation and low birthweight. This variable was not included in the multiple regression model due to

data being unavailable on more than 1/3 of the infants. On the advice of epidemiologist (Dr P MacKinney) all data was collected from maternal records rather than maternal questioning, to avoid recollection bias. Unfortunately records from referring hospitals were often unavailable, reducing the amount of information which could be achieved. A model of the reduced dataset excluded antenatal steroids at $p=0.055$. This is difficult to interpret as the dataset is small, and given the overwhelming benefits of antenatal steroids on neonatal lung maturation (Crowley, 1999) this finding has minimal relevance.

There is increasing evidence that there is no advantage in administering multiple doses of maternal steroids and they may even have detrimental effects (Banks et al, 1999; French et al, 1999). An adverse effect of antenatal steroid administration may be potentiated in infants exposed to multiple doses. This data suggests that follow-up studies on the effect on fetal mineralised bone mass on infants delivered following multiple steroid doses is warranted.

The demonstration of absent or reversed end-diastolic flow on doppler ultrasound scan was also associated with reduced BMC and BMD. However this was associated with low birth weight and did not improve the regression model. In the regression models excluding birthweight as a potential predictor, this variable was also non-significant.

In all 3 models excluding birthweight as a predictor (BMC, BMD and RW), placental abnormality remained a significant predictor. Unlike antenatal steroids,

the presence of placental abnormality was not related to birthweight. However, the definition was a clinical one assigned by the obstetrician or midwife present at delivery and was not based on a pathological examination. Descriptions recorded as “abnormality” included “small”, “gritty” or the presence of an infarct or haemorrhage. As there were only 5 infants with placental abnormality on whom PTH concentrations were available, it was not possible to analyse the presence/absence of placental abnormality in relation to PTH concentrations.

Previous studies have shown elevated PTH concentrations in infants delivered by caesarean section (Bruchi et al, 1984). It is not clear if this was seen as an acute response to the operative procedure or fetal distress, or if delivery by caesarean section could be used as a marker for chronic *in utero* compromise, potentially resulting in impaired mineralised bone mass. We therefore included mode of delivery in the regression equation. We found no relationship between mode of delivery and BMC, BMD, RW.

Finally, the hypothesis stated that impaired placental function associated with growth restriction would result in reduced mineral accretion manifesting as diminished mineralised bone mass, and that such an infant would exhibit increased production of PTH in order to enhance plasma calcium concentrations. We found that term infants (>37 weeks PCA) have low venous cord PTH concentrations, below the limit of detectability by this assay, and that infants with high PTH concentrations were of lower birthweight and of lower gestation. Term low birth weight infants did not have elevated PTH concentrations (Figure 14).

Some SGA infants were found to have low PTH concentrations, and elevated concentrations were seen in AGA preterm infants. These results do not support the hypothesis that PTH production by the growth-restricted fetus is increased. PTH concentrations are detectable in the second trimester and fall below the normal adult range at term.

Although physiological *in utero*, the presence of circulating PTH may result in renal phosphorus losses in the first days of life, explaining the early phosphorus deficiency seen in preterm infants (Holland et al, 1990). As well as the renal effects, PTH acts on osteoclasts to increase bone resorption. Low PTH concentrations seen in some SGA and preterm infants could prevent increased resorption and therefore demineralisation of the skeleton, and be viewed as a protective effect.

The portable single photon absorptiometer has permitted measurement of radial BMC, BMD and RW in extremely low birth weight infants during the first few days of life. This technique has confirmed that radial BMC, BMD and RW is linearly related to birthweight from 23 to 41 weeks gestation. BMC measurements are comparable to previous studies using SPA (Minton et al, 1979; Pohlandt & Mathers, 1989). However, DXA is now seen as the “gold standard” for bone mineral estimation in the newborn period. Given the practical constraints of performing DXA measurements on the extremely preterm infant at birth, this technique has added to the current literature by demonstrating the linear relationship between

birthweight and mineralised bone mass to the limits of viability in the liveborn infant.

5.1.2 Critique of the Methodology

This cohort study could have been significantly improved both in the study design and in the study procedure. Most importantly, the number of infants recruited should have been increased significantly as outlined below, with both cord blood sampling, data collection and bone images completed on all recruited infants, and precise documentation of reasons for non-recruitment of eligible infants. Increasing the size and completeness of the datasets would have significantly improved the ability to broaden the analyses and explore the hypothesis.

5.1.2i Population

Recruitment was targeted at preterm infants and infants who were expected to be small for gestational age. This was a significant flaw in the study design, as this cohort study should have been inclusive of all deliveries. By having more available researchers, all infants delivered during the recruitment period could have been targeted for cord blood collection. Where recruitment did not take place, the reason for non-inclusion or non-consent should have been recorded.

5.1.2ii Consent

Attempts should have been made to approach parents of all eligible infants about inclusion in the study. Therefore, having multiple researchers involved in the consent process may have increased the number of infants recruited, and aided collection of data on those eligible infants not enrolled in the study.

5.1.2iii Measured variables

The measured variables should be defined at the start of the data collection as: BMC, BMD, RW, PTH, PTHrP, 1,25-DHCC, birthweight, gestation, maternal PIH, placental abnormality, antenatal steroids, gender, method of delivery, onset of labour, antenatal doppler studies, maternal smoking.

5.1.2iv Data Sampling

8 ml of umbilical cord blood should have been obtained for each infant, permitting analysis of PTH, PTHrP and 1,25-DHCC. Venous cord blood samples were drawn from the clamped umbilical cord following extraction of the placenta. By drawing mixed blood “milked” from the placental bed, the number of samples may have been increased, particularly in growth-restricted infants on whom the umbilical cords were thin and difficult to sample from.

Clearly defined criteria for the diagnosis of pregnancy induced hypertension and placental abnormality should have been implemented. Data sets could have been improved by extending the data collection process from medical record extraction to maternal history taking. As an epidemiological research process this is considered inaccurate due to recollection bias, and was therefore not done.

An inherent problem with testing the hypothesis is defining the growth-restricted infant exposed to placental insufficiency. We attempted to strengthen our regression model by including factors which may influence the growth of the fetus or act as markers of impaired placental function: maternal smoking, pregnancy induced

hypertension, placental abnormality, and absent/reversed end diastolic flow. Data was collected on the presence of pre-existing maternal illness and growth of the fetus by ultrasound scan. Insufficient complete data sets were available in relation to the number of variables in the model, and they were therefore excluded from the analysis. Furthermore, the likelihood of statistical error increases along with the increasing number of variables included from a small dataset.

Mineralised bone mass was measured using single photon absorptiometry, using a portable machine which had been adapted for use in the preterm infant. The data available from these images was limited, and could now be improved by using DEXA. X-rays may have provided further information.

5.1.2v Study Design

The cohort study is a collection of overlapping datasets, with one substudy exploring the effect of maternal and pregnancy factors on mineralised bone mass, and one looking at the relationship between cord PTH and mineralised bone mass. This should have been collected as one dataset, with bone images and cord blood collected on all infants, as well as complete data extraction from the maternal charts, supplemented with history taking.

5.1.2vi Data Collection

As previously stated, data collection was poor, with details of all non-eligible infants, and eligible infants not recruited or consented being unrecorded.

Further information may have been obtained by measuring 25-hydroxycholecalciferol concentrations in maternal or cord blood. 25-HCC is an intermediate metabolite which is thought to reflect overall vitamin D, as it is the most abundant circulating form. The aim of this study was to explore potential fetal mechanisms implicated in the control of materno-fetal calcium transport, and as limited blood was likely to be available, we therefore chose to focus on 1,25-DHCC, the active metabolite, rather than 25-HCC. 25-HCC is thought to cross the placenta directly (Delvin et al, 1982; Hollis & Pittard, 1984; Zeghoud et al, 1997), and might therefore have been drawn from maternal blood samples.

5.1.3 Limitations of single photon absorptiometry

A water bolus was used as a background on the assumption that the forearm of the infant is comprised of soft tissues with a density comparable to water. Although this applies to term infants, in the extreme preterm infant the soft tissue mass is negligible, such that the radius and ulna occupy a substantial volume of the forearm. In these infants the use of the background water bolus may result in a lower estimate of BMC and BMD.

Interpretation of BMD measurements is limited by the necessity to assume that the bone is cylindrical, which is only true in small infants. Using this assumption it would be possible to estimate a true bone mineral density using BMD and RW. It was felt that this assumption was inappropriate and was therefore not applied. In interpreting the results using area-normalised BMD rather than true density it must be remembered that a bone of larger radial width but of the same true density will have a larger area-normalised density. By using BMC of a 1mm segment of radius and ulna, area-normalised BMD of radius, and radial width together, we are able to observe the changes in mineral deposition within the bone and in mineralised-bone growth, but cannot measure true changes in bone mineral density. Similarly our results are applicable only to forearm bone and cannot be extrapolated to total body mineralised bone mass.

This technique provides an estimation of bone mineral content and areal density based on the detection of hydroxyapatite, providing a measurement of crystal

deposition within the bone matrix, and cannot detect unmineralised osteoid. MBD is characterised by reduced matrix formation and decreased osteoblast activity, although rickets is caused by the accumulation of unmineralised osteoid at the growth plates. SPA provides early detection of reduced hydroxyapatite deposition, but may fail to identify the earliest changes in abnormalities of mineralised bone mass.

5.1.4 Effect on future research

With the increasing emphasis on the “fetal origin of adult disease”, techniques for identifying infants at risk of disease in adult life, and an understanding of the mechanism of disease pathogenesis and potential intervention are attracting attention.

Osteoporosis is responsible for significant morbidity in the adult population. Our study has shown that low birthweight infants have reduced radial mineralised bone mass at birth. This data can be used to explore a link between adult and newborn mineralised bone mass, using low birthweight as a marker for low mineral status at birth. Inevitably environmental and potentially other genetic factors will play a role in bone mineralisation in adult life, but an association between birthweight and adult bone mineral content would support the theory of nutritional programming in bone development.

We explored a hormonal variation to explain differences in bone mineralised bone mass, hypothesising that SGA infants would have an elevated PTH concentration in response to reduced mineral availability. Although this was not demonstrated, a high PTH concentration was found in low birth weight infants as a result of immaturity. We hypothesise that exposure to elevated PTH concentrations in the perinatal period may upregulate receptors, “resetting” at a higher level, such that elevated PTH concentrations are required throughout life to maintain “normal” mineral homeostasis. Such children would be expected to exhibit reduced 1,25-

DHCC in the presence of elevated PTH and low calcium concentrations. In the presence of low plasma calcium concentrations, an increase in PTH production might fail to stimulate bone resorption, thereby resulting in failure to reduce mineralised bone mass in the presence of relative hypocalcaemia (Figure 1), effectively resulting in “bone sparing”. Follow-up studies of preterm infants fed unsupplemented human milk have shown catch-up mineralisation at 8-12 years of age (Fewtrell et al, 1999). Infants who were thinner at 1 year of age were also seen to have increased catch-up in mineralisation by 6-7 years of age (Kurl et al, 1998). Over-mineralisation would not be seen if excessive mineral supplementation were supplied, as this effect of suppressing PTH concentrations would not impact on mineralised bone mass. Assessment of PTH, 1,25-DHCC in association with measurement of mineralised bone mass would be required to further this hypothesis.

We hypothesised that reduced calcium availability in the fetus would result in increased PTH production, potentially as a means of increasing placental calcium transfer. Although this is not a proven action of PTH, the hormone PTHrP has been shown to increase placental calcium transfer in the sheep (Rodda et al, 1988). As a pilot study we measured PTHrP in a number of infants, but found no relationship between PTH or mineralised bone mass and PTHrP. The assays for PTHrP are continuing to evolve and further research in this area may be warranted.

5.2 Randomised controlled trial

It is well recognised that demineralisation in premature infants can be reduced by mineral supplementation (Greer & McCormick, 1988; Gross, 1987, Abrams et al, 1989; Schanler et al, 1992). We sought to further improve mineralised bone mass by individual calcium and phosphorus supplementation based on plasma concentrations and urinary excretion as suggested by Pohlandt (Pohlandt, 1994a). However in this randomised controlled trial we were unable to show that supplementation of calcium and phosphorus based on urinary excretion improved mineralised bone mass in this population of preterm infants.

5.2.1 Mineralised bone mass at discharge

The longitudinal data on the preterm infants has been analysed in relation to the data collected on newborn infants in study 1. As with previous studies (Horsman et al, 1989a; Lapillonne et al, 1994) BMC and BMD remained below weight-adjusted values according to weight at term.

Using the portable single photon absorptiometer we were able to perform longitudinal bone measurements on a cohort of very low birthweight infants. This study demonstrated that in both trial and control infants there was a failure to demonstrate any increase in mineralised bone mass from birth to term. A further unexpected finding in the post hoc analysis was that even stable larger infants, who had not received parenteral nutrition or had respiratory complications had failed to

mineralise. In fact a greater increase in BMC and BMD was seen in the smaller infants, although they had more time to achieve this increment.

In contrast, a greater increment in radial width was seen in the mature infants. Given that the mineral content in a 1mm section of bone was the same in the 2 groups, this suggests that true volumetric bone density, which is not measurable by this technique, may actually be lower in the larger infants. The greater increase in radial width than BMC or BMD suggests that bone growth is taking place without mineralised bone mass.

5.2.2 Follow-up

The numbers of infants followed beyond term is small, however these infants appear to remain significantly demineralised at 6 months corrected gestational age. In this study the lack of mineralised bone mass is even more marked when compared to reports of catch-up mineralisation seen in similar preterm populations (Horsman et al, 1989b). In this cohort there did not seem to be a relationship between birthweight and mineral status at 6 months as seen by Rubinacci (Rubinacci et al, 1993). Previous studies have suggested that reduced mineralised bone mass is a result of both reduced growth and reduced density of mineralisation (Horsman et al, 1989a), however at 6 months our infants had a radial BMD expected in a newborn infant of 2.3 kg.

5.2.3 Individualised supplementation

This trial confirms that oral bolus and continuous intravenous phosphorus supplementation reduces the percentage tubular resorption of phosphorus as seen in the trial group. The effect of calcium supplementation on urinary calcium excretion is difficult to assess, as calcium excretion may be increased both by calcium supplementation and phosphorus depletion (Senterre et al, 1983), possibly accounting for the lack of observed difference in calcium excretion index between the trial and control groups. Although failing to enhance mineralised bone mass, the use of concomitant plasma and urinary monitoring may aid the assessment of dietary supplementation in minerally-depleted infants.

5.2.4 Alkaline Phosphatase

The study provides further evidence that alkaline phosphatase is unhelpful as a marker of bone mineral status (Walters et al, 1986; Evans et al, 1989; Pittard et al, 1992; Faerk et al, 2002). There was no correlation between AP and our measures of mineralised bone mass. However, higher concentrations of AP were seen in the control group, despite a failure to demonstrate improved mineralised bone mass with enhanced supplementation in this group. Lucas (Lucas et al, 1989) has suggested that an increase in plasma activity of AP may be seen in substrate deficiency despite a reduction in mineralised bone mass. These results suggest that individual supplementation provides a more adequate substrate level, as indicated by increased urinary bone mineral excretion, but this is not translated into measurable hydroxyapatite within the bone matrix.

5.2.5 Cortical Width

In the term infant, the single photon absorptiometer produces images of the forearm bones permitting delineation of the cortex of the bones (Figure 5). In the preterm infant at birth the cortex is often difficult to distinguish, and this become more ill-defined in the ex-preterm infant at term. In one infant we were able to obtain cortical width assessments from the SPA images(Figure 31, Figure 32), using the profile of bmc/pixel, where cortical width was assumed to be the distance from the outside edge to the peak pixel density (Figure 33). In this example, cortical width remained the same at term as at birth, with the distance from outside edge to peak pixel density of 0.11 cm in both images. In the majority of images of preterm infants it was not possible to distinguish the peak pixel density in order to estimate cortical width.

Figure 31: SPA image of 25 week gestation infant at birth

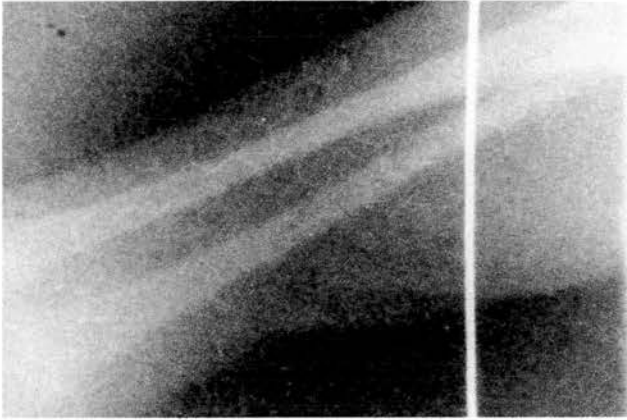


Figure 32: SPA image of 25 week gestation infant at term

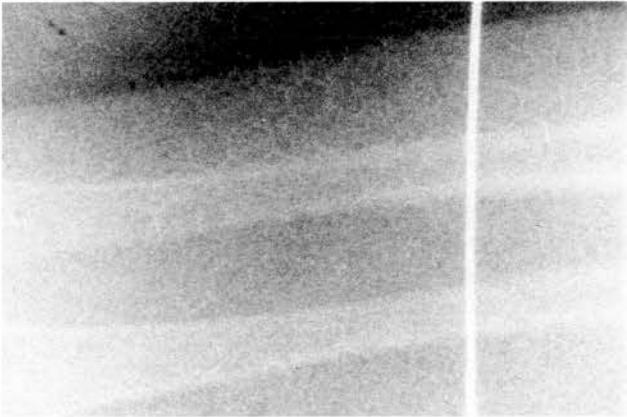
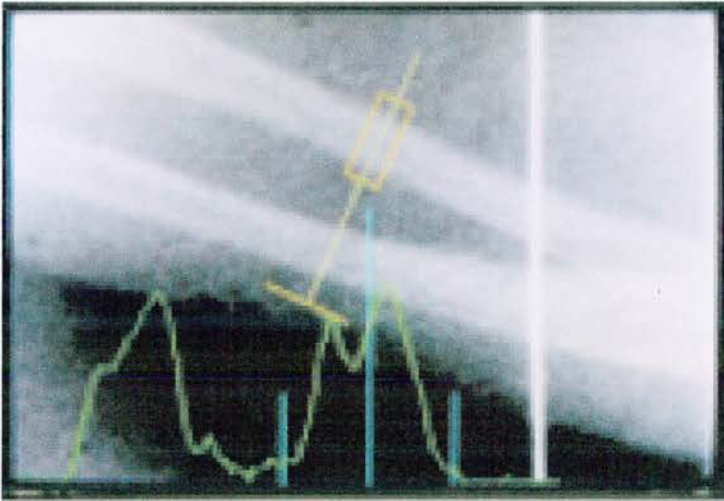


Figure 33: Determination of cortical width

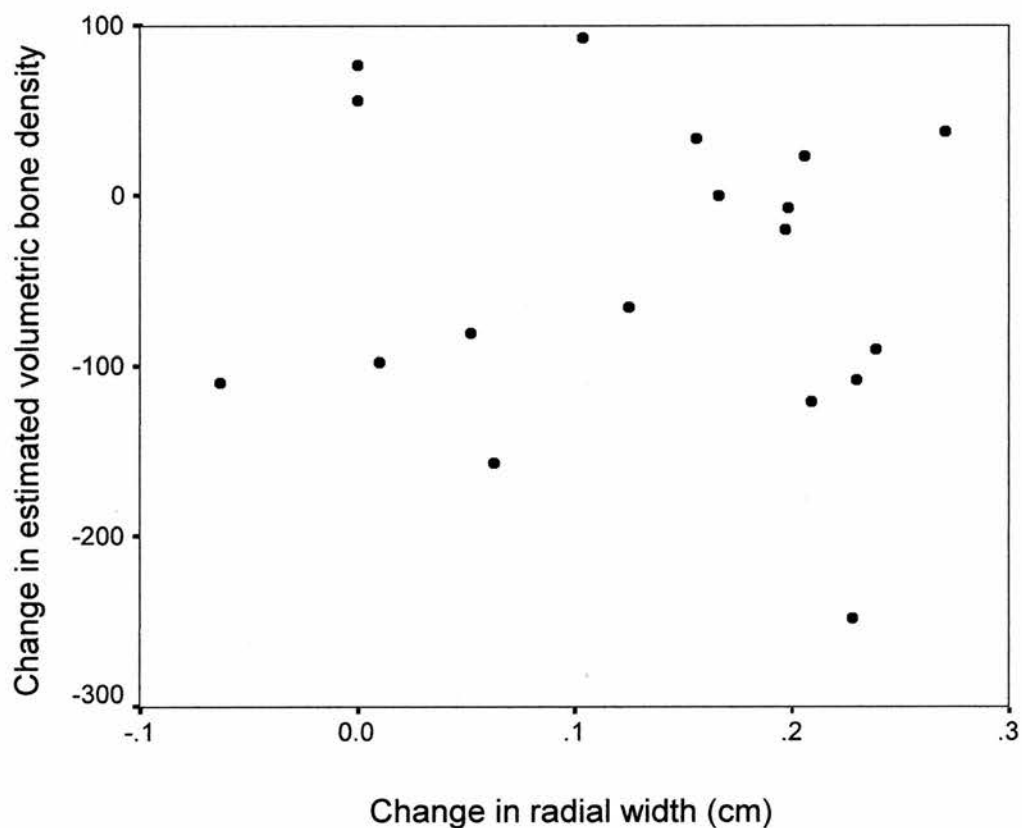


cortical width estimation

SPA provides a measure of mineralised bone mass. In the bone which has increased in radial width, it does not distinguish the bone with an increase in cortical thickness with increased porosity from one with the same (or decreased) cortical width of comparable density (Prof N.J. Bishop, personal communication). Making assumptions that cortical width remained the same for each infant between birth and term, then a linear relationship should exist between the change in cortical volume

and the change in radial width. As an estimate of cortical volume, areal bone mineral density divided by the radial width was used (excluding constants), and the change in BMD/RW between birth and term was plotted against the change in RW between birth and term (Figure 34).

Figure 34: Relationship between change in estimated volumetric bone density and change in radial width



Although the numbers are few, this does not demonstrate a linear relationship, suggesting that cortical width does not remain constant, with a change only in mineralisation of the cortex, between birth and term. The lack of increased mineralised bone mass, seen in these infants, could therefore be due to changes in cortical width, homogeneous changes in mineralisation of the cortex, increased areas of porosity within the cortex, or a combination of all three.

This in an area in which improvements in the image resolution obtained by the SPA scanner may provide further information.

5.2.6 Critique of the Methodology

As with the cohort study, there were major errors in the design of this randomised controlled trial and in its execution. Most significantly, a low recruitment rate and a failure to adhere to a methodology that would meet the reporting guidelines of a randomised controlled trial as set out in the CONSORT statement (Begg et al, 1996). The optimal reporting of a trial should include 5 subheadings in the report, with the 3 subheadings of “protocol”, “assignment” and “masking (blinding)” under the methods section, and “participant flow and follow-up”, and “analysis” in the results section, and I have followed these headings to highlight the major deficits in this study.

5.2.6i Protocol

The setting of the study in a single centre had the advantage of ensuring that feeding and supplementing protocols remained the same for all infants in the study.

However, as a confounder, this could have been minimised by stratifying the randomisation by centre. The limited ability to move the scanner for bone images was an obstacle to carrying out the study on multiple sites, which would have increased the recruitment rate, allowing the target to be reached within the 2 year period.

The study required a participant to remain in the neonatal unit until discharge home, and as such, only those parents of infants with local addresses were approached.

Following commencement of the study there was a change in the admission profile

to the neonatal unit resulting in a reduction in the number of preterm infants eligible for entry to the study. Numbers of extremely preterm infants were further reduced by the problems of approaching parents of critically ill infants for a long term study within the first days of life. Few infants less than 28 weeks gestation were recruited to the study. The design of the study failed to incorporate a means of recording the total number of infants assessed for eligibility, and those not meeting the inclusion criteria, as recommended in the CONSORT statement (Begg et al, 1996; Moher et al, 2001).

The primary outcome should be clearly stated. In this case the bone mineral content of radius and ulna at 40 weeks PMA.

The secondary outcomes are those outcomes which are also measured and analysed at the completion of the study. In this case, bone mineral areal density and radial width at term; BMD, RW and BMC at 64 weeks PMA; alkaline phosphatase concentrations. Lower leg length measurements at birth and term would have been a useful adjunct.

Infants randomised to the control arm received the neonatal unit protocol of mineral supplementation at the discretion of the neonatal unit medical staff, following guidelines (Bremer et al, 1987). We did not want to change current management in the unit, and allowed the physicians to control the level of supplementation given to the infants in the control arm of the study.

5.2.6ii Assignment

Computer-generated randomisation would have been a more robust method of randomising infants. The randomisation process and storage of records should not be carried out by the researcher collecting the data.

5.2.6iii Masking

Details were taken from the medical and nursing records by the single researcher. This information should be gathered by a separate researcher from the one aware of study group allocation. Mineralised bone mass was measured using single photon absorptiometry, using a portable machine which had been adapted for use in the preterm infant. The data available from these images was limited, and could now be improved by using DEXA. Regular x-rays may have provided further information.

As well as blinding of the researcher extracting data from the bone images, the researcher collecting data and prescribing supplements should be blind to study group allocation. This produced practical problems in terms of funding and available personnel, and was not done.

5.2.6iv Participant Flow and Follow-up

Details of all non-eligible infants, and eligible infants not recruited or consented should be recorded.

5.2.6v Analysis

Initial power calculations were based on detecting a 20% (1 s.d.) difference in BMC at term with 90% power, based on Horsman's data (Horsman et al, 1989a). With 12 infants in each group the study has 80% power to detect 25% difference and 90% power to detect 28% difference between the 2 groups. In order for a regimen of supplementation based on urinary excretion to be worth implementing in a NICU setting, we felt that an improvement in mineralised bone mass of at least 25% should be obtainable. Therefore it was felt that by failing to show improved mineralised bone mass in the individually supplemented infants of 25% this study demonstrated that this method of supplementation was not an improvement on the current practice of supplementing according to plasma mineral concentrations.

These initial calculations were based on previous work by Horsman (Horsman et al, 1989a) using an earlier version of the single photon absorptiometer and measuring mineralised bone mass at the middle of the right forearm. They found no increment in mineralised bone mass between 32 weeks and discharge in their preterm population. The BMC results we obtained were comparable at birth (although expressed in different units), but significant increments were seen in both trial and control groups.

5.2.7 What does this study add?

Despite low recruitment, randomisation resulted in comparable groups at entry to the trial. The 2 groups received comparable protein, carbohydrate and fat intakes both parenteral and enteral, and achieved the same discharge weights. Trial infants received more oral phosphorus over the first five weeks, and had a lower TRP than control infants, as would be expected from additional supplementation. Calcium and vitamin D supplementation was not significantly different between the 2 groups. Although administering calcium supplements according to the protocol, the decision to maintain Ca_E greater than 0.3 mg/100ml GFR was based on extrapolation from adult data (Nordin et al, 1967; Brion et al, 1994). We were cautious not to provide excessive amounts of calcium in the diet in order to avoid gastrointestinal and renal complications, but the level of calcium supplementation in the trial group may have been insufficient as it did not exceed supplements provided to the control group.

Given Horsman's data (Horsman et al, 1989a), which showed no improvement in mineralised bone mass at discharge, and assuming that his infants were receiving the same level of supplementation as our control group as they were in the same NICU (although he did not look at this), the study aimed to look for improved mineralised bone mass when supplementing on the basis of urinary excretion, which it was assumed (correctly for phosphate) would increase the level of supplementation. The level of supplementation may certainly have blunted the differences, but given current practice it was not ethical to reduce the current amount of supplementation given to the control group. The conclusion drawn from this is that the level of

supplementation based on urinary excretion shows no benefit in mineralised bone mass over the supplementation already used in the NICU for this population of infants.

The effects of diuretics on urinary mineral losses did not confound the supplementation protocol. As supplementation in the trial group was commenced on the basis of plasma concentrations, the effect of high urinary losses secondary to diuretics resulted in reduced plasma concentrations requiring supplementation irrespective of TRP or Ca_E .

In this study we looked at the actual nutritional, phosphorus and calcium intake by the infants rather than the prescribed intake. As anticipated the actual nutrition received by the infants was less than prescribed. In infants receiving parenteral nutrition this was usually due to other intravenous infusions and in these infants we included all dextrose infusions in the carbohydrate intake. Nutritional and mineral content of breast milk was taken from published figures (Appendix A) and was not measured. Accurate recording was only possible in the first weeks of life, as more mature infants in the study were taking breast feeds by 5 weeks of age. As the aim of the study was to investigate the effect of early individualised mineral supplementation in order to prevent mineral depletion this was felt to be the most appropriate feed data to collect.

No attempt was made to perform balance studies on these infants. The aim was to investigate the effect of a simple supplementation regimen on bone mineralised bone

mass, with a view to application in the clinical setting. The trial group received more phosphorus with a significant reduction in TRP indicating absorption of enteral phosphate. Individually phosphorus supplementation led to a reduction in Ca_E . Similarly, increased calcium supplementation resulted in a rise in Ca_E and an increase in TRP, suggesting enteral absorption of calcium. However balance studies would have provided more data, perhaps helping to explain the failure to improve mineralised bone mass in the trial group.

Accurate linear growth data may have also provided useful information. Previous studies have suggested that bone mineralisation is enhanced by adequate weight gain (Horsman et al, 1989a). However, we found the preterm infants to be significantly behind weight-matched controls in both BMC and BMD, but not in RW, suggesting bone growth without mineralised bone mass. The use of knemometry to record lower leg length was attempted in a small group of infants but insufficient data was obtained for analysis.

Each infant had bone measurements taken at 4 weekly intervals throughout their course in the neonatal unit. Having failed to recruit larger numbers there is insufficient data to analyse the results of the 4 weekly bone mineral measurements, or to compare outcomes on breast versus formula fed infants. Data has been analysed according to BMC, BMD and RW at discharge or term. Some infants exhibited marked losses initially in BMC and BMD, whereas others showed increases and subsequent loss (Appendix B). There was no relationship between

bone mineralised bone mass changes and gestation or birthweight. However the number of recruited infants was insufficient to perform detailed analyses.

The portable single photon absorptiometer allowed the assessment of radial BMC, BMD and RW in the first days of life, even in unstable extremely preterm infants. This provided a baseline measurement in each infant in the study. Further assessment of maternal mineral status, both from dietary history and biochemical measurements was not obtained. In the cohort study we found no relationship between antenatal or maternal factors and mineralised bone mass at birth, and so would be unlikely to do so in this small group of infants.

Due to the nature of the supplemental regimen in the trial group it was not possible to blind the investigator to the randomisation process. As the supplements were prescribed on the infants' medication sheet, other health professionals caring for the infant, as well as the parents, were aware of the group allocation. The effects of supplementation on plasma phosphorus, calcium and alkaline phosphatase concentrations was readily observed, and as such may have begun to influence practice in the neonatal unit during the course of the study. This may have resulted in more supplementation being prescribed to non-study and control infants, although urinary monitoring was only performed within the study setting. As seen from the results, the trial group continued to receive higher phosphorus supplementation than the control group.

Follow-up after discharge from the neonatal unit was limited. Infants returning to the out-patient clinic for review by the Neonatologist had repeat BMD and RW measurements. The baby scanner was modified for this purpose to accommodate increased forearm soft tissue, but was not validated in this setting. BMC of radius and ulna could not be made as the 2 bones could no longer be incorporated within the imaged area. There were no term-born controls, and as such inferential interpretation of bone measurements was made. The BMD measurements remained within the range seen in the cohort data, allowing reverse application of the regression equation to obtain expected infant weight based on radial BMD. The regression equation for radial width was less robust because of the wider confidence interval.

Opportunistic follow-up in this setting meant that the infants with few perinatal complications were not seen, as they had been discharged from clinic. This may have affected our limited results by missing infants with higher BMD. Follow-up of all study infants with DXA scanning is required to observe potential long term effects of early individualised mineral supplementation in these preterm-born infants.

5.2.8 Effect on future research

In order to reduce the incidence of metabolic bone disease, researchers are continuing to investigate the potential of urinary calcium and phosphorus excretion (Catache & Leone, 2003) and individualised mineral supplementation based on plasma and urinary concentrations (Trotter & Pohlandt, 2002). However, there are no published randomised controlled trials addressing supplementation based on the measurement of urinary mineral excretion. As this method of titrating phosphorus and calcium supplementation to urinary excretion failed to improve mineralised bone mass to that of a newborn infant of comparable weight, it may be that factors other than substrate supply are important in the promotion of bone mineral deposition in the preterm infant. The lack of increased BMD in the larger preterm infants was unexpected and may support this theory, suggesting that the change from *in utero* to *ex utero* environment may have a more profound influence than the cessation of transfer of minerals from mother to infant. Placental, fetal and maternal hormones, growth factors or intrinsic bone stresses may play a role in modulating mineralised bone mass, and warrant further investigation.

5.3 Implications of reduced mineralisation

The implications of reduced bone mineralisation in the preterm population is currently unknown. Increasing supplementation seems the most appropriate means of enhancing bone mineral density, reducing the complications of rickets of prematurity and fractures. With current nutritional regimens it seems inevitable that the extremely low birth weight infant suffers an obligatory period of growth failure in the immediate postnatal period, and this data shows that the majority of preterm infants endure a prolonged period of mineralisation failure.

Epidemiological studies have now demonstrated a clear link between early growth failure and cardiovascular disease in adult life (Barker et al, 1990), and the concept of the “thrifty” fetus/infant later exposed to an increased nutritional load is the suggested explanation. In terms of bone development, it has been suggested that an adverse prenatal environment may reprogramme the epiphyseal growth plate towards a lower level of responsiveness to IGF-I or other growth factors (de Zegher et al, 2000), although currently there is little supporting evidence.

The effects of nutritional supplementation on the hormonal environment is potentially as significant as the dietary manipulations themselves (Jobe, 2001). In light of the evolving research the effects of enhancing nutritional supplementations in the post-neonatal period should be carefully monitored into adult life for potential adverse as well as beneficial outcomes.

5.4 Conclusion

This study has demonstrated that mineralised bone mass remains primarily related to birthweight at the limits of viability, and that individualised mineral supplementation of preterm infants does not improve mineralisation over standard supplementation regimens, such that preterm-born infants have a reduced mineralised bone mass at term compared to expected weight-adjusted values.

This work was limited primarily by the inability to achieve the proposed number of subjects to the study (RCT), and by failure to complete data collection on those recruited (cohort study). At the time of this study, the portability of SPA had advantages over DXA imaging in this population of infants. However, with the advent of more portable DXA scanners, the questions posed in this study will be better approached using whole body assessment of bone mineral density.

6 Appendix

Appendix A: Phosphorus and calcium content of preterm formula and breast milk

Nutrient Values (per 100 ml)

		Cow and Gate Premium	SMA Gold	Farleys First	Pregestimil	Similac Special Care Study Formula
Energy	(kcal)	66	67	68	68	80
Protein	(g)	1.41	1.5	1.45	1.9	2.2
Fat	(g)	3.6	3.6	3.82	3.8	4.4
Carbohydrate	(g)	7.1	7.2	6.96	6.9	8.6
Phosphorus	(mmol)	0.9				
	(mg)	26.7	33	27	42	90
Calcium	(mmol)	1.3				
	(mg)	53.3	46	39	63	143.8
Vitamin D	(IU)					120
	(ug)	1.1	1.1	1.0	1.1	

		Cow and Gate Nutriprem	SMA Low Birth Weight formula	Farleys Osterprem	Milupa Prematil	Preterm breast milk
Energy	(kcal)	80	80	80	70	70
Protein	(g)	2.2	2.0	2.0	2.0	1.8
Fat	(g)	4.4	4.4	4.6	3.5	4.0
Carbohydrate	(g)	8.0	8.6	7.65	7.7	7.0
Phosphorus	(mmol)	1.7				0.45
	(mg)	54	41	63	42	14
Calcium	(mmol)	2.7				0.55
	(mg)	108	77	110	70	22
Vitamin D	(IU)					n.s.
	(ug)	2.4	1.2	2.4	2.1	

		Fortifier (2 sachets in 100 ml preterm EBM)	Thixo D (per 100 g)	Duocal (per 100g)	Maxijul (per 100 g)	Carobel (per 100g)
Energy	(kcal)	80	392	492	380	42
Protein	(g)	2.5	0.5			6
Fat	(g)	4.0	0.2	22		2
Carbohydrate	(g)	9.0	97	73	95	
Phosphorus	(mmol)	1.7				
	(mg)	54	2.0	5.0	5.0	
Calcium	(mmol)					
	(mg)	82		5.0		500
Vitamin D	(IU)					
	(ug)	5.0				

Appendix B: Individual variation in bone mineral content and density

Bone measurements were taken every 4 weeks throughout the study period. Each graph represents measurements on a single infant.

Figure 35: Individual bone mineral content changes throughout the study period

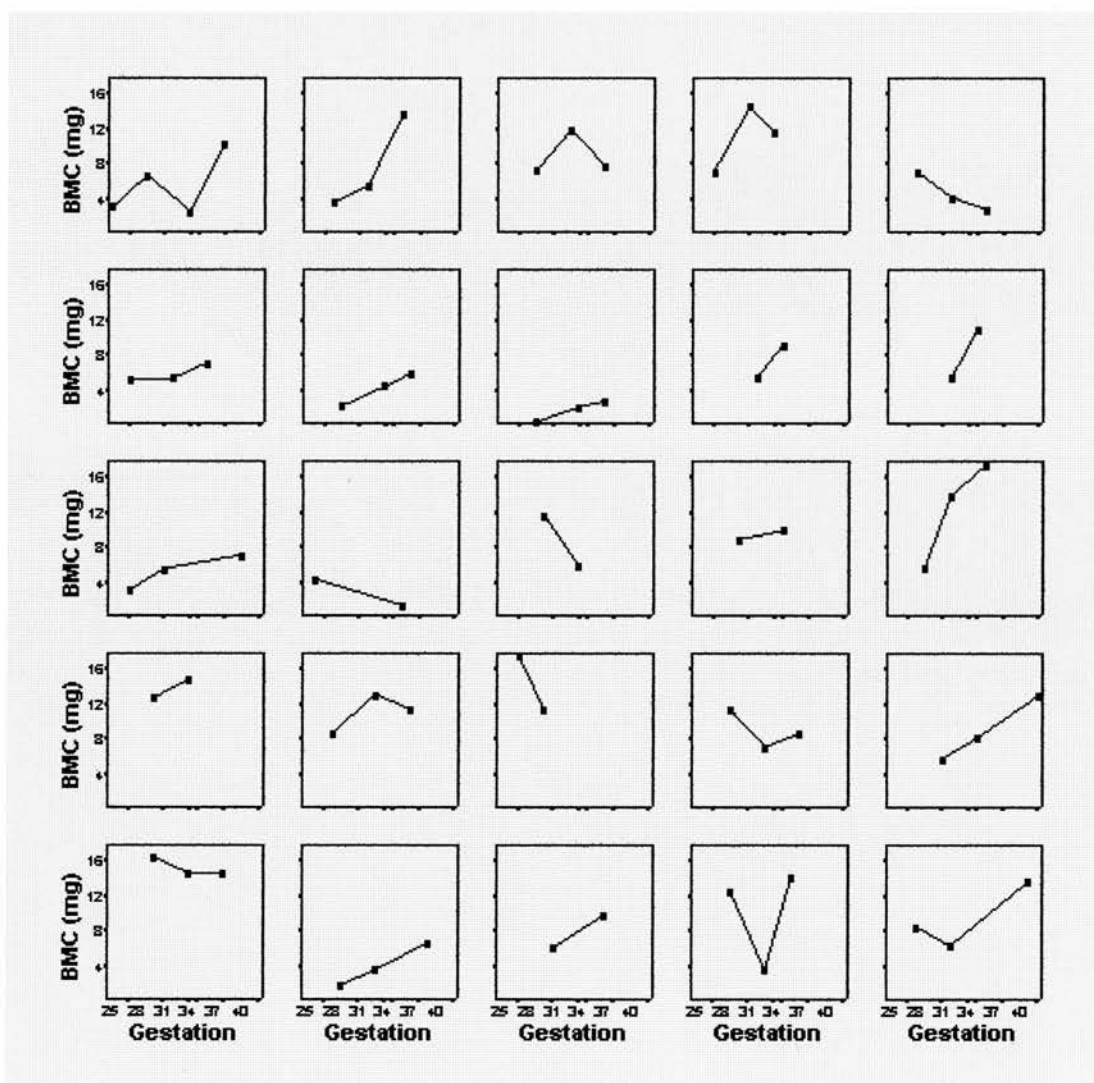
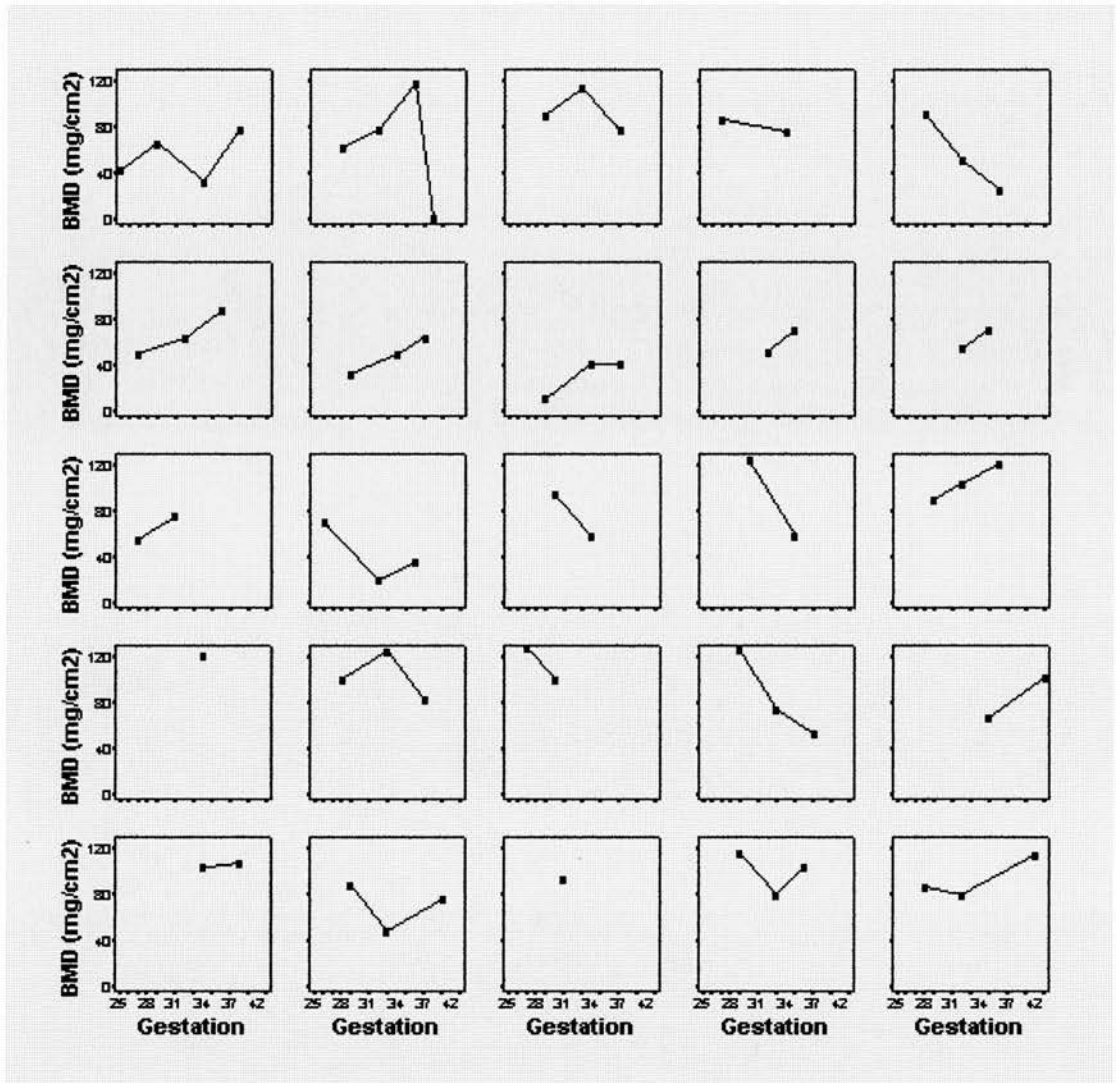


Figure 36: Individual bone mineral density changes throughout the study period



Appendix C: Structured form used for obstetric and neonatal note extraction

Bone Mineral Density of the New Born DATA COLLECTION FORM

Multiple Birth 1=Singleton , 2=Twin , 3=Triplet

CONTENTS

<u>SECTION A</u>	<u>Page no.</u>
1 MOTHERS DETAILS / 2 HOSPITAL DETAILS	2
3 CHILDS / 4 RESERVE DETAILS & GP DETAILS	3
5 OTHER PREGNANCIES	4
6 FIRST ANTENATAL VISIT	5
7 ILLNESSES AND OPERATIONS / 8 MEDICATION (antenatal steroids)	6
9 FETAL ASSESSMENT	7
10 AMNIOCENTESIS / 11 X-RAYS / 12 HAEMOGLOBIN	8
13 TESTS AND INVESTIGATIONS	9
14 LABOUR RECORD	9-10
15 NEONATAL RECORD	10-12
<u>SECTION B</u>	
STUDY 1	13
STUDY 2	14-21

IS INVOLVED IN STUDY 1	YES	<input type="checkbox"/>	NO	<input type="checkbox"/>
IS INVOLVED IN STUDY 2	YES	<input type="checkbox"/>	NO	<input type="checkbox"/>
IF YES:	TRIAL	<input type="checkbox"/>	CONTROL	<input type="checkbox"/>

IF THE CHILD HAS BEEN TRANSFERRED TO L.G.I. THEN SECTION A MAY BE INCOMPLETE (SEE HOSPITAL DETAILS).

1. MOTHERS DETAILS

Title _____

First Name _____

Surname _____

Previous Surnames _____

Current Address

Post Code

Date of Birth

Ethnic Group

2. HOSPITAL DETAILS

L.G.I. Unit Number

C
M

IF TRANSFERRED FROM ANOTHER HOSPITAL:

When transferred ?

In Utero =1 Postnatal = 2

Hospital Name

Hospital Unit Number

Mother:

Baby:

Date Transferred to L.G.I.

3. CHILDS DETAILS

Childs Name _____

Childs Surname _____

Childs change of Surname _____

Childs Address _____

Post Code _____

Childs Date of Birth

4. RESERVE ADDRESS / GP DETAILS

Name of Reserve Address _____

Reserve Address _____

Post Code _____

GP Name _____

GP Address _____

Post Code _____

5. OTHER PREGNANCIES

Total pregnancies following

--	--

(DO NOT RECORD DETAILS OF INDEX CHILD)

Pregnancy no	<table border="1"><tr><td style="width: 20px; height: 20px;"></td><td style="width: 20px; height: 20px;"></td></tr></table>			<table border="1"><tr><td style="width: 20px; height: 20px;"></td><td style="width: 20px; height: 20px;"></td></tr></table>			<table border="1"><tr><td style="width: 20px; height: 20px;"></td><td style="width: 20px; height: 20px;"></td></tr></table>																																
Multiple Birth	1=Singleton , 2=Twin , 3=Triplet <input type="checkbox"/>	1=Singleton , 2=Twin , 3=Triplet <input type="checkbox"/>	1=Singleton , 2=Twin 3=Triplet <input type="checkbox"/>																																				
Source	1 =Hospital original obstetric record 2=Hospital history taking 3=GP original obstetric record <input type="checkbox"/> 4=GP history taking	1 =Hospital original obstetric record 2=Hospital history taking 3= GP original obstetric record <input type="checkbox"/> 4=GP history taking	1 =Hospital original obstetric record 2=Hospital history taking 3= GP original obstetric record <input type="checkbox"/> 4=GP history taking																																				
Date of Birth or pregnancy end	<table border="1"><tr><td style="width: 20px; height: 20px;"></td><td style="width: 20px; height: 20px;"></td><td style="width: 20px; height: 20px;"></td><td style="width: 20px; height: 20px;"></td></tr><tr><td style="text-align: center;">Day</td><td style="text-align: center;">Mth</td><td style="text-align: center;">Yr</td><td></td></tr></table>					Day	Mth	Yr		<table border="1"><tr><td style="width: 20px; height: 20px;"></td><td style="width: 20px; height: 20px;"></td><td style="width: 20px; height: 20px;"></td><td style="width: 20px; height: 20px;"></td></tr><tr><td style="text-align: center;">Day</td><td style="text-align: center;">Mth</td><td style="text-align: center;">Yr</td><td></td></tr></table>					Day	Mth	Yr		<table border="1"><tr><td style="width: 20px; height: 20px;"></td><td style="width: 20px; height: 20px;"></td><td style="width: 20px; height: 20px;"></td><td style="width: 20px; height: 20px;"></td></tr><tr><td style="text-align: center;">Day</td><td style="text-align: center;">Mth</td><td style="text-align: center;">Yr</td><td></td></tr></table>					Day	Mth	Yr													
Day	Mth	Yr																																					
Day	Mth	Yr																																					
Day	Mth	Yr																																					
Gestation	Weeks <table border="1"><tr><td style="width: 20px; height: 20px;"></td><td style="width: 20px; height: 20px;"></td></tr></table>			Weeks <table border="1"><tr><td style="width: 20px; height: 20px;"></td><td style="width: 20px; height: 20px;"></td></tr></table>			Weeks <table border="1"><tr><td style="width: 20px; height: 20px;"></td><td style="width: 20px; height: 20px;"></td></tr></table>																																
Outcome	1=livebirth 2=miscarriage 3=stillbirth 4=termination 5=ectopic 6=hydatidiform mole <input type="checkbox"/>	1=livebirth 2=miscarriage 3=stillbirth 4=termination 5=ectopic 6=hydatidiform mole <input type="checkbox"/>	1=livebirth 2=miscarriage 3=stillbirth 4=termination 5=ectopic 6=hydatidiform mole <input type="checkbox"/>																																				
Delivery	1=normal 2=assisted 3=caesarian 8=N/A 9=not known <input type="checkbox"/>	1=normal 2=assisted 3=caesarian 8=N/A 9=not known <input type="checkbox"/>	1=normal 2=assisted 3=caesarian 8=NIA 9=not known <input type="checkbox"/>																																				
Sex	1=male 2=female 9=not known <input type="checkbox"/>	1=male 2=female 9=not known <input type="checkbox"/>	1=male 2=female 9=not known <input type="checkbox"/>																																				
Birthweight	<table border="1"><tr><td style="width: 20px; height: 20px;"></td><td style="width: 20px; height: 20px;"></td><td style="width: 20px; height: 20px;"></td><td style="width: 20px; height: 20px;"></td></tr><tr><td style="text-align: center;">lb</td><td style="text-align: center;">oz</td><td></td><td></td></tr><tr><td style="width: 20px; height: 20px;"></td><td style="width: 20px; height: 20px;"></td><td style="width: 20px; height: 20px;"></td><td style="width: 20px; height: 20px;"></td></tr></table> gm					lb	oz							<table border="1"><tr><td style="width: 20px; height: 20px;"></td><td style="width: 20px; height: 20px;"></td><td style="width: 20px; height: 20px;"></td><td style="width: 20px; height: 20px;"></td></tr><tr><td style="text-align: center;">lb</td><td style="text-align: center;">oz</td><td></td><td></td></tr><tr><td style="width: 20px; height: 20px;"></td><td style="width: 20px; height: 20px;"></td><td style="width: 20px; height: 20px;"></td><td style="width: 20px; height: 20px;"></td></tr></table> gm					lb	oz							<table border="1"><tr><td style="width: 20px; height: 20px;"></td><td style="width: 20px; height: 20px;"></td><td style="width: 20px; height: 20px;"></td><td style="width: 20px; height: 20px;"></td></tr><tr><td style="text-align: center;">lb</td><td style="text-align: center;">oz</td><td></td><td></td></tr><tr><td style="width: 20px; height: 20px;"></td><td style="width: 20px; height: 20px;"></td><td style="width: 20px; height: 20px;"></td><td style="width: 20px; height: 20px;"></td></tr></table> gm					lb	oz						
lb	oz																																						
lb	oz																																						
lb	oz																																						
Abnormalities or problems with baby	1=yes 2=no 9=not known <input type="checkbox"/>	1=yes 2=no 9=not known <input type="checkbox"/>	1=yes 2=no 9=not known <input type="checkbox"/>																																				
If yes: please describe	_____ _____	_____ _____	_____ _____																																				
ICD	1 2 <table border="1"><tr><td style="width: 20px; height: 20px;"></td><td style="width: 20px; height: 20px;"></td><td style="width: 20px; height: 20px;"></td><td style="width: 20px; height: 20px;"></td></tr><tr><td style="width: 20px; height: 20px;"></td><td style="width: 20px; height: 20px;"></td><td style="width: 20px; height: 20px;"></td><td style="width: 20px; height: 20px;"></td></tr></table>									<table border="1"><tr><td style="width: 20px; height: 20px;"></td><td style="width: 20px; height: 20px;"></td><td style="width: 20px; height: 20px;"></td><td style="width: 20px; height: 20px;"></td></tr><tr><td style="width: 20px; height: 20px;"></td><td style="width: 20px; height: 20px;"></td><td style="width: 20px; height: 20px;"></td><td style="width: 20px; height: 20px;"></td></tr></table>									<table border="1"><tr><td style="width: 20px; height: 20px;"></td><td style="width: 20px; height: 20px;"></td><td style="width: 20px; height: 20px;"></td><td style="width: 20px; height: 20px;"></td></tr><tr><td style="width: 20px; height: 20px;"></td><td style="width: 20px; height: 20px;"></td><td style="width: 20px; height: 20px;"></td><td style="width: 20px; height: 20px;"></td></tr></table>																				

6. FIRST ANTENATAL VISIT

Available & complete = 1
 incomplete = 2

Date of first visit Day / Mth / Yr
 □□ □□ □□

Gestation at first visit Weeks
 □□

Date last menstrual period Day / Mth / Yr
 □□ □□ □□

Expected date delivery (by Imp) Day / Mth / Yr
 □□ □□ □□

Revised EDD ? Yes = 1 No = 2
 (If no skip the next question) □

Revised EDD date by scan < 15 weeks Day / Mth / Yr
 □□ □□ □□

Age at menarche Years
 □□

Height cm OR ft in
 □□□ □□□□

Weight kg OR st lb
 □□□ □□□□

Blood pressure systolic diastolic
 □□□ □□□

Smoking Yes = 1 No = 2 NK = 9
 (If no skip the next question) □

Number of cigarettes smoked a day □□
 If not known use code "99".

7. ILLNESSES AND OPERATIONS

Available & complete = 1
incomplete= 2

Total illnesses following

Condition	Date From	Date To	ICD Code
_____	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
_____	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
_____	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
_____	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
_____	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>

8. MEDICATION

Available & complete = 1
incomplete = 2

Total drugs following

Antenatal Steroids > 12 hours before delivery

Yes = 1 No = 2 NK = 9

	Date from Day / Mth / Yr	No. of doses	ICD Code
_____	<input type="checkbox"/> <input type="checkbox"/> / <input type="checkbox"/> <input type="checkbox"/> / <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
_____	<input type="checkbox"/> <input type="checkbox"/> / <input type="checkbox"/> <input type="checkbox"/> / <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
_____	<input type="checkbox"/> <input type="checkbox"/> / <input type="checkbox"/> <input type="checkbox"/> / <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
_____	<input type="checkbox"/> <input type="checkbox"/> / <input type="checkbox"/> <input type="checkbox"/> / <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>

Other Drugs

	Date from	Date to	ICD Code
_____	<input type="checkbox"/> <input type="checkbox"/> / <input type="checkbox"/> <input type="checkbox"/> / <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> / <input type="checkbox"/> <input type="checkbox"/> / <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
_____	<input type="checkbox"/> <input type="checkbox"/> / <input type="checkbox"/> <input type="checkbox"/> / <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> / <input type="checkbox"/> <input type="checkbox"/> / <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
_____	<input type="checkbox"/> <input type="checkbox"/> / <input type="checkbox"/> <input type="checkbox"/> / <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> / <input type="checkbox"/> <input type="checkbox"/> / <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
_____	<input type="checkbox"/> <input type="checkbox"/> / <input type="checkbox"/> <input type="checkbox"/> / <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> / <input type="checkbox"/> <input type="checkbox"/> / <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>

9. FETAL ASSESSMENT							
Available & complete = 1 incomplete = 2					Sheet	Of	
Test no	1	2	3	4	5		
Date	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	
Gest age (wks)	<input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/>	
HC (mm)	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	
Abdo circ (mm)	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	
Fem Length (mm)	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	
BPD (mm)	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	
Liquor vol. 1=Increased 2=Normal	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
3=Sub reduced 4=Severe reduced 5=Absent							
Umbilical Artery A/B ratio	<input type="checkbox"/> . <input type="checkbox"/> : <input type="checkbox"/> . <input type="checkbox"/>	<input type="checkbox"/> . <input type="checkbox"/> : <input type="checkbox"/> . <input type="checkbox"/>	<input type="checkbox"/> . <input type="checkbox"/> : <input type="checkbox"/> . <input type="checkbox"/>	<input type="checkbox"/> . <input type="checkbox"/> : <input type="checkbox"/> . <input type="checkbox"/>	<input type="checkbox"/> . <input type="checkbox"/> : <input type="checkbox"/> . <input type="checkbox"/>	<input type="checkbox"/> . <input type="checkbox"/> : <input type="checkbox"/> . <input type="checkbox"/>	
Umbilical Artery Flow 1=Normal 2=Sub reduced 3=Severely reduced	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
4=Absent 5=Reversed							
Ech fet bowel 1=Yes 2=No	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	

10. AMNIOCENTESIS OR CHORIONIC VILLUS SAMPLING

Available & complete =1 incomplete =2

Total amniocentesis following

REASON	Amnio=1 CVS=2 Chordo- centesis=3	Gestation (wks)	Day / Mth / Yr	Code
_____	<input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/>
_____	<input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/>

11. X-RAYS

Available & complete =1 incomplete =2

Total x-rays following

Gestation (wks)	Day / Mth / Yr	Code
<input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/>
<input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/>

12. HAEMOGLOBIN

Total Haemoglobin's following

grams per dl	Day / Mth / Yr
<input type="checkbox"/> <input type="checkbox"/> . <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
<input type="checkbox"/> <input type="checkbox"/> . <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
<input type="checkbox"/> <input type="checkbox"/> . <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
<input type="checkbox"/> <input type="checkbox"/> . <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
<input type="checkbox"/> <input type="checkbox"/> . <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
<input type="checkbox"/> <input type="checkbox"/> . <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>

13. TESTS AND INVESTIGATIONS
NOT ELSEWHERE CLASSIFIED

Available & complete =1 incomplete =2

Total tests following

Description

Day / Mth / Yr

Code

 / /

 / /

 / /

14. LABOUR RECORD

Yes =1 No =2 NK =9

1 Spontaneous labour ?

2 Prostaglandin pessaries ?

3 Artificial rupture of membranes ?

If YES : before labour = 1 during labour = 2

4 Oxytocic infusion ?

If YES : to induce labour = 1 to augment labour = 2

5 Caesarean ?

If YES : emergency before onset =1 emergency after onset =2
 planned before onset =3 planned after onset =4 uncertain =9

6 Complications in labour ?

Total complications following

ICD Code

A _____

B _____

C _____

D _____

4	Birthweight	<div style="display: flex; justify-content: space-around;"> <div style="text-align: center;">kg □ . □ □ □</div> <div style="text-align: center;">O R</div> <div style="text-align: center;">lb oz □ □ □ □</div> </div>
5	Head circumference	<div style="display: flex; justify-content: space-around;"> <div style="text-align: center;">cm □ □ □ □</div> <div style="text-align: center;">O R</div> <div style="text-align: center;">in □ □ . □ □</div> </div>
6	Length	<div style="display: flex; justify-content: space-around;"> <div style="text-align: center;">cm □ □ □ □</div> <div style="text-align: center;">O R</div> <div style="text-align: center;">in □ □ . □ □</div> </div>
7	Time to first gasp or cry less than one minute	<div style="display: flex; justify-content: space-between;"> <div>Yes =1 No =2 NK =9</div> <div style="border: 1px solid black; width: 20px; height: 20px;"></div> </div>
8	Time to regular respirations (if < one minute code = 00)	minutes
9	Apgar score	<div style="display: flex; justify-content: space-between;"> <div>1 minute </div> <div>5 minutes </div> <div>Use Code 99 if not known 10 minutes </div> </div>
10	Number of umbilical vessels	Use Code 99 if not known
11	Baby's blood group if known	<div style="display: flex; align-items: center;"> <div style="margin-right: 20px;">□ □</div> <div>Rhesus: +ve =1 -ve =2</div> <div style="margin-left: 20px; border: 1px solid black; width: 20px; height: 20px;"></div> </div>
12	Problems at birth (specify)	ICD Code

13	Congenital malformations (specify)	ICD Code

14	Other problems (specify)	ICD Code

15 Baby admitted to SCBU ? Yes =1 No =2 NK =9

If YES : Duration of stay (days)

16 Child's date of discharge Day / Mth / Yr

Discharged to : Home =1 GPU =2 Other Hospital = 3

17 If baby transferred to another Hospital for treatment please give Hospital name and address

ANY OTHER RELEVANT INFORMATION (SPECIFY DATES AND ICD CODE)

STUDY 1

CORD BLOODS

Available & complete =1 incomplete =2

PTH (pg/ml)

1,25 dihydroxycholecalciferol (pmol/l)

25 hydroxycholecalciferol (ng/ml)

PTHrP (pmol/l)

Total calcium (mmol/l)

Corrected calcium (mmol/l)

Phosphate (mmol/l)

pH

A=

V=

Base excess

A=

V=

Albumin (g/dl)

Other

INITIAL BONE MINERAL MEASUREMENTS

Arm used

Right =1 Left =2 NK =9

Date

Day of age

BMC mg/cm

BMD radius mg/cm²

BMD ulnar mg/cm²

Measure A

Measure B

STUDY 2

Sheet

of

WEEKLY RESULTS

Biochemistry

Date	Serum calcium (mmol/l)	Serum phosphate (mmol/l)	Serum creatinine (umol/l)	Alkaline Phosphatase (iu/l)	Urine calcium (mmol/l)	Urine phosphate (mmol/l)	Urine creatinine (mmol/l)
<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> . <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> . <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> . <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> . <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> . <input type="text"/> <input type="text"/>
<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> . <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> . <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> . <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> . <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> . <input type="text"/> <input type="text"/>
<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> . <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> . <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> . <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> . <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> . <input type="text"/> <input type="text"/>
<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> . <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> . <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> . <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> . <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> . <input type="text"/> <input type="text"/>
<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> . <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> . <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> . <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> . <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> . <input type="text"/> <input type="text"/>
<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> . <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> . <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> . <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> . <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> . <input type="text"/> <input type="text"/>
<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> . <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> . <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> . <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> . <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> . <input type="text"/> <input type="text"/>

STUDY 2

Sheet

of

WEEKLY RESULTS

Measurements

Date	Weight (kg)	OFC (cm)	Knemometry (mm)
<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> . <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> . <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> . <input type="text"/> <input type="text"/>
<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> . <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> . <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> . <input type="text"/> <input type="text"/>
<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> . <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> . <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> . <input type="text"/> <input type="text"/>
<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> . <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> . <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> . <input type="text"/> <input type="text"/>
<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> . <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> . <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> . <input type="text"/> <input type="text"/>
<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> . <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> . <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> . <input type="text"/> <input type="text"/>
<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> . <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> . <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> . <input type="text"/> <input type="text"/>
<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> . <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> . <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> . <input type="text"/> <input type="text"/>

MONTHLY RESULTS

Bone Mineral Density

Arm used Right =1 Left =2 NK =9

Arm	Date	BMC mg/cm	BMD radius mg/cm ²	BMD ulnar mg/cm ²
<input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> . <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> . <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> . <input type="text"/> <input type="text"/> <input type="text"/>
<input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> . <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> . <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> . <input type="text"/> <input type="text"/> <input type="text"/>
<input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> . <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> . <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> . <input type="text"/> <input type="text"/> <input type="text"/>
<input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> . <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> . <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> . <input type="text"/> <input type="text"/> <input type="text"/>
<input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> . <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> . <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> . <input type="text"/> <input type="text"/> <input type="text"/>

STUDY 2

Sheet of

DAILY RESULTS

Enteral intake (minerals and nutrition)

Date	Calc mmol/ day	Phos mmol/ day	Vit D i.u.	Milk Type	Additive (state% where needed)	Volume received (mls/day)	Volume received (mls/kg/day)	Weight (kg)
<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>

--	--	--	--

STUDY 2

DAILY RESULTS

Parenteral Intravenous intake

Date	Weight (kg)	Dex rate pres (ml/hr)	CHO (g/kg/day)	Amino acid (g/kg/day)	Calc (mmol/day)	Phos (mmol/kg/day)	Dex received (mls/day)	Lipid received (mls/day)
□□ □□ □□	□.□□□□	□□□□.□□	□□ □□	□□.□□	□□.□□	□□.□□	□□□□□.□□	□□□□.□□
□□ □□ □□	□.□□□□	□□□□.□□	□□ □□	□□.□□	□□.□□	□□.□□	□□□□□.□□	□□□□.□□
□□ □□ □□	□.□□□□	□□□□.□□	□□ □□	□□.□□	□□.□□	□□.□□	□□□□□.□□	□□□□.□□
□□ □□ □□	□.□□□□	□□□□.□□	□□ □□	□□.□□	□□.□□	□□.□□	□□□□□.□□	□□□□.□□
□□ □□ □□	□.□□□□	□□□□.□□	□□ □□	□□.□□	□□.□□	□□.□□	□□□□□.□□	□□□□.□□
□□ □□ □□	□.□□□□	□□□□.□□	□□ □□	□□.□□	□□.□□	□□.□□	□□□□□.□□	□□□□.□□
□□ □□ □□	□.□□□□	□□□□.□□	□□ □□	□□.□□	□□.□□	□□.□□	□□□□□.□□	□□□□.□□
□□ □□ □□	□.□□□□	□□□□.□□	□□ □□	□□.□□	□□.□□	□□.□□	□□□□□.□□	□□□□.□□

STUDY 2

DAILY RESULTS

Parenteral Intravenous intake

Date	Weight (kg)	Volume received (mls/day)	Dextrose solution	Standard calcium (1 = Yes, 2 = No, 9 = NK)
<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> . <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> . <input type="text"/>	<input type="text"/>
<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> . <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> . <input type="text"/>	<input type="text"/>
<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> . <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> . <input type="text"/>	<input type="text"/>
<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> . <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> . <input type="text"/>	<input type="text"/>
<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> . <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> . <input type="text"/>	<input type="text"/>
<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> . <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> . <input type="text"/>	<input type="text"/>
<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> . <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> . <input type="text"/>	<input type="text"/>
<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> . <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> . <input type="text"/>	<input type="text"/>
<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> . <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> . <input type="text"/>	<input type="text"/>
<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> . <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> . <input type="text"/>	<input type="text"/>
<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> . <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> . <input type="text"/>	<input type="text"/>
<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> . <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> . <input type="text"/>	<input type="text"/>
<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> . <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> . <input type="text"/>	<input type="text"/>
<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> . <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> . <input type="text"/>	<input type="text"/>
<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> . <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> . <input type="text"/>	<input type="text"/>

STUDY 2

Sheet

of

NEONATAL DRUGS

Diuretics

Name

Code

Start date

Stop date

Name	Code	Start date	Stop date
_____	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>
_____	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>
_____	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>
_____	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>
_____	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>
_____	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>
_____	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>
_____	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>
_____	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>
_____	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>
_____	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>
_____	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>
_____	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>
_____	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>
_____	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>
_____	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>

STUDY 2

NEONATAL DRUGS

Dexamethasone

Dose (mg/kg)	Start date	Stop date
<input type="text"/> . <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>
<input type="text"/> . <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>
<input type="text"/> . <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>
<input type="text"/> . <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>
<input type="text"/> . <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>
<input type="text"/> . <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>
<input type="text"/> . <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>

Other Drugs

Name	Code	Start date	Stop date
_____	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>
_____	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>
_____	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>
_____	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>
_____	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>
_____	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>

STUDY 2

RESPIRATORY SUPPORT

	Start date			Stop date		
HFOV	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
HFOV	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
SIMV	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
SIMV	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
SIMV	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
CPAP (Flow Driver)	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
CPAP (Flow Driver)	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
CPAP (Flow Driver)	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
CPAP (Other)	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
CPAP (Other)	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
CPAP (Other)	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
Supplemental Oxygen	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
Supplemental Oxygen	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
Supplemental Oxygen	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>

PAEDIATRIC EPIDEMIOLOGY GROUP -: PA . MCKINNEY / L.PRYDE

Appendix D: Parent Information Sheets

Study 1

Study on Bone Strength in Babies

Dear Parent

We would like to invite you to take part in a research study.

In this study we want to find out more about the factors that influence the strength of babies' bone. We wish to do this so that in future we may be able to identify those babies with a tendency to have weak bones and give them extra minerals they need to strengthen their bones.

We would do this by measuring the density of babies' bones and relating the measurements we obtain to the information we gather from the mothers' and babies' medical records.

If you agree to your baby taking part in the study, we would scan your baby's forearm twice within 72 hours of the birth.

The scan is taken by a machine which will not disturb or hurt your baby at all. It should take no longer than 10 seconds to take the picture. It is like having an Xray but uses 6 times less radiation than a routine chest Xray and is therefore very safe.

Taking part in this study will not help your baby directly but may help babies in the future. If you feel able to help we would be happy to discuss this with you in more detail. Participation is entirely voluntary. You can refuse to take part, or withdraw from the study at any stage, and this will have no effect on the care of your baby.

Adele Harrison

Study 2

Study on Bone Strength in Premature Babies

Dear Parent

We would like to invite you to take part in a research study.

Nutrition plays a very important role in the growth and development of your premature babies. As premature babies are born early it is known that they miss out on the normal transfer of the minerals calcium and phosphorus from the mother. These minerals are needed to make strong bones. In this study we would like to compare two different feeding regimens aimed at improving the strength of babies' bones.

We would randomly allocate your baby to one of the two regimens. This means that the treatment is allocated by chance, which avoids biased results and means that we can be certain at the end of the study which treatment is best.

Your baby would receive one of the following feeding regimens:

- a. Our usual feeding regimen which is already in use in the neonatal unit.
- b. A special feeding regimen tailored to the individual needs of your baby. We would give extra calcium and phosphorus supplements based on blood and urine results which are already done on the baby. No extra blood tests would be required.

Both groups of babies may receive the minerals calcium and phosphate, only the amounts may differ.

To find out which regimen is better we would like to obtain information from mothers' and babies' notes, and then measure the development of your baby's bones by scanning your baby's forearm at the following times:

1. Within 96 hours of the birth.
2. Every 4 weeks until discharge from the neonatal unit.
3. When your baby is 6 months past the date he/she is due.

The scan is taken by a machine which will not disturb or hurt your baby at all. It should take no longer than 10 seconds to take the picture. It is like having an Xray but uses 6 times less radiation than a routine chest Xray and is therefore very safe.

Taking part in this study will not help your baby directly but may help babies in the future. If you feel able to help we would be happy to discuss this with you in more detail. Participation is entirely voluntary. You can refuse to take part, or withdraw from the study at any stage, and this will have no effect on the care of your baby.

Adele Harrison

Appendix E: Assessment of calcium and phosphorus solubility

After completion of the study, an assessment of the compatibility of formula and human milk with calcium and phosphorus additives were made with the assistance of V Lalari (dietician, Children's and Women's Health Centre of British Columbia). The original calcium and phosphorus formulations were no longer available, and the milk formula used (Similac Special Care 24, SSC24) in these experiments had higher phosphorus (72 mg/100ml) and calcium (132 mg/100ml) content than Nutriprem, the most commonly used formula in the study. Fortifier was similar to that used in the study, but was available in smaller sachets, such that 1 sachet of study fortifier was equivalent to 2 sachets of the similac fortifier used below.

0.5 mmol calcium gluconate (0.23mmol/ml) and 1 mmol sodium phosphate (3mmol/ml) were added separately first to 5ml Similac Special Care formula 24 and then to 5ml donated human milk with added similac fortifier (1 sachet/25cc).

In each of the 4 experiments (SSC24 + calcium, SSC24 + phosphorus, EBM+HMF + calcium, and EBM+HMF +phosphorus) there was no precipitation seen in the test tube. Due to the high fat content it was not possible to pass the milk through filter paper.

When 0.5mmol of calcium gluconate and 1mmol sodium phosphorus were mixed together, precipitate was immediately visible in the test tube. The addition of 5cc of SSC24 appeared to dissolve the precipitate. However, with the addition of 5cc of EBM with HMF (1 sachet/25cc) precipitated particles remained visible on the test

tube and marked sludging at the bottom of the tube occurred on emptying. The finding of sludging and precipitation in this case supports the evidence that the addition of calcium or phosphate alone did not result in precipitation.

The volume of milk used in this experiment was much less than administered to the infants in the study, although the calcium and phosphorus supplementation was comparable. In view of the increased plasma phosphorus concentrations, and urinary excretion of calcium and phosphorus seen in the supplemented infants, this supports the thesis that, in the doses used, calcium and phosphorus administered separately are not precipitated in the milk, and are absorbed to some extent by the infants.

Appendix F: Abidec

Yellow liquid containing in 0.6ml:

Vitamin C (ascorbic acid)	40mg
Vitamin A palmitate	1333iu
Vitamin B1(thiamine hydrochloride)	0.4mg
Vitamin B6 (pyridoxine hydrochloride)	0.8mg
Nicotinamide (niacin)	8mg
Vitamin B2 (riboflavin)	0.8mg
Vitamin D2 (ergocalciferol)	400iu

7 Presentations/Publications

Harrison A, Holland P, Truscott J. Relationship of bone mineralisation to antenatal factors in the newborn infant. Platform presentation, Western Perinatal Research Meeting, Banff, Alberta, February 2001.

Harrison A, Holland P, Truscott J. Randomised controlled trial of the effect of individualised phosphorus and calcium supplementation on bone mineralisation in preterm infants. Poster presentation, Pediatric Academic Societies Annual Meeting, Baltimore, April 2001.

Harrison A, Holland P, Truscott J. Development of regression equations for estimation of bone mineralisation in the newborn infant. Poster presentation, Canadian Pediatric Society Annual Meeting, Vancouver, June 2001.

Harrison A, Holland P, Truscott J (2001) Randomised controlled trial of the effect of individualised phosphorus and calcium supplementation on bone mineralisation in preterm infants (abstract). *Pediatr Res*, 49 Part 2(2) suppl, p. 294A.

Harrison A, Truscott J, Holland P (2001) Randomised controlled trial of individualised mineral supplementation in preterm infants (abstract). *J Perinatol*, 21, p. 314.

Harrison AM (2001) Mineral requirements in the preterm infant. *Current Medical Literature Clinical Nutrition*, 1, p. 1-6.

Harrison AM (2001) Mineral requirements in the preterm infant. *Current Medical Literature Paediatrics*, 14, p. 25-30.

8 References

- Abbas S, Pickard DW, Rodda CP, Heath JA, Hammonds RG, Wood WI, Caple IW, Martin TJ, Care AD (1989) Stimulation of ovine placental calcium transport by purified natural and recombinant parathyroid hormone-related protein (PTHrP) preparations. *Q J Exp Physiol*, 7, p. 549-52.
- Abrams SA, Schanler RJ, Tsang RC, Garza C (1989) Bone mineralisation in former very low birth weight infants fed either human milk or commercial formula: one year follow-up observation. *J Pediatr*, 114, p. 1041-4.
- Ardran GM (1951) Bone destruction not demonstrable by radiography. *Brit J Radiol*, 24, p. 107-9.
- Atkinson SA, Shah JK, McGee C, Steele BT (1988) Mineral excretion in premature infants receiving various diuretic therapies. *J Pediatr*, 113, p. 540-5.
- Balabanova S, Lang T, Wolf AS, Henrichs I, Homoki J, Gaedicke G, Teller WM (1986) Placental transfer of parathyroid hormone. *J Perinat Med*, 14, p. 243-50.
- Bailie MD (1993) Diuretic Treatment Agents. *Advances in Pediatrics*, 40, p. 273-85.
- Banks BA, Cnaan A, Morgan MA, Parer JT, Merrill JD, Ballard PL, Ballard RA, the North American Thyrotropin-Releasing Hormone Study Group (1999) Multiple courses of antenatal corticosteroids and outcome of premature neonates. *Am J Obstet Gynecol*, 181, p. 709-17.
- Barker DJP, Osmond C (1992) Infant mortality, childhood nutrition, and ischaemic heart disease in England and Wales. In: *Fetal and infant origins of adult disease*, ed. Barker DJP, BMJ, London.
- Barker DJP, Bull AR, Osmond C, Simmonds SJ (1990) Fetal and placental size and risk of hypertension in adult life. *BMJ*, 301, p. 259-62.
- Barker DJP, Meade TW, Fall CHD, Lee A, Osmond C, Phipps K, Stirling Y (1992) Relation of fetal growth and infant growth to plasma fibrinogen and factor VII concentrations in adult life. *BMJ*, 304, p. 148-52.
- Begg C, Cho M, Eastwood S, Horton R, Moher D, Olkin I, Pitkin R, Rennie D, Schulz KF, Simel D, Stroup DF (1996) Improving the quality of reporting of randomized controlled trials: The CONSORT statement. *JAMA*, 276(8), p. 637-9.
- Bell MJ, Ternberg JL, Feigin RD, Keating JP, Marshall R, Barton L, Brotherton T (1978) Neonatal necrotising enterocolitis. Therapeutic decisions based upon clinical staging. *Ann Surg*, 187, p. 1-7.

Bellido T, Jilka RL, Boyce BF, Girasole G, Broxmeyer H, Dalrymple SA, Murray R, Manolagas SC (1995) Regulation of interleukin-6, osteoclastogenesis, and bone mass by androgens. The role of the androgen receptor. *J Clin Invest*, 95, p. 2886-95.

Benjamin HR, Gordon HH, Maples E (1943) Calcium and phosphorus requirements of premature infants. *Am J Dis Child*, 65, p. 412-5.

Bentur L, Alon U, Berant M (1987) Bone and Mineral Homeostasis in the Preterm Infant: A Review. *Pediatric Rev Commun*, 1, p. 291-310.

Bishop NJ (1989) Bone disease in preterm infants. *Arch Dis Child*, 64, p. 1403-9.

Bishop NJ, King FJ, Lucas A (1993) Increased bone mineral content of preterm infants fed with a nutrient enriched formula after discharge from hospital. *Arch Dis Child*, 68, p. 573-8.

Bishop NJ, Dahlenburg SL, Fewtrell MS, Morley R, Lucas A (1996) Early diet of preterm infants and bone mineralisation at age 5 years. *Acta Paediatr*, 85, p. 230-6.

Bland M (1997) An introduction to medical statistics. Oxford University Press, Oxford. Second Edition.

Bremer HJ, Brooke OG, Orzalesi M, Putet G, Raiha NCR, Senterre J, Shaw JCL, Wharton BA (1987) Nutrition and Feeding of Preterm Infants. *Acta Paediatr Suppl*, 336, p. 3-14.

Brickman AS, Massry SG, Coburn JW (1972) Changes in serum and urinary calcium during treatment with hydrochlorothiazide: Studies on mechanisms. *J Clin Invest*, 51, p. 945-54.

Brion LP, Satlin LM, Edelmann CM Jr (1994) Renal disease, in: Avery GB, Fletcher MA, MacDonald MG. *Neonatology: Pathophysiology and management of the newborn*. JB Lippincott Company, Philadelphia. Fourth Edition, p. 792-886.

Brodehl J, Gellissen K, Weber HP (1982) Postnatal development of tubular phosphate reabsorption. *Clin Nephrol*, 17, p. 163-71.

Brodehl J, Krause A, Hoyer PF (1988) Assessment of maximal tubular phosphate reabsorption: comparison of direct measurement with the nomogram of Bijvoet. *Pediatr Nephrol*, 2, p. 183-9.

Brooke OG, Lucas A (1985) Metabolic bone disease in preterm infants. *Arch Dis Child*, 60, p. 682-5.

Bruchi S, Scattoni M, Buonocore G, Zani S, Bagnoli F (1984) Concentrazioni sieriche di calcitonina e paratormone nel sangue funicolare del prematuro. *Boll Soc It Biol Sper*, 60, p. 1703-8.

Budayr AA, Halloran BP, King JC, Diep D, Nissenson RA, Strewler GJ (1989) High levels of a parathyroid hormone-like protein in milk. *Proc Natl Acad Sci (USA)*, 86, p. 7183-5.

Callenbach JC, Sheehan MB, Abramson SJ, Hall RT (1981) Etiologic factors in rickets of very low-birth-weight infants. *J Pediatr*, 98, p.800-5.

Cameron JR, Sorenson J (1963) Measurement of bone mineral in vivo: An improved method. *Science*, 142, p. 230-6.

Campbell DE, Fleischman AR (1988) Rickets of prematurity: Controversies in causation and prevention. *Current Controversies in Perinatal Care*, 15(4) , p. 879-90.

Campfield T, Braden G, Flynn-Valone, Powell S (1997) Effect of diuretics on urinary oxalate, calcium, and sodium excretion in very low birth weight infants. *Pediatrics*, 99, p. 814-8.

Carey DE, Goetz CA, Horak E, Rowe JC (1985) Phosphorus wasting during phosphorus supplementation of human milk feedings in preterm infants. *J Pediatr*, 107, p. 790-794.

Carter DR, Bouxsein ML, Marcus R (1992) New approaches for interpreting projected bone densitometry data. *J Bone Miner Res*, 7, p. 137-45.

Casey PH, Kraemer HC, Bernbaum J, Tyson JE, Sells JC, Yogmam MW, Bauer CR (1990) Growth patterns of low birthweight preterm infants: A longitudinal analysis of a large varied sample. *J Pediatr*, 117, p. 298-307.

Casey PH, Kraemer HC, Bernbaum J, Yogman MW, Sells JC (1991) Growth status and growth rate of a varied sample of low birthweight, preterm infants: A longitudinal cohort from birth to three years of age. *J Pediatr*, 119, p. 599-605.

Catache M, Leone CR (2003) Role of plasma and urinary calcium and phosphorus measurements in early detection of phosphorus deficiency in very low birthweight infants. *Acta Paediatr*, 92, p.76-80.

Caufriez A, Frankenne F, Englert Y, Golstein J, Cantraine F, Hennen G, Copinschi G (1990) Placental growth hormone as a potential regulator of maternal IGF-I during human pregnancy. *Am J Physiol*, 258, p. E1014-9.

Chambers EL, Gordan GS, Goldman L, Reifenshtein EC (1956) Tests for hyperparathyroidism: Tubular reabsorption of phosphate, phosphate deprivation and calcium infusion. *J Clin Endocrinol Metab*, 16, p. 1507-21.

Chamoux E, Coxam V, Lebecque P, Davicco MJ, Miller SC, Barlet JP (1997) Influence of sex steroids on development of cultured fetal rat metatarsal bones. *Growth, Develop, Ageing*, 61, p. 79-91.

Chan GM, Roberts CC, Polland D, Jackson R (1982) Growth and bone mineralisation of normal breast-fed infants and the effects of lactation on maternal bone mineral status. *Am J Clin Nutr*, 36, p. 438-43.

Chan GM (1993) Growth and bone mineral status of discharged very low birth weight infants fed different formulas or human milk. *J Pediatr*, 123, p. 439-43.

Charles P, Poser JW, Mosekilde L, Jensen FT (1985) Estimation of bone turnover evaluated by ⁴⁷-Ca kinetics. Efficiency of serum bone gamma-carboxyglutamic acid containing protein, serum alkaline phosphatase, and urinary hydroxyproline excretion. *J Clin Invest*, 76, p. 2254-8.

Chessex P, Pineault M, Zebiche H, Ayotte A (1985) Calciuria in parenterally fed preterm infants: role of phosphorus intake. *J Pediatr*, 107, p. 794-6.

Clément-Lacroix P, Ormandy C, Lepescheux L, Ammann P, Damotte D, Goffin V, Bouchard B, Amling M, Gaillard-Kelly M, Binart N, Baron R, Kelly PA (1999) Osteoblasts are a new target for prolactin: analysis of bone formation in prolactin receptor knockout mice. *Endocrinology*, 140, p. 96-105.

Coburn JW, Hartenbower DL, Massry SG (1971) Modification of calciuric effect of extracellular volume expansion by phosphate infusion. *Am J Physiol*, 220, p. 377-83.

Corey HE, Spitzer A (1992) Renal blood flow and glomerular filtration rate during development, in Edelman CM Jr (ed): *Pediatric Kidney Disease*. Boston, Little, Brown, p 3-18.

Crofton PM, Shrivastava A, Wade JC, Stephen R, Kelnar CJH, Lyon AJ, McIntosh N (1999) Bone and collagen markers in preterm Infants: Relationship with growth and bone mineral content over the first 10 weeks of life. *Pediatr Res*, 46, p. 581-7.

Crowley P (1999) Corticosteroids prior to preterm delivery. *The Cochrane Review*. Oxford: The Cochrane Library.

Cruikshank DP, Pitkin RM, Varner MW, Williams GA, Hargis GK (1983) Calcium metabolism in diabetic mother, fetus, and newborn infant. *Am J Obstet Gynecol*, 145, p. 1010-6.

Cushing H (1932) The basophil adenomas of the pituitary body and their clinical manifestations (pituitary basophilism). *Bull. Johns Hopkins Hosp*, 50, p. 137-95.

David L, Salle BL, Putet G, Grafmeyer DC (1981) Serum immunoreactive calcitonin in low birth weight infants. Description of early changes; effect of intravenous calcium infusion; relationships with early changes in serum calcium, phosphorus, magnesium, parathyroid hormone, and gastrin levels. *Pediatr Res*, 15, p. 803-8.

Day GM, Chance GW, Radde IC, Reilly BJ, Park E, Sheepers J (1975) Growth and mineral metabolism in very low birth weight infants. II. Effects of calcium supplementation on growth and divalent cations. *Pediatr Res*, 9, p. 568-75.

- Delmas PD, Wilson DM, Mann KG, Riggs BL (1983) Effect of renal function on plasma levels of bone Gla-protein. *J Clin Endocrinol Metab*, 57, p. 1028-30.
- Delvin EE, Glorieux FH, Salle BL, David L, Varenne JP (1982) Control of vitamin D metabolism in preterm infants: feto-maternal relationships. *Arch Dis Child*, 57, p. 754-7.
- Dempster D (1989) Bone histomorphometry in glucocorticoid-induced osteoporosis. *J Bone Miner Res*, 4, p. 137-41.
- Dincsoy MY, Tsang RC, Laskorzewski P, Ho M, Chen IW, Davis N (1982) Serum calcitonin response to administration of calcium in newborn infants during exchange blood transfusion. *J Pediatr*; 100, p. 782-6.
- Dobnig H, Turner R (1995) Evidence that intermittent treatment with parathyroid hormone increases bone formation in adult rats by activation of bone lining cells. *Endocrinology*, 136, p. 3632-8.
- Dobnig H, Turner R (1997) The effects of programmed administration of human parathyroid hormone fragment (1-34) on bone histomorphometry and serum chemistry in rats. *Endocrinology*, 138, p. 4607-12.
- Downing GJ, Egelhof JC, Daily DK, Thomas MK, Alon U (1992) Kidney function in very low birth weight infants with furosemide-related renal calcification at ages 1 to 2 years. *J Pediatr*, 120, p. 599-604.
- Drummond BK, Ryan S, O'Sullivan EA, Congdon P, Curzon MEJ (1992) Enamel defects of the primary dentition and osteopenia of prematurity. *Pediatr Dent*, 14, p. 119-21.
- Duarte CG (1968) Effects of ethacrynic acid and furosemide on urinary calcium, phosphate and magnesium. *Metabolism*, 17, p. 867-76.
- Dwyer T, Blizzard L, Morley R, Ponsonby AL (1999) Within pair association between birth weight and blood pressure at age 8 in twins from a cohort study. *BMJ*, 319, p. 1325-9.
- Eastell R, Wahner HW (1990) Methods for measuring bone mineral density. *Bone*, 7(3), p.81-3.
- Ehrenkranz RA, Ackerman BA, Nelli CM, Janghorbani M (1985) Absorption of calcium in premature infants as measured with a stable isotope ⁴⁶Ca extrinsic tag. *Pediatr Res*, 19, p. 178-84.
- Ehrenkrantz RA, Younes N, Lemons JA, Fanaroff AA, Donovan EF, Wright Katsikiotis V, Tyson JE, Oh W, Shankaran S, Bauer CR, Korones SB, Stoll BJ, Stevenson DK, Papile LA (1999) Longitudinal growth of hospitalized very low birth weight infants. *Pediatrics*, 104, p. 280-9.

- Erenberg A, Weinstein MM, Chen I, Tsang RC (1978) Placental impermeability to parathormone (PTH) in the ewe. *Pediatr Res*, 12A, p. 394.
- Evans JR, Allen AC, Stinson DA, Hamilton DC, Brown BS, Vincer MJ, Raad MA, Gundberg CM, Cole DEC (1989) Effect of high-dose vitamin D supplementation on radiographically detectable bone disease of very low birth weight infants. *J Pediatr*, 115, p. 779-86.
- Ezzedein F, Adelman RD, Ahlfors CE (1988) Renal calcification in preterm infants: pathophysiology and long-term sequelae. *J Pediatr*, 113, p. 532-9.
- Faerk J, Peitersen B, Petersen S, Michaelsen KF (2002) Bone mineralisation in premature infants cannot be predicted from serum alkaline phosphatase or serum phosphate. *Arch Dis Child Fetal Neonatal Ed*, 87, p.F133-6.
- Fall CHD, Barker DJP, Osmond C, Winter PD, Clark PMS, Hales CN (1992) Relation of infant feeding to adult serum cholesterol concentration and death from ischaemic heart disease. *BMJ*, 304, p. 801-5.
- Fewtrell MS, Prentice A, Jones SC, Bishop NJ, Stirling D, Buffenstein R, Lunt M, Cole TJ, Lucas A (1999) Bone mineralization and turnover in preterm infants at 8-12 years of age: The effect of early diet. *J Bone Miner Res*, 14, p. 810-20.
- Frankenne F, Rentier-Delrue F, Scippo ML, Martial J, Hennen G (1987) Expression of the growth hormone variant gene in human placenta. *J Clin Endocrinol Metab*, 64, p. 635-7.
- Fraser WD, Robinson J, Lawton R, Durham B, Gallacher SJ, Boyle IT, Beastall GH, Logue FC (1993) Clinical and laboratory studies of a new immunoradioactive assay of parathyroid hormone-related protein. *Clin Chem*, 39, p. 414-9.
- Freeman JV, Cole TJ, Chinn S, Jones PRM, White EM, Preece MA (1995) Cross-sectional stature and weight reference curves for the UK, 1990. *Arch Dis Child*, 73, p. 17-24.
- French NP, Hagan R, Evans SF, Godfrey M, Newnham JP (1999) Repeated antenatal corticosteroids: Size at birth and subsequent development. *Am J Obstet Gynecol*, 180, p. 114-21.
- Gibson AT, Pearse RG, Wales JKH (1993) Growth retardation after dexamethasone administration: assessment by knemometry. *Arch Dis Child*, 69, p. 505-9.
- Giles MM, Fenton MH, Shaw B, Elton RA, Clarke M, Lang M, Hume R (1987) Sequential calcium and phosphorus balance studies in preterm infants. *J Pediatr*, 110, p. 591-8.
- Gillham B, Papachristodoulou DK, Thomas JH (1997) *Wills' Biochemical Basis of Medicine*. Butterworth-Heinemann, Oxford. Third Edition, p. 106-15.

Girasole G, Jilka RL, Passeri G, Boswell S, Boder G, Williams DC, Manolagas SC (1992) 17 β -estradiol inhibits interleukin-6 production by bone marrow-derived stromal cells and osteoblasts in vitro: a potential mechanism for the antiosteoporotic effect of estrogens. *J Clin Invest*, 89, p. 883-91.

Givens MH, Macy IG (1933) The chemical composition of the human fetus. *J Biol Chem*, 102, p. 7-17.

Glasgow JFT, Thomas PS (1977) Rachitic respiratory distress in small preterm infants. *Arch Dis Child*, 52, p. 268-73.

Glorieux FH, Salle BL, Delvin EE, David L (1981) Vitamin metabolism in preterm infants: Serum calcitriol values during the first five days of life. *J Pediatr*, 99, p. 640-3.

Gluckman (1989) Fetal growth: an endocrine perspective. *Acta Paediatr Scand (Suppl)*, 349, p. 21-5.

Gowen M, Wood DD, Ihrie EJ, McGuire MKB, Russell GG (1983) An interleukin 1 like factor stimulates bone resorption in vitro. *Nature*, 306, p. 378-80.

Greer FR, Steichen JJ, Tsang RC (1982) Effects of increased calcium, phosphorus and vitamin D intake on bone mineralization in very low-birth-weight infants fed formulas with Polycose and medium-chain triglycerides. *J Pediatr*, 100, p. 951-5.

Greer FR, McCormick A (1987) Bone mineral content and growth in very-low-birth-weight premature infants. Does bronchopulmonary dysplasia make a difference? *Am J Dis Child*, 141, p.179-83.

Greer FR, McCormick A (1988) Improved bone mineralisation and growth in premature infants fed fortified own mother's milk. *J Pediatr*, 112, p. 961-9.

Greer FR (1994) Osteopenia of Prematurity. *Annu Rev Nutr*, 14, p. 169-85.

Gross SJ (1987) Bone mineralization in preterm infants fed human milk with and without mineral supplementation. *J Pediatr*, 111, p. 450 -8.

Hales CN, Barker DJP, Clark PMS, Cox LJ, Fall C, Osmond C, Winter PD (1991) Fetal and infant growth and impaired glucose tolerance at age 64. *BMJ*, 303, p. 1019-23.

Handwerger S, Freemark M (2000) The roles of placental growth hormone and placental lactogen in the regulation of human fetal growth and development. *J Pediatr Endocrinol Metab*, 13, p. 343-56.

Hanning RM, Mitchell MK, Atkinson SA (1989) In vitro solubility of calcium glycerophosphate versus conventional mineral salts in pediatric parenteral nutrition solutions. *J Pediatr Gastroenterol Nutr*, 9, p. 67-72.

Hill DJ, Riley SC, Bassett NS, Waters MJ (1992) Localisation of the growth hormone receptor, identified by immunocytochemistry, in second trimester human fetal tissues and in placenta throughout gestation. *J Clin Endocrinol Metab*, 75, p. 646-50.

Hillman LS, Haddad JG (1975) Perinatal vitamin D metabolism. II. Serial 25-hydroxyvitamin D concentrations in sera of term and premature infants. *J Pediatr*, 86, p. 928-935.

Hillman LS, Rojanasathit S, Slatopolsky E, Haddad JG (1977) Serial measurements of serum calcium, magnesium, parathyroid hormone, calcitonin, and 25-hydroxyvitamin D in premature and term infants during the first week of life. *Pediatr Res*, 11, p. 739-44.

Hillman LS, Slatopolsky E, Haddad JG (1978) Perinatal vitamin D metabolism. IV. Maternal and cord serum 24,25-dihydroxyvitamin D concentrations. *J Clin Endocrinol Metab*, 47, p. 1073-1077.

Hillman LS, Hoff N, Salmons S, Martin L, MacAlister W, Haddad J (1985a) Mineral homeostasis in very premature infants: Serial evaluation of serum 25-hydroxyvitamin D, serum minerals, and bone mineralisation. *J Pediatr*, 106, p. 970-80.

Hillman LS, Hollis B, Salmons S, Martin L, Slatopolsky E, McAlister W, Haddad J (1985b) Absorption, dosage, and effect on mineral homeostasis of 25-hydroxycholecalciferol in premature infants: Comparison with 400 and 800IU vitamin D₂ supplementation. *J Pediatr*, 106, p. 981-9.

Hillman LS, Forte LR, Throne PK, Johnson LS, Allen SH (1990) Elevated PTH-related peptide in cord blood of term and premature neonates (Abstract). *J Bone Miner Res*, 5 Suppl 2, p. 469.

Holland PC, Wilkinson AR, Diez J, Lindsell DRM (1990) Prenatal deficiency of phosphate, phosphate supplementation, and rickets in very low birthweight infants. *Lancet*, 335, p. 697-701.

Hollis BW, Pittard WB III (1984) Evaluation of the total fetomaternal vitamin D relationships at term: evidence for racial differences. *J Clin Endocrinol Metab*, 59, p. 652-7.

Horsman A, Ryan SW, Congdon PJ, Truscott JG, James JR (1989a) Osteopenia in extremely low birthweight infants. *Arch Dis Child*, 64, p. 485-8.

Horsman A, Ryan SW, Congdon PJ, Truscott JG, Simpson M (1989b) Bone mineral content and body size 65 to 100 weeks' postconception in preterm and full term infants. *Arch Dis Child*, 64, p. 1579-86.

Hufnagle KG, Khan SN, Penn D, Cacciarelli A, Williams P (1982) Renal calcifications: A complication of long term furosemide therapy in preterm infants. *Pediatrics*, 70, p. 360-3.

- Ichiba H, Hirai C, Fujimaru M, Shintaku H, Yamano T, Funato M (2001) Measurement of bone mineral density of lumbar spine and whole body in low-birth-weight infants: comparison of two methods. *J Bone & Miner Metab*, 19, p. 52-5.
- James JR, Truscott J, Congdon PJ, Horsman A (1986) Measurement of bone mineral content in the human fetus by absorptiometry. *Early Hum Dev*, 13, p. 169-81.
- Jilka RL, Hangoc G, Girasole G, Passeri G, Williams DC, Abrams JS, Boyce B, Broxmeyer H, Manolagas (1992) Increased osteoclast development after estrogen loss: mediation by interleukin-6. *Science*, 257, p. 88-91.
- Jilka RL, Weinstein RS, Bellido T, Roberson P, Parfitt AM, Manolagas SC (1999) Increased bone formation by prevention of osteoblast apoptosis with parathyroid hormone. *J Clin Invest*, 104, p. 439-46.
- Jobe AH (2001) Predictors of outcomes in preterm infants: which ones and when? *J Pediatr*, 138, p. 153-6.
- Jobert AS, Zhang P, Couvineau A, Bonaventure J, Roume J, Le Merrer M, Silve C (1998) Absence of functional receptors for parathyroid hormone and parathyroid hormone-related peptide in Blomstrand chondrodysplasia. *J Clin Invest*, 102, p. 34-40.
- Jørgensen HL, Scholler J, Sand JC, Bjuring M, Hassager C, Christiansen C (1996) Relation of common allelic variation at Vitamin D receptor locus to bone mineral density and postmenopausal bone loss: cross sectional and longitudinal population study. *BMJ*, 313, p. 586-90.
- Karlén J, Aperia A, Zetterström R (1985) Renal excretion of calcium and phosphate in preterm and term infants. *J Pediatr*, 106, p. 814-9.
- Katz L, Hamilton JR (1974) Fat absorption in infants of birth weight less than 1300gm. *J Pediatr*, 85, p. 608-14.
- Khatab AK, Forfar JO (1971) The interrelationship between calcium, phosphorus, and glucose levels in mothers and infants in conditions commonly associated with "placental insufficiency". *Biol Neonate*, 18, p. 1-16.
- Khosla S, Johansen KL, Ory SJ, O'Brien PC, Kao PC (1990) Parathyroid hormone-related peptide in lactation and in umbilical cord blood. *Mayo Clin Proc*, 65, p. 1408-14.
- Koo WWK, Gupta JM, Nayanar VV, Wilkinson M, Posen S (1982) Skeletal changes in preterm infants. *Arch Dis Child*, 57, p. 447-52.
- Koo WWK, Sherman R, Succop P, Oestreich AE, Tsang RC, Krug-Wispe SK, Steichen JJ (1988) Sequential bone mineral content in small preterm infants with and without fractures and rickets. *J Bone Miner Res*, 3, p. 193-7.

- Koo WWK, Kaplan LA, Krug-Wispe SK, Succop P, Bendon R (1989) Response of preterm infants to aluminium in parenteral nutrition. *J P E N*, 13, p. 516-9.
- Koo WWK, Wispe SKK, Succop P, Champlin A, Sherman R, Berry H (1990) Urinary hydroxyproline in infants with and without fractures/rickets. *Clin Chem*, 36, p. 642-4.
- Koo WWK, Walters J, Bush AJ, Chesney RW, Carlson SE (1996) Dual-Energy X-Ray Absorptiometry Studies of Bone Mineral Status in Newborn Infants. *J Bone Miner Res*, 11, p. 997-1002.
- Kovar I, Mayne P, Barltrop D (1982) Plasma alkaline phosphatase activity: a screening test for rickets in preterm neonates. *Lancet*, 1, p. 308-10.
- Kovar IZ, Mayne PD, Robbé I (1983) Hypophosphataemic rickets in the preterm infant: hypocalcaemia after calcium and phosphorus supplementation. *Arch Dis Child*, 58, p. 629-31.
- Kuschel CA, Harding JE (1998) Multicomponent fortified human milk for promoting growth in preterm infants. In: *The Cochrane Library*, Issue 4. Oxford:Update Software.
- Kuschel CA, Harding JE (2001) Calcium and phosphorus supplementation of human milk for preterm infants. In: *The Cochrane Library*, Issue 4. Oxford: Update Software.
- Kurl S, Heinonen K, Länsimies E, Launiala K (1998) Determinants of bone mineral density in prematurely born children aged 6-7 years. *Acta Paediatr*, 87, p. 650-3.
- Kyle LH, Schaaf M, Canary JJ (1958) Phosphate clearance in the diagnosis of parathyroid dysfunction. *Am J Med*, 24, p. 240-8.
- Laake H (1960) The action of corticosteroid on the renal reabsorption of calcium. *Acta Endocrinol*, 34, p. 60-4.
- Lanske B, Karaplis AC, Lee K, Luz A, Vortkamp A, Pirro A, Karperien M, Defize LHK, Ho C, Mulligan RC, Abou-Samra AB, Juppner H, Segre GV, Kronenberg HM (1996) PTH/PTHrP receptor in early development and Indian hedgehog-regulated bone growth. *Science*, 273, p. 663-6.
- Lapillonne AA, Glorieux FH, Salle BL, Braillon PM, Chambon M, Rigo J, Putet G, Senterre J (1994) Mineral balance and whole body bone mineral content in very low-birth-weight infants. *Acta Paediatr Suppl*, 405, p. 117-22.
- Lapillonne A, Braillon P, Claris O, Chatelain PG, Delmas PD, Salle BL (1997) Body composition in appropriate and small for gestational age infants. *Acta Paediatr*, 86, p. 196-200.
- Lapillonne A, Salle BL (1999) Whole-body bone mineral content in premature infants measured at full-term age (letter). *Am J Clin Nutr*, 69, p. 154-6.

Lau K, Goldfarb S, Goldberg M, Agus ZS (1982) Effects of phosphate administration on tubular calcium transport. *J Lab Clin Med*, 99, p. 317-24.

Leger J, Levy-Marchal C, Bloch J, Pinet A, Chevenne D, Porquet D, Collin D, Czernichow (1997) Reduced final height and indications for insulin resistance in 20 year olds born small for gestational age: regional cohort study. *BMJ*, 315, p. 341-7.

Lichtenstein P, Gormley C, Poser J, Tsang RC, Specker B (1987) Serum osteocalcin concentrations in infancy: Lower values in those fed cow milk formula vs. breast feeding. *J Pediatr*, 110, p. 910-1.

Lin YJ, Yeh TF, Lin HC, Win JM, Lin CH, Yu CY (1998) Effects of early postnatal dexamethasone therapy on calcium homeostasis and bone growth in preterm infants with respiratory distress syndrome. *Acta Paediatr*, 87, p. 1061-5.

Lindroth M, Westgren U, Laurin S (1986) Rickets in very low birthweight infants. Influence of supplementation with vitamin D, phosphorus and calcium. *Acta Paediatr*, 75, p. 927-31.

Lindsay R, Nieves J, Formica C, Henneman E, Woelfert L, Shen V, Dempster D, Cosman F (1997) Randomised controlled study of effect of parathyroid hormone on vertebral bone mass and fracture incidence among postmenopausal women on oestrogen with osteoporosis. *Lancet*, 350, p. 550-5.

Lucas A, Brooke OG, Baker BA, Bishop N, Morley R (1989) High alkaline phosphatase activity and growth in preterm neonates. *Arch Dis Child*, 64, p. 902-9.

Lucas A, Morley R, Cole TJ, Gore SM, Davis JA, Bamford MFM, Dossetor JFB (1990) Early diet in preterm babies and developmental status at 18 months. *Lancet*, 335, p. 1477-81.

Lucas A (1991) Programming by early nutrition in man. In: *The Childhood Environment and Adult Disease*, CIBA Foundation Symposium 156, Wiley, Chichester, p. 35-38.

Lucas A, Quinlan P, Abrams S, Ryan S, Meah S, Lucas PJ (1997) Randomised controlled trial of a synthetic triglyceride milk formula for preterm infants. *Arch Dis Child*, 77, p. F178-184.

Lyon AJ, McIntosh N, Wheeler K, Brooke OG (1984) Hypercalcaemia in extremely low birthweight infants. *Arch Dis Child*, 59, p. 1141-4.

MacDonald BR, Gowan M (1992) Scientific review in rheumatology. *Cytokines and Bone*. *Brit J Rheumatol*, 31, p. 149-55.

MacGillivray MH, Morishima A, Conte F, Grumbach M, Smith EP (1998) Pediatric endocrinology update: an overview. The essential roles of estrogens in pubertal growth, epiphyseal fusion and bone turnover: lessons from mutations in the genes for aromatase and the estrogen receptor. *Horm Res*, 49 (suppl 1), p. 2-8.

- Maierhofen WJ, Gray RW, Cheung HS, Lemann J Jr (1983) Bone resorption stimulated by elevated serum 1-25-(OH)₂-vitamin D concentrations in healthy men. *Kidney Int*, 24, p. 555-60.
- Manolagas SC (1998) The role of IL-6 type cytokines and their receptors in bone. *Ann NY Acad Sci*, 840, p. 194-204.
- Markestad T, Aksnes L, Finne PH, Aarskog D (1983a) Vitamin D nutritional status of premature infants supplemented with 500 IU Vitamin D₂ per day. *Acta Paediatr*, 72, p. 517-20.
- Markestad T, Aksnes L, Finne PH, Aarskog D (1983b) Plasma concentrations of vitamin D metabolites in a case of rickets of prematurity. *Acta Paediatr*, 72, p. 759-61.
- Martin TJ, Moseley JM (1990) The pathology and physiology of parathyroid hormone-related protein. *Bone*, 7(2), p. 70-2.
- Massry SG, Friedler RM, Coburn JW (1973) Excretion of Phosphate and Calcium. Physiology of Their Renal Handling and Relation to Clinical Medicine. *Arch Intern Med*, 131, p. 828-59.
- Mawer EB, Berry JL (1995) Biochemistry of calcium regulation, in: Tovey FI, Stamp TCB. The measurement of metabolic bone disease. Parthenon publishing Group Ltd, UK, p. 49-75.
- McIntosh N, Livesey A, Brooke OG (1982) Plasma 25-hydroxyvitamin D and rickets in infants of extremely low birth weight. *Arch Dis Child*, 57, p. 848-50.
- McIntosh N, Williams JE, Lyon AJ, Wheeler KA (1984) Diagnosis of rickets of prematurity. *Lancet*, 2, p. 869.
- McKinney PA, Parslow R, Gurney K, Law G, Bodansky HJ, Williams DRR (1997) Antenatal risk factors for childhood diabetes mellitus; a case-control study of medical record data in Yorkshire, UK. *Diabetol*, 40, p. 933-9.
- Michaelsson K, Bergstrom R, Mallmin H, Holmberg L, Wolk A, Ljunghall S (1996) Screening for osteopenia and osteoporosis – selection by body composition. *Osteoporosis Int*, 6, p. 120-6.
- Mimouni F, Loughhead JL, Tsang RC, Khoury J (1990) Postnatal surge in serum calcitonin concentrations: no contribution to neonatal hypocalcemia in infants of diabetic mothers. *Pediatr Res*, 28, p. 493-5.
- Minton SD, Steichen JJ, Tsang RC (1979) Bone mineral content in term and preterm appropriate-for-gestational-age infants. *J Pediatr*, 95, p. 1037-42.
- Minton SD, Steichen JJ, Tsang RC (1983) Decreased bone mineral content in small for gestational age infants compared with appropriate for gestational age infants:

normal serum 25-hydroxyvitamin D and decreasing parathyroid hormone. *Pediatrics*, 71, p. 383-8.

Mirlesse V, Frankenne F, Alsat E, Poncelet M, Hennen G, Evain-Brion D (1993) Placental growth hormone levels in normal pregnancy and in pregnancies with intrauterine growth retardation. *Pediatr Res*, 34, p. 439-42.

Mize CE, Uauy R, Waidelich D, Neylan MJ, Jacobs J (1995) Effect of phosphorus supply on mineral balance at high calcium intakes in very low birth weight infants. *Am J Clin Nutr*, 62, p. 385-91.

Moher D, Schulz KF, Altman DG, CONSORT Group (2001) The CONSORT statement: revised recommendations for improving the quality of reports of parallel-group randomised trials. *Lancet*, 357, p. 1191-4.

Moore KL, Persaud TVN (1993) *Before we are born: essentials of embryology and birth defects*, 4th ed. Saunders, Philadelphia.

Morgan MEI, Evans SE (1986) Osteopenia in very low birthweight infants (Letter). *Lancet*, 2 (8520), p. 1399-400.

Morley R, Lucas A (1994) Influence of early diet on outcome in preterm infants. *Acta Paediatr Suppl*, 405, p. 123-6.

Morrison NA, Qi JC, Tokita A, Kelly PJ, Crofts L, Nguyen TV, Sambrook PN, Eisman JA (1994) Prediction of bone density from vitamin D receptor alleles. *Nature*, 367, p. 284-7.

Moseley JM, Kubota M, Diefenbach-Jagger H, Wettenhall REH, Kemp BE, Suva LJ, Rodda CP, Ebeling PR, Hudson PJ, Zajac JD, Martin TJ (1987) Parathyroid hormone-related protein purified from a human lung cancer cell line. *Proc Natl Acad Sci*, 84, p. 5048-52.

Moya M, Doménech E (1982) Role of calcium-phosphate ratio of milk formulae on calcium balance in low birth weight infants during the first 3 days of life. *Ped Res*, 16, p. 675-81.

Moyer-Mileur L, Luetkemeier M, Boomer L, Chan GM (1995) Effect of physical activity on bone mineralisation in premature infants. *J Pediatr*, 127, p. 620-5.

Mughal MZ, Langton CM, Utretch G, Morrison J, Specker BL (1996) Comparison between broadband ultrasound attenuation of calcaneum and total body bone mineral density in children. *Acta Paediatr*, 85, p. 663-5.

Mundy GR (1995) *Bone remodeling and its disorders*, Martin Dunitz Ltd, UK.

Namgung R, Tsang RC, Specker BL, Sierra RI, Ho ML (1993) Reduced serum osteocalcin and 1,25-dihydroxyvitamin D concentrations and low bone mineral content in small for gestational age infants: Evidence of decreased bone formation rates. *J Pediatr*, 122, p. 269-75.

Namgung R, Tsang RC, Specker BL, Sierra RI, Ho ML (1994) Low bone mineral content and high serum osteocalcin and 1,25-dihydroxyvitamin D in summer- versus winter-born newborn infants: an early fetal effect? *J Pediatr Gastroenterol Nutr*, 19: 220-7.

Namgung R, Tsang RC, Sierra RI, Ho ML (1996) Normal serum indices of bone collagen biosynthesis and degradation in small for gestational age infants. *J Pediatr Gastroenterol Nutr*, 23: 224-8.

Namgung R, Tsang RC, Lee C, Han DG, Ho ML, Sierra RI (1998) Low total body bone mineral content and high bone resorption in Korean winter-born versus summer-born newborn infants. *J Pediatr*, 132, p. 421-5.

Naylor KE, Eastell R, Shattuck KE, Alfrey AC, Klein GL (1999) Bone turnover in preterm infants. *Pediatr Res*, 45, p. 363-6.

Nordin BEC (1976) Plasma calcium and plasma magnesium homeostasis. In: Nordin BEC, ed. *Calcium, phosphate and magnesium metabolism*. London: Churchill Livingstone, p. 190-191.

Nordin BEC, Hodgkinson A, Peacock M (1967) The Measurement and meaning of urinary calcium. *Clin Orthop*, 52, p. 293-322.

Papantoniou NE, Papapetrou PD, Antsaklis AJ, Kontoleon PE, Mesogitis SA, Aravantinos D (1996) Circulating levels of immunoreactive parathyroid hormone-related protein and intact parathyroid hormone in human fetuses and newborns. *Eur J Endocrinol*, 134, p. 437-42.

Passeri G, Girasole G, Jilka RL, Manolagas SC (1993) Increased interleukin-6 production by murine bone marrow and bone cells after estrogen withdrawal. *Endocrinol*, 133, p. 822-8.

Petersen S, Gotfredsen A, Knudsen FU (1989) Total body bone mineral in light-for-gestational-age infants and appropriate-for-gestational-age infants. *Acta Paediatr*, 78, p. 347-50.

Pfeilschifter J, Chenu C, Bird A, Mundy GR, Roodman GD (1989) Interleukin-I and tumour necrosis factor stimulate the formation of human osteoclastlike cells in vitro. *J Bone Miner Res*, 4, 113-8.

Pittard WB III, Geddes KM, Hulsey TC, Hollis BW (1992) Osteocalcin, skeletal alkaline phosphatase, and bone mineral content in very low birth weight infants: A longitudinal assessment. *Pediatr Res*, 31, p. 181-5.

Pocock NA, Eisman JA, Hopper JL, Yeates MG, Sambrook PN, Eberl S (1987) Genetic determinants of bone mass in adults: a twin study. *J Clin Invest*, 80, p. 706-10.

- Pohlandt F, Mathers N (1989) Bone mineral content of appropriate and light for gestational age preterm and term newborn infants. *Acta Paediatr*, 78, p. 835-9.
- Pohlandt F (1994a) Prevention of postnatal bone demineralisation in very low-birth-weight infants by individually monitored supplementation with calcium and phosphorus. *Pediatr Res*, 35, p. 125-9.
- Pohlandt F (1994b) Bone mineral deficiency as the main factor of dolichocephalic head flattening in very-low-birth-weight infants. *Pediatr Res*, 35, p. 701-3.
- Pohlandt F (1994c) Hypothesis: myopia of prematurity is caused by postnatal mineral deficiency. *Eur J Pediatr*, 153, p. 234-6.
- Potts JT, Jüppner H (1998) Parathyroid hormone and parathyroid hormone-related peptide in calcium homeostasis, bone metabolism, and bone development: The proteins, their genes, and receptors, in: Avioli LV, Krane SM. *Metabolic bone disease and clinically related disorders*. Academic Press, San Diego. Third Edition, p. 51- 94.
- Poulter NR, Chang CL, MacGregor AJ, Snieder H, Spector TD (1999) Association between birth weight and adult blood pressure in twins: historical cohort study. *BMJ*, 319, p. 1330-3.
- Prestridge LL, Schanler RJ, Shulman RJ, Burns PA, Laine LL (1993) Effect of parenteral calcium and phosphorus therapy on mineral retention and bone mineral content in very low birth weight infants. *J Pediatr*, 122, p. 761-8.
- Puschett JB, Goldberg M (1968) The acute effects of furosemide on acid and electrolyte excretion in man. *J Lab Clin Med*, 71, p. 666-77.
- Raupp P, Poss G, von Kries R, Schmidt E (1997) Effect of a calcium and phosphorus-enriched formula on bone mineralisation and bone growth in preterm infants after discharge from hospital. *Ann Nutr Metab*, 41, p. 358-64.
- Robinson MJ, Merret AL, Tetlow VA, Compston JE (1981) Plasma 25-hydroxyvitamin D concentrations in preterm infants receiving oral vitamin D supplements. *Arch Dis Child*, 56, p. 144-5.
- Rodda CP, Kubota M, Heath JA, Ebeling PR, Moseley JM, Care AD, Caple IW, Martin TJ (1988) Evidence for a novel parathyroid hormone-related protein in fetal lamb parathyroid glands and sheep placenta; comparisons with a similar protein implicated in humeral hypercalcaemia. *J Endocrinol*, 117, p. 261-71.
- Rodríguez JI, Garcia-Alix A, Palacios J, Paniagua R (1988) Changes in the long bones due to fetal immobility caused by neuromuscular disease. A radiographic and histological study. *J Bone Joint Surg*, 70A, p. 1052-9.
- Romagnoli C, Zecca E, Tortorolo G, Diodato A, Fazzini G, Sorcini-Carta M (1987) Plasma thyrocalcitonin and parathyroid hormone concentrations in early neonatal hypocalcaemia. *Arch Dis Child*, 62, p. 580-4.

Rowe JC, Wood DH, Rowe DW, Raisz LG (1979) Nutritional hypophosphataemic rickets in a premature infant fed breast milk. *N Engl J Med*, 300, p. 293-6.

Rowe J, Rowe D, Horak E, Spackman T, Saltzman R, Robinson S, Phillips A, Raye J (1984) Hypophosphatemia and hypercalciuria in small premature infants fed human milk: Evidence for inadequate dietary phosphorus. *J Pediatr*, 104, p. 112-7.

Rubinacci A, Sirtoli P, Moro G, Galli L, Minoli I, Tessari L (1993) Is there an impact of birth weight and early life nutrition on bone mineral content in preterm born infants and children? *Acta Paediatr*, 82, p. 711-3.

Ryan S, Congdon PJ, James J, Truscott J, Horsman A (1988) Mineral accretion in the human fetus. *Arch Dis Child*, 63, p. 799-808.

Salle B, David L, Glorieux F, Delvin EE, Louis JJ, Troncy G (1982a) Hypocalcemia in infants of diabetic mothers. Studies in circulating calcitropic hormone concentrations. *Acta Paediatr Scand*, 71, p. 573-7.

Salle BL, David L, Glorieux FH, Delvin E, Senterre J, Renaud H (1982b) Early oral administration of vitamin D and its metabolites in premature neonates: Effect on mineral homeostasis. *Pediatr Res*, 16, p. 75-8.

Salle B, Senterre J, Putet G, Rigo J (1986) Effects of calcium and phosphorus supplementation on calcium retention and fat absorption in preterm infants fed pooled human milk. *J Pediatr Gastroenterol Nutr*, 5, p. 638-42.

Schanler RJ, Garza C, O'Brien Smith E (1985) Fortified mother's milk for very low birth weight infants: Results of macromineral balance studies. *J Pediatr*, 107, p. 767-74.

Schanler RJ, Burns PA, Abrams SA, Garza C (1992) Bone mineralization outcomes in human milk-fed preterm infants. *Pediatr Res*, 31, p. 583-6.

Schanler RJ, Abrams SA (1995) Postnatal attainment of intrauterine macromineral accretion rates in low birth weight infants fed fortified human milk. *J Pediatr*, 126, p. 441-7.

Schipani E, Kruse K, Juppner H (1995) A constitutively active mutant PTH-PTHrP receptor in Jansen-type metaphyseal chondrodysplasia. *Science*, 268, p. 98-100.

Seeman E, Wahner HW, Offord KP, Kuman P, Johnson WJ, Riggs BL (1982) Differential effects of endocrine dysfunction on the axial and appendicular skeleton. *J Clin Invest*, 69, p. 1302-9.

Seino Y, Ishii T, Shimotsuji T, Ishida M, Yabuuchi H (1981) Plasma active vitamin D concentration in low-birth-weight infants with rickets and its response to vitamin D treatment. *Arch Dis Child*, 56, p. 628-32.

Seki K, Furuya K, Makimura N, Mitsui C, Hirata J, Nagata I (1994a) Cord blood levels of calcium-regulating hormones and osteocalcin in premature infants. *J Perinat Med*, 22, p. 189-94.

Seki K, Wada S, Nagata N, Nagata I (1994b) Parathyroid hormone-related protein during pregnancy and the perinatal period. *Gynecol Obstet Invest*, 37, p. 83-6.

Senterre J, Salle B (1982) Calcium and phosphorus economy of the preterm infant and its interaction with vitamin D and its metabolites. *Acta Paediatr*, 296 (suppl), p. 85-92.

Senterre J, Putet G, Salle B, Rigo J (1983) Effects of vitamin D and phosphorus supplementation on calcium retention in preterm infants fed banked human milk. *J Pediatr*, 103, p. 305-7.

Shaw JCL (1976) Evidence for defective skeletal mineralisation in low-birthweight infants: the absorption of calcium and fat. *Pediatrics*, 57, p. 16-25.

Shaw NJ, Bishop NJ (1995) Mineral accretion in growing bones - a framework for the future? *Arch Dis Child*, 72, p. 177-9.

Short MA, Brooks-Brunn JA, Reeves DS, Yeager J, Thorpe JA (1996) The effects of swaddling versus standard positioning on neuromuscular development in very low birth weight infants. *Neonatal Network*, 15, p. 25-31.

Smith DA, Nance WE, Won Kan K, Christian JC, Johnston CC Jr (1973) Genetic factors in determining bone mass. *J Clin Invest*, 52, p. 2800-8.

Smith EP, Boyd J, Frank GR, Takahishi H, Cohen RM, Specker B, Williams TC, Lubahn DB, Korach KS (1994) Estrogen resistance caused by a mutation in the estrogen-receptor gene in a man. *New Engl J Med*, 331, p. 1056-61.

Sørensen HT, Sabroe S, Olsen J, Rothman KJ, Gillman MW, Fischer P (1997) Birth weight and cognitive function in young adult life: historical cohort study. *BMJ*, 315, p. 401-4.

Spector TD, Keen RW, Arden NK, Morrison NA, Major PJ, Nguyen TV, Kelly PJ, Baker JR, Sambrook PN, Lanchbury JS, Eisman JA (1995) Influence of vitamin D receptor genotype on bone mineral density in postmenopausal women: a twin study in Britain. *BMJ*, 310, p. 1357-60.

Stephanou A, Ross R, Handwerger S (1994) Regulation of human placental lactogen expression by 1,25-dihydroxyvitamin D₃. *Endocrinol*, 135, p. 2651-6.

Stevenson DK, Wright LL, Lemons JA, Oh W, Korones SB, Papile LA, Bauer CR, Stoll BJ, Tyson JE, Shankaran S, Fanaroff AA, Donovan EF, Ehrenkranz RA, Verter J (1998) Very low birth weight outcomes of the National Institute of Child Health and Human Development Neonatal Research Network, January 1993 through December 1994. *Am J Obstet Gynecol*, 179, p. 1632-9.

- von Sydow G (1946) A study of the development of rickets in premature infants. *Acta Paediatr (Suppl 2)*, 33, p. 1-122.
- Thiébaud D, Janish S, Koebl H, Hanzal E, Jacquet AF, Leodolter S, Burckhardt P, Pecherstorfer M (1993) Direct evidence of a parathyroid-related protein gradient between the mother and the newborn in humans. *Bone Miner*, 23, p. 213-21.
- Trotter A, Maier L, Grill H-J, Kohn T, Heckmann M, Pohlandt F (1999) Effects of postnatal estradiol and progesterone replacement in extremely preterm infants. *J Clin Endocrinol Metab*, 84, p. 4531-7.
- Trotter A, Pohlandt F (2002) Calcium and phosphorus retention in extremely preterm infants supplemented individually. *Acta Paediatr*, 91, p. 680-3.
- Truscott JG, Milner R, Wood C, Smith MA (1996) A portable system for measuring bone mineral density in the pre-term neonatal forearm. *Brit J Radiol*, 69, p. 532-8.
- Tsang RC, Chen IW, Friedman MA, Giger M, Steichen J, Koffler H, Fenton L, Brown D, Pramanik A, Keenan , Strub R, Joyce T (1975) Parathyroid function in infants of diabetic mothers. *J Pediatr*, 86, p. 399-404.
- Tsang RC, Greer F, Steichen JJ (1981) Perinatal metabolism of vitamin D. Transition from fetal to neonatal life. *Clin Perinatol. Symposium on Perinatal Nephrology*, 8(2), p. 287-306.
- Tulchinsky D, Hobel CJ, Yeager E, Marshall JR (1972) Plasma estrone, estradiol, estriol, progesterone, and 17-hydroxyprogesterone in human pregnancy. I. Normal pregnancy. *Am J Obstet Gynecol*, 112, p. 1095-100.
- Van't Veen SJGA, Hagen JW, Van Ginkel FC, Prahl-Andersen B, Burger EH (1995) Intermittent compression stimulates cartilage mineralisation. *Bone*, 17, p. 461-5.
- Venkataraman PS, Blick KE, Fry HD, Rao RK (1985) Postnatal changes in calcium-regulating hormones in very low-birth-weight infants. Effect of early neonatal hypocalcaemia and intravenous calcium infusion on serum parathyroid hormone and calcitonin homeostasis. *Am J Dis Child*, 139, p. 913-6.
- Venkataraman PS, Tsang RC, Chen I-W, Sperling MA (1987) Pathogenesis of early neonatal hypocalcaemia: Studies of serum calcitonin, gastrin, and plasma glucagon. *J Pediatr*, 110, p. 599-603.
- Vortkamp A, Lee K, Lanske B, Segre GV, Kronenberg HM, Tabin CJ (1996) Regulation of rate of cartilage differentiation by Indian hedgehog and PTH-related protein. *Science*, 273, p. 613-22.
- Walters EG, Murphy JF, Henry P, Gray OP, Helder G (1986) Plasma alkaline phosphatase activity and its relation to rickets in pre-term infants. *Ann Clin Biochem*, 23, p. 652-6.

- Walton RJ, Bijvoet OLM (1975) Nomogram for derivation of renal threshold phosphate concentration. *Lancet*, 2 (7929), p. 309-10.
- Warner JT, Linton HR, Dunstan FD, Cartlidge PH (1998) Growth and metabolic responses in preterm infants fed fortified human milk or a preterm formula. *Int J Clin Prac*, 52, p. 236-40.
- Wauben IP, Atkinson SA, Grad TL, Shah JK, Paes B (1998) Moderate nutrient supplementation of mother's milk for preterm infants supports adequate bone mass and short-term growth: a randomized controlled trial. *Am J Clin Nutr*, 67, p. 465-72.
- Weinstein RS, Jilka RL, Parfitt AM, Manolagas SC (1998) Inhibition of osteoblastogenesis and promotion of apoptosis of osteoblasts and osteocytes by glucocorticoids. Potential mechanisms of their deleterious effects on bone. *J Clin Invest*, 102, p. 274-82.
- Weinstein RS, Nicholas RW, Manolagas SC (2000) Apoptosis of osteocytes in glucocorticoid-induced osteonecrosis of the hip. *J Clin Endocrinol Metab*, 85, p. 2907-12.
- Weiler HA, Wang Z, Atkinson SA (1995) Dexamethasone treatment impairs calcium regulation and reduces bone mineralization in infant pigs. *Am J Clin Nutr*, 61, p. 805-11.
- Wilson J (1999) The Barker hypothesis: an analysis. *Aus N Z J Obstet Gynaecol*, 39, p. 1-7.
- de Zegher F, Francois I, Ibáñez L, Dunger D (2000) Prenatal growth and paediatric endocrinology. *Current Medical Literature. Growth hormone and growth factors*, 15, p. 1-7.
- Zeghoud F, Vervel C, Guillozo H, Walrant-Debray O, Boutignon H, Garabédian M (1997) Subclinical vitamin D deficiency in neonates: definition and response to vitamin D supplements. *Am J Clin Nutr*, 65, p.771-8.
- Ziegler EE, O'Donnell AM, Nelson SE, Fomon SJ (1976) Body composition of the reference fetus. *Growth*, 40, p. 329-41.
- Ziegler EE (1985) Infants of low birth weight: special needs and problems. *Am J Clin Nutr*, 41 (2 suppl) , p. 440-6.