INFANT GROWTH AND BONE MINERALIZATION: THE DETERMINATION OF RELATIVE EFFECTS OF PARENTAL CHARACTERISTICS, GESTATIONAL AGE AT BIRTH AND NUTRITION

BY

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A Thesis Submitted to the Faculty of Graduate Studies in Partial Fulfillment of the Requirements for the Degree of

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Abstract

Fetal growth and development of bones is affected by the intrauterine environment and by the genetic potential. This study was undertaken to determine the relative effects of parental characteristics, gestational age at birth and nutrition on infant growth and bone mineralization. A secondary objective was to investigate the effects of programming in the third trimester due to preterm birth on overall growth and bone mass at term age as measured by anthropometry and bone mass. Twenty-one very preterm infants (gestational age 28.5 ± 1.8 weeks) were compared to 21 term born infants (gestational age 39.1 ± 1.2 weeks) for anthropometry, and bone mass at term age. Blood and urine samples were collected throughout very preterm infant hospital stay and at birth in term infants for the measurement of markers of bone metabolism. Nutrient intake of mothers and very preterm infants were assessed and a morning maternal blood sample was obtained for assessment of bone metabolism. Bone mass of parent's and infant's hip/femur, spine and whole body were assessed using dual energy x-ray absorptiometry. Very preterm infant femur, spine and whole body bone mineral content (BMC) were significantly lower than infants born at term age despite adjustment for body size. Maternal diet, parental anthropometry and parental bone mass were more predictive of preterm infant BMC than term infant BMC. These results suggest that early maternal diet and genetics strongly influence bone mass of infants born preterm and that the trajectory of bone growth is programmed early in gestation.

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List of Abbreviations

ANOVA	Analysis of Variance
BCE	Bone Collagen Equivalents
BMC	Bone Mineral Content
BMD	Bone Mineral Density
CV	Coefficient of Variance
DRI	Dietary Reference Intake
DXA	Dual-Energy X-ray Absorptiometry
GA	Gestational Age
GH	Growth Hormone
GH-IGF-I	Growth Hormone-Insulin-Like Growth Factor I
GIT	Gastrointestinal Tract
25(OH)D	25-hydroxy Vitamin D
$1,25(OH)_2D$	1,25-dihydroxy Vitamin D
IGF-I	Insulin-Like Growth Factor I
IGF-II	Insulin-Like Growth Factor II
IGFBP	Insulin-Like Growth Factor Binding Proteins
mRNA	Messenger Ribonucleic Acid
NTx	N-telopeptide
OC	Osteocalcin
PNA	Postnatal Age
P-RNI	Premature Infant Recommended Nutrient Intake
RNI	Recommended Nutrient Intake

1.0 Present State of Knowledge

1.1 Preterm Birth

Interest in studying preterm infants has increased due to their improved survival rate. Preterm birth is defined as a gestational age (GA) less than 37 completed weeks while very preterm births are those with fewer than 32 completed weeks of gestation (Health Canada, 1999). Preterm birth accounts for approximately 7.1 % of all pregnancies and is responsible for 75-85 % of all neonatal deaths in Canada (Health Canada, 1999). The rate of preterm birth is increased among multiple births with 51.1 % of twins being born prematurely compared to 6.1 % of singleton births (Health Canada, 1999). Almost all higher order multiple births are premature (Health Canada, 1999). The rate of preterm birth has been increasing in Canada in part due to the larger number of multiple births, increased obstetrical intervention, improved recording of extremely preterm births (20-27 weeks) and the use of ultrasound to estimate GA (Health Canada, 1999; Kramer et al, 1998).

Further research in the area of preterm birth is needed due to the increasing incidence of preterm birth, the high mortality rate and the long-term complications that can accompany these infants. Common morbidities associated with preterm birth include neurocognitive, respiratory and ophthalmological problems (Kramer et al, 1998). Delays in overall growth and delays in bone mineralization have also been seen in infants born preterm. These morbidities associated with preterm birth are largely affected by the medical and nutritional management of these infants and therefore much research is focused on the optimization of these intervention strategies. Preterm infants and in particular very preterm infants are susceptible to multiple health problems due to their

immaturity. Nutrition is one of the most important interventions with premature infants for if they are to grow and overcome the obstacles of prematurity they require nourishment. The feeding of preterm infants is far more varied and complex than that of term born infants and deserves careful consideration.

1.2 Bone Growth and Development *in utero*

Bone growth and modeling are regulated by intricate interactions between an individual's genetics, environmental influences and nutritional factors (Watkins, 1998). All bone is derived from mesenchymal tissue that can either directly produce bone or indirectly synthesize bone through a cartilage model (Watkins, 1998; Martin et al, 1989). Cells from the various lineages migrate to their respective sites in the embryo and condense to form regions of high cell density representing outlines of future skeletal elements (Olsen, 1999; Olsen et al, 2000; Hall, 1987). A cartilagenous model of bone can be seen as early as five weeks of gestation (Javid & Cooper, 2002). Early in the second month of development limb buds appear (Baron, 1999) and the formation of skeletal elements is completed by the end of embryonic development in humans (Salle et al, 2002). These condensed cells differentiate into either osteoblasts or chondrocytes depending on their location in the body (Olsen et al, 2000). By 19 weeks of gestation the mineralization of bone has begun with a linear matrix organization similar to that of mature bone architecture (Glorieux et al, 1991). A materno-fetal calcium gradient develops as early as 20 weeks to deliver calcium to the fetus for bone mineralization (Javid & Cooper, 2002). During the embryonic phase (first trimester) of development the axial skeleton and spine is developing most rapidly whereas during the placental or the

second trimester the appendicular skeleton is developing most rapidly (Godfrey et al, 2001). Therefore by 24 weeks of age, when infants are sufficiently developed to become viable, a distinct cartilaginous outline of the skeleton has been formed however much of the mineralization of the bones has yet to take place. The development of the skeleton involves two main processes, intramembranous ossification and endochondral ossification.

1.2.1 Intramembranous Ossification

During embryonic development mesenchymal cells proliferate and differentiate within the connective tissue to form regions of high cell condensation (Baron, 2003). The osteoblasts which differentiate from the mesenchymal cells produce a woven bone matrix with irregular collagen fibre bundles and areas of calcification that contain high numbers of osteocytes. Blood vessels become incorporated into the woven bone and form the bone marrow. The woven bone produced through intramembranous ossification is eventually replaced by mature bone through remodelling (Baron, 2003).

1.2.2 Endochondral Ossification

The formation of long bones through endochondral ossification involves three components including the formation of a cartilage model, vascular invasion with longitudinal growth and finally growth in diameter and shape modification. As with intramembranous ossification, endochondral ossification begins with the proliferation and differentiation of mesenchymal cells however rather than the differentiation into osteoblasts mesenchymal cells involved in endochondral ossification differentiate into

chondroblasts. Chondroblasts secrete a cartilaginous matrix in which they become embedded and are then referred to as chondrocytes. A plate of cartilage between the epiphyseal and metaphyseal regions of the bone allow for growth in length as endochondral ossification spreads towards the ends of the bone (Watkins, 1998). The synthesis of cartilage occurs both appositionally at the periphery of the cartilaginous bone and in the growth plate of long bones. During early development of long bones a ring of woven bone produced through intramembranous ossification is formed where the midshaft of the future long bone will be. Blood vessels then penetrate the cartilage to form the hematopoietic bone marrow. The cartilage matrix is eventually resorbed and osteoblasts form a layer of woven bone on the cartilaginous remnant. Lastly, the deposition of membranous bone beneath the periosteum allows for growth and shape modification of long bones (Baron, 2003). Growth in diameter of long bones results from the resorption of the diaphysis cortex and bone formation on the outside (Baron, 1999). The woven bone and cartilaginous remnant of long bones is eventually replaced with mature lamellar bone through a series of bone remodelling cycles.

1.2.3 Bone Modelling/Remodelling

Both modelling and remodelling are the consequence of specific cellular actions within the bone itself. Bone remodelling is a cyclical sequence of events including activation, resorption and formation. Remodelling occurs on approximately 20 % of bone surface in adults and formation and resorption occur in balance with one another (Martin et al, 1989; Parfitt, 1990). During periods of rapid growth such as during infancy and childhood bones undergo modelling which is distinct from remodelling. During modelling the cyclical sequence of bone remodelling is not present (Parfitt, 1990). Bone

modelling occurs on approximately 90 % of bone surface resulting in net bone formation (Parfitt, 1990). While developing *in utero* the fetal skeleton accumulates minerals and bone begins to be selectively calcified.

1.3 Mineral Accretion *in utero*

Bone mineral accretion *in utero* is not linear and does not increase proportionally to weight gain during normal development (Ryan et al, 1988). Ziegler and colleagues (1976) demonstrate that fetal calcium accumulation was the greatest between 36 and 38 weeks of gestation followed by a marked decrease in mineral accretion to 40 weeks. The fetuses in the Ziegler study were between the 5^{th} and 50^{th} percentiles of Kloosterman growth curves (Ziegler et al, 1976) raising the question of whether or not these fetuses are truly representative of the normal growth *in utero*. Despite their small size, almost half (45 %) of calcium accretion occurred during the last trimester of gestation with more than three quarters (26 % overall) of that accumulating in the last 7 weeks (Ziegler et al, 1976). Using metabolic balances Shaw (1976) found calcium accretion rates similar to those of Ziegler and co-workers over the first 30 to 60 days of life in infants 24 to 36 weeks of gestation. They found that after 25 days calcium accumulation corresponded more closely with maturity of the infant rather than body weight (Shaw, 1976). Subsequently Ryan and colleagues (1988) attempted to develop an equation for the calcium accretion rate *in utero* and measured the mid-forearm bone mineral content (BMC) of over 100 infants (live and stillborn) using single photon absorptiometry. When stillborn fetuses were compared with the live born infants a significant difference in BMC was found cautioning people when interpreting results used for the estimation of

calcium needs of preterm infants (Ryan et al, 1988). As bone mineral accretion is not linear, it is also likely that calcium accretion occurs at different rates in distinct locations of the fetus during development. With the development of dual energy x-ray absorptiometry and infant whole body software, the measurement of BMC of the entire fetus rather than a single site would be a more appropriate estimation of mineral accretion *in utero*. It is also important to consider that intrauterine accretion of bone mass may not parallel extrauterine accretion even if the gestational period is identical. These differences in intrauterine and extrauterine growth are largely due to differences in nourishment. Premature infants do not have the benefit of nutrient transport from the placenta and instead rely on an immature gut and metabolic systems for growth. These changes in nutrient supply along with other common morbidities of premature birth put very premature infants at risk for delayed growth which may include decreased bone mineralization.

1.4 Preterm Bone Mass

Infants born preterm miss the period of maximal calcium deposition during the third trimester of gestation. Meeting calcium and other nutrient needs of the preterm infant is challenging and osteopenia of prematurity is a recognized complication of preterm birth. Few studies have examined the long-term effects of preterm birth on bone mineralization and fewer yet have accounted for variations seen due to genetics.

Pittard and colleagues (1990) in a study of 12 very low birth weight infants born between 32 and 36 weeks gestation found a significant correlation between radial BMC and GA at birth, 8 and 16 weeks postnatal age (PNA). These differences however did not

remain significant after correction for birth weight being the first study of that time to recognize that BMC appears to be in proportion to body size. Similarly the BMC and bone mineral density (BMD) of the lumbar spine (L1-L4) in 21 preterm infants (birth weight 1764 ± 467 g; GA 32 ± 2 weeks) was initially different between term and preterm infant at term age (Hori et al, 1995). However these differences did not remain significant when the infants reached ages three to four years (Hori et al, 1995), demonstrating that catch-up growth may be possible. However when bone mass (lumbar spine BMC) was measured in 153 adult women (age 21 years) who were born at term age, a significant association with weight at one year of age was found even after correction for current body weight (Cooper et al, 1995). These researchers concluded that growth in infancy was one of the important determinants of peak bone mass in women (Cooper et al, 1995). In a later study Cooper and co-workers (1997) reported similar results in an older cohort (men and women ages 63-73 years). This study also included measurement of serum osteocalcin (OC) which had a negative relationship with BMC at the spine and femur of both men and women (Cooper et al, 1997). These authors suggested that skeletal growth may be programmed during early life. In a descriptive follow-up study of subjects born preterm (birth weight <1500 g) Weiler and co-workers (2002) found that subjects born preterm were significantly shorter and had lower whole body BMC compared to those born at term age. Young adults who were born preterm had lower whole body BMC and were significantly shorter than those born at term age (Weiler et al, 2002). Analysis revealed that whole body and regional BMC was negatively correlated with the time required to recover birth weight potentially suggesting life-long effects on skeletal size (Weiler et al, 2002). Differing results between studies

may be attributed to nutritional and medical management of infants, site of bone mineral determination, complications during hospitalization, size at birth, GA, diet and genetics. In general, it does appear that a relationship exists between growth during infancy and subsequent bone mass suggesting a potential mechanism of programming.

1.5 Measuring Bone Density and Body Composition

X-ray absorptiometry is a relatively new method of bone mineral measurement and can achieve better precision, image resolution, faster scan times and longer source life while exposing the subject to minimal radiation. Dual energy x-ray absorptiometry uses two different x-ray energies allowing the detector to record the attenuation (reduction in the number of energy) at two different photon energies. The difference in attenuation is proportional to the density and amount of the tissues through which it passes enabling the various tissues to be distinguished including bone, lean and fat. The Hologic 4500 Acclaim Series Elite system available for this thesis uses fast kV_p switching of the x-ray tube to generate both high- and low-energy photon beams (70 and 140 kV_p). DXA however is limited to two-dimensional display and so does not measure a true density but rather an areal density only (Kleerekoper & Nelson, 1997). The mean differences between Holigic DXA machines have been reported between -1.0 to 1.4 % for all body compartments except fat which was reported to be 6.4 %. The precision of DXA has reported to be between 0.68 % and 1.8 % in adults, depending on the site of measurement, (according to manufacturer's specifications) and < 2.3 % for infants (Brunton et al, 1997). Previous research shows that diapers and blankets on infants do not significantly interfere with the precision and accuracy of BMC measurements

(Brunton et al, 1997; Koo et al, 1995). The Hologic QDR 4500A emits 6 μSv of radiation during an infant whole body scan making it a safe method of determining BMC in infants when repeated measures are necessary. With the updated infant whole body software the measurement of bone density and body composition is even more accurate and precise than the previous pediatric whole body software (Picaud et al, 1999; Brunton et al, 1997). This method of bone density measurement is safe, non-invasive and acceptable for repeated measures in neonates and infants.

1.6 Measuring Bone Metabolism

Indirect measurement of the dynamic changes in bone growth and metabolism can be determined through the measurement of biochemical bone markers present in blood and urine. Blood and urine collection permit easy and frequent measurements over time without undue risk or discomfort to the subject (Calvo et al, 1996). These markers of bone metabolism are under both environmental and genetic influences (Koo, 1996).

1.6.1 Pyridinium Crosslink Amino-Terminal Telopeptides

The formation of crosslinks within the bone matrix is initiated enzymatically following aggregation of collagen molecules into fibrils (Eriksen et al, 1995). Crosslinked peptides are derived specifically from bone collagen degradation and are not metabolized (Hanson et al, 1992). Type I collagen has three or four potential cross-linking sites with one locus in each of the non-helical C- and N-terminal regions (telopeptide regions) per molecule (Eriksen et al, 1995). It has been suggested that the measurement of crosslinks is not specific to type I collagen and that the determination of

the telopepide crosslink fragment would be more specific (Price & Thompson, 1995). Type I collagen telopeptides like crosslinks follow a circadian rhythm which needs to be considered when collecting specimens for analyses along with renal function since telopeptides are cleared by the kidneys (Eriksen et al, 1995; Price & Thompson, 1995). These diurnal rhythms of urinary NTx are important to consider when collecting spot urines (Bollen et al, 1995). Crosslinked aminoterminal telopeptide of type I collagen (N-telopeptide) has been shown to be a specific and sensitive marker of bone resorption (Price & Thompson, 1995). Osteoclasts generate NTx as they resorb bone in vitro (Apone et al, 1997). NTx has been shown to be higher in preterm infants compared to those born at term (Naylor et al, 1999; Mora et al, 1997). NTx follows a progressive decrease in concentration reaching a nadir between 38 and 42 weeks of gestation (Mora et al, 1997). NTx may be a more specific marker of bone resorption than all others that have been reported (Hanson et al, 1992).

1.6.2 Osteocalcin

Osteocalcin or bone γ-carboxy glutamic acid containing protein is the most abundant non-collagenous protein found in bone and dentine (1-2 % of total bone protein) and the most common bone formation marker (Naylor et al, 1999; Price & Thompson, 1995; Eriksen et al, 1995; Koo, 1996; Bhandari et al, 1999). Biosynthesis of OC occurs in the osteoblast where it is incorporated into the extracellular matrix of the bone (Naylor et al, 1999; Price & Thompson, 1995; Delmas, 1993). OC that is not incorporated into bone and bound to hydroxyapatite is released into circulation where it can be measured (Price & Thompson, 1995; Naylor et al, 1999; Delmas, 1990, Delmas,

1993; Bhandari et al, 1999). Although physiological functions of OC are still unknown, serum levels of this bone protein may represent a marker of osteoblast activity, OC being higher at ages when the bone formation is increased (Bhandari et al, 1999; Gundberg et al, 1985). The average serum concentration of OC is approximately 6 to 10 times higher during infancy and decreases to near 2 to 3 times adult concentrations by one year of age (Koo, 1996). Recent data suggest there are differences in OC concentrations by age, gender and ethnicity in adults, but these differences have yet to be observed in infants (Gundberg et al, 2002; Koo, 1996). Interestingly, infants fed human milk have been observed to have higher OC concentrations than those fed infant formula (Lichtenstein et al, 1987). Both circadian rhythms and seasonal variations of OC have been reported with higher concentrations being observed during early morning hours and summer and autumn months (Lichtenstein et al, 1987; Parviainen, 1991 Eriksen et al, 1995). OC is cleared by the kidneys and therefore serum levels depend on their function (Eriksen et al, 1995). Increased levels of OC are found in conditions of rapid bone turnover (Naylor et al, 1999; Price & Thompson, 1995). A decrease of serum OC however may be an indicator of lowered bone formation and turnover (Koo 1996; Parviainen et al, 1991). The precise function of OC is unknown, but it has been suggested to be involved in the recruitment of osteoclasts chemotactically (Eriksen et al, 1995; Delmas, 1990), inhibition of hydroxyapatite precipitation (Eriksen et al, 1995), as a messenger for vitamin D in bone resorption (Price & Thompson 1995), as an inhibitor of growth factor activity (Price & Thompson 1995) and in the regulation of bone mineralization (Price & Thompson 1995). The expression of OC is known to be enhanced by vitamin D (Price & Thompson

1995; Eriksen et al, 1995; Parviainen et al, 1991). Both serum and urinary OC have been used as markers of bone formation (Taylor et al. 1990).

Due to the inherent instability of the OC molecule it becomes fragmented either during osteoclastic degradation of the bone matrix or from the catabolic breakdown of the circulating protein after synthesis (Price & Thompson, 1995; Taylor at al, 1990). Intact and N-terminal OC are the forms found in highest concentration in blood (Calvo et al, 1996). Assays that detect intact OC are sensitive to *in vivo* degradation, while assays detecting fragments may overestimate the concentration of intact OC depending on the fragments recognized (Lee et al, 2000).

1.7 Hormones and Growth Factors Involved in Bone Metabolism

1.7.1 Vitamin D

Vitamin D is a fat-soluble vitamin necessary for bone development and maintenance. It plays an integral role in calcium and phosphorous homeostasis, thereby affecting bone. Vitamin D can be obtained from either the diet or by the action of ultraviolet sunlight on skin. The major circulating metabolite of vitamin D is 25-hydroxy vitamin D_3 (25(OH)D) and the most active form being 1,25-dihydroxyvitamin D ($1,25(OH)_2D$). $1,25(OH)_2D$ enhances the absorption and transportation of calcium and phosphorous from the intestine to maintain mineral homeostasis (Brown et al, 1999). The active form of vitamin D also exerts both positive and negative effects on bone growth. $1,25(OH)_2D$ can either induce bone formation through increased levels of serum calcium and phosphorous thereby aiding the formation of bone matrix proteins and

mineralization, or stimulate osteoclast activity resulting in bone resorption (Brown et al, 1999).

Plasma 25(OH)D is the best indicator of vitamin D nutritional status (Paulson & Deluca, 1986, Institute of Medicine, 1997; Salle et al, 1987). During pregnancy the levels of this form of vitamin D remain constant however whether or not they are higher than those in non-pregnant women has yet to be elucidated (Paulson & Deluca, 1986). "The requirements of the fetus for a growing skeleton place great demands on maternal mineral stores such that hormonal adjustments occur . . . to prevent calcium depletion" (Paulson & Deluca, 1986). As previously mentioned circulating 25(OH)D levels depend on dietary intake and ultraviolet B light 290-320 nm, but geographic location and season are also common influences in pregnant women (Paulson & Deluca, 1986; Salle et al, 1987; Institute of Medicine, 1997; Salle et al, 1988). Maternal transfer of 25(OH)D to the fetus has been demonstrated and it is known that the human fetus is capable of producing limited amounts of $1,25(OH)_2D$ from this precursor (Paulson & Deluca, 1986; Salle et al, 1987; Salle et al, 1988; Delvin et al, 1982). Despite the transfer of nutrients and the ability of the fetus to produce the active form of vitamin D our research group has found that 43 % of term infants to be deficient in 25(OH)D, as defined by a concentration < 27.5 nM, and 50 % of mothers to be deficient in 25(OH)D as defined by a concentration < 40 nM (Weiler et al, 2005). Until birth, infants are completely reliant on their mothers for a source of 25(OH)D. Activation of vitamin D is operative as early as 24 hours postpartum in preterm infants 28 weeks of gestation and older (Salle et al, 1987). Infants however are still at risk of vitamin D deficiency due to their limited ability

to produce the hormone. After birth vitamin D levels depend on the nutrition they receive from breast milk, infant supplements, or infant formula.

1.7.2 Cortisol and Glucocorticoids

Cortisol is an endogenous glucocorticoid that affects glucose, fat and protein metabolism and has a mineralocorticoid activity. Cortisol is released from the anterior pituitary in response to stress. Glucocorticoids can have a negative effect on bone through decreased calcium absorption and renal tubular resorption. Glucocorticoids are also known to regulate both bone formation and resorption through its effects on gene expression, replication and differentiation (Delany et al, 1995). Glucocorticoids alter the number and function of osteoblasts in that they decrease the rate of replication and increase the rate of apoptosis resulting in a shortened lifespan along with a decrease in the synthesis of type I collagen and OC (Lukert, 2003). Bone resorption is enhanced by glucocortioids as suggested by calcium kinetic studies and histomorphometry (Lukert, 2003). During most of gestation fetal glucocorticoid concentrations including cortisol are regulated by placental 11β-hydroxysteroid dehydrogenase type 2 which converts cortisol to its active form (Seckyl, 2001). The placental activity of this enzyme is regulated by nutrition and endocrine factors (Clark et al, 2002; Seckl, 2001). When concentrations of glucocorticoids are elevated fetal growth rate has been seen to decline due to decreased tissue accretion and altered cellular differentiation (Seckl, 2001; Fowden et al, 1998). In late gestation glucocorticoid exposure *in utero* can alter various tissue receptors, enzymes, ion channels and transports. The cellular effects of cortisol depend on GA and are tissue specific. Tissue gene expression of insulin-like growth factor-I (IGF-I) is

affected by the endogenous rise in cortisol seen in infants close to term age (Fowden, 2003). Glucocorticoids are also known to affect the number of IGF binding proteins (Price et al, 1992) and IGF-I gene transcription in osteoblasts (Lukert, 2003) resulting in decreased bone cell growth.

1.7.3 Insulin-Like Growth Factor-1

IGF is a non-glycosylated polypeptide hormone that is structurally related to insulin and is involved in the development of the fetus. These hormones circulate in the blood bound to one of six IGF binding proteins (IGFBP) that play a role in regulating the distribution of IGF (Langford et al, 1998; Croucher & Russell, 1999; Lian, et al, 1999; Jones & Clemmons, 1995). IGF-I is produced mainly in the liver and extrahepatically in bone and cartilage by osteoblasts where it acts in a paracrine/autocrine fashion (Croucher & Russell, 1999; Yakar et al, 2002; McCarthy et al, 1989). Both hormones and skeletal growth factors control IGF-I production. For example, parathyroid hormone and growth hormone (GH) increase IGF-I production whereas glucocorticoids inhibit the synthesis of IGF-I (Lian et al, 1999). The GH-IGF-I axis is one of the major hormonal systems that effects postnatal skeletal growth (Gertner, 1999).

IGFs play a central role in longitudinal bone growth and the maintenance of bone matrix and bone mass (Lian et al, 1999; McCarthy & Centrella, 2001). IGF-I stimulates the formation of bone matrix through direct enhancement of bone collagen production from differentiated affects on osteoblasts, and by indirectly increasing the number of functional osteoblasts per bone unit (Hock et al, 1988; Lian et al, 1999). IGF-I enhances the mRNA expression of type I collagen and is therefore thought to regulate its synthesis

(McCarthy et al, 1989). Type I collagen transcription is increased by IGFs with a concurrent decrease in transcription of a collagen-degrading protease (Lian et al, 1999). Overall these actions result in increased net collagen through increased formation and decreased degradation. Lower IGF-I levels are consistent with decreased bone growth since it is known that IGF-I stimulates chondrocyte proliferation (Yakar et al, 2002). IGF-I has been shown to enhance the recruitment and activation of osteoclasts and potentially act as a mediator between osteoblast and osteoclasts (Mochizuki et al, 1992). When administered to humans there is a generalized increase in bone remodelling (Lian et al, 1999). IGF-I is found in calcified bone matrix where it is thought to reactivate local osteoblasts to aid in formation of bone (McCarthy & Centrella, 2001).

IGF-I is expressed in most tissues during fetal development and increases with GA as does circulating maternal IGF-I concentrations (Langford et al, 1998). IGF-I has also been correlated with birth weight, cord blood cortisol, insulin, insulin-like growth factor-II (IGF-II) concentrations as well as postnatal height and weight (Christou et al, 2001; Özkan et al, 1999). IGF-I increases with GA unlike its binding proteins (Langford et al, 1998) and is significantly higher in appropriate for gestational age infants and in small for gestational age infants who show catch-up growth (Özkan et al, 1999). It has been suggested that IGF-I is essential for postnatal catch-up growth and that infants with lower circulating levels of IGF-I may show growth delays. IGF-II is the predominate growth promoting factor during early gestation whereas IGF-I is most predominate during late gestation, possibly due to production of IGF-I by the fetal placental unit (Christou et al, 2001; Daughaday & Rotwein, 1989; Rosen 1999). Concentrations of IGF-I increase two to three fold between 33 weeks gestation and birth (Lassarre et al,

1991) meaning that infants who are born preterm do not experience this increase in growth factors necessary for normal growth. IGF-I concentrations do not show an age effect among term born infants, however a significant effect of study age has been found among preterm infants (Rajaram et al, 1995). In the later part of the third trimester and after birth, the GH-IGF-I axis begins to assume the primary role in driving linear growth (Cooper et al, 1995; Barker, 1998). Term infants show a progressive decline in IGF-I concentration during the first year of life whereas preterm infants demonstrate significant increases in IGF-I from birth until 2 months corrected age (Rajaram et al, 1995). Preterm circulating IGF-I concentrations have been seen to be significantly higher compared to term infants at 2, 4, 6 and 12 months corrected age (Rajaram et al, 1995). Reference values of IGF-I have yet to be established in preterm infants and the study by Rajaram and co-workers (1995) is one of the first to report longitudinal data of a large sample of infants. The pattern of changes in IGF-I concentrations had yet to be fully elucidated however the differences seen among results thus far may be due to feeding practices, severity of illness of preterm infants and other unknown agents.

1.8 Contribution of Parental Factors to Infant Bone Mass

The effects of genetics and environment on subsequent bone mass are difficult to unravel due to their interrelationships with each other. Variation in birth weight due to intrauterine environment is thought to account for 62 % of variation with maternal genes contributing 20 % and fetal genes 18 % (Penrose, 1954). Environmental effects which play a role in the determination of bone mass include maternal diet and smoking during pregnancy.

1.8.1 Maternal Diet

It has been shown that when undernourished mothers are given calcium supplements during pregnancy the resulting bone density of their neonates is improved. Jones and colleagues (2000) reported a substantial association between maternal diet during the third trimester of pregnancy (and hence *in utero* diet) and bone mass of their children at age eight. These authors suggest that the results seen were likely to be a programming phenomenon "whereby cells respond differently to genetic and environmental stimuli based on early exposure" (Jones et al, 2000). It has yet to be determined if these differences in bone mass persist into adulthood and affect the risk for osteoporosis.

1.8.2 Maternal Smoking

Maternal smoking during pregnancy has been linked to decreased birth weight, increased susceptibility to respiratory diseases and altered immune function (Nelson et al, 1999). Nicotine and other tobacco products have been shown to depress osteoblast activity in animal and in *in vitro* studies (Çolak et al, 2002). Passive tobacco smoke exposure significantly reduced rat pup body weight in a dose dependent manner (Nelson et al, 1999). A retardation of ossification was also observed in rat pups of dams exposed to passive sidestream smoke (Nelson et al, 1999). Infants of mothers who smoked during pregnancy have been observed to have significantly lower concentrations of cord blood OC and alkaline phosphatase than infants of non-smoking mothers suggesting that smoking during pregnancy has negative effects on fetal bone formation (Çolak et al, 2002). Çolak and colleagues (2002) suggest that the effects of smoking during pregnancy

may be due to reduced utero-placental blood flow from hypoxia resulting in diminished transport of nutrients across the placenta thereby causing reduced growth and fetal bone formation. An Australian group also reported a disproportionate deficit in bone mass, at the lumbar spine and femoral neck, of children whose mothers smoked during pregnancy (Jones et al, 1999). These associations however were diminished when adjustment was made for placental weight suggesting that the effects of smoking are mediated through placental size and function (Jones et al, 1999). These combined results indicate that smoking does have a profound effect on fetal growth including bone development and therefore needs to be considered when assessing bone mass in infants and children.

1.8.3 Parental Height, Weight and Bone Mineral Density

The relationship between parental and infant characteristics has been investigated since the early 1950s. Penrose and colleagues (1954) and Morton (1955) found strong correlations between infant birth weight and the birth weigh of people on their maternal, but not paternal side of the family. More recently maternal and paternal heights were found to be associated with infant length at birth (Godfrey et al, 2001). Cooper et al (2001) found an increased fracture risk in men and women born of taller mothers independent of childhood growth in height or weight. Parental height and paternal birth weight have been shown to be positively associated with infant spine BMC and BMD (Godfrey et al, 2001). However paternal, but not maternal height was associated with neonatal whole body BMC and BMD after adjustment for GA (Godfrey et al, 2001). Lonzer and colleagues (1996) found that the mean parental BMD Z-scores were significantly correlated with the BMD Z-scores of their children ages 5 to 20 years, even

after adjustment for the children's height and weight. The relationship between parental bone mass and infant bone mass have not yet been reported.

1.9 Feeding Preterm Infants

Feeding preterm infants is complicated due to immaturity of the gastrointestinal tract (GIT) and sucking reflexes. The main goal of feeding preterm infants is to mimic *in utero* growth. Nutritional needs of preterm infants depend on their maturity and clinical status (Cooke, 2000). Preterm infants have limited body stores of nutrients as most nutrients including calcium, accumulate during the third trimester of gestation. Maturity of the intestinal tract, feeding route, tolerance and nutrients all must be considered when determining feeding needs of the preterm infant.

Parenteral nutrition is often the first form of nutrition support established in the preterm infant. There is some evidence that early use of parenteral nutrition may improve growth outcomes by minimizing initial weight loss after birth (Deene et al, 2002a). The use of parenteral nutrition may result in cholistasis or sepsis and other consequences such as the atrophy of GIT mucosa, significant decrease in intestinal mass, decreased mucosal enzymes and increased gut permeability (Denne et al, 2002a) and therefore should not be used long term. Enteral nutrition should be established as soon as considered safe in the preterm infant so that parenteral nutrition can be discontinued.

The human GIT does not reach maturity until 38 weeks of gestation (Grand et al, 1976). Despite the immaturity of the GIT, studies looking at the motor patterns of the GIT in preterm infants have determined that early feeding enhances the maturation of the small intestine motor activity and that delays in feeding prevent normal maturation

(Berseth, 1992; Berseth & Nordyke, 1993). The presence of enteral nutrients stimulates growth of the GIT mucosa thereby enhancing its functional maturity (Berseth & Nordyke, 1993). Enteral nutrition is often initiated by providing minimal enternal feeds which do not provide sufficient calories to sustain somatic growth, but are thought to help mature the GIT both structurally and functionally, reduce liver dysfunction and improve feeding tolerance (Deene et al, 2002b). Minimal enternal feeds involve "priming" the GIT with small intermittent boluses of either fortified human breast milk or formula designed for preterm infants. These boluses increase in volume and frequency as tolerated until full feeds are established (150 ml/kg/d).

Suckling in infants does not develop fully until approximately 37 weeks of gestation however immature suckling has been seen as young as 32 to 33 weeks gestation (Lau et al, 2000). Some studies suggest that non-nutritive sucking during feedings may mature the sucking reflex, decrease intestinal transit time and improve transition to oral feeds however these results have not been reproducible in all settings (Denne et al, 2002b). When suckling is established infants are gradually weaned to oral feeding by bottle and/or breast.

Human milk is better tolerated in preterm infants and fortification does not affect gastric emptying and is tolerated well (Schandler et al, 1999; McClure & Newell, 1996). Preterm infants fed fortified human milk attain tube feeding faster and hence parenteral nutrition use is reduced (Schandler et al, 1999). Studies also show that preterm mother's milk is preferable to banked mature breast milk. At two weeks PNA, preterm infants fed banked breast milk were only 94 % of their initial birth weight whereas infants fed preterm mother's milk had regained or surpassed their birth weight (Atkinson, 1981).

Preterm infants fed mother's milk also have lower incidences of necrotizing enterocolitis and improved neurocognitive development (Atkinson, 2000).

As with term infants, human breast milk is the preferred form of feeding due to the protective non-nutrient components present, decreased incidence of necrotizing enterocolitis, improved gastric emptying and possible better neurocognitive development (Denne et al, 2002b). Breast milk is considered the "gold standard" for term infants, however nutrient composition may not be adequate to support optimal growth of preterm infants.

Preterm breast milk composition differs from that of term milk. Breast milk of mothers who delivered between 32 and 36 weeks of gestation has milk compositions similar to that of term breast milk. However preterm milk from mothers who delivered at 28 to 32 weeks of gestation has significantly higher nitrogen, sodium, fatty acids, calories, calcium and vitamin D (Atkinson, 2000). These differences in breast milk composition are seen most readily during early lactation and mirror term breast milk after several weeks (Atkinson, 2000; Denne et al, 2002b). Despite these higher concentrations of some nutrients early in lactation, human breast milk does not completely meet the nutritional needs of the preterm infant (Denne et al, 2002b). Protein, calcium, phosphorous, sodium, zinc, vitamins and potentially energy are insufficient to support optimal growth of the infant born preterm (Denne et al, 2002b). For this reason human milk fortifiers have been developed to enrich breast milk to meet the unique needs of preterm infants.

1.9.1 Preterm Infant Diet and Bone Mineral Content

Greer and McCormick (1988) found that infants (birth weight <1600 g; GA <32 weeks) fed fortified human milk or preterm formula supplemented with calcium, phosphorous and protein showed improved bone mineralization compared to infants fed standard formula or unfortified breast milk. In this study no feeding group reached radial BMCs similar to those of "*in utero* values" and all infants were less than the 50th percentile for weight at time of discharge (Greer & McCormick, 1988). These results point to the fact that bone mineralization in preterm infants is altered and that catch-up growth is not apparent before term age, however the long-term effects of this had not been established at the time of publication. Faerk and colleagues (2000), in a randomized control trial of 127 preterm infants (< 32 weeks GA) fed either human milk supplemented with phosphate, human milk fortified with protein, calcium and phosphorous or preterm formula found differences in whole body BMC at term age. All infants however showed whole body BMCs and BMC/kg body weight below values for infants born at term (Faerk et al, 2000). Preterm infants fed fortified human milk have been noted to have slower rates of weight gain and length increment than infants fed preterm formula (Schandler et al, 1999). Schandler and colleagues (1999) concluded that despite the slower rate of growth observed in preterm infants fed fortified human milk the benefits of improved host defence and gastrointestinal function justified its use.

1.10 Assessment of Maternal Nutrient Intake

There are several methods of estimating food consumption of an individual including 24-hour recalls, food records, dietary history and food frequency questionnaires (Gibson, 1990). Selection of a dietary assessment tool should be based on the objectives

of the study. To estimate the usual intake of an individual, multiple 24-hour recalls or estimated food intake methods should be used (Gibson, 1990).

Dietary history attempts to assess an individual's usual food intake over an extended period of time (Gibson, 1990). The dietary history is labour intensive and not suitable for studies of large numbers of people (Gibson, 1990). Dietary histories overall show good precision and correlation among dietary methods used within interview.

Food frequency questionnaires are used to estimate usual food consumption patterns and do not provide quantitative data about food or nutrient intake (Gibson, 1990). Focused questionnaires can be used to predict specific nutrient intakes (Gibson, 1990). Food frequency questionnaires are generally considered to be easy to administer, have a low burden on the individual and represent usual intakes of subjects over an extended period of time with good precision (Gibson, 1990).

Food records require the subject to record all foods and beverages consumed either with or without weighing. The duration of food records varies, but usually three, five or seven days are used. Seven day weighed food records are considered to be an appropriate estimation of usual food intake, however due to the high burden on the subject short periods of time are often used (Gibson, 1990). Generally estimated food records are considered to be less accurate than weighted food records but have a lesser burden on the subject. Subject motivation, literacy and numeracy affect the accuracy of this estimate of food consumption (Gibson, 1990). According to Basiotis and co-workers (1987) three days of intake measurements are required to estimate true average energy intake for a group of 29 adults.

A single 24-hour recall is appropriate for assessing average food and nutrient intakes for large groups of people, but is not suitable for the nutritional assessment of an individual (Gibson, 1990). A single 24-hour recall has a low precision of estimating the usual pattern of eating of an individual due to intra-subject variability (Gibson, 1990, Guenther, 1994). The accuracy of a 24-hour recall depends on the subject's memory, ability to estimate portion sizes and degree of motivation as well as the interviewers' ability to probe the subject for further details regarding his/her food intake (Gibson, 1990). Advantages of using 24-hour recalls for nutrition assessment are that they do not rely on long-term memory, they are inexpensive, have a low subject burden and can provide the details of foods and preparations needed for analysis. Additional props can be used to enhance the quality of these measures including food models, photographs of portion sizes and standard measures such as measuring cups and rulers to describe food portions. 24-hour recall can also be performed either in person or via telephone. Studies using doubly labelled water indicate that 24-hour food recalls can be administered in person or by telephone without any significant differences in estimates of group energy intake (Tran et al, 2000). Repeated 24-hour food recalls can be used to estimate the typical food intake of an individual over a prolonged period of time (Gibson, 1990). If an adequate number of 24-hour recalls are obtained to account for the influence of weekends, seasons and holidays on food intake the results can be used as an estimate of usual food consumption.

1.11 Programming

Fetal growth and development is affected by the intrauterine environment (nutrient availability to the fetus via placenta) and by the genetic potential (Gallaher et al,

1998). Programming is a term used to describe "persistent changes in structure and function caused by environmental stimuli acting at critical periods during early development" (Cooper et al, 2000). The concept of programming stems from several earlier studies which noted that people who were undernourished early in life and subsequently had low birth weight were at higher risk of coronary heart disease, hypertension, non-insulin dependent diabetes hypercholesterolemia and osteoporosis. Thus there is growing evidence that intrauterine nutrition plays a role in programming health and disease outcomes.

According to Cooper and colleagues (2000) there are three cellular mechanisms for the induction of programming including altered gene expression, reduced cell numbers and altered clones of cells. All three of these mechanisms are thought to be affected by nutrition and/or environmental adversities during development causing permanent effects on body structure, physiology and metabolism (Cooper et al, 2000). The rapidly growing fetus and neonate are most vulnerable to the effects of undernutrition and the extent of this adversity is dependent on the developmental stage of the fetus and/or neonate (Cooper et al, 2000). Undernutrition late in gestation can result in altered gene expression causing reduced cell numbers due to reduced cell replication, an imbalance between cell types, altered organ structure and changes in the patterns of hormonal release and tissue sensitivity to these hormones (Cooper et al, 2000; Barker, 1998). Another manifestation of metabolic programming might be the allocation of cells during critical early periods to different body compartments, including fat, muscle, and bone (Gale et al, 2001).

Barker (1998) postulates that undernutrition early in life can become translated into pathology and thereby determine disease later in life. When fetal nutrient demands are greater than that supplied by the placenta, fetal undernutrition and subsequent programming can occur. Fetal programming may be due to adaptations to undernutrition such as hypoxemia, metabolic and endocrine changes (Barker, 1998; Figure 1.1). The fetal response to undernutrition is to catabolize its own substrates for energy and this leads to a reduction in growth and enhances the fetus' ability to survive (Barker, 1998). Blood flow may be redistributed in efforts to protect vital organs such as the brain, this however results in reduced growth of abdominal viscera in humans due to the large requirements for brain growth (Barker, 1998). Barker (1998) points out that birth weight has some severe limitations as a measure of fetal growth in that different patterns of fetal growth can result in similar birth weights.

The majority of research thus far has focused on endocrine changes in particular IGFs in relation to disease states. Changes in fetal nutrition may alter insulin, IGFs and cortisol which are thought to play a central role in the regulation of growth and cell differentiation (Barker, 1998). When nutrient availability is restricted anabolic hormones such as insulin and IGF-I fall while catabolic hormones including glucocorticoids increase (Barker, 1998). Low birth weight children have been observed to have raised plasma IGF-I concentrations with the highest being observed among those with the lowest birth weights but that attained the largest body size during childhood and therefore may be related to catch-up growth (Barker, 1998). Persistent changes have been postulated to play a major role in the development of insulin resistance later in life (Barker, 1998).

It has been shown in sheep that undernutrition during late gestation and at time of conception results in a significant reduction in fetal and maternal IGF-I at 115 days of gestation (Gallaher et al, 1998). IGFBP-3 was also significantly depressed at 115 days of gestation in the ewes that were undernourished late in gestation; this observation was significantly greater in ewes that were undernourished both at the time of conception and late in gestation (Gallaher et al, 1998). These results suggest that undernutrition during the periconceptual period may reprogram fetal IGF-I and IGFBP-3 systems and their abilities to respond to changes in substrate supply. Gallaher and colleagues (1998) report that these physiological responses may have significant long-term alterations in regulation of homeostasis via changes in plasma glucose and insulin.

Cortisol, insulin, IGF-I and growth hormone are all important in the growth and development of bone as previously discussed. Alterations in cortisol may program glucose, fat and protein metabolism or affect the absorption of calcium. During gestation insulin is permissive to growth however during the third trimester IGF-I takes over its role in fetal development.

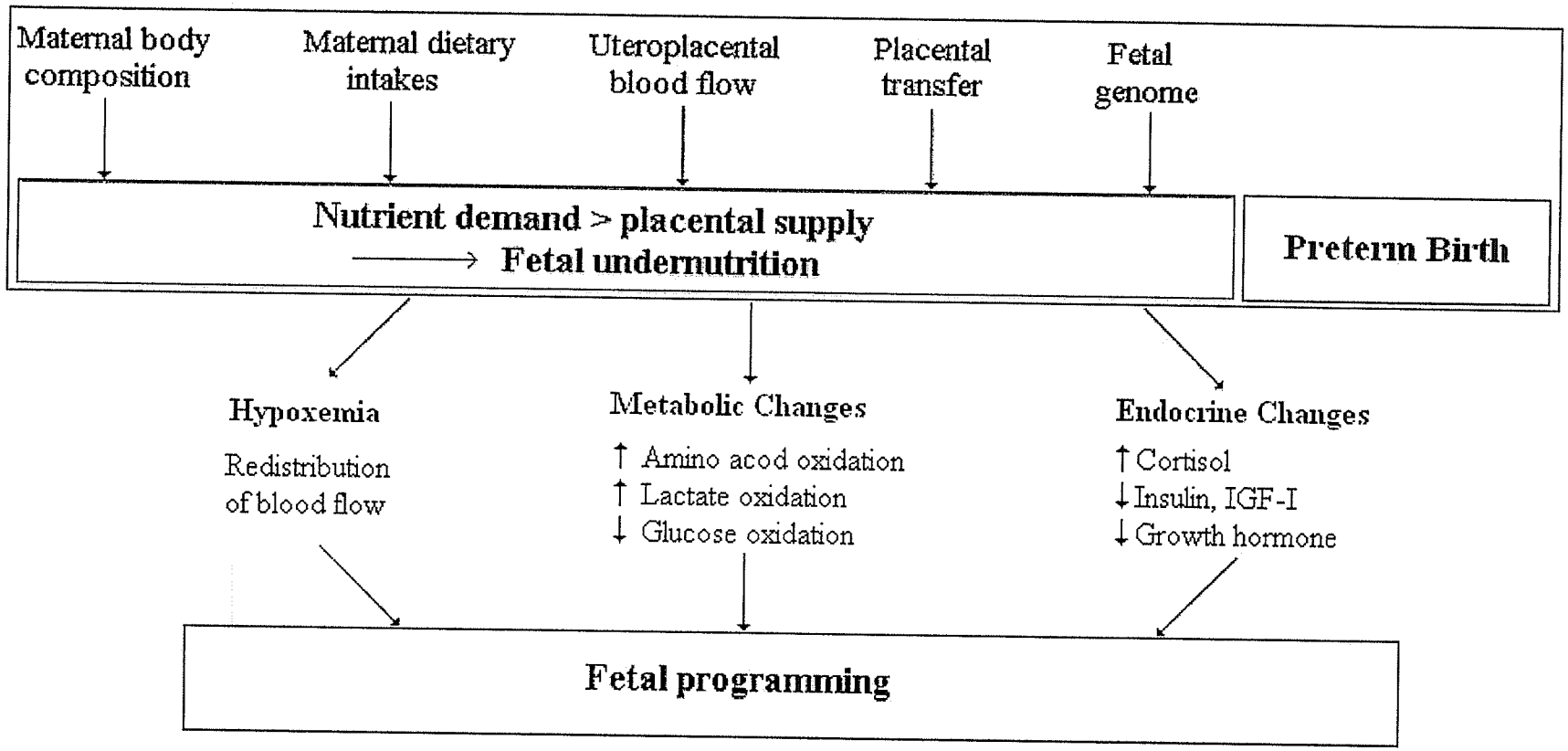

Figure 1.1: Framework for fetal adaptation to undernutrition and preterm birth (Adapted from Barker, 1998). IGF-I, insulin-like growth factor-I

1.11.1 Programming of Skeletal Growth

Different tissues of the body undergo critical periods of rapid cell division at various points during fetal growth. Long bone growth rate increases during the second trimester of gestation (Cooper et al, 2000). Genetic markers only explain a small proportion of variation seen in bone mass and therefore it is likely that environmental influences early in life interact with genomes involved in skeletal growth (Cooper et al, 2000). Evidence that the risk of osteoporosis may be modified by environmental effects during early life stems from research of: (i) adult bone mineral measurements of those with detailed birth and/or childhood records; (ii) detailed physiological studies exploring the relationship between candidate endocrine systems which might be programmed (GH/IGF-I; hypothalamic-pituitary-adrenal, gonadal steroids) and age-related bone loss; (iii) pregnant women, their nutrition, body build and lifestyle related to the bone mass of their offspring; and (iv) childhood growth rates and risk of hip fractures later in life (Copper et al, 2000). Highly significant relationships between weight at one year of age and adult hip and spine bone area have been noted even after adjustment for genetic markers and lifestyle factors (Cooper et al, 1997). Significant positive associations have been found between birth weight, placental weight and whole body BMC and BMD of infants (Godfrey et al, 2001). Positive and significant correlations have been noted between birth weight and adult whole body bone mass, and BMC at the femoral neck and lumbar spine (Gale et al, 2001). The relationship between birth weight and adult BMC at the lumbar spine, proximal femur and whole body remained significant after adjustment for age, sex, adult height (Gale et al, 2001). Studies of men and women whose birth records were preserved revealed that birth weight in infancy was predictive of basal

growth hormone and cortisol levels in adulthood (Cooper et al, 2000). These results are compatible with the theory that environmental stressors during intrauterine or early postnatal life may alter the sensitivity of the growth plate to growth hormone and cortisol (Cooper et al, 2000). Cooper and colleagues (2000) indicate that these changes or programming of the endocrine system could reduce peak skeletal size and mineralization and lead to a predisposition to accelerated rates of bone loss (Fall et al, 1998; Dennison et al, 1999; Phillips et al, 1998). The GH-IGF-I axis may be programmed by adverse intrauterine environmental conditions and may manifest as alterations in the rates of skeletal growth and loss later in life (Godfrey et al, 2001). Neonatal bone mass after correction for gender and GA has been shown to be strongly and positively correlated with birth weight, birth length and placental weight (Cooper et al, 2000). "The independent affect of maternal and paternal birth weight on fetal skeletal development support the notion that paternal influences . . . contribute strongly to the establishment of the early skeletal growth trajectory, while maternal nutrition and body build modify fetal nutrient supply and subsequent bone accretion, predominately through influences on placentation" (Cooper et al, 2000). Mathematical analysis of postnatal birth suggest a transition between the fetal and childhood growth phases that occurs around one year of age and that is influenced by the intrauterine growth trajectory (Godfrey et al, 2001). Data of Gale and colleagues (2001) indicate that genetic and/or intrauterine environmental factors that alter the fetal growth trajectory and are reflected in birth weight have long-term consequences on adult bone mass which may affect fracture risk later in life. Studies of childhood growth rates and subsequent risk of hip fractures show after adjustment for socioeconomic status tall maternal height and low rate of childhood

growth are both independent predictors of hip fracture risk (Cooper et al, 1999). These data are consistent with the theory of endocrine programming affecting risk of hip fracture. According to Cooper and co-workers (2000) the observation that adults with higher risk of hip fracture were shorter at birth but of average height by age 7 years suggests that risk may be higher among those whose skeletal envelop is forced ahead of the capacity to mineralize. Slowing of growth is thought to be a major adaptation of undernutrition.

Preterm birth is a situation in which large changes in the neonatal environment occur. Preterm infants no longer have the advantage of placental transfer to meet their demanding nutritional needs resulting in a period of time where undernutrition occurs that might be synonymous with fetal undernourishment. This situation along with the infant's immaturity and clinical status may result in programming of growth and bone mineralization with potential life long effects on bone health.

2.0 Hypothesis

Preterm infants will undergo programming of bone mineralization via the GH-IGF-I axis resulting in lower BMC at term age.

3.0 Objectives

The objectives of this research in premature infants are to:

1. Determine the relative effects of parental characteristics, GA at birth and nutrition on infant growth and bone mineralization.

2. Determine the effects of programming in the third trimester due to preterm birth on overall growth and bone mass at term age as measured by weight, length, head circumference, body composition and BMC of the whole body, femur and lumbar spine (L1-L4).

4.0 Methods

4.1 Sample Size

A sample size of 64 was calculated based on a whole body BMC estimated population mean of 74 ± 13 g (Atkinson & Randall-Simpson, 2000) and a 95 % confidence interval (Colton, 1974). An estimated difference of 3 g was calculated based on a 4 % error in estimating the population mean (Atkinson & Randall-Simpson, 2000) and a difference of 1.4 % between Hologic machines previously reported in adults and children (Ellis & Shypailo, 1998) and a precision in measuring infants of < 2.3 %. Based on a sample estimation of 29 infants and an estimated attrition rate of 10 % the attempt was to recruit 32 infants per group.

4.1.1 Recruitment

Infants were recruited from the Health Sciences Centre Women's Hospital and St. Boniface General Hospital between May 2001 and September 2003 for measurement of anthropometry and bone mass. No monetary remuneration was provided to the families, however in efforts to standardize infant nutrition, families were provided with Enfalac with Iron (provided in kind by Mead Johnson) upon request. Small token gifts, breast pumps and the cost of parking of transportation costs were provided.

4.1.2 Inclusion Criteria

Preterm infants who were less than or equal to 32 weeks gestation and who were an appropriate weight for GA (as defined by >3rd percentile Usher& McLean, 1969) with a weight of 1600 g or less were eligible for study. Term infants born between 37 and 40 6/7 weeks of gestation who were an appropriate weight for GA were eligible for study. Infants were excluded if they had known congenital abnormalities or if they developed necrotizing enterocolitis during the course of the study. Infants born of a mother who used alcohol or illicit drugs such as cocaine or heroine during pregnancy, had diabetes or other endocrine diseases known to affect bone metabolism, or took medications known to affect bone metabolism were also excluded from the study.

4.2 Ethical Approval

This study was reviewed by the University of Manitoba Ethics Committee. Written informed consent was obtained from parents prior to enrolment in the study.

4.3 Medical and Nutritional Management in Hospital

The nutritional and medical management of all infants (both term and preterm) was not altered at any point during the course of the study. Feeding type (breast milk or formula) was determined by the parents and nutritional management (timing of nutrition administration) was determined by hospital medical staff for preterm infants while in hospital. Information regarding feeding type, volume and other medical information was collected from preterm infant hospital charts. Parenteral and enteral nutrition intakes of

preterm infants were recorded daily until full enteral feeds (150 ml/kg/d) were established, after which intakes were recorded twice weekly. Medications administered to infants including vitamin and mineral supplements were recorded daily for the full duration of hospitalization. Information regarding ventilation and oxygen support were recorded twice weekly.

4.4 Infant Nutritional Intake After Hospital Discharge

Upon discharge nutrient intake of infants was estimated using a 3-day food record to be completed at term age. Nutrition intake of breast fed infants was estimated using test weighing (Scanlon et al, 2002). Mothers were instructed to record the weight of their infants prior to breast feeding and immediately after, using the provided scale (SB32000; Mettler-Toledo Inc, Greifensee, Switzerland) with a dynamic weighing program. The difference between the two weights was taken as the volume of breast milk consumed assuming a density of approximately 1.0 g/ml. When feedings were not recorded properly, the average feed volume was used in place of the value recorded. Parents were requested to record any other supplementary feedings of either breast milk or formula from a bottle, or vitamin supplements. All food records were analyzed by the same investigator (H.K.) using Nutrient Analysis Program (E. Warwick, PEI, Canada) that included the 1996 Canadian Nutrient File. Foods not included in the nutrient analysis program were added to the program using nutrition information from food labels and manufacturers.

4.5 Infant Growth Measurement

Growth of preterm infants, including weight, length and head circumference was measured. Weight was measured daily to the nearest gram in hospital using a digital scale (Olympic Medical 56320, WA, USA) and again at subsequesent research visits after hospital discharge using a digital scale (SB32000; Mettler-Toledo Inc, Greifensee, Switzerland) with a dynamic weighing program to account for movement. Length and head circumference were measured weekly while in hospital by research or nursing staff until discharge and again at follow-up research visits. Infant crown-heel length was measured to the nearest centimetre by two examiners using a Plexiglas recumbent preemie, newborn or pediatric statiometer (O'Learly LengthBoards™; Ellard Instrumentation Ltd, WA, USA). Crown-heel length was measured with infants wearing only a diaper and a soft blanket beneath them with one examiner holding the infant head touching the fixed headboard while the other examiner extended the infants legs fully and placed the movable footboard against the infant's heels to obtain the measurement. Head circumference was measured using a non-stretch tape measure (Perspective Enterprises, MI, USA) placed at the most prominent part above the supraorbital ridges and over the part of the occiput that gave the maximum circumference. Growth of preterm infants while in hospital was expressed in kg/week and cm/week. Growth velocity was expressed as g/kg/d to account for size differences among infants using the following formula:

Growth velocity (g/kg/d) = $\frac{\text{(weight gained in hospital)/average weight)}}{\text{postnatal age at discharge}}$

Where:

a) weight gain is in grams as the difference between birth weight and discharge weight;
b) average weight is in kilograms using birth weight and weight at discharge; and
c) postnatal age is in days

Term and preterm infant anthropometry was measured within two weeks of term age to coincide with BMC measurements. Z-scores were created using the Centre for Disease Control database (Center for Disease Control, 2000).

4.6 Infant Blood and Urine Sampling

Cord blood samples were collected at delivery from all term infants and preterm infants where possible. While in hospital four blood samples (approximately 500 μl) were obtained from preterm infants at weeks one, three, five and at time of discharge using a heel prick and heparized microtainers. Blood samples (with the exception of cord blood) were collected between 0400 h and 0900 h to control for diurnal variations in some measurements. Blood samples were centrifuged at 2000 g at 4 °C for 10 minutes and plasma removed and stored under nitrogen at -80 °C until analysis.

Infant urine samples were obtained between 0200 h and 0900 h to control for circadian rhythms. At birth both term and preterm urine samples were obtained within the first 48 h. Preterm infant urine was also collected at weeks one, three, five and at discharge. Urine was collected using a pediatric urine collection bag that was placed in the infant's diaper. All urine samples were stored at -80 °C until analysis.

4.7 Biochemical Measurements

4.7.1 Osteocalcin

Radioimmunoassay (DiaSorin, MN, USA) was used to measure plasma OC as a reflection of bone formation. Maternal, term infant and week one preterm plasma samples were assayed directly. All other samples were diluted 2-fold (week 3 preterm samples) or 4-fold (week 5 and discharge preterm samples) with the 0 standard provided in the kit. In borosilicate glass tubes calibrator or sample was combined with OC antiserum containing rabbit anti-bovine OC antibody and ^{125}I bovine OC. Competitive binding between the sample or calibrator OC, ^{125}I bovine OC and the antibody was allowed to proceed according to manufacturer's directions. Phase separation was accomplished by the addition of goat anti-rabbit serum which bound with rabbit serum contained in the rabbit anti-bovine ostecalcin antibody. Tubes were centrifuged and decanted leaving the precipitate that was measured for 60 seconds using a gamma scintillation counter (Wallac 1470 Wizard Automatic Gamma Counter, Turku, Finland). Counts are inversely proportional to the concentration of OC present in the sample. Unknown concentrations were interpolated from the standard curve. Coefficient of variation (CV) of ≤ 20 % or a difference of ≤ 200 cpm between duplicates was considered acceptable precision. This assay had a minimum detectable limit of 0.035 nM.

4.7.2 N-Telopeptide

As a marker of bone resorption NTx was determined by enzyme-linked immunosorbent assay (Osteomark, WA, USA). Infant urine samples were diluted 10-fold

with deionized water. Turbid urine samples were centrifuged prior to dilution to remove particulates. Maternal plasma samples were assayed directly. A standard curve was prepared using the calibrators provided in the kit. Calibrators or samples were combined with conjugate solution in purified human NTx antigen coated microwells. Competitive binding between the sample NTx and solid phase NTx with the conjugate containing a monoclonal antibody labelled with horseradish peroxidase was allowed to proceed. The microwells were then decanted, washed and chromogen/buffered solution added. A blue colour was produced from the binding of the horseradish peroxidase and the peroxide contained in the chromogen/buffered solution. The intensity of the blue colour that developed is inversely proportional to the concentration of NTx present in the sample. A stopping reagent was added to terminate the reaction and resulted in a solution colour change from blue to yellow. The microwells were allowed to stand for 5 minutes to allow for uniform colour development before the absorbance was read at 450 nm using a microplate scanning spectrophotometer (Powerwave X, Bio-Tek Instruments Inc, VT, USA). The CV for reproducibility was ≤ 20 % for all sample duplicates. This assay had a minimum detectable limit of 20 nM bone collage equivalents.

4.7.3 Creatinine

Creatinine was determined to account for renal function and lean mass in order to standardize urinary NTx, cortisol, calcium and phosphorous concentrations. This assay was an adapted microassay based on the Jaffe reaction (Sigma Diagnostics, Inc, MO, USA). Infant urine samples were diluted 2-fold to 10-fold with deionized water (typical dilution 5-fold). Twenty microlitres of urine, creatinine standard (Sigma Diagnostics,

Inc, MO, USA) or deionized water (blank) was pipetted in triplicate into a 96-well microplate. Alkaline picrate solution was prepared by combining five parts creatinine colour reagent with one part sodium hydroxide and 200 µl was pipetted into each well using a multi-channel pipette. The assay was allowed to incubate for 10 minutes at room temperature during which time a yellow/orange colour developed. Absorbance was read at 500 nm using a microplate scanning spectrophotometer (Powerwave X, Bio-Tek Instruments Inc, VT, USA). Absorbance was proportional to the concentration of creatinine present in the sample. Immediately after absorbance reading, 7 µl of acid reagent was pipetted into each well to stop the reaction. Absorbance was read again after allowing the mixture to stand for 5 minutes. A standard curve was created from the standards with five concentrations (2.5, 3.0, 5.0, 7.5 and 10.0 mg/dl). Creatinine concentration was calculated using the following formula:

$$\text{Creatinine (mM)} = \left(\frac{\text{initial-final sample absorbance}}{\text{initial-final standard absorbance}} \times \text{standard concentration} \right) \times 88.4$$

Final creatinine concentration was obtained by multiplying the above value by the dilution factor. The CV for reproducibility was ≤ 10 %.

4.7.4 Insulin-Like Growth Factor-1

Plasma IGF-1 was measured by a solid-phase enzyme linked immunosorbant assay (R&D Systems, MN, USA). Undiluted plasma samples (5 µl) were pretreated with 95 µl acid dislocation solution to release the IGF-1 from its binding proteins according to manufacturer's directions. Twenty-five microlitres was transferred to a new polypropylene tube and combined with 100 µl of a buffered protein with blue dye. Pretreated plasma (50 µl) or standards were pipetted in duplicate into 96-well microplate

coated with a mouse monoclonal antibody against IGF-1. Assay diluent was added to each well using a multi-channel pipette and incubated. During incubation any IGF-1 present in the samples became bound and immobilized by the antibody coating on the microwells. Microwells were then aspirated and washed with a wash buffer solution to remove any unbound substances. A conjugate containing a polyclonal antibody against IGF-1 bound to horseradish peroxidase was added to each well. The polyclonal antibody became bound to the IGF-1 that was bound to the microwell coating during incubation. Microwells were then aspirated and washed an additional time and a substrate solution containing stabilized hydrogen peroxide and chromogen was added. The peroxide in the substrate solution interacted with the horseradish peroxidase resulting in colour development that is proportional to the concentration of IGF-1 present in the sample. Following an incubation 50 µl of sulphuric acid stop solution was added and absorbance read at 450 nm using microplate scanning spectrophotometer (Bio-Tek Instruments Inc, VT, USA). The CV for reproducibility was ≤ 5 % and the assay had a minimum detectable limit of 0.0034 nM.

4.7.5 25-hydroxy vitamin D

Plasma 25(OH)D was measured using an equilibrium RIA (DiaSorin, MN, USA). Briefly, 25(OH)D was extracted from plasma samples, controls and standards using acetonitrile. In disposable glass tubes standards, controls and samples were combines with ^{125}I 25-OH-D and goat 25-OH-D antiserum. Competitive binding of sample 25-OH-D and ^{125}I 25-OH-D with the goat antiserum was allowed to proceed followed by the addition of donkey anti-goat precipitating complex. Tubes were incubated and a

phosphate-gelatin buffer added before centrifugation. Supernatant was decanted ensuring all the liquid was removed. Each tube was counted in a gamma scintillation counter (Wallac 1470 Wizard Automatic Gamma Counter, Turku, Finland) for one minute. Coefficient of variation of ≤ 20 % or a difference of ≤ 500 cpm between duplicates was considered acceptable precision. This assay had a minimum detectable limit of 0.005 nM.

4.7.6 Cortisol

Urinary cortisol was measured using a competitive binding RIA (DiaSorin, MN, USA). Most urine samples were assayed undiluted however some samples required dilutions up to 10-fold with deionized water. Cortisol serum blank, standard or samples were pipetted into GammaCoat tubes in duplicate. Trace-buffered reagent was then added to each tube and vortexed to mix and the competitive binding reaction was then allowed to proceed. Tubes were then decanted and care taken to ensure any adhering liquid was removed. Tubes were then counted for 60 seconds using a gamma scintillation counter (Cobra II Auto-Gamma, Canberra Packard, Canada). Unknown cortisol concentrations were interpolated from the standard curve created from cortisol serum standards which were assayed simultaneously. The calculated sensitivity of the assay was 0.21 μg/dl. The average CV for reproducibility was 2.1 % with a maximum of 8.2 %.

4.7.7 Calcium and Phosphorous

Urine (0.25 ml) was combined with 0.5 ml concentrated HNO_3 (Fisher Scientific, trace metal grade, ON, Canada) and allowed to dissolved overnight in glass test tubes. Once dissolved, deionized water was added to the test tubes to reach a concentration of 5 % HNO_3. Dissolved and diluted urine was then transferred to scintillation vial and analyzed by emission spectrometry (Varian Liberty 200 ICP, Varian Canada, ON, Canada). The minimum detectable limit of calcium was 2.5 $x10^{-4}$ mM and for phosphorous 6.5 x 10^{-4} mM.

4.8 Infant Dual-Energy X-ray Absorptiometry (DXA)

BMC of infant whole body, femur and spine were determined by DXA using a Hologic QDR 4500 Acclaim Series Elite (MA, USA). Body composition, including fat and lean mass, were also determined for all infants at term age. Infants were clothed in gowns without snaps, zippers or buttons and were swaddled in a thin cotton blanket to minimize movement. All infants were scanned with clean dry diapers on. When asleep infants were placed in the centre of the DXA table as most of the infant data is acquired during the central scan pass. Whenever possible femur and spine scans were performed in array mode however if staff and parents were unable to get the infant to sleep scan were performed in turbo mode while infants were gently restrained. Measurement of spine bone area (cm^3) was derived from the projected bone area (cm^2) using the following formula: bone area = $(\text{projection area})^{1.5}$ (Carter et al, 1992). Whole body scans were analysed using infant whole body software (version 11.2:3). Scans were analyzed by a single investigator (H.K.).

4.9 Maternal and Paternal Data

Maternal and paternal data was collected to assist in interpreting infant outcome measures and to account for genetics. Weight was measured to the nearest 0.1 kg using an upright digital scale (Health-O-Meter, IL, USA) and height determined to the nearest millimetre using a wall-mounted electronic stadiometer (Seca 242, Hamburg, Germany) at the time of DXA. Z-scores for height and weight and BMI (kg/m^2) were produced using the Center for Disease Control growth charts (Center for Disease Control, 2000).

BMD of the hip, spine and whole body were determined using DXA. For the hip DXA scan parents were positioned on their backs with a foot positioner (Hologic, MA, USA) under their legs. The left leg was then rotated 25° inward from the hip and the median edge of the foot was placed against the foot positioner and secured with Velcro straps. The femur was then aligned parallel with the table edge to allow sufficient space for the neck box during analysis. In some cases the lesser trochanter of the hip was visible despite proper rotation of the leg, in no case was this due to osteoarthritis of the subjects. Both total hip, including lesser trochanter, and lumbar spine (L1-L4) scans were performed in fast array mode as all parents were healthy and to standardize the method of measurement. Parents were positioned on their back with a knee positioner (Hologic, MA, USA) under their lower legs to reduce the lordotic curve of the spine. For the whole body DXA scan parents were asked to lie on their backs with their arms at their sides with palms facing down. Their feet were pointing up and rotated 25° inward as in the hip scan. Care was taken to ensure that the whole body was within the scan limit boarder line. In some cases when parents were larger than the width of the exam table parts of the arm were excluded from the scan. The whole body scans also provided body

composition including lean and fat mass. Paternal whole body T-scores were created from the data generated in the MINOS study (Szulc et al, 2004). Maternal DXA measurements were determined after at least 6 months of breast feeding cessation. Parents were asked about their education to help account for socioeconomic status. Age and ethnicity were self-reported.

Other maternal demographics including parity, weight gain during pregnancy, vitamin and/or mineral supplementation, smoking and medications used during pregnancy were obtained from medical records. Fasting morning (between 0800 h and 1000 h) maternal blood was obtained in heparinized vacutainers prior to hospital discharge for determination of OC, 25(OH)D status and NTx as described previously.

Mothers were asked to fill out 3-day food records around the time of delivery, 6 months and 12 months post-partum to help establish dietary habits that might affect bone health. Mothers were instructed to record all food and beverages consumed on three non-consecutive day (two week days and one weekend day) including the amount consumed and brand names where possible. When information was missing or unclear mothers were ask for clarification either at the DXA visit or with a follow-up phone call. Twenty-four-hour food recalls were also conducted in co-ordination with DXA visits. Portion sizes were determined using standard household measurements and food models. Both 3-day food record and 24-hour recalls were analyzed using Nutrient Analysis Program (E. Warwick, PEI, Canada) that included the 1996 Canadian Nutrient File. All food records were analyzed by a single investigator (H.K.). Fathers were also requested to complete 3-day food records and 24h hour recalls. This data however was excluded from analysis due to the low return rate of 3-day food records (8 %) and the difficulty the

fathers experienced in identifying foods and portion sizes that they ate which was felt to contribute to the large variation seen in analysis.

4.10 Statistical Analysis

Statistical analysis was done using SAS (SAS Institute Inc, Cary, NC) and GraphPad Prism 3.0 (GraphPad Software Inc., San Diego, CA). All data presented has been trimmed to 2.5 standard deviations for statistics analysis. Repeated measures ANOVA was performed to determine differences between biochemical measurements over time in preterm infants and Tukey's post-hoc test was used with Bonferroni correction factor to determine differences between means. Student's t-test was performed to determine differences between term and preterm biochemical measures where only one preterm measurement was made. Correlation analysis was performed to detect relationships between BMC and infant anthropometrics, infant biochemistry, infant diet, maternal anthropometrics and bone mass, maternal diet, maternal biochemistry, and paternal anthropometrics and bone mass. Regression analysis was then used to determine predictors of infant bone mass. Variables were entered into regression model in groups to reduce the number of variables being entered into the model at one time. Term and preterm data was analyzed separately. Infant anthropometrics included weight, length and head circumference at time of DXA scan. Infant weight and length were entered into all models in order to allow for comparison with other published literature where anthropometrics have been used to adjust for infant size. Term infant nutrition was not assessed as it was not believed that nutrition would contribute an appreciable amount to infant BMC between birth and the time of DXA. Very preterm infant nutrition

was evaluated at the time of hospital discharge or DXA visit depending on the amount of time which lapsed between the two time points. Very preterm infant nutrition variable entered into the regression model were the same as those for mothers. Average parental data included infant anthropometry, maternal and infant dietary factors, and average parental height, weight, average parental BMD T-score and average whole body % fat. Maternal and Paternal data were then entered into the model individually rather than as an average to ascertain if one parent was driving the relationship. Maternal data included infant weight and length, maternal and infant dietary factors and maternal anthropometrics, average maternal BMD T-score and whole body % fat. Paternal factors included infant and paternal anthropometrics, infant and maternal dietary factors and paternal average BMD T-score and percent whole body fat.

Due to small sample size factors found to be related by more than $r = 0.8$ from correlation analysis were considered collinear and therefore were not entered into the regression model simultaneously (Dohoo et al, 1996). Due to the co-linearity of maternal and infant dietary calories, carbohydrate and fat, dietary carbohydrates and dietary fat were not included as dietary factors in the regression models. Dietary phosphorous was co-linear with calcium and moderately co-linear with protein so the decision was made to remove phosphorous from the regression analysis as well. Infant and parental weight and length/height were allowed to be entered into the regression model together despite their high degree of co-linearity as previous literature has used both these factors to adjust for body size and as predictors of infant bone mass. Due to the co-linearity of the various parental sites of bone mass measurement an average BMD T-score was used as a composite value to represent the overall bone mass of each parent

individually and together. Parental whole body percent fat was used instead of whole body lean or fat mass to avoid co-linearity with whole body weight. Significance was set at ≤ 0.05.

5.0 Results

5.1 Subjects

A total of 29 term and 23 very preterm infants and parents were recruited to the study. Six infants were excluded due to incomplete data. One infant was excluded due to a congenital disease not known at birth. A mother was excluded due to the diagnosis of celiac disease which was thought to affect her nutrition during pregnancy and two fathers were excluded due to skeletal deformities (fused spine and amputated leg). The resulting population of 21 very preterm and 21 term infants underwent assessment of growth and BMC. Descriptive characteristics of infants, mothers and fathers are described in Tables 5.1, 5.4 and 5.5. Gestational ages of term born infants ranged between 36.9 and 40.9 weeks and very preterm infants ranged between 25.1 and 32.0 weeks. Nine of the 21 very preterm infants were of multiple births (three sets of twins and one set of triplets). Ten (48 %) term infants and four (19 %) very preterm infants were female. Sixty-seven percent of term infants and 71 % of very preterm infants were born of Caucasian parents. For the term infant born to non-Caucasian parents one was born from African American parents, three were born of Asian parents, and one from First Nations parents, one infant had a Caucasian mother and an Asian father. For the very preterm infants not born from Caucasian parents, one was born of African American parents, one infant had a Portuguese mother and an African American father, two infants had a First Nations

mother and a Caucasian father and two infants had a Caucasian mother and an Asian father. All preterm infants were an appropriate size for GA as defined by > 3rd and < 97th percentile on infant growth charts (Usher& McLean, 1969, Arbuckle et al, 1993). All term infants had Z-score for weight, length and head circumference within two standard deviation of the normal mean as defined by the Center of Disease Control (2000). Seventy-six percent of term births were standard vaginal deliveries whereas only 29 % of very preterm infants were delivered vaginally. The average age of mothers at time of delivery was 29.2 years (range 19.1-39.7) for term mothers and 29.5 years (range 18.3-38.4) for very preterm mothers. Seventy-one percent of term mothers and 62 % of very preterm mothers were Caucasian. All but two term mothers (90.5 %) had grade 12 education or higher with 12 of mothers having taken some post-secondary education and five mothers having university degrees. Five very preterm mothers had not completed grade 12 high school, seven mothers had taken some form of post-secondary education and only two had completed university degrees. Fathers of term born infants on average were 32.3 years (range 23.1-46.2) of age at time of DXA measurement and fathers of very preterm infants 34.0 years (range 25.3-50.0). Seventy-one percent of fathers of term born infants were Caucasian while 62 % of fathers of very preterm infants were Caucasian. Three fathers of term infants had not completed grade 12 high school. The level of education attained by one father was unknown. Thirteen fathers of term infants had taken some form of post secondary education of which six had completed a university degree. Two fathers of very preterm infants had not completed grade 12 high school. The level of education of one father of a preterm infant was unknown. Eight

fathers of very preterm infants had taken some post secondary education and three had attained a university degree.

5.2 Preterm Infant In-Hospital Growth

Gestational age of very preterm infant at birth ranged from 25.1 to 32.0 weeks (Table 5.1) with average of 28.5 ± 1.8 weeks (± SD). Mean birth weight of very preterm infants was 1145 ± 251 g. Very preterm infants spent an average of 73 ± 30 days in hospital with a mean in-hospital weight gain of 1417 ± 680 g. Very preterm infants lost an average of 126 ± 59 g after birth with maximal weight loss occurring on average 5 ± 2 days after birth. Average time to regain birth weight was 11 ± 4 days with an overall in-hospital rate of growth of 11.1 ± 1.7 g/kg/d and 3.3 ± 2.0 cm/m/d. Eight very preterm infants developed bronchopulmonary dysplasia of which two required treatment with dexamethasone.

5.3 Nutrient Intake of Infants

Nutrient intake was assessed in very preterm infants during hospitalization and prior to term age when BMC was measured (Table 5.2). Very preterm infants spent an average of 15 ± 10 days on TPN and enteral nutrition was introduced at 6 ± 8 days PNA. All very preterm infants received breast milk for some period of time with average corrected age at cessation of breastfeeding being 2.9 months. All but two very preterm infants received human milk fortifier with the average introduction time of 19 ± 6 days after birth. Full enteral feeds (150 ml/kg/d) were reached at 25 ± 16 days with a range of 7 to 87 days. During the "transition" period (birth to 7 days) where nutrition support is

being established only ten (48 %) very preterm infants met the energy requirements of 70-80 kcal/kg set by the Canadian Pediatric Society Nutrition Committee (1995). The average caloric intake of the very preterm infants during the first seven days of life was 68 ± 13 kcals/kg. Calcium, phosphorous, magnesium intakes did not reach the minimum recommended nutrient intake (RNI) for the "transition" period with only one infant meeting the recommendations during this time. The Canadian Paediatric Society defines the "stable-growing" period as day 8 of life until hospital discharge. This stable growing period was divided into three other time periods (days 8-21 PNA, days 22-35 PNA and days 36 PNA until discharge) based on study blood and urine sampling. Average energy intake remained below recommendation (105-135 kcal/kg) during days 8-21 of the "stable-growing" but reached minimum recommendations for days 22-35 and continued through to discharge. The median PNA when very preterm infants reached 105 kcal/kg was 6 days (range 4 to 34 d) with both parental and enteral nutrition. When only enteral nutrition is accounted for the median day on which very preterm infants reached 105 kcal/kg increased to 22 days. The minimum for all macronutrients were met during the "stable-growth" period despite the deficit in total energy. At no time during hospitalization did the average intake of calcium of phosphorous meet the minimum RNI. Magnesium intake was sufficient throughout the "stable-growing" period. Average vitamin D intake increased during the "stable-growing" period but never met the premature infant recommended nutrient intake (P-RNI). Vitamin K intake was not assessed as the nutrient analysis program lacked this nutrient.

5.4 Dual-Energy X-ray Absorptiometry Measurements of Infants

Term and very preterm infant bone mass and body composition measurements are summarized in Table 5.1. Term infants had significantly higher femur BMC, spine BMC and whole body BMC (Figure 5.1) at birth than very preterm infants did at term age even when adjusted for infant body weight (BMC/kg) and length (BMC/cm). Term and very preterm infant whole body fat did not differ at term age. Term infants had significantly more lean mass than very preterm infants did at term age ($p = 0.018$), however this relationship did not remain significant when adjusted for body weight.

5.5 Measurement of Infant Bone Markers

Urinary NTx was measured as a marker of bone resorption in very preterm infants at birth, day 7, 21, 35 and at discharge (usually near term age) and in term infants at birth. Very preterm infant NTx was significantly lower at both birth and day 7 than at days 21, day 35 and at discharge (Figure 5.2). Very preterm infant NTx concentrations at days 21 and 35 and at hospital discharge were significantly higher than term infant NTx at birth ($p \leq 0.002$).

Very preterm infant urinary calcium/creatinine was measured while in hospital at birth, day 7, day 21, day 35 and at discharge. Average urinary calcium/creatinine increased from birth until day 35 after which it stabilized. Very preterm infant calcium/creatinine at birth was significantly lower than all other time points (Figure 5.3). Term and very preterm infant urinary calcium/creatinine at birth were not significantly different. Very preterm infant urinary calcium/creatinine was however significantly

higher than term infant concentrations at days 7, 21, 35 and at hospital discharge ($p \leq 0.002$).

Urinary phosphorous/creatinine was significantly higher at birth in very preterm infants than any other time point while in hospital (Figure 5.4). Term infant urinary phosphorous/creatinine at birth and very preterm infant concentrations from birth to discharge were not significantly different.

Very preterm infant urinary creatinine at birth was not significantly lower than at day 7, day 35 or at discharge but is however significantly lower than urinary creatinine on day 21 (Figure 5.5). Term infant urinary creatinine at birth was significantly higher than very preterm infant urinary creatinine at all time points ($p \leq 0.0004$).

Very preterm infant plasma 25(OH)D was not significantly different between days 7 and 21 but did increase significantly by day 35 and remained different at hospital discharge (Figure 5.6). Term infant plasma 25(OH)D at birth and very preterm infant plasma 25(OH)D at day 7 were not significantly different. Very preterm infant plasma 25(OH)D was however significantly higher at day 21, 35 and at hospital discharge than term infant concentrations at birth ($p \leq 0.001$).

Very preterm infant plasma OC increased in concentrations through hospital stay. Plasma OC concentrations at days 35 and discharge were significantly higher than at days 7 and 21 (Figure 5.7). Term infant plasma OC concentrations were significantly lower than very preterm concentrations at all time points ($p \leq 0.01$).

Very preterm infant plasma cortisol concentrations at discharge were significantly higher than term plasma cortisol at birth (Figure 5.8).

Very preterm plasma IGF-I was significantly lower at hospital discharge compared to term infant IGF-I at birth (Figure 5.9).

5.6 Nutrient Intake of Mothers

Results of correlation analysis show significant positive correlations ($p \leq 0.01$) between 3-day food records and 24-hour intake nutrient analysis therefore when 3-day food records were unavailable 24-hour recalls were used in their place for analysis. All term mothers has nutrient intake analysis completed at least one of the three time points. A total of seven nutrient analysis were not completed over the course of the three nutrient intake analysis time points. Sixteen 24-hour recalls were used in lieu of 3-day food records when none were provided by the mothers. As a result 89 % of nutrition information was completed for term mothers. The average energy intake of term mothers was 1945 ± 659 kcals with 18 % energy from protein, 32 % energy from fat and 50 % energy from carbohydrate. On average term mothers exceeded the RNI and dietary reference intake (DRI) recommendations for micronutrients except the calcium and magnesium DRI (Table 5.3). However 67 % of term mothers met the RNI for calcium and only four (19 %) met the DRI for calcium. Only one term mother did not meet the RNI or DRI for phosphorous. Three term mothers did not meet the RNI for magnesium however only three mothers (14 %) met the DRI for magnesium. Ninety percent of term mothers met or exceeded the RNI for vitamin D while only 48 % met the newer DRI recommendations.

All very preterm mothers has nutrient intake analysis completed at least one of the three time points. Five nutrient analysis were not completed over the course of the three

nutrient intake analysis time points. Eighteen 24-hour recalls were used in lieu of 3-day food records when none were completed. As a result 92 % of nutrition information was completed for very preterm mothers. The average energy intake of very preterm mothers was 1969 ± 492 kcal with 16 % energy from protein, 33 % energy from fat and 51 % energy from carbohydrate. On average, very preterm mothers exceeded the RNI and DRI recommendations for micronutrients except the calcium and magnesium DRI (Table 5.3). Four very preterm mothers did not meet the RNI of calcium, while nine mothers did not meet the DRI for calcium. Five (31 %) very preterm mothers did not meet the phosphorous RNI however all very preterm mothers met or exceeded the DRI for phosphorous. Two very preterm mothers did not meet the RNI for magnesium and nine did not meet the magnesium DRI. Only two very preterm mothers did not meet the old RNI for vitamin D while four mothers did not meet the DRI for vitamin D.

5.7 Dual-Energy X-ray Absorptiometry Measurements in Mothers

Term and very preterm mother DXA measurements are presented in Table 5.4. Term mothers hip BMD ranged from 0.768 to 1.212 g/cm^2 with average T-score of 0.0 and Z-score of 0.1. Term mother spine BMD ranged from 0.839 to 1.283 g/cm^2 and had an average T-score of -0.5 and Z-score of -0.4. The whole body BMD of term mothers ranged from 1.037 to 1.314 g/cm^2 and had an average T-score of 0.2 and Z-score of 0.4. Very preterm mothers hip BMD had an average T-score and z-score of 0.1 with range of 0.645 to 1.269 g/cm^2. Very preterm mother spine BMD ranged from 0.783 to 1.276 g/cm^2 and had an average T-score of -0.3 and Z-score of -0.2. The whole body BMD of very preterm mothers ranged from 1.021 to 1.271 g/cm^2 with average T-score of 0.1 and

Z-score of 0.3. Mothers of term born infants and preterm born infants had an average T-score of the hip, spine and whole body combined of -0.1 and 0.0 respectively.

5.8 Measurement of Maternal Bone Markers

Term and very preterm maternal biochemistry is summarized in Table 5.4. Very preterm maternal plasma OC was significantly lower than term mother concentrations ($p \leq 0.05$). Term and very preterm maternal plasma NTx and plasma 25(OH)D did not differ significantly.

5.9 Dual-Energy X-ray Absorptiometry Measurements in Fathers

The bone mass and anthropometric measures of fathers of term and very preterm infants are summarized in Table 5.5. Fathers of term infants had hip BMD with range of 0.723 to 1.243 g/cm^2 and average T-score of -0.2 and average Z-score of -0.1. Fathers of term infants had spine BMD ranging from 0.765 to 1.189 g/cm^2 with average T-score of -1.0 and average Z-score of -1.0. The whole body BMD of fathers of term infants ranged from 0.990 to 1.390 g/cm^2, with average T-score of -0.3. Fathers of very preterm infants had hip BMD ranging from 0.780 to 1.276 g/cm^2 and average T-scores of -0.3 and average Z-scores of -0.2. Father of very preterm infants had a range of spine BMD from 0.815 to 1.320 g/cm^2 with average T-scores and Z-scores of -1.0. Whole body BMD of fathers of very preterm infants ranged from 1.022 to 1.498 g/cm^2 with average T-score of -0.3. Average T-score of the hip, spine and whole body of both fathers of term and preterm infants were -0.6 and -0.4 respectively.

5.10 Correlation Analysis

Correlation analysis was performed to look for relationships between infant biochemistry and bone mass (Table 5.6). Infant urinary creatinine, phosphorous/creatinine, urinary cortisol/creatinine, plasma OC and plasma IGF-1 did not yield any significant relationships with bone mass. Term infant urinary NTx/creatinine was related to infant whole body BMC. Urinary calcium/creatinine was correlated with very preterm infant spine BMC. Very preterm infant plasma 25(OH)D was most significantly related to infant lumbar spine BMC.

Very preterm infant diet was associated with bone mass at term age (Table 5.7). Very preterm infant femur BMC was positively correlated with dietary calcium and phosphorous, while infant spine BMC was negatively correlated with dietary carbohydrate and whole body BMC was positively correlated with dietary magnesium. Dietary magnesium and infant very preterm infant femur BMC were approaching a significant relationship ($p = 0.07$). Very preterm infant dietary protein, fat, calories and vitamin D did not prove to be significantly related to very preterm infant BMC at term age.

Maternal height was significantly correlated with all term but no very preterm infant bone mass measurements (Table 5.8). No significant relationships were found between maternal weight, hip, spine or average BMD T-score bone mass measurements and term or preterm infant bone mass. Whole body BMD of mothers of very preterm infants was correlated with infant whole body BMC ($r = -0.56$, $p = 0.01$). Maternal whole body fat, lean and percent whole body fat were not significantly correlated with infant bone mass.

Maternal plasma biochemistry only yielded a significant relationship with very preterm infant bone mass (Table 5.9). Very preterm maternal NTx was negatively related to infant spine BMC at term age ($r = -0.56$, $p = 0.008$). Maternal OC was positively correlated with very preterm infant femur BMC ($r = -0.45$, $p = 0.05$). Osteocalcin of motheres of very preterm infants approached significance with infant whole body BMC ($p = 0.06$). Maternal plasma 25(OH)D was not significantly associated with either term or very preterm infant bone mass.

Maternal diet did not yielded significant relationships with either term or very preterm infant bone mass measurements (Table 5.10).

Paternal height, weight and hip BMD were significantly positively associated with term infant femur BMC (Table 5.11). Very preterm infant femur BMC however was significantly negatively correlated with paternal height, spine BMD and whole body fat. Height of fathers of term infants was also positively associated with term infant spine BMC. Very preterm infant whole body BMC was also related to paternal factors including whole body fat and percent whole body fat.

5.11 Regression Analysis

In the models for term infant whole body BMC infant weight was selected into each model (Tables 5.12). Average parental BMD T-score was selected into the model, however when maternal factors were entered into the model only infant weight remained significant. Paternal average BMD T-score was a significant predictor of term infant whole body BMC.

In models for very preterm infant whole body BMC infant weight was selected into all models (Table 5.13). Average parental height and weight were significant predictors of very preterm infant whole body BMC. Infant dietary magnesium was also a significant factor in the average parental model. When maternal data was entered into the model as opposed to average parental data, only infant weight remained significant. Paternal whole body percent fat was a significant predictor of very preterm infant whole body BMC.

Term infant femur BMC had few variables enter into the models but included infant weight, average parental BMD T-score and maternal height (Table 5.14). No other variables were seen to have a significant relationship.

Both infant length and weight were selected individually into the various models of very preterm infant femur BMC (Table 5.15). Infant dietary calcium was selected into the model as significant with both the average parental data and the maternal data but not with paternal data.

Infant weight was selected into the average parental data model and the paternal data model as a significant predictor of term infant lumbar spine BMC (Table 5.16). Average parental whole body percent fat was a negative but significant predictor of term infant spine BMC. Maternal height as with term infant femur was a positive significant predictor of term infant spine BMC. When spine bone area to the exponent 1.5 was entered into the models to account for bone size, all other variables were removed from the model and only spine bone area remained significant.

Very preterm infant lumbar spine BMC was the most complicated of all models. Very preterm infant length was the most significant anthropometric measure related to

spine BMC in both the average parental and maternal data models(Table 5.17). Infant dietary factors including protein, calcium, and vitamin D were significant predictors of very preterm infant spine BMC in the average parental data model. Infant dietary calories replaced infant dietary protein as being significant in the maternal data model, with calcium and vitamin D remaining significant. Maternal dietary protein and calories were significant predictors of spine BMC in very preterm infants in the average parental data model. Maternal protein as well as magnesium, and vitamin D were also significant in the maternal data model. Despite the fact that no parental anthropometrics or bone mass indices were significant in the average parental data model, both maternal whole body percent fat and average maternal BMD T-score were significant predictors of very preterm infant spine BMC. There were no significant predictive factors in the paternal data model. When bone area was entered into the models there were significant changes in the variables which predicted very preterm infant spine BMC. In the average parental data model infant length was no longer significantly predictive of spine BMC however infant weight was then selected into the model along with bone area to the exponent 1.5. The only other difference in the average parental data model was that infant dietary magnesium did not remain a significant predictor of very preterm infant spine BMC. When bone area was entered into the maternal data model infant dietary protein became significant along with calcium, magnesium and vitamin D but calories did not remain a significant predictor of spine BMC. Maternal dietary factors remained the same when bone area was entered into and selected into the model. With spine bone area included in the model however maternal whole body percent fat was no long significant but maternal weight became a significant predictor of very preterm infant spine BMC. Lastly when

spine bone area was entered into the paternal data model infant body weight was selected into the model in combination with bone area. Also the same dietary factors (infant protein, calcium, magnesium and vitamin D, maternal protein and calories) entered into the model as did in the average paternal data model without spine bone area.

Table 5.1: Characteristics of term and very preterm infants at birth and term dual-energy x-ray absorptiometry (DXA) visit.

	Term (n = 21)	Very Preterm (n = 21)	P-value
At Birth			
Gestational age (wks)	39.10 ± 1.22	28.47 ± 1.78	nt
Gender (males/females)	11/10	17/4	nt
Ethnicity (Caucasian/Non-Caucasian)	14/7	15/6	nt
Weight (g)	3511 ± 436	1145 ± 251	nt
Recumbent length (cm)	52.3 ± 2.7	37.1 ± 3.4	nt
Head circumference (cm)	35.2 ± 1.1	26.9 ± 1.9	nt
APGAR at 1 minute	9 (7-9)	5 (0-9)	nt
APGAR at 5 minutes	9 (8-10)	8 (1-9)	nt
At Term Age DXA			
Gestational age (wks)	40.32 ± 1.36	39.79 ± 2.14	0.4375
Weight (g)	3583 ± 359	2989 ± 820	0.0038
Recumbent length (cm)	51.7 ± 2.0	47.4 ± 2.8	<.0001
Head circumference (cm)	35.9 ± 1.1	35.2 ± 2.7	0.3521
Femur BMC (g)	2.85 ± 0.61*	1.36 ± 0.43*	<.0001
Spine BMC (g)	2.27 ± 0.38	1.09 ± 0.26	<.0001
Spine BMD (g/cm^2)	0.263 ± 0.038	0.148 ± 0.046	<.0001
Whole Body BMC (g)	72.43 ± 11.60*	47.78 ± 13.91*	<.0001
Whole Body Fat (g)	391.5 ± 178.9*	457.5 ± 313.0*	0.4185
Whole Body Lean (g)	3265.8 ± 389.3*	2665.4 ± 639.3*	0.0009

Data shown as mean ± SD except gender and ethnicity which are shown as number of subjects and APGAR data which is shown as median (range).
nt, not tested
*n = 20

Table 5.2: Nutrient intake of very preterm infants during hospitalization compared to premature infant recommended nutrient intakes (P-RNI)[a].

Nutrient	Transition Period		Stable Growing Period			
	Birth- Day 7	P-RNI [b]	Day 8-21	Day 22-35	Day 36-Discharge	P-RNI [b]
Energy (kcals/kg)	68.0 ± 13.5 (10)	70.0-80.0	99.4 ± 13.4 (7)	109.2 ± 15.4 (10)	114.2 ± 13.5 (16)	105.0-135.0
Protein (g/kg)	1.9 ± 0.5 (21)	1.0-3.0	2.6 ± 0.4 (1)	2.8 ± 0.5 (3)	2.9 ± 0.4 (2)	3.5-4.0
Fat (g/kg)	2.3 ± 1.3 (21)	0.5-3.6	7.1 ± 2.6 (17)	9.9 ± 2.5 (20)	9.7 ± 2.6 (21)	4.5-6.8
Carbohydrate (g/kg)	11.7 ± 2.0 (21)	5-20	10.9 ± 2.6 (19)	7.9 ± 3.7 (9)	8.8 ± 3.2 (13)	7.5-15.5
Calcium (mg/kg)	29.6 ± 13.6 (1)	60.1-80.2	61.3 ± 39.8 (0)	125.7 ± 51.2 (5)	135.3 ± 35.5 (7)	160.3-240.5
Phosphorous (mg/kg)	18.9 ± 8.3 (1)	31.0-46.5	41.0 ± 18.2 (1)	67.9 ± 23.2 (6)	73.1 ± 17.1 (9)	77.4-117.7
Magnesium (mg/kg)	4.0 ± 1.3 (3)	4.9-6.1	5.2 ± 1.3 (11)	6.4 ± 2.1 (16)	7.4 ± 2.1 (18)	4.9-9.7
Vitamin D (IU/d)	138.9 ± 55.9 (21)	40.0-120.0	133.5 ± 56.3 (0)	296.7 ± 141.7 (12)	260.4 ± 114.1 (15)	400.0

[a] Data shown as mean ± SD (number of infants meeting P-RNI), n = 21.
[b] Canadian Paediatric Society, Nutrition Committee. Nutrient needs and feeding of premature infants. Can Med Assoc J 1995;152:1765-1785.

Table 5.3: Average nutrient intake of mothers of term and very preterm born infants as determined by 3-day food record and 24-hour food recalls compared to Recommended Nutrient Intakes (RNI)[a] and Dietary Reference Intakes (DRI)[b]

	Term Mothers (n = 21)	Very Preterm Mothers (n = 16)	RNI	DRI
Energy (kcal/d)	1945.4 ± 659.2	1969.2 ± 492.5		
Protein (g/d)	85.3 ± 31.6	80.3 ± 22.7	15 % energy	10-35 % energy
Fat (g/d)	69.8 ± 32.9	72.1 ± 20.1	≤30 % energy	20-35 % energy
Carbohydrate (g/d)	245.2 ± 89.8	254.3 ± 71.2	>55 % energy	45-65 % energy
Calcium (mg/d)	891.7 ± 457.6	979.1 ± 350.5	700	1000
Phosphorus (mg/d)	1160.3 ± 425.2	1235.0 ± 480.4	850	700
Magnesium (mg/d)	254.6 ± 101.2	292.6 ± 79.3	200	310-320
Vitamin D (IU/d)	217.0 ± 149.2	285.3 ± 148.7	100	200

Data shown are mean ± SD
RNI values shown are for women aged 16-49 years.
DRI values shown are the adequate intakes or recommended dietary allowance for women aged 19-50 years.
[a] Minister of National Health & Welfare, Health Canada. Nutrition Recommendations. The report of the scientific review committee. Canadian Government Publishing Center, Ottawa, Ontario, Canada. 1990.
[b] American Dietetic Association /Dietitians of Canada. Manual of Clinical Dietetics, 6th Edition. 2000.

Table 5.4: Descriptive characteristics, anthropometry and bone mass of mothers of term and very preterm born infants.

	Term (n = 21)	Preterm (n = 16)	P-value
Age (yrs)	28 ± 7	29 ± 6	0.7948
Gravida	2	2	0.2722
Parity	1	1	0.5625
Pregnancy weight gain (kg)	15.4 ± 8.6	8.9 ± 3.9	0.0119
Smoking (yes/no)	7/15 (18 %)	3/13 (19 %)	nt
Vitamin/mineral supplement use (yes/no)	18/4 (82 %)	14/2 (88 %)	nt
Height (cm)	164.3 ± 8.4	164.9 ± 7.8	0.6794
Weight (kg)	78.8 ± 20.1	75.1 ± 16.0	0.5880
Body mass index (kg/m^2)	29.2 ± 7.3	27.6 ± 5.7	0.4562
Total left hip BMD (g/cm^2)	0.941 ± 0.108	0.954 ± 0.148	0.8777
Total spine (L1-4) BMD (g/cm^2)	1.000 ± 0.110	1.010 ± 0.120	0.7701
Whole body BMD (g/cm^2)	1.114 ± 0.080	1.123 ± 0.068	0.8587
Whole body lean (kg)	46.9 ± 7.7	45.2 ± 6.9	0.5381
Whole body fat (kg)	29.6 ± 0.1	27.4 ± 9.8	0.5990
Whole body % fat	36.2 ± 7.2	35.4 ± 7.6	0.7686
Plasma 25(OH)D (nM)	52.8 ± 30.5	66.0 ± 26.9	0.2313
Plasma osteocalcin (nM)	0.5 ± 0.2	0.2 ± 0.2	<.0001
Plasma N-telopeptide (nM BCE)	133.6 ± 42.6	126.0 ± 53.9	0.6602

Data shown as mean ± SD except parity and gravida which are shown as medians and smoking and prenatal vitamin/mineral supplement use, which are shown as number of subjects (percentage smokers).
BMD, bone mineral density; 25(OH)D, 25-hydroxy vitamin D; BCE, bone collagen equivalents

Table 5.5: Descriptive characteristics, anthropometry and bone mass of fathers of term and very preterm born infants.

	Term (n = 21)	Preterm (n = 16)	P-value
Age (yrs)	32 ± 7	34 ± 7	0.4760
Height (cm)	177.2 ± 0.1	175.8 ± 0.1	0.5978
Weight (kg)	80.8 ± 14.0	85.9 ± 16.6	0.2116
Body mass index (kg/m^2)	25.6 ± 3.6	27.7 ± 4.8	0.0730
Total left hip BMD (g/cm^2)	0.984 ± 0.131	1.019 ± 0.134	0.4241
Total spine (L1-4) BMD (g/cm^2)	0.976 ± 0.137	1.010 ± 0.145	0.4791
Whole body BMD (g/cm^2)	1.197 ± 0.114	1.214 ± 0.132	0.7813
Whole body lean (kg)	61.0 ± 8.9	62.7 ± 9.6	0.1583
Whole body fat (kg)	17.7 ± 7.0	20.0 ± 7.8	0.5672
Whole body % fat	21.3 ± 6.3	22.7 ± 5.4	0.2422

Data shown as mean ± SD
BMD, bone mineral density

Table 5.6: Relationships between infant bone mass and biochemical indices at term age.

	Femur BMC (g)	Spine BMC (g)	Whole Body BMC (g)
	Term Infants		
Urinary Creatinine (mM)	0.30048 0.2257 18	0.27319 0.2578 19	0.26484 0.2882 18
Urinary NTx/Creatinine (nM/mM)	0.16740 0.5067 18	0.35626 0.1344 19	**0.48130** **0.0432** **18**
Urinary Ca/Creatinine (mM/mM)	-0.19974 0.4268 18	0.06983 0.7764 19	-0.41059 0.0905 18
Urinary P/Creatinine (mM/mM)	0.14395 0.5688 18	0.31657 0.1867 19	0.17625 0.4842 18
Urinary Cortisol/Creatinine (nM/mM)	0.10538 0.6773 18	0.09510 0.6986 19	0.21995 0.3805 18
Plasma 25(OH)D (nM)	0.07468 0.7544 20	0.00799 0.9726 21	0.30851 0.1857 20
Plasma Osteocalcin (nM)	0.09828 0.6802 20	0.13338 0.5644 21	-0.21267 0.3680 20
Plasma IGF-I (nM)	-0.06763 0.7770 20	-0.15229 0.5099 21	0.03481 0.8842 20

Table 5.6: Relationships between infant bone mass and biochemical indices at term age continued.

	Femur BMC (g)	Spine BMC (g)	Whole Body BMC (g)
	Very Preterm Infants		
Urinary Creatinine (mM)	-0.06805 0.7756 20	-0.35745 0.1117 21	-0.04301 0.8571 20
Urinary NTx/Creatinine (nM/mM)	0.00077 0.9974 20	-0.04856 0.8344 21	-0.13940 0.5578 20
Urinary Ca/Creatinine (mM/mM)	-0.06800 0.7758 20	**-0.46285** **0.0346** **21**	-0.10559 0.6577 20
Urinary P/Creatinine (mM/mM)	0.08361 0.7260 20	0.21514 0.3490 21	0.02321 0.9226 20
Urinary Cortisol/Creatinine (nM/mM)	0.10371 0.6635 20	0.34406 0.1267 21	-0.14887 0.5311 20
Plasma 25(OH)D (nM)	0.38530 0.0934 20	**-0.60934** **0.0034** **21**	0.43721 0.0539 20
Plasma Osteocalcin (nM)	-0.15278 0.5202 20	0.09094 0.6950 21	-0.15546 0.5128 20
Plasma IGF-I (nM)	0.16758 0.4801 20	-0.40400 0.0693 21	0.06301 0.7918 20

Data are presented as r, p-value and sample size
Data in bold are significant $p \leq 0.05$
BMC, bone mineral content; NTx, N-telopeptide; 25(OH)D, 25-hydroxy vitamin D; IGF-I, insulin-like growth factor-I

Table 5.7: Relationships between very preterm infant bone mass and nutrition at term age.

	Femur BMC (g)	Spine BMC (g)	Whole Body BMC (g)
	Very Preterm Infants		
Dietary Protein (g/d)	0.46455 0.0391 20	-0.03671 0.8745 21	0.40265 0.0784 20
Dietary Fat (g/d)	-0.19047 0.4212 20	-0.31025 0.1711 21	-0.05719 0.8107 20
Dietary Carbohydrate (g/d)	0.15279 0.5202 20	**-0.43303** **0.0499** **21**	0.38609 0.0927 20
Dietary Calories (kcal/d)	-0.03211 0.8931 20	-0.39335 0.0777 21	0.16035 0.4995 20
Dietary Calcium (mg/d)	**0.48674** **0.0295** **20**	0.19017 0.4090 21	0.27453 0.2414 20
Dietary Phosphorous (mg/d)	**0.46629** **0.0382** **20**	0.16073 0.4864 21	0.24474 0.2984 20
Dietary Magnesium (mg/d)	0.41537 0.0686 20	-0.07461 0.7479 21	**0.44761** **0.0478** **20**
Dietary Vitamin D (IU/d)	0.16561 0.4853 20	0.07046 0.7615 21	-0.01991 0.9336 20

Data are presented as r, p-value and sample size
Data in bold are significant $p \leq 0.05$
BMC, bone mineral content

Table 5.8: Relationships between infant anthropometry and bone mineral content at term age and maternal anthropometry and bone mass.

	Femur BMC (g)	Spine BMC (g)	Whole Body BMC (g)
	Term Infants		
Maternal Height (m)	**0.66629** **0.0013** **20**	**0.68598** **0.0006** **21**	**0.59493** **0.0057** **20**
Maternal Weight (kg)	0.15642 0.5102 20	0.13940 0.5467 21	0.07855 0.7420 20
Maternal Hip BMD (g/cm^2)	0.30393 0.1927 20	0.01890 0.9352 21	0.03443 0.8854 20
Maternal Spine BMD (g/cm^2)	0.29088 0.2134 20	-0.00355 0.9878 21	-0.15547 0.5128 20
Maternal Whole Body BMD (g/cm^2)	0.29215 0.2113 20	-0.10012 0.6659 21	0.00342 0.9886 20
Maternal Average T-score	0.31694 0.1733 20	-0.02663 0.9088 21	-0.06619 0.7816 20
Maternal Whole Body Fat (g)	0.01172 0.9609 20	0.01140 0.9609 21	-0.01163 0.9612 20
Maternal Whole Body Lean (g)	0.42177 0.0640 20	0.34532 0.1252 21	0.23541 0.3177 20
Maternal Whole Body % Fat	-0.15535 0.5131 20	-0.14829 0.5212 21	-0.10572 0.6573 20

Table 5.8: Relationships between infant anthropometry and bone mineral content at term age and maternal anthropometry and bone mass continued.

	Femur BMC (g)	Spine BMC (g)	Whole Body BMC (g)
	Very Preterm Infants		
Maternal Height (m)	0.01576 0.9474 20	0.03788 0.8705 21	0.24360 0.3007 20
Maternal Weight (kg)	0.08739 0.7141 20	-0.11563 0.6177 21	0.17832 0.4520 20
Maternal Hip BMD (g/cm^2)	-0.12923 0.5871 20	-0.27029 0.2360 21	-0.01411 0.9529 20
Maternal Spine BMD (g/cm^2)	-0.06171 0.7960 20	-0.13744 0.5524 21	-0.07214 0.7625 20
Maternal Whole Body BMD(g/cm^2)	-0.40913 0.0733 20	0.12593 0.5865 21	**-0.55928** **0.0104** **20**
Maternal Average T-score	-0.20950 0.3754 20	-0.14072 0.5429 21	-0.26364 0.2614 20
Maternal Whole Body Fat (g)	0.11725 0.6225 20	-0.04997 0.8297 21	0.20248 0.3919 20
Maternal Whole Body Lean (g)	0.04365 0.8550 20	-0.20720 0.3675 21	0.14317 0.5471 20
Maternal Whole Body % Fat	0.11943 0.6160 20	0.04476 0.8472 21	0.21447 0.3639 20

Data are presented as r, p-value and sample size N
Data in bold are significant $p \leq 0.05$
BMC, bone mineral content; BMD, bone mineral density

Table 5.9: Relationships between infant anthropometry and bone mass and maternal biochemistry at term age.

	Femur BMC (g)	Spine BMC (g)	Whole Body BMC (g)
	Term Infants		
Maternal Plasma NTx (nM BCE)	-0.37561 0.1027 20	-0.20088 0.3826 21	-0.34730 0.1335 20
Maternal Plasma 25(OH)D (nM)	0.08781 0.7128 20	0.14569 0.5286 21	0.07722 0.7462 20
Maternal Plasma OC (nM)	0.02032 0.9322 20	-0.16999 0.4613 21	-0.32168 0.1667 20
	Very Preterm Infants		
Maternal Plasma NTx (nM BCE)	-0.08677 0.7160 20	**-0.56530** **0.0076** **21**	-0.13368 0.5742 20
Maternal Plasma 25(OH)D (nM)	0.34575 0.1354 20	0.05273 0.8204 21	0.29667 0.2040 20
Maternal Plasma OC (nM)	**0.44853** **0.0473** **20**	0.03519 0.8796 21	0.42415 0.0623 20

Data are presented as r, p-value and sample size N
Data in bold are significant $p \leq 0.05$
BMC, bone mineral content; NTx, N-telopeptide; BCE, bone collagen equivalents; 25(OH)D, 25-hydroxy vitamin D; OC, osteocalcin

Table 5.10: Relationships between infant bone mass and maternal diet.

	Femur BMC (g)	Spine BMC (g)	Whole Body BMC (g)
	Term Infants		
Maternal Dietary Protein (g/d)	-0.17675 0.4560 20	-0.15908 0.4910 21	-0.08463 0.7228 20
Maternal Dietary Fat (g/d)	0.16713 0.4813 20	-0.10452 0.6521 21	-0.08014 0.7370 20
Maternal Dietary Carbohydrate (g/d)	0.27819 0.2350 20	0.17784 0.4406 21	0.35871 0.1204 20
Maternal Dietary Calories (kcal/d)	0.21499 0.3627 20	0.01188 0.9593 21	0.15554 0.5126 20
Maternal Dietary Calcium (mg/d)	-0.06082 0.7990 20	-0.08249 0.7222 21	-0.10733 0.6524 20
Maternal Dietary Phosphorous (mg/d)	0.07650 0.7485 20	-0.09683 0.6763 21	0.05262 0.8256 20
Maternal Dietary Magnesium (mg/d)	0.06064 0.7995 20	0.14226 0.5385 21	-0.18350 0.4387 20
Maternal Dietary Vitamin D (IU/d)	-0.21193 0.3697 20	-0.17599 0.4454 21	-0.25067 0.2864 20

Table 5.10: Relationships between infant bone mass and maternal diet continued.

	Femur BMC (g)	Spine BMC (g)	Whole Body BMC (g)
	Very Preterm Infants		
Maternal Dietary Protein (g/d)	0.19339 0.4140 20	0.05516 0.8123 21	0.37783 0.1005 20
Maternal Dietary Fat (g/d)	-0.16251 0.4936 20	-0.21596 0.3471 21	-0.07566 0.7512 20
Maternal Dietary Carbohydrate (g/d)	-0.01961 0.9346 20	-0.28114 0.2170 21	0.25538 0.2772 20
Maternal Dietary Calories (kcal/d)	-0.02913 0.9030 20	-0.20658 0.3689 21	0.19396 0.4126 20
Maternal Dietary Calcium (mg/d)	0.00397 0.9867 20	-0.14869 0.5201 21	0.08648 0.7170 20
Maternal Dietary Phosphorous (mg/d)	0.12986 0.5853 20	-0.00720 0.9753 21	0.29439 0.2077 20
Maternal Dietary Magnesium (mg/d)	0.06977 0.7701 20	-0.14129 0.5413 21	0.37885 0.0995 20
Maternal Dietary Vitamin D (IU/d)	0.05067 0.8320 20	-0.13993 0.5452 21	0.00700 0.9766 20

Data are presented as r, p-value and sample size N
Data in bold are significant $p \leq 0.05$
BMC, bone mineral content

Table 5.11: Relationships between infant anthropometry and bone mineral content at term age and paternal anthropometry and bone mass.

	Femur BMC (g)	Spine BMC (g)	Whole Body BMC (g)
	Term Infants		
Paternal Height (m)	**0.49254** **0.0274** **20**	**0.48467** **0.0260** **21**	0.42477 0.0619 20
Paternal Weight (kg)	**0.48443** **0.0304** **20**	0.29002 0.2022 21	0.31366 0.1781 20
Paternal Hip BMD (g/cm^2)	**0.48126** **0.0317** **20**	0.28199 0.2156 21	0.06365 0.7898 20
Paternal Spine BMD (g/cm^2)	0.40600 0.0757 20	0.19401 0.3994 21	0.25699 0.2740 20
Paternal Whole Body BMD (g/cm^2)	0.36013 0.1299 19	0.11145 0.6399 20	0.04361 0.8593 19
Paternal Average T-score	0.40097 0.0798 20	0.19870 0.3879 21	0.12167 0.6094 20
Paternal Whole Body Fat (g)	0.32801 0.1704 19	0.01073 0.9642 20	0.20032 0.4109 19
Paternal Whole Body Lean (g)	0.40377 0.0865 19	0.16670 0.4824 20	0.24928 0.3034 19
Paternal Whole Body % Fat	0.23595 0.3308 19	-0.02192 0.9269 20	0.14624 0.5502 19

Table 5.11: Relationships between infant anthropometry and bone mineral content at term age and paternal anthropometry and bone mass continued.

	Femur BMC (g)	Spine BMC (g)	Whole Body BMC (g)
	Very Preterm Infants		
Paternal Height (m)	0.09016 0.7054 20	0.24349 0.2875 21	-0.03349 0.8885 20
Paternal Weight (kg)	**-0.46122** **0.0407** **20**	0.29976 0.1868 21	-0.48604 0.0298 20
Paternal Hip BMD (g/cm^2)	-0.10437 0.6615 20	0.06854 0.7678 21	0.00078 0.9974 20
Paternal Spine BMD (g/cm^2)	**-0.23182** **0.3254** **20**	0.10566 0.6485 21	-0.15365 0.5178 20
Paternal Whole Body BMD (g/cm^2)	-0.13079 0.5826 20	0.11870 0.6083 21	-0.05154 0.8291 20
Paternal Average T-score	-0.15193 0.5226 20	0.11269 0.6267 21	-0.09765 0.6821 20
Paternal Whole Body Fat (g)	**-0.47734** **0.0333** **20**	0.18776 0.4151 21	**-0.50974** **0.0217** **20**
Paternal Whole Body Lean (g)	-0.40529 0.0763 20	0.34925 0.1207 21	-0.40631 0.0755 20
Paternal Whole Body % Fat	-0.43757 0.0537 20	0.03191 0.8908 21	**-0.46325** **0.0397** **20**

Data are presented as r, p-value and sample size N
Data in bold are significant $p \leq 0.05$
BMC, bone mineral content; BMD, bone mineral density

Table 5.12: Regression analysis of term infant whole body bone mineral content.

Predictor	R^2	Coefficient	P-value
Infant Anthropometrics	0.5359		0.0002
Intercept		-12.27869	0.5191
Infant Weight (g)		0.02364	0.0002
Average Parental Data	0.6367		0.0002
Intercept		-24.59783	0.1877
Infant Weight (g)		0.02755	<.0001
Average Parental BMD T-score		6.23517	0.0443
Maternal Data	0.5359		0.0002
Intercept		-12.27869	0.5191
Infant Weight (g)		0.02364	0.0002
Paternal Data	0.6512		0.0002
Intercept		-22.16589	0.2261
Infant Weight (g)		0.02700	<.0001
Paternal Average BMD T-score		4.27475	0.0436

BMD, bone mineral density; n = 21

Table 5.13: Regression analysis of very preterm infant whole body bone mineral content.

Predictor	R^2	Coefficient	P-value
Infant Anthropometrics	0.8619		<.0001
Intercept		-0.13322	0.9776
Infant Weight (g)		0.01591	<.0001
Average Parental Data	0.9389		<.0001
Intercept		-90.81356	0.0114
Infant Weight (g)		0.01420	<.0001
Infant Dietary Magnesium (mg/d)		0.68233	0.0108
Average Parental Height (m)		60.12239	0.0059
Average Parental Weight (kg)		-0.21070	0.0293
Maternal Data	0.8619		<.0001
Intercept		-0.13322	0.9776
Infant Weight (g)		0.01591	<.0001
Paternal Data	0.9077		<.0001
Intercept		14.61388	0.0360
Infant Weight (g)		0.01485	<.0001
Paternal Whole Body % Fat		-0.54575	0.0098

n = 21

Table 5.14: Regression analysis of term infant femur bone mineral content.

Predictor	R^2	Coefficient	P-value
Infant Anthropometrics[a]	-	-	-
Average Parental Data	0.5144		0.0022
Intercept		-0.03382	0.9755
Infant Weight (g)		0.000867	0.0109
Average Parental BMD T-score		0.61007	0.0024
Maternal Data	0.4439		0.0013
Intercept		-5.37259	0.0236
Height (m)		5.00507	0.0013
Paternal Data[a]	-	-	-

[a] No significant effects seen in model.
BMD, bone mineral density; n = 21

Table 5.15: Regression analysis of very preterm infant femur bone mineral content.

Predictor	R^2	Coefficient	P-value
Infant Anthropometrics	0.5192		0.0003
Intercept		-4.08855	0.0040
Infant Length (cm)		0.11457	0.0003
Average Parental Data	0.6115		0.0003
Intercept		-0.26453	0.4390
Infant Weight (g)		0.000329	0.0008
Infant Dietary Calcium (mg/d)		0.00236	0.0339
Maternal Data	0.6115		0.0003
Intercept		-0.26453	0.4390
Infant Weight (g)		0.000329	0.0008
Infant Dietary Calcium (mg/d)		0.00236	0.0339
Paternal Data	0.5192		0.0003
Intercept		-4.08855	0.0040
Infant Length (cm)		0.11457	0.0003

n = 21

Table 5.16: Regression analysis of term infant spine bone mineral content.

Predictor	R^2	Coefficient	P-value
Infant Anthropometrics [a]	-	-	-
Average Parental Data	0.3658		0.0166
Intercept		1.39195	0.0985
Infant Weight (g)		0.00054	0.0165
Average Parental % Body Fat		-0.03652	0.0362
Maternal Data	0.4706		0.0006
Intercept		-2.87077	0.0336
Height (m)		3.13979	0.0006
Paternal Data	0.2334		0.0309
Intercept		0.73114	0.2964
Infant Weight (g)		0.00044	0.0309

[a] No significant effects seen in model.
n = 21

Table 5.17: Regression analysis of very preterm infant spine bone mineral content.

Predictor	R^2	Coefficient	P-value
Infant Anthropometrics [a]	-	-	-
Average Parental Data	0.6618		0.0091
Intercept		3.92909	0.0002
Infant Length (cm)		-0.04112	0.0246
Infant Dietary Protein (g/d)		-0.26135	0.0295
Infant Dietary Calcium (mg/d)		0.00452	0.0203
Infant Dietary Vitamin D (IU/d)		-0.000839	0.0161
Maternal Dietary Protein (g/d)		0.01478	0.0040
Maternal Dietary Calories (kcal/d)		-0.000738	0.0039
Maternal Data	0.8122		0.0060
Intercept		3.04914	0.0007
Infant Length (cm)		-0.08615	0.0005
Infant Dietary Calories (kcal/d)		0.00432	0.0422
Infant Dietary Calcium (mg/d)		0.00220	0.0312
Infant Dietary Vitamin D (IU/d)		-0.00140	0.0018
Maternal Dietary Protein (g/d)		0.03242	0.0006
Maternal Dietary Magnesium (mg/d)		-0.01015	0.0006
Maternal Dietary Vitamin D (IU/d)		-0.00162	0.0021
Maternal Whole Body % Fat		0.05293	0.0005
Maternal Average BMD T-score		-0.14299	0.0381
Paternal Data [a]	-	-	-

[a] No significant effects seen in model.
BMD, bone mineral density; n = 21

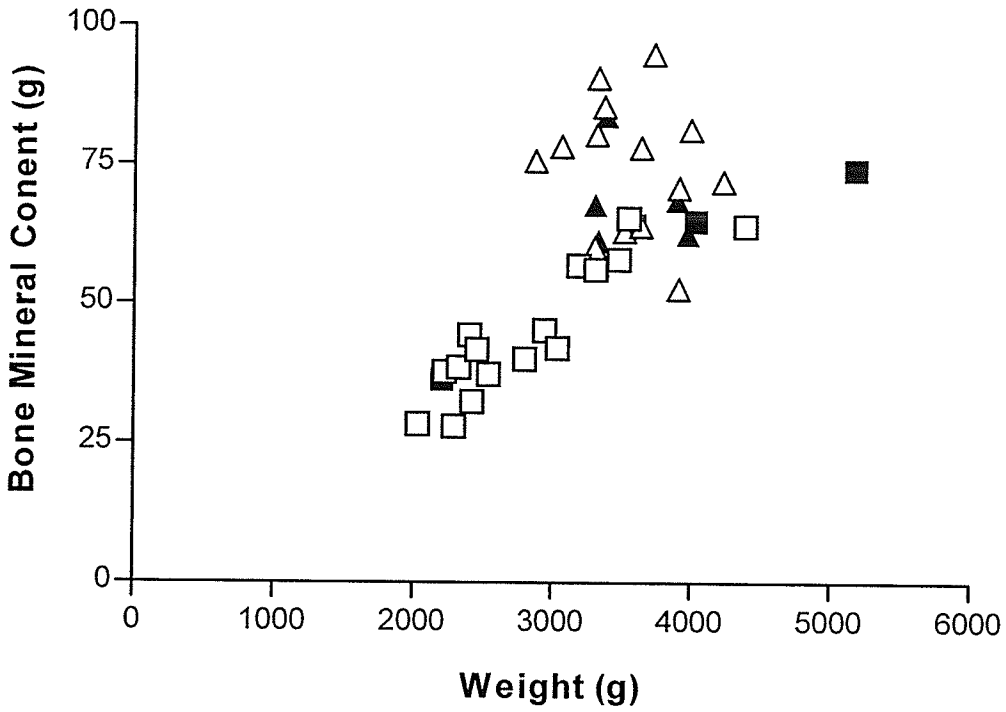

Figure 5.1: Relationship between weight and whole body bone mineral content of term and very preterm infants.

Open triangles represent Caucasian term born infants, n = 16; solid triangles represent non-Caucasian term born infants, n = 5; open square represent Caucasian very preterm infants, n = 18; solid squares represent non-Caucasian very preterm infants, n = 3.

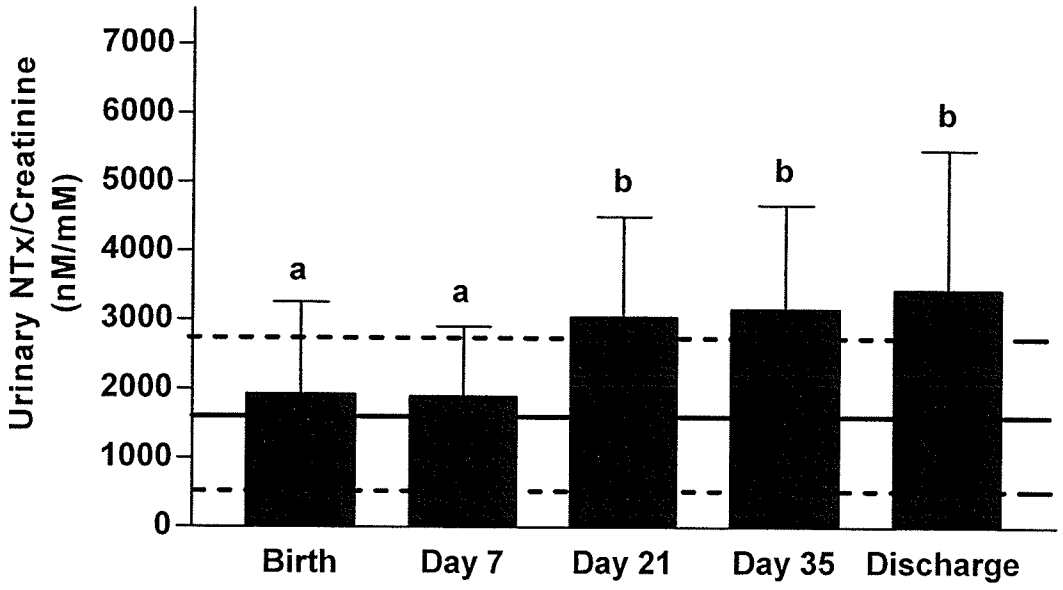

Figure 5.2: Urinary N-telopeptide/creatinine in very preterm infants at birth, day 7, 21, 35 and at hospital discharge.

Columns with different subscripts are significantly different at $p \leq 0.05$ as analyzed by repeated measures ANOVA.
n = 18 at birth; n = 20 at day 7; n = 21 at day 21, 35 and at discharge
Solid horizontal line represents mean urinary N-telopeptide (NTx)/creatinine of term born infants
Dashed horizontal lines represents ± 2 standard deviations from the mean NTx/creatinine of term born infants.

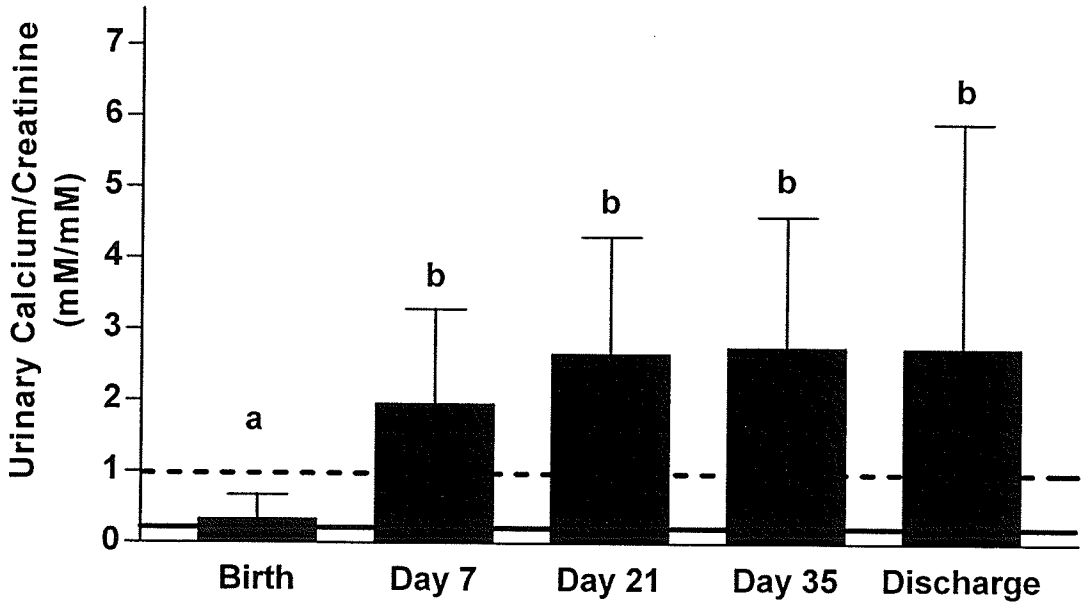

Figure 5.3: Urinary calcium/creatinine in very preterm infants at birth, day 7, 21, 35 and at hospital discharge.

Columns with different subscripts are significantly different at $p \leq 0.05$ as analyzed by repeated measures ANOVA.
n = 18 at birth; n = 21 at day 7, 21, 35 and at discharge.
Solid horizontal line represents mean urinary calcium/creatinine of term born infants
Dashed horizontal lines represents ± 2 standard deviations from the mean calcium/creatinine of term born infants.

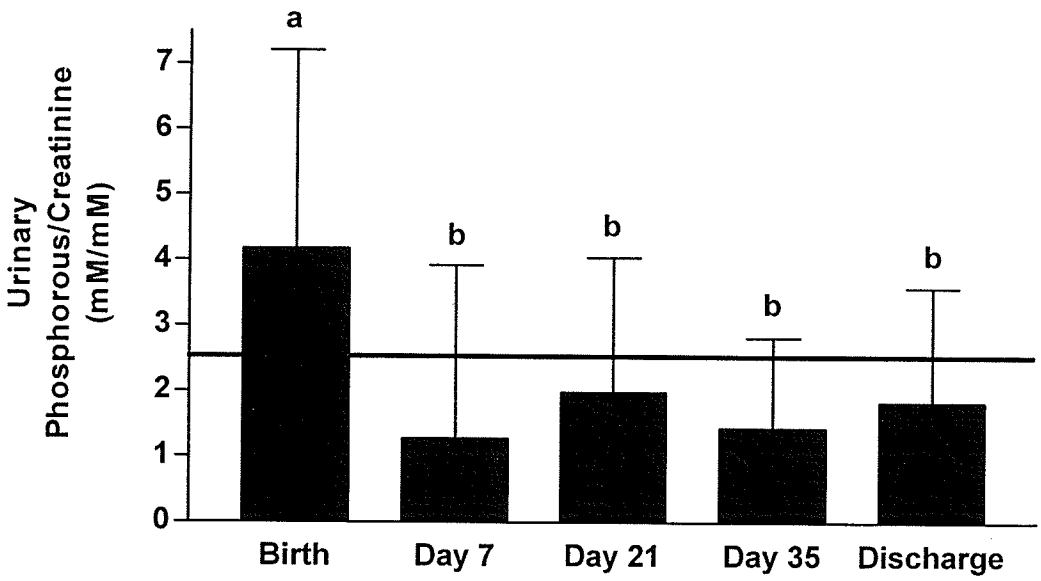

Figure 5.4: Urinary phosphorous/creatinine in very preterm infants at birth, day 7, 21, 35 and at hospital discharge.

Columns with different subscripts are significantly different at $p \leq 0.05$ as analyzed by repeated measures ANOVA.
n = 18 at birth; n = 21 at day 7, 21, 35 and at discharge
Solid horizontal line represents mean urinary phosphorous /creatinine of term born infants

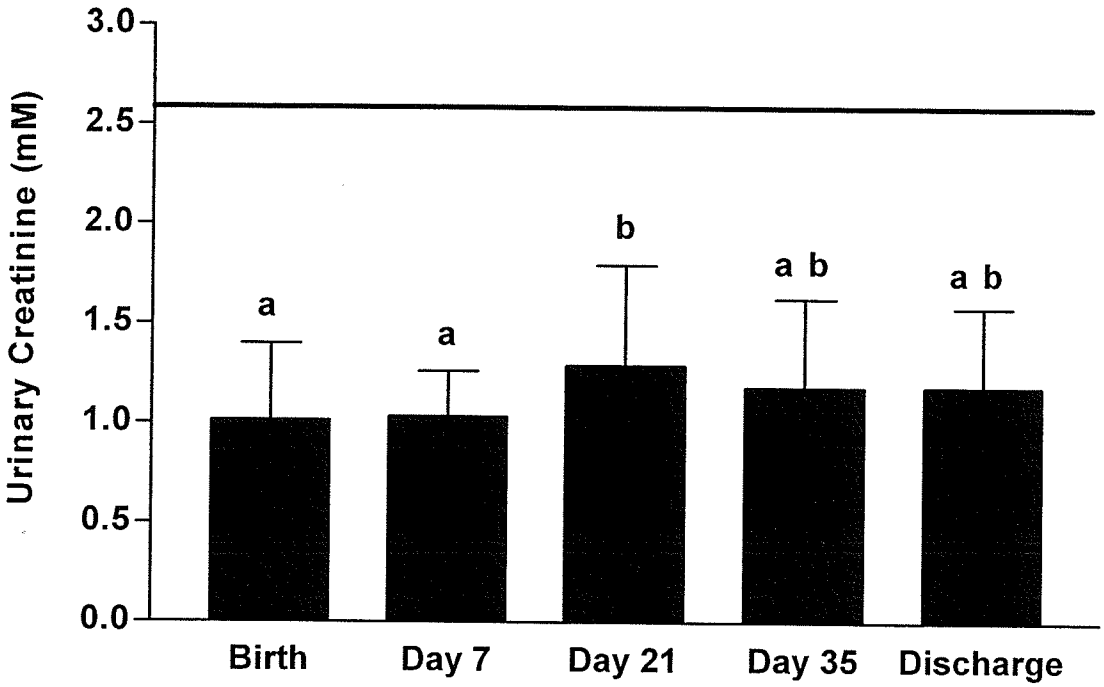

Figure 5.5: Urinary creatinine in very preterm infants at birth, day 7, 21, 35 and at hospital discharge.

Columns with different subscripts are significantly different at $p \leq 0.05$ as analyzed by repeated measures ANOVA.
n = 18 at birth; n = 21 at day 7, 21, 35 and at discharge.
Solid horizontal line represents mean urinary creatinine of term born infants

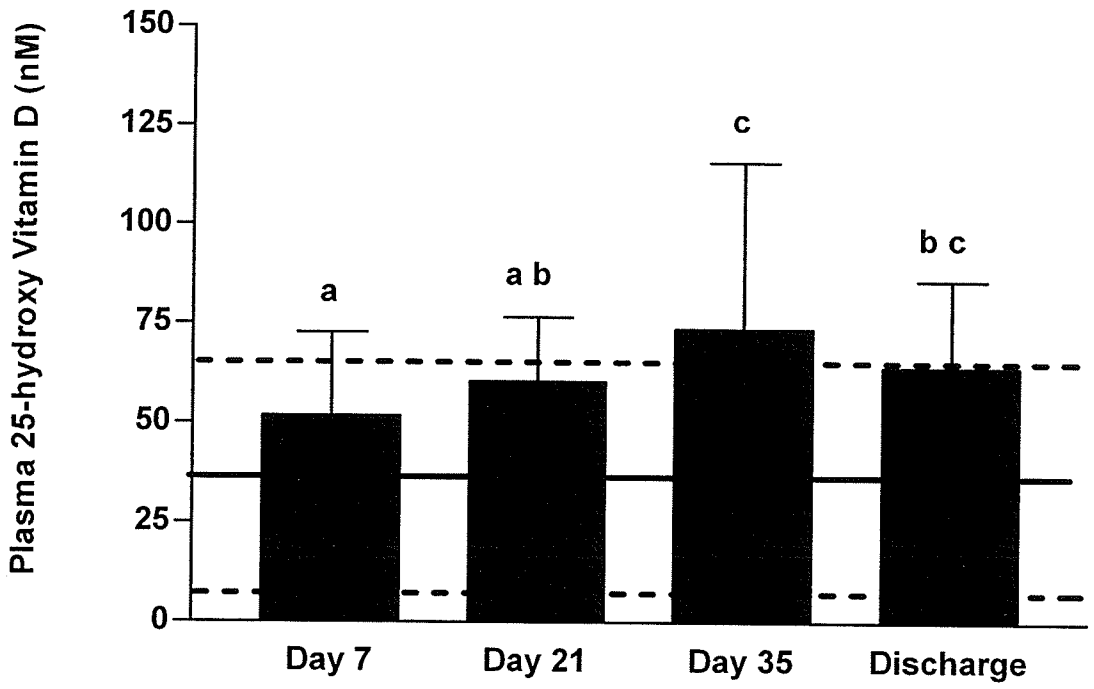

Figure 5.6: Plasma 25-hydroxy vitamin D in very preterm infants at day 7, 21, 35 and at hospital discharge.

Columns with different subscripts are significantly different at $p \leq 0.05$ as analyzed by repeated measures ANOVA.
n = 20 at day 7; n = 21 at day 21, 35 and at discharge.
Solid horizontal line represents mean plasma 25-hydroxy vitamin D of term born infants
Dashed horizontal lines represents ± 2 standard deviations from the mean plasma 25-hydroxy vitamin D of term born infants.

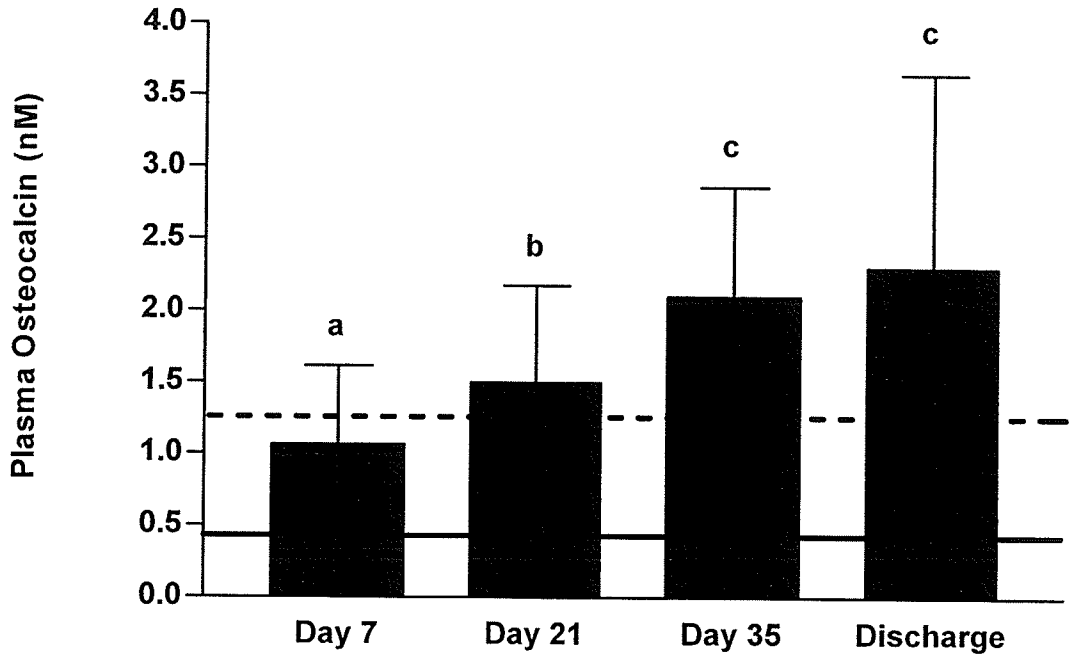

Figure 5.7: Plasma osteocalcin in very preterm infants at day 7, 21, 35 and at hospital discharge.

Columns with different subscripts are significantly different at $p \leq 0.05$ as analyzed by repeated measures ANOVA.
n = 20 at day 7 and 35; n = 21 at day 21 and at discharge.
Solid horizontal line represents mean plasma osteocalcin of term born infants
Dashed horizontal lines represents ± 2 standard deviations from the mean plasma osteocalcin of term born infants.

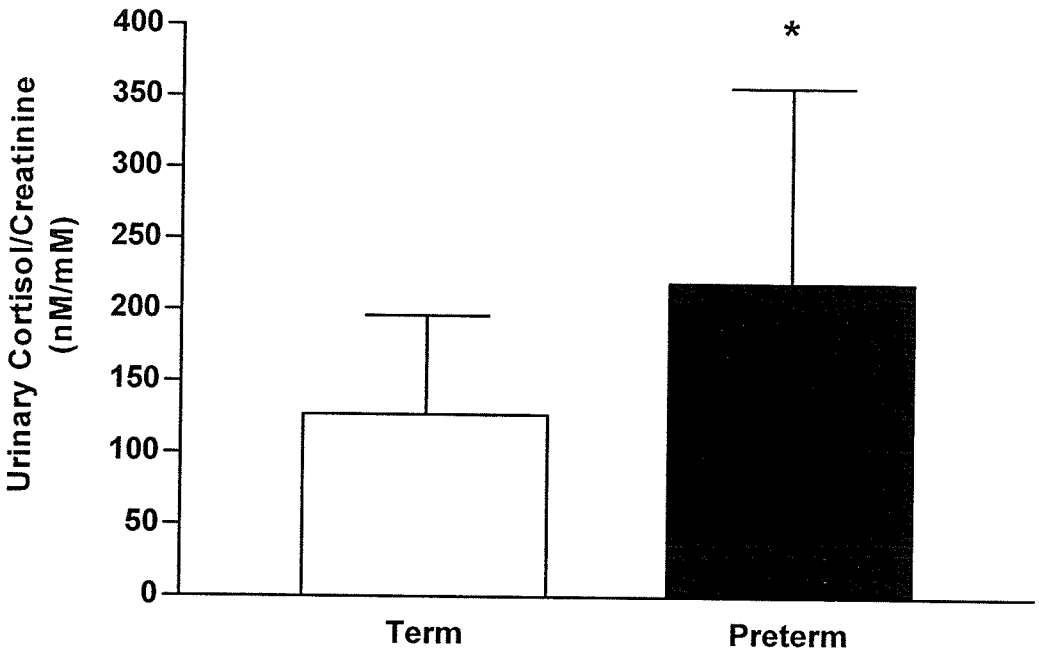

Figure 5.8: Urinary cortisol/creatinine concentrations of term infants at birth and very preterm infants at hospital discharge.

* significantly different at $p \leq 0.05$ as measured by student's t-test.
n = 19 term infants; n = 21 very preterm infants

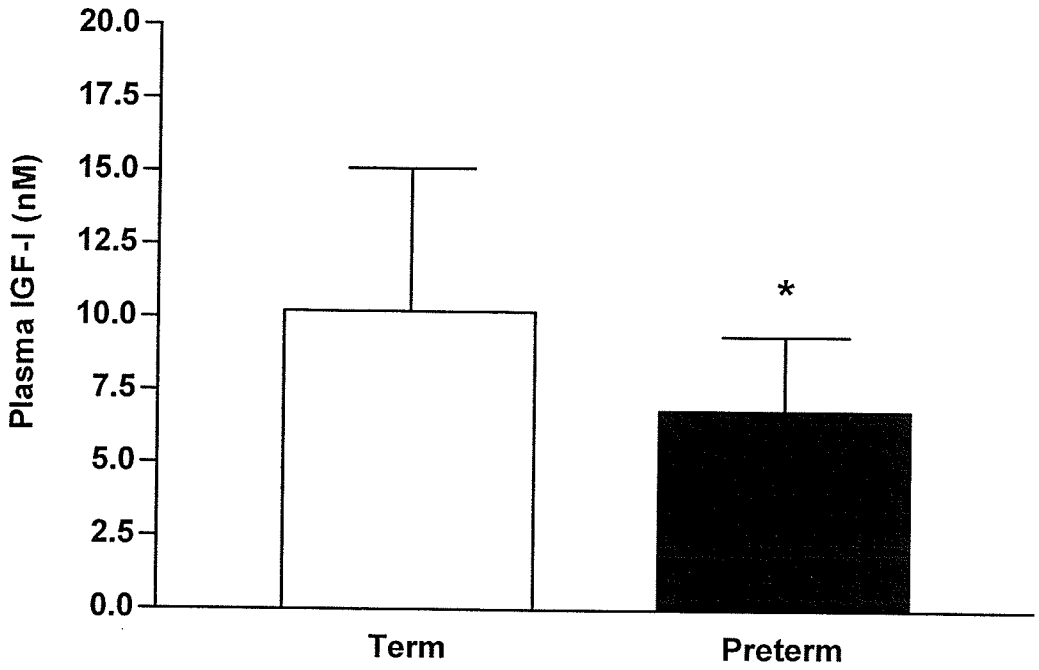

Figure 5.9: Plasma insulin-like growth factor-I concentrations of term infants at birth and very preterm infants at hospital discharge.

* significantly different at $p \leq 0.05$ as measured by student's t-test.
n = 21 term infants, n = 21 very preterm infants.

6.0 Discussion

The main objective of this study was to determine the relative effects of parental characteristics, GA at birth and nutrition on infant growth and bone mineralization and to investigate the effects of programming. Bone growth and modeling are regulated by interactions between an individual's genetics, environmental influences including nutritional factors. Infants born preterm miss the period of maximal calcium deposition during the third trimester of gestation necessary for normal bone development. In the present study very preterm infants had 52 % lower BMC of femur and spine, and 34 % lower whole body BMC at term age than did the term born infants even when adjusted for infant size. These differences may potentially suggest that the postnatal environment has reset the growth trajectory of infant born preterm or that there is a form of *in utero* programming which is realized beyond preterm birth. The elevated cortisol combined with reduced IGF-1 in the very preterm infants is strikingly parallel to the intrauterine programming observed in infants born at term but with intrauterine growth restriction (Barker, 1998). Such modifications in the endocrine axis are well known to result in reduced bone mass (Fowden & Forhead, 2004).

Alterations in IGF-I concentrations can be caused by a number of conditions including acromegaly, dwarfism, intrauterine growth retardation and constitutionally delayed growth (Rajaram et al, 1995). A summary of factors affecting circulating IGF-I concentrations is summarized in Table 6.1. IGF-I circulates in blood bound to a high molecular weight specific binding protein therefore if the concentrations of these binding proteins are altered the concentration of IGF-I measured may be altered. Some studies indicate that IGF-I production is influenced by both genetics and nutritional factors

(Özkan et al, 1999). Several studies indicate that serum IGF-I is a heritable phenotype which may be controlled by the pituitary (Rosen, 1999). Protein energy malnutrition, a known problem in some hospitalized preterm infants, has been shown to be related to lower plasma IGF-I concentrations which can subsequently be reversed with refeeding (Berry et al, 1997; Rajaram et al, 1995; Gallaher et al, 1998). Protein energy malnutrition causes reduced IGF-I concentrations by decreasing its synthesis in the liver and bone (Estivarez & Zigler, 1997; Rosen, 1999). Growth hormone stimulates osteoblast and chondrocyte production of IGF-I (Rosen, 1999). Since only 76% of very preterm infants were meeting the P-RNI for energy and less than 10% of infants were meeting the recommendations for protein it is possible that this is one of the mechanisms which resulted in the lower IGF-I concentrations observed in this population. Increases in visceral body fat may feedback negatively on the hypothalamic GH-GH-releasing hormone (possibly via leptin) resulting in decreased circulating IGF-I concentrations (Rosen, 1999). Very preterm infants had significantly more whole body fat compared to term born infants despite their significantly smaller size which may have also contributed to a reduction in IGF-I concentrations. Decreased circulating IGF-I has also been associated with pO_2 concentrations and maternal hypoxia (a potential cause of fetal programming) has been demonstrated to reduce IGF-I concentrations by up to 50% (Fowden & Forhead, 2004; Fowden, 2003). Pharmacological doses of glucocortisoids cortisol and dexamethasone has been shown to directly lower the basal expression of IGF-I (McCarthy et al, 2000; Fowden 2003). Although only two very preterm infants received dexamethasone, on average very preterm infants had significantly higher urinary

Table 6.1: Factors that affect circulating IGF-I concentrations.

Major direct determinants of circulating IGF-I concentrations
Growth hormone
Protein-calorie intake
Catabolic stressors
Illness
Sepsis
Trauma
Thyroxine
Insulin
Binding affinity of Acid-labile subunit for IGFBP-I/IGF-I
Indirect determinants operating through the GH/IGF-I axis
Body fat (possibly via leptin)
Estrogens
Androgens
Adrenal androgens
Inflammatory cytokines
Other determinants that could directly affect circulating IGF-I
Zinc
Parathyroid hormone
Parathyroid hormone-related peptide
Estrogens
Androgens
Adrenal androgens
Platelet-derived growth factor
Inflammatory cytokines

(adapted from Rosen, 1999)

cortisol concentrations which may or may not have been elevated enough to cause a reduction in IGF-I expression.

Fetal cortisol concentrations are know to increase due to adverse conditions causing fetal stress. When glucocorticoid concentrations are increased either endogenously or exogenously the rate of growth is reduced and the differentiation of various tissues including lungs, liver, kidneys, muscle, fat and gut are altered (Fowden & Forhead, 2004). Exogenous administration of glucocorticoids to women delivering preterm infants is common practice due to its maturation effects on the fetal lungs. The administration of prenatal glucocorticoids may permanently increase basal plasma concentrations of corticosterone through reduced expression of glucocorticoid and mineralcorticoid receptors (Seckl, 2001).

Infant bone mass is strongly influenced by infant body size, specifically weight and length in this and previous studies (Avila-Díaz et al, 2001; Godfrey et al, 2001; Koo & Hockman, 2000; Faerk et al, 2000, Jones & Dwyer, 2000). In the current study infant weight was strongly correlated with term infant femur BMC ($r = 0.7$, $p = 0.0006$) and whole body ($r = 0.9$, $p < 0.0001$) and very preterm infant femur BMC ($r = 0.7$, $p = 0.0006$) and whole body BMC ($r = 0.9$, $p < 0.0001$). Length was likewise correlated however not as strongly as weight. This supports work performed by Godfrey and colleagues (2001), which found strong positive correlations between term infant birth weight and whole body BMC. The same research groups (Godfrey et al, 2001) however found correlations between infant birth weight and spine BMC as well but this was not evident in the present data set. A larger sample size plus adjustment of spine BMC measurements for the duration of gestation might have made the relationship with infant

body weight more clear as was seen in the work of Godrey and co-workers (2001). Similarly, Avila-Díaz and co-workers demonstrate that BMC in a group of term and preterm infants has a high degree of correlation with infant weight and length. When entered into a regression model Avila-Díaz and colleagues found that current weight was the only significant predictor of infant whole body BMC. Koo and Hockman (2000) found infant weight to be a dominant predictor of term and preterm infant lumbar spine (L1-L4) BMC with infant length also making a contribution in a regression model. The subjects of the Koo and Hockman study (2000) were primarily African American whereas in the current study only one term and one preterm infant was African American, therefore ethnicity may account for differences in results if a stronger relationship exists between African American infant weight and spine BMC. Jones and Dwyer (2000) studied term born infants at age eight and found similar correlations between infant size at one month of age and BMD at age eight. Jones and Dwyer (2000) commented that the associations between bone density and anthropometrics were mediated through bone size rather than density suggesting that the trajectory of bone growth is determined early in life. However, no other research group has reported on these relationships plus parental bone mass, infant and maternal makers of bone turnover, infant and maternal diets all in combination with infant bone mass.

Parental height is one of the strongest predictive measures of subsequent preterm infant height at ages nine to 12 years (Fewtrell et al, 2000). Average parental height was a significant predictor of very preterm but not term infant whole body BMC. However maternal height correlated strongly with term but not very preterm infant bone mass in the present study potentially suggesting that the growth trajectory of preterm infants has

been reset. In regression maternal height was not a significant predictor of term infant whole body BMC which is consistent with previous reports (Godfrey et al, 2001). Maternal height was however a significant predictor of term infant femur and spine BMC, however this effect on the spine was removed with spine bone area was entered into the model. Paternal height correlated with term infant femur BMC and spine BMC but not whole body BMC. This relationship however was not evident in the regression model where paternal height did not predict term infant whole body, femur or spine BMC as opposed to previous reports where paternal ($r = 0.22$) but not maternal ($r = -0.01$) height was associated with term infant whole body BMC (Godfrey et al, 2001). Paternal height was not correlated with any very preterm infant bone mass measurements. Parental heights have been shown to be strong predictors of a child's height at age three suggesting that there may be a long-term genetic effect on bone growth beyond term age (Fewtrell et al, 2000). Perhaps given time, this relationship would have evolved in the present study.

Birth weight of parents has also been found to correlate with term infant whole body BMC (Godfrey et al, 2001) but few authors have investigated the relationship between a parent's adult weight and infant bone mass. The current study found only paternal weight correlated with both term and very preterm infant femur BMC. Maternal weight did not correlate with either term or very preterm infant bone mass. However in the regression analysis average parental weight predicted very preterm infant whole body BMC but not femur or spine BMC.

Body composition may be a better indicator of genetic and environmental effects on achieving peak bone mass as it may relate to level of physical activity, diet and weight

loading response in bones. Godfrey and co-workers (2001) reported that maternal thinness (as measured by triceps skinfold thickness) was associated with lower bone mineral apparent density in term born infants. The current study found that maternal percent whole body fat was not correlated with infant bone mass. Paternal whole body percent fat was negatively correlated with very preterm infant whole body BMC and was also a significant predictor in the regression model. Thus it could be postulated that a negative relationship with paternal fat may imply higher propensity for leanest (including bone) that is being inherited by the infant. Maternal whole body percent fat was positively predictive of very preterm infant spine BMC when spine bone area is not included in the model. The positive association with maternal fat and lean mass may also reflect energy and nutrient stores necessary to support rapid fetal growth. Average parental whole body percent fat was predictive of term infant spine BMC. The current study is one of the first to examine bone density of both parents as genetic predictor of infant bone mass.

Previous research has reported a positive correlation between mean parental spine BMD Z-score, maternal and paternal spine BMD Z-scores and the spine Z-scores of children ages five to 20 years (Lonzer et al, 1996). McKay and colleagues (1994) found that the lumbar spine BMD Z-scores of mothers and daughters, and mothers and grandmothers showed a strong familial resemblance. The present study results support previously published literature in that average parental BMD T-scores positively predict term infant whole body and femur BMC, maternal average BMD T-score predicts very preterm infant spine BMC and paternal average BMD T-score predicts term infant whole body BMC. From our data set it is apparent that parental skeletal size and density is an

important factor in determining infant bone mass. Very preterm infant whole body BMC may not have shown a relationship with parental bone mass due to the fact that these infants have not yet achieved a bone mass which is appropriate for their size. In this case where growth has continued despite delays in bone mineralization parental height and weight are better predictors as they likely relate to infants size. At the site of the femur term infants showed a similar genetic prediction of bone mass demonstrating again that parental skeletal BMD is an important factor that should be considered when assessing infant bone mass. In very preterm infants this genetic influence is not apparent and once more this may be due to the fact that growth has superseded bone mineralization. Preterm birth has such as strong environmental influence that it may mask the genetics effects on bone seen in term infants. At the lumbar spine there does not appear to be as much of a genetic effect of BMD of parents but rather when bone area is not included in the equation there appears to be a size adjustment with either infant or parental size. In very preterm infant spine BMC there still appears to be a genetic effects of maternal bones. This site of bone measurement may not be as sensitive to environmental influences as there is no growth plate. The observation that term infant bone mass is not related to maternal bone mass but was in the preterm group might be related to the more diverse environment of the term infants during late gestation (i.e. variable nutrition, uteroplacental flow, maternal activity etc.) compared to the preterm infants who were in a standardized environment (neonatal care including nutrition).

Variance within a phenotypic characteristic depends on both genetics and environmental influences, of which early nutrition is one of the strongest. Many studies suggest that poor *in utero* growth is related to lower bone mass in adulthood (Cooper et

al, 1995; Cooper et al, 1997; Gale et al, 2001; Jones & Dwyer, 2000). A potential cause of poor growth during fetal life could be maternal diet. Analysis of maternal diet during pregnancy and term born infant bone mass at age nine showed positive associations between maternal protein, magnesium, phosphorous, iron and vitamin C intakes and BMD of children at various sites of measurement (Jones et al, 2000; Tobias et al, 2005). In the present study maternal diet, in particular protein, calories, magnesium and vitamin D, seemed to only affect very preterm infant spine BMC. Differences among results could be due to the method of data collection and type of food tables used to determine nutrient content of various foods. In the present study 3-day food records and 24-hour recalls were used with Canadian food tables to assess diet whereas food frequency questionnaire and British and Australian food tables were used by Jones and co-workers (2000) and Tobias and colleagues (2005). Also, regional differences in food preferences, selection and food availability may have contributed to differences seen. The effect of diet in very preterm infants appeared to be much stronger than those in term born infants reinforcing the importance of proper nutrition during early pregnancy. In the very preterm group, maternal protein was positively associated with preterm infant spine BMC. Overall energy however was negatively related to preterm infant spine BMC. It may be possible that in the mothers who consumer high caloric diets may have a larger portion of calories were derived from fat and hence the positive association was not maintained as expected. There is evidence that lower income women consume a diet high in fat (Havas et al, 2003). Since a lower income status is a risk factor for delivering prematurely, diet may be a contributing factor in this risk. A study of rats shows analogous results with low maternal protein intake resulting in lower adult BMC of

offspring (Mehta et al, 2002). Mehta and colleagues (2002) also found changes in tibial ephiphyseal growth plate morphology including increased width of the growth plate itself. Changes in growth plate morphology may suggest programming of skeletal development and help to explain the reduced stature seen in children born prematurely. Maternal dietary calcium may not be associated with preterm infant bone mass as these infants have yet to reach the period of gestation where maximal mineral accretion occurs and hence the amount of dietary calcium retained may depend more on maternal metabolic adjustments than actual amount of nutrient intake. During the third trimester of gestation the growing fetus relies heavily on maternal body stores and a mother's ability to physiologically adapt to increased mineral needs. The composition of the maternal diet is often reflected in the nutrient composition of breast milk which may affect the bone mineralization of infant fed mother's milk.

The breast milk of mother's who gave birth prematurely differs from those who deliver at term age (Anderson et al., 1981). Preterm mother's milk during early lactation has a higher concentration of fat and protein and higher overall energy density compared to term mother's milk (Anderson et al, 1981). Despite preterm mother's milk higher energy density it fails to meet the energy needs of preterm infants (Anderson et al, 1981) so commercial formulas and human milk fortifiers are commonly used to help prevent energy and mineral deficiencies commonly seen in preterm infants with metabolic bone disease. Femur BMC and BMD have been demonstrated to be increased in a group of preterm infants fed commercial formulas with higher calcium and phosphorous contents (Narbona et al, 1998). More recently in a study of 127 preterm infants phosphorous was found to be significantly associated with weight at term age, but BMC was not

significantly different between preterm infants and a reference term group when adjusted for body size (Faerk et al, 2000). Similarly Backström and colleagues (1999) showed that premature infants receiving diets supplemented with calcium and phosphorous had radial BMC 36 % higher than those unsupplemented at three months of age. However when the same group of infants reached the ages of 9 to 12 years this relationship was no longer apparent as measured at lumbar spine L1-L4, suggesting only short-term benefits of supplementation (Backström et al, 1999). In the present study calcium was found to positively predict preterm infant femur BMC and spine BMC. In very preterm infant spine BMC regression there were more dietary factors related to bone mass than at the femur. These differences in nutritional effects on various sites of BMC measurement may indicate that the timing of bone mineralization is different at various centers of ossification. Gale and colleagues (2001) suggests that the fetal growth trajectory is influence by the intrauterine environment resulting in long term changes in bone and muscle mass in adulthood.

Few infant biochemical indices were related to bone mass in the present study. The strongest correlation was seen between very preterm infant plasma 25(OH)D and spine BMC. Infant and maternal plasma 25(OH)D are highly correlated (Weiler et al, 2005) and therefore blood concentrations early in life before supplementation has begun may reflect maternal transfer and relative availability of this nutrient for the growing fetus. The relationship between very preterm infant plasma 25(OH)D and bone mass at other sites of measurement may not be as evident as most infants received vitamin D supplementation while in hospital. All very preterm infants had adequate (> 27.5 nmol/L) plasma 25(OH)D concentrations at hospital discharge and therefore any

relationship with 25(OH)D may have been masked (Institute of Medicine, 1997). The timing, amount and duration of vitamin D supplementation may be important factors to consider when evaluating dietary vitamin D intake. The negative relationship between spine BMC and very preterm infant urinary calcium may be a reflection of calcium wasting or alteration in calcium metabolism due to a high turnover state of bone resulting in increased urinary losses. Also the amount of calcium and phosphorous supplemented above the infants metabolic ability to use the mineral may alter the excretion of mineral and therefore may only reflect the degree of fortification of the diet and not a true relationship with bone.

IGF-I stimulates growth plate chondrocytes necessary for long bone growth and stimulates osteoblast proliferation and differentiation required for the production of bone matrix (Zhang et al, 2002). In the present study very preterm infants were shown to have significantly lower IGF-1 concentrations at hospital discharge compared to term born infants which may have contributed to the lower BMC seen in these infants. Similarly, tibia and lumbar vertebrae of mice deficient in IGF-I deficient mice have been shown to be significantly smaller and have reduced mineral content compared to wild-type animals (Bikle et al, 2001). IGF-I has also been shown to increase two to three fold between weeks 33 and birth (Lassarre et al, 1991). It appears that concentrations of IGF-I in infants born very preterm never reach those of term born infants and therefore may affect fetal size and bone growth. Infants with intrauterine growth retardation show a similar pattern in IGF-I concentrations. Özkan and colleagues (1999) found that those infants with intrauterine growth retardation that showed catch-up growth had higher concentrations of IGF-I that those who did not show catch-up growth. This may suggest

that infants born very preterm are at a disadvantage for adequate catch-up growth or are not displaying adequate catch-up growth.

Maternal markers of bone metabolism were related to very preterm infant bone mass at term age. NTx a marker of bone resorption was negatively correlated with very preterm infant spine BMC. Maternal plasma OC, a marker of bone formation was positively correlated with very preterm infant femur BMC. During pregnancy OC concentrations decline and NTx concentrations increase to reduce bone formation and increase bone resorption in order to supply minerals to the growing fetus. A negative correlation between maternal NTx and infant bone mass may be suggestive that a mother is heavily dependent on her bones to maintain mineral homeostasis as fetal demands increase. OC could be suppressed concurrently to help support the growing fetus, or in the case of mothers consuming an adequate diet the relationship between infant BMC and OC could be positive.

7.0 Study Limitations

The largest limitation in analyzing the data of the present study was the inability to recruit sufficient study subjects; particularly the fathers. This study therefore, did not achieve an adequate sample size. As a result, there was limited power to detect differences, and differences may exist where in fact this study detected none.

In this study, nine of 21 very preterm infants were born of multiple gestations. There is speculation that differences exist in bone mineralization between multiple gestation preterm infants and singleton preterm infants; however, there is limited published research that determines whether significant differences exist. More than half

of calcium accretion and bone mineralization occurs in the third trimester of pregnancy (Ziegler et al, 1976). Preterm infants miss this critical time of calcium accretion and consequently do not tax a mother's calcium stores and physiological ability to adapt to the increased calcium needs during pregnancy. Therefore, differences in bone mineralization in multiple gestation preterm infants may not be as apparent, as bone accretion is limited due to premature parturition.

Some researchers indicate that there are differences in body composition between male and female infants at birth (Rigo et al, 1998). A greater proportion of preterm infants are male in gender (Cooperstock & Campbell, 1996). If size differences between male and female preterm infants exist, this would skew the data collected. In this study, four very preterm infants were female and 17 were male, which would increase the overall weight of the group, increasing the average calculated BMC. This study included weight in regression to models as an attempt to account for potential size differences that may or may not exist. Avila-Díaz and colleagues (2001) did not find gender differences in bone BMC or BMD in preterm or term born infants. Godfrey et al (2001) only found small and non-significant differences between male and female term infant bone mineral measurements.

This study used an average of three, 3-day food records to estimate the mother's intake. When 3-day food records were not available, 24-hour food recalls were used instead to estimate intake. A correlation analysis indicated that 24-hour food recalls were significantly correlated with the three, 3-day food records. However, the food records and recalls were completed postpartum, and may not reflect actual food habits of the mothers during pregnancy. Food habits may change dramatically in the postpartum

period as some mothers may no longer adhere to strict dietary requirements. Additionally, food records successfully estimate nutrient content of diets to varying degrees. For example, 3-day food records may accurately reflect intake of macronutrients, however, studies indicate that it takes approximately 74-88 days of food records to accurately estimate calcium intake (Basiotis et al, 1987). Calcium intake varies greatly on a daily basis due to the relatively limited number of foods where calcium is in abundance, hence the large number of days required to accurately estimate intake compared to other nutrients (Basiotis et al, 1987). A food frequency questionnaire may have been a more appropriate tool for measuring food habits during pregnancy. It was not possible to collect information pre-parturition due to the nature of the study.

Activity level, with particular reference to weight bearing activity, influences bone density. BMD is determined not only by genetic but by environmental influences such as activity level. Presently, accurate and objective measures of activity do not exist. Available questionnaires are largely subjective in nature and the accuracy of such tools is not adequate to account for the differences in activity/genetic interaction that may influence BMD in parents of infants. Activity level of parents was thus not accounted for in this study.

8.0 Future Research

The current study suggests that preterm birth programs for low bone mass as evidenced by the low BMC accompanied by elevated cortisol, reduced IGF-1 as well as high turnover bone metabolism. Future research should investigate if these programming effects persist beyond term age and if the stress of preterm birth can be attenuated

through diet or medical interventions. Studies need to be designed to include better measures of maternal and paternal diet as well as activity level of parents to help account for differences in parental bone due to lifestyle. Ideally a larger longitudinal prospective study is necessary to follow subject until skeletal maturity when peak bone mass is achieved. It might also be interesting to investigate the possibility that adults who were bone preterm may lose bone mass at a different rate than those born at term due to changes in bone morphology as this may place them at even higher risk of bone disease in later life. Future research is necessary to develop better premature infant care to help optimize bone health especially if growth trajectory has been changed or full genetic potential cannot be achieved.

9.0 Conclusion

In conclusion infants born very preterm continue to have lower BMC at term age despite advances in nutritional and medical management. Very preterm infant BMC appears to be influenced not only by environmental factors such as nutrition but also by genetics as seen through the relationship between parental size and bone mass with very preterm infant femur, spine and whole body bone mass at term age. Whether this difference in BMC between term and very preterm born infants continues beyond term age has yet to be established unequivocally.

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Appendix A

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RESEARCH PARTICIPANT INFORMATION AND CONSENT FORM

PRETERM INFANTS

Title of Study: Does earlier introduction of feeds stimulate catch-up growth, bone mineralization and neurodevelopment in preterm infants.

Protocol Number: B2000:212

Principal Investigator: Dr. H. Weiler, RD, PhD
Co-Investigator: Dr. M.M. Seshia, MBChB

Sponsor: University of Manitoba, Winnipeg, Manitoba, R3T 2N2

You are being asked to participate in a human research study. Please take your time to review this consent form and discuss any questions you may have with the study staff. You may take your time to make your decision about participating in this research study and you may discuss it with your regular doctor, friends and family before you make your decision. This consent form may contain words that you do not understand. Please ask the study doctor or study staff to explain any words or information that you do not clearly understand.

Purpose of Study

This study is being conducted to study feeding regimens, nutritional status, growth, bone growth and neurodevelopment of infants. You are being asked to take part in this study because you are soon to or have just delivered a newborn infant. A total of 236 infants will participate in this study.

The purpose is to study if the timing of delivery of feeds to infants affects the growth patterns, bone growth and neurodevelopment of babies that are born early (prematurely). The results of growth, bone growth and neurodevelopment will also be compared with that of babies born at the expected due date (full-term).

This research is being done because research suggests that introduction of amino acids (which are used to make protein/muscle) within the first 24 hours of life may be better than if introduced after 72 hours of life. These amino acids are routinely introduced to your infant at approximately 72 hours of life. The other aspect of the trial is that introduction of small volumes of human milk or formula into an infant's stomach may help prepare the intestinal tract for later feeding. We wish to compare starting these feeds within the first 72 hours compared to the standard 73-120 hours of life on the time required by your infant to reach targeted milk volumes and on growth and development. The type of nutrients and starting amounts will not be altered.

Thus this study will help to determine if prematurely born babies would benefit from earlier delivery of nutrients/feeds than is routine as demonstrated by growth, bone growth and neurodevelopment.

Study Procedures

The study will begin at the time of your baby's birth and will continue until he/she is 18 months corrected age.

In order to participate in this study, you must not have a personal history of diabetes before or during your pregnancy and your doctor will determine whether or not any medications you are currently taking will interfere with the proposed measurements. Your baby will be examined at birth as part of routine clinical care for any medical conditions that may exclude him/her from participating in this study.

Your infant will be randomly assigned to receive one of four feeding combinations and this is controlled by the study design so you will not be able to choose which approach is used to feed your infant. The four combinations are: 1) usual introduction of amino acids and milk feeds; 2) early amino acids starting on day one of life but usual introduction of milk feeds; 3) early introduction of milk feeds starting on day one of life but usual amino acid feeding; or 4) early introduction of amino acids and milk feeds starting on day one of life.

If you take part in this study, the following tests and procedures will be done:

Your Infant's Involvement in Hospital

We request your permission to obtain the following from your premature baby:

- a sample of blood from the placenta (afterbirth) taken routinely at birth;
- measurements of your baby's length, weight, and head size at birth and once a week starting from one week of age;
- a urine sample from your baby at birth, one, three and five weeks of age and at term;

- At one, three and five weeks of age and at term, a blood sample (approximately 0.5 ml or 1/10 a teaspoonful) from your baby will be collected;
- we will also collect information from your infants medical record including the amount and type of feeding provided, body weight, measurements made in blood such as protein, medications prescribed, medical conditions that develop and results of tests such as chest x-rays or head ultrasounds as ordered by the health care team.

These samples and measurements will help us learn if earlier introduction of nutrients helps infants grow while in hospital and the additional information from the medical record will help us interpret the results of growth and development later on.

Your Infant's Involvement after Discharge from Hospital

We also ask your permission to:

- measure your infants weight, length and head size at 6, 12 and 18 months corrected age;
- scan your baby's whole body, lower (lumbar) spine and thigh bone (femur) using a low dose x-ray technique called dual energy x-ray absorptiometry at the research facility immediately after hospital discharge and again at 6, 12 and 18 months corrected age;
- collect information from neurodevelopmental tests routinely performed at the Growth and Development Clinic at 18 months corrected age as well as information regarding hearing and sight measured within the first 18 months after discharge from hospital.

These measurements will help us learn about the relationships among earlier introduction of nutrients and growth, bone growth and neurodevelopment after discharge from hospital.

Your Own Involvement

We request your permission to take a blood sample (approximately 10 ml or 2 teaspoonfuls) from you prior to your discharge from hospital. We also ask if you would keep a three-day record of food intake at various intervals after you have been discharged. This will be reviewed with you approximately one week later by telephone call at a convenient time and should take no more than half an hour. We ask that we collect information from your hospital record about your pregnancy and medications. At the first visit after discharge we will request that we measure height of both parents and ask you some general questions about your education level, range of income and to indicate which group you identify with (First Nations, Filipino, Black, Asian, White).

You will decide on your own whether you wish to breast-feed or to feed your baby infant formula. If breast-feeding your premature baby, you will be asked to provide a sample of your breast milk on two days of each week, to be collected at 9:00 a.m. and 9:00 p.m. by manual expression or with a breast pump, for nutrient analysis. The amount required is small (approximately 5 ml or 1 teaspoonful) and should not interfere with the normal feeding of your infant.

Both Your and Your Infant's Involvement

At the follow-up visits, at your infant's discharge from hospital, 6, 12 and 18 months, we will ask you about the amounts and types of food that you and your infant consume to help us learn about nutrition and growth. If you are no longer breast feeding or chose not to you do not have to record the foods you ate at these times. At 6, 12 and 18 months we will mail you a brief questionnaire to complete regarding your infant's development. This questionnaire takes minutes to complete and will be accompanied by a self-addressed stamped envelop in which to mail to the investigators.

Participation in the study will be from the time you join the study until your infant reaches 18 months corrected age.

The researcher may decide to take you off this study if you or your infant develop illnesses that affect growth, bone or neurodevelopment.

Risks and Discomforts

There are no risks associated with measurements of growth or collection of the urine, and breast milk samples. There is low risk associated with sampling of blood such as bruising but we will try to take blood when samples are taken for routine monitoring to limit the number of times blood is sampled. The earlier introduction of amino acids is thought to be safe since these were delivered already through the placenta. The intravenous catheter does have risks associated with its placement and use. However, the preterm infants studied would have a catheter in place regardless of participation in this study because this is a routine method of initially providing nutrients to preterm infants. An infant without a catheter in place at birth would not participate in the study. Most infants born 8 weeks early need to be fed milk through a feeding tube that is placed from the nose or mouth into the stomach. This process usually begins close to birth but may not begin until later in the first week of life. We wish to feed milk within the first 72 hours of life. Feeding of milks into the stomach within the first 72 hours of life is thought to be safe as indicated by research at other hospitals but is not usually started until after 72 hours in small infants at this hospital. The physical risks associated with the scan of your infant's whole body, lumbar spine and femur are considered to be low. The device uses an x-ray source to measure bone density. However, the amount of radiation is much less than a regular chest x-ray and equivalent to the radiation accumulated during one normal day.

Benefits

There are no monetary benefits to you or others in this study. You will be provided a breast pump kit if you choose to breastfeed. The knowledge gained through this study will help us to improve nutritional care for infants who are born prematurely.

Costs

All laboratory tests which will be performed as part of this study are provided at no cost to you and your infant. There will be no cost for the study measurements that you participate in.

Payment for Participation

There will be no financial remuneration for participating in this study with exception of coverage of parking at the follow-up visits after discharge.

Confidentiality

Information gathered in this research study may be published or presented in public forums, however your name and your infant's name will not be used or revealed. Medical records that contain your identity will be treated as confidential in accordance with the Personal Health Information Act of Manitoba. Despite efforts to keep your personal information confidential, absolute confidentiality cannot be guaranteed. Your personal information may be disclosed if required by law. Organizations that may inspect and/or copy your research records for quality assurance and data analysis include groups such as the Biomedical Research Ethics Board of the University of Manitoba.

Voluntary Participation/Withdrawal From the Study

Your decision to take part in this study is voluntary. You may refuse to participate or you may withdraw from the study at any time. Your decision to not participate or to withdraw from the study will not affect your or your infant's other medical care at this site.

Questions

You are free to ask any questions that you may have about your rights as a research participant. If any questions come up during or after the study, contact the study doctor and the study staff:

Dr. H. Weiler (474-6798) OR Dr. M. Seshia (787-1827)

For questions about your rights as a research participant, you may contact The University of Manitoba's Biomedical Research Ethics Board at (204) 789-3389.

Do not sign this consent unless you have had a chance to ask questions and have received satisfactory answers to all of your questions.

Statement of Consent

I have read this consent form. I have had the opportunity to discuss this research study with either of Drs. Weiler and Seshia and or their study staff. I have had my questions answered by them in language I understand. The risks and benefits have been explained to me. I understand that I will be given a copy of this consent form after signing it. I understand that my participation in this clinical trial is voluntary and that I may choose to withdraw at any time. I freely agree to participate in this research study.

I understand that information regarding my personal identity will be kept confidential, but that confidentiality is not guaranteed. I authorize the inspection of my, and my infant's, medical records by the University of Manitoba's Biomedical Research Ethics Board.

By signing this consent form, I have not waived any of the legal rights that I have as a participant in a research study.

Participant signature:_____ Date:_____

Participant printed name:_____

I, the undersigned, attest that the information in the Participant Information and Consent Form was accurately explained to and apparently understood by the participant or the participant's legally acceptable representative and that consent to participate in this study was freely given by the participant or the participant's legally acceptable representative.

I, the undersigned, have fully explained the relevant details of this research study to the participant named above and believe that the participant has understood and has knowingly given their consent.

Printed Name:_____ Date:_____

Signature:_____

Role in the study:_____

Appendix B

UNIVERSITY OF MANITOBA

FACULTY OF HUMAN ECOLOGY

Human Nutritional Sciences

Duff Robin Building
Winnipeg, Manitoba
Canada RAT 2N2
(204) 474-6798
(204) 474-7593 FAX
Email:
hweiler@ms.umanitoba

RESEARCH PARTICIPANT INFORMATION AND CONSENT FORM

TERM INFANTS

Title of Study: Does earlier introduction of feeds stimulate catch-up growth, bone mineralization and neurodevelopment in preterm infants.

Protocol Number: B2000:212

Principal Investigator: Dr. H. Weiler, RD, PhD
Co-Investigator: Dr. M.M. Seshia, MBChB

Sponsor: University of Manitoba, Winnipeg, Manitoba, R3T 2N2

You are being asked to participate in a human research study. Please take your time to review this consent form and discuss any questions you may have with the study staff. You may take your time to make your decision about participating in this research study and you may discuss it with your regular doctor, friends and family before you make your decision. This consent form may contain words that you do not understand. Please ask the study doctor or study staff to explain any words or information that you do not clearly understand.

Purpose of Study

This study is being conducted to study feeding regimens, nutritional status, growth, bone growth and neurodevelopment of infants. You are being asked to take part in the observational part of this study because you are soon to or have just delivered a newborn infant at term. A total of 60 term infants and 176 preterm infants will participate in this study.

The purpose of the observational part of this study is to gather information regarding growth, bone growth and neurodevelopment from term born infants so that we can interpret the results of these measurements made in infants who are born early (premature) and learn if the intervention was successful. The purpose of the study in infants who are born early is to study if the timing of delivery of feeds to infants affects the growth patterns, bone growth and neurodevelopment.

This research is being done because research in premature infants suggests that introduction of amino acids (which are used to make protein/muscle) within the first 24 hours of life may be better than if introduced after 72 hours of life. These amino acids are routinely introduced to preterm infants at approximately 72 hours of life. The other aspect of the trial is that introduction of small volumes of human milk or formula into a preterm infant's stomach may help prepare the intestinal tract for later feeding. We wish to compare starting these feeds within the first 72 hours compared to the standard 73-120 hours of life on the time required by a preterm infant to reach targeted milk volumes and on growth and development. The type of nutrients and starting amounts will not be altered.

Thus this study will help to determine if prematurely born babies would benefit from earlier delivery of nutrients/feeds than is routine as demonstrated by growth, bone growth and neurodevelopment.

Study Procedures

The study will begin at the time of your baby's birth and continue until he/she is 18 months of age.

In order to participate in this study, you must not have a personal history of diabetes before or during your pregnancy and your doctor will determine whether or not any medications you are currently taking will interfere with the proposed measurements. Your baby will be examined at birth as part of routine clinical care for any medical conditions that may exclude him/her from participating in this study.

If you take part in this study, the following tests and procedures will be done:

Your Infant's Involvement in Hospital

We request your permission to obtain the following from your infant:

- a sample of blood from the placenta (afterbirth) taken routinely at birth;
- measurements of your baby's length, weight, and head size at birth;
- a urine sample from your baby at birth.

These samples and measurements will help us learn about the expected size and nutritional status of infants when born at term age.

Your Infant's Involvement after Discharge from Hospital

We also ask your permission to:

- measure your infants weight, length and head size at 6, 12 and 18 months of age;
- scan your baby's whole body, lower (lumbar) spine and thigh bone (femur) using a low dose x-ray technique called dual energy x-ray absorptiometry at the research facility immediately after hospital discharge and again at 6, 12 and 18 months corrected age;
- collect information from neurodevelopmental tests that we will conduct in the research facility when you make the last visit at 18 months. This test is a standard test used to assess infant development.

These measurements will help us learn about the expected rates of growth, bone growth and neurodevelopment in term born infants.

Your Own Involvement

We request your permission to take a blood sample (approximately 10 ml or 2 teaspoonfuls) from you prior to your discharge from hospital. We also ask if you would keep a three-day record of food intake at various intervals after you have been discharged. This will be reviewed with you approximately one week later by telephone call at a convenient time and should take no more than half an hour. We ask that we collect information from your hospital record about your pregnancy and medications. At the first visit after discharge we will request that we measure height of both parents and ask you some general questions about your education level, range of income and to indicate which group you identify with (First Nations, Filipino, Black, Asian, White).

You will decide on your own whether you wish to breast-feed or to feed your baby infant formula. If breast-feeding your baby, you will be asked to provide a sample of your breast milk at the end of the first, second, third and fourth weeks after your infant was born. The amount required is small (approximately 5 ml or 1 teaspoonful) and should not interfere with the normal feeding of your infant.

Both Your and Your Infant's Involvement

At the follow-up research visits, at your infant's discharge from hospital, 6, 12 and 18 months, we will ask you about the amounts and types of food that you and your infant consume to help us learn about nutrition and growth. If you are no longer breastfeeding or chose not to you do not have to record the foods you ate at these times. At 6, 12 and 18 months we will mail you a brief questionnaire to complete regarding your infant's development. This questionnaire takes a few minutes to complete and will be accompanied by a self-addressed stamped envelop in which to mail to the investigators.

Participation in the study will be from the time you join the study until your infant reaches 18 months corrected age.

The researcher may decide to take you off this study if you, or your infant, develop illnesses that affect growth, bone or neurodevelopment.

Risks and Discomforts

There are no risks associated with measurements of growth or collection of the urine, and breast milk samples. The physical risks associated with the scan of your infant's whole body, lumbar spine and femur are considered to be low. The device uses an x-ray source to measure bone density. However, the amount of radiation is much less than a regular chest x-ray and equivalent to the radiation accumulated during one normal day.

Benefits

There are no monetary benefits to you or others in this study. You will be provided a breast pump kit if you choose to breastfeed. The knowledge gained through this study will help us to improve nutritional care for infants who are born prematurely.

Costs

All laboratory tests which will be performed as part of this study are provided at no cost to you and your infant. There will be no cost for the study measurements that you participate in.

Payment for Participation

There will be no financial remuneration for participating in this study with exception of coverage of parking at the follow-up visits after discharge.

Confidentiality

Information gathered in this research study may be published or presented in public forums, however your name and your infant's name will not be used or revealed. Medical records that contain your identity will be treated as confidential in accordance with the Personal Health Information Act of Manitoba. Despite efforts to keep your personal information confidential, absolute confidentiality cannot be guaranteed. Your

personal information may be disclosed if required by law. Organizations that may inspect and/or copy your research records for quality assurance and data analysis include groups such as the Biomedical Research Ethics Board of the University of Manitoba.

Voluntary Participation/Withdrawal From the Study

Your decision to take part in this study is voluntary. You may refuse to participate or you may withdraw from the study at any time. Your decision to not participate or to withdraw from the study will not affect your or your infant's other medical care at this site.

Questions

You are free to ask any questions that you may have about your rights as a research participant. If any questions come up during or after the study, contact the study doctor and the study staff:

Dr. H. Weiler	Dr. M. Seshia
474-6798	787-1827

For questions about your rights as a research participant, you may contact The University of Manitoba's Biomedical Research Ethics Board at (204) 789-3389.

Do not sign this consent unless you have had a chance to ask questions and have received satisfactory answers to all of your questions.

Statement of Consent

I have read this consent form. I have had the opportunity to discuss this research study with either of Drs. Weiler and Seshia and or their study staff. I have had my questions answered by them in language I understand. The risks and benefits have been explained to me. I understand that I will be given a copy of this consent form after signing it. I understand that my participation in this clinical trial is voluntary and that I may choose to withdraw at any time. I freely agree to participate in this research study.

I understand that information regarding my personal identity will be kept confidential, but that confidentiality is not guaranteed. I authorize the inspection of my, and my infant's, medical records by the University of Manitoba's Biomedical Research Ethics Board.

By signing this consent form, I have not waived any of the legal rights that I have as a participant in a research study.

Participant signature:_____ Date:_____

Participant printed name:_____

I, the undersigned, attest that the information in the Participant Information and Consent Form was accurately explained to and apparently understood by the participant or the participant's legally acceptable representative and that consent to participate in this study was freely given by the participant or the participant's legally acceptable representative.

I, the undersigned, have fully explained the relevant details of this research study to the participant named above and believe that the participant has understood and has knowingly given their consent.

Printed Name:_____ Date:_____

Signature:_____

Role in the study:_____

Appendix C

UNIVERSITY
OF MANITOBA

FACULTY OF HUMAN ECOLOGY

Human Nutritional Sciences

Duff Robin Building
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(204) 474-6798
(204) 474-7593 FAX
Email:
hweiler@ms.umanitoba.ca

RESEARCH PARTICIPANT INFORMATION AND CONSENT FORM

Title of Study: Growth and bone mineralization in premature infants

Protocol Number: B2000:204

Principal Investigator: Dr. H. Weiler, RD, PhD
Co-Investigator: Dr. M.M. Seshia, MBChB; Dr. C.K. Yuen, MD, MBA;
Sponsor: University of Manitoba, Winnipeg, Manitoba, R3T 2N2

You are being asked to participate in a human research study. Please take your time to review this consent form and discuss any questions you may have with the study staff. You may take your time to make your decision about participating in this clinical trial and you may discuss it with your regular doctor, friends and family before you make your decision. This consent form may contain words that you do not understand. Please ask the study doctor or study staff to explain any words or information that you do not clearly understand.

Purpose of Study

This study is being conducted to study nutritional status and growth of infants. You are being asked to take part in this study because you are soon to or have just delivered a newborn infant. A total of 64 mother infant pairs will participate in this study.

The purpose is to study the growth patterns and bone development of babies that are born early (prematurely) and to examine if specific nutrients are linked to infant growth and bone development. The growth and bone development of these babies will also be compared with that of babies born at the expected due date (full-term). This research is being done because research suggests that growth in height, weight, and head size and the amount of bone mineral are different in babies born prematurely compared to those born at the expected term date. The nutrient of interest is arachidonic acid. This nutrient is normally made by the liver and is essential for growth. Thus this study will help to determine if prematurely born babies with greater amounts of arachidonic acid, as indicated in a small blood sample, experience improved growth and bone mineral content and will help to advance nutritional practices. In addition, the influence of growth over the first year of life on body size at one year of age will also be studied.

Study Procedures

Phase One: Since your baby was born at term, you and your baby are being asked to participate in the term phase of the study. You and your baby will be followed in this phase of study from the time of birth to the time of discharge from hospital and also in Phase Two – see below.

In order to participate in this study, you must not have a personal history of diabetes before or during your pregnancy and your doctor will determine whether or not any medications you are currently taking will interfere with the proposed measurements. Your baby will be examined at birth as part of routine clinical care for any medical conditions that may exclude him/her from participating in this study.

Phase Two: at discharge from hospital, 6 and 12 months thereafter, we ask that you and your infant visit our research facility to enable measurement of growth and bone as well as collect information on what you and your infant are eating. We would also like to measure your bones at the 12-month visit.

If you take part in this study, you will have the following tests and procedures:

Your Infant's Involvement

We request your permission to obtain the following samples from your baby: a sample of blood from the placenta (afterbirth) taken routinely at birth; measurement of your baby's length, weight, and head size and a urine sample before discharge from hospital.

We also ask your permission to scan your baby's whole body, lower (lumbar) spine and thigh bone (femur) using a low dose x-ray technique called dual energy x-ray absorptiometry at the research facility immediately after hospital discharge. For the second part of the study we wish to measure your baby's weight, length and head size as well as the same measurements on bone at 6 and 12 months. This will help us learn about the relationships among birth size, nutrition, growth and growth by one year after discharge from hospital.

Your Own Involvement

We request your permission to take a blood sample (approximately 10 ml or 2 teaspoonfuls) from you prior to your discharge from hospital. If breastfeeding your baby, you will be asked to keep a three-day record of food intake after you have been discharged. This will be reviewed with you approximately one week later at a convenient time over the telephone when you are at home. This should take no more than half an hour.

You will decide on your own whether you wish to breast-feed or to feed your baby infant formula. If you choose to breastfeed your infant, we ask for a sample of milk at the end of the first, second, third and fourth weeks after delivery with collection at 9:00 am and 9:00 pm (approximately 5 ml or 1 teaspoonful).

At the follow-up visits, at your infant's discharge from hospital, 6 and 12 months, we will ask you about the amounts and types of food that you and your infant consume to help us learn about nutrition and growth. In addition, we ask that at the 12 month visit that we measure your height, weight and bones using the same approach as for your infant. This will help us learn if maternal size and bone are also related to your infant's growth over the first year.

Participation in the study will be from the time you join the study until your infant reaches one year of age or one year corrected age.

The researcher may decide to take you off this study if you or your infant develop illnesses that affect growth and bone.

Risks and Discomforts

There are no risks associated with measurements of growth or collection of the urine, and breast milk samples. The sampling of blood from you and the placenta pose minimal or no risk with bruising a potential with sampling from you. The physical risks associated with the scan of you and your infant's whole body, lumbar spine and femur are considered to be low. The device uses an x-ray source to measure bone density. However, the amount of radiation is much less than a regular chest x-ray and equivalent to the radiation accumulated during one normal day.

Benefits

There are no monetary benefits to you or others in this study. You will be provided a breast pump kit if you choose to breastfeed. The knowledge gained through this study will help us to improve nutritional care for infants who are born prematurely.

Costs

All laboratory tests which will be performed as part of this study are provided at no cost to you and your infant. There will be no cost for the study measurements that you participate in.

Payment for Participation

There will be no financial remuneration for participating in this study with exception of coverage of parking at the follow-up visits at 6 and 12 months after discharge.

Confidentiality

Information gathered in this research study may be published or presented in public forums, however your name and your infant's name will not be used or revealed. Medical records that contain your identity will be treated as confidential in accordance with the Personal Health Information Act of Manitoba. Despite efforts to keep your personal information confidential, absolute confidentiality cannot be guaranteed. Your personal information may be disclosed if required by law. Organizations that may inspect and/or copy your research records for quality assurance and data analysis include groups such as the University of Manitoba Research Ethics Board.

Voluntary Participation/Withdrawal From the Study

Your decision to take part in this study is voluntary. You may refuse to participate or you may withdraw from the study at any time. Your decision to not participate or to withdraw from the study will not effect your other medical care at this site.

Questions

You are free to ask any questions that you may have about your rights as a research participant. If any questions come up during or after the study, contact the study doctor and the study staff:

Dr. H. Weiler	Dr. M. Seshia	Dr. K. Yuen
975-7716	787-1827	788-5710

For questions about your rights as a research participant, you may contact The University of Manitoba Faculty of Medicine Research Ethics Board at (204) 789-3389.

Do not sign this consent unless you have had a chance to ask questions and have received satisfactory answers to all of your questions.

Statement of Consent

I have read this consent form. I have had the opportunity to discuss this research study with either of Drs. Weiler, Seshia, Yuen and/or their study staff. I have had my questions answered by them in language I understand. The risks and benefits have been explained to me. I understand that I will be given a copy of this consent form after signing it. I understand that my participation in this clinical trial is voluntary and that I may choose to withdraw at any time. I freely agree to participate in this research study.

I understand that information regarding my personal identity will be kept confidential, but that confidentiality is not guaranteed. I authorize the inspection of my, and my infant's, medical records by the University of Manitoba and the University of Manitoba Research Ethics Board.

By signing this consent form, I have not waived any of the legal rights that I have as a participant in a research study.

Participant signature:_____ Date:_____

Participant printed name:_____

I, the undersigned, attest that the information in the Participant Information and Consent Form was accurately explained to and apparently understood by the participant or the participant's legally acceptable representative and that consent to participate in this study was freely given by the participant or the participant's legally acceptable representative.

I, the undersigned, have fully explained the relevant details of this research study to the participant named above and believe that the participant has understood and has knowingly given their consent.

Printed Name:_____ Date:_____

Signature:_____

Role in the study:_____

Appendix D

UNIVERSITY
OF MANITOBA

FACULTY OF HUMAN ECOLOGY

Human Nutritional Sciences

Duff Robin Building
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RESEARCH PARTICIPANT INFORMATION AND CONSENT FORM

Title of Study: Growth and bone mineralization in premature infants

Protocol Number: B2000:204

Principal Investigator: Dr. H. Weiler, RD, PhD
Co-Investigator: Dr. M.M. Seshia, MBChB; Dr. C.K. Yuen, MD, MBA;
Sponsor: University of Manitoba, Winnipeg, Manitoba, R3T 2N2

You are being asked to participate in a human research study. Please take your time to review this consent form and discuss any questions you may have with the study staff. You may take your time to make your decision about participating in this clinical trial and you may discuss it with your regular doctor, friends and family before you make your decision. This consent form may contain words that you do not understand. Please ask the study doctor or study staff to explain any words or information that you do not clearly understand.

Purpose of Study

This study is being conducted to study nutritional status and growth of infants. You are being asked to take part in this study because you are soon to or have just delivered a newborn infant. A total of 64 mother infant pairs will participate in this study.

The purpose is to study the growth patterns and bone development of babies that are born early (prematurely) and to examine if specific nutrients are linked to infant growth and bone development. The growth and bone development of these babies will also be compared with that of babies born at the expected due date (full-term). This research is being done because research suggests that growth in height, weight, and head size and the amount of bone mineral are different in babies born prematurely compared to those born at the expected term date. The nutrient of interest is arachidonic acid. This nutrient is normally made by the liver and is essential for growth. Thus this study will help to determine if prematurely born babies with greater amounts of arachidonic acid, as indicated in a small blood sample, experience improved growth and bone mineral content and will help to advance nutritional practices. In addition, the influence of growth over the first year of life on body size at one year of age will also be studied.

Study Procedures

Phase One: Since your baby was born early, you and your baby are being asked to participate in this research study as part of the preterm group of the study. The study will begin at the time of your baby's birth and will continue until he/she is discharged from the hospital or reaches the age he/she would have been had they been delivered at expected due date. Your infant will also be followed in Phase Two – see below.

In order to participate in this study, you must not have a personal history of diabetes before or during your pregnancy and your doctor will determine whether or not any medications you are currently taking will interfere with the proposed measurements. Your baby will be examined at birth as part of routine clinical care for any medical conditions that may exclude him/her from participating in this study.

Phase Two: at discharge from hospital, 6 and 12 months thereafter, we ask that you and your infant visit our research facility to enable measurement of growth and bone as well as collect information on what you and your infant are eating. We would also like to measure your bones at the 12-month visit.

If you take part in this study, you will have the following tests and procedures:

Your Infant's Involvement

We request your permission to obtain the following samples from your premature baby: a sample of blood from the placenta (afterbirth) taken routinely at birth; measurement of your baby's length, weight, and head size once a week starting from one week of age; a urine sample from your baby at birth, one, three and five weeks of age and at term. At one, three and five weeks of age and at term, a blood sample (approximately 0.5 ml or 1/10 a teaspoonful) from your baby will be collected at the same time as for routine monitoring.

We also ask your permission to scan your baby's whole body, lower (lumbar) spine and thigh bone (femur) using a low dose x-ray technique called dual energy x-ray absorptiometry at the research facility immediately after hospital discharge. For the second part of the study we wish to measure your baby's weight, length and head size as well as the same measurements on bone at 6 and 12 months. This will help us learn about the relationships among birth size, nutrition, growth and growth by one year after discharge from hospital.

Your Own Involvement

We request your permission to take a blood sample (approximately 10 ml or 2 teaspoonfuls) from you prior to your discharge from hospital. To help us learn about mother's diet and breast milk, you will be asked to keep a three-day record of food intake after you have been discharged. This will be reviewed with you approximately one week later at a convenient time, for example, while you are visiting your baby in hospital or over the telephone when you are at home. This should take no more than half an hour.

You will decide on your own whether you wish to breast-feed or to feed your baby infant formula. If breast-feeding your baby, you will be asked to provide a sample of your breast milk on two days of each week, to be collected at 9:00 a.m. and 9:00 p.m. by manual expression or with a breast pump, for nutrient analysis. The amount required is small (approximately 5 ml or 1 teaspoonful) and should not interfere with the normal feeding of your infant.

At the follow-up visits, at your infant's discharge from hospital, 6 and 12 months, we will ask you about the amounts and types of food that you and your infant consume to help us learn about nutrition and growth. In addition, we ask that at the 12 month visit that we measure your height, weight and bones using the same approach as for your infant. This will help us learn if maternal size and bone are also related to your infant's growth over the first year.

Participation in the study will be from the time you join the study until your infant reaches one year of age or one year corrected age.

The researcher may decide to take you off this study if you or your infant develop illnesses that affect growth and bone.

Risks and Discomforts

There are no risks associated with measurements of growth or collection of the urine, and breast milk samples. There is low risk associated with sampling of blood such as bruising but we will try to take blood when samples are taken for monitoring to limit the number of times blood is sampled. The sampling of blood from you and the placenta pose minimal or no risk with bruising a potential with sampling from you. The physical risks associated with the scan of you and your infant's whole body, lumbar spine and femur are considered to be low. The device uses an x-ray source to measure bone density. However, the amount of radiation is much less than a regular chest x-ray and equivalent to the radiation accumulated during one normal day.

Benefits

There are no monetary benefits to you or others in this study. You will be provided a breast pump kit if you choose to breastfeed. The knowledge gained through this study will help us to improve nutritional care for infants who are born prematurely.

Costs

All laboratory tests which will be performed as part of this study are provided at no cost to you and your infant. There will be no cost for the study measurements that you participate in.

Payment for Participation

There will be no financial remuneration for participating in this study with exception of coverage of parking at the follow-up visits at 6 and 12 months after discharge.

Confidentiality

Information gathered in this research study may be published or presented in public forums, however your name and your infant's name will not be used or revealed. Medical records that contain your identity will be treated as confidential in accordance with the Personal Health Information Act of Manitoba. Despite efforts to keep your personal information confidential, absolute confidentiality cannot be guaranteed. Your personal information may be disclosed if required by law. Organizations that may inspect and/or copy your research records for quality assurance and data analysis include groups such as the University of Manitoba Research Ethics Board.

Voluntary Participation/Withdrawal From the Study

Your decision to take part in this study is voluntary. You may refuse to participate or you may withdraw from the study at any time. Your decision to not participate or to withdraw from the study will not effect your other medical care at this site.

Questions

You are free to ask any questions that you may have about your rights as a research participant. If any questions come up during or after the study, contact the study doctor and the study staff:

Dr. H. Weiler	Dr. M. Seshia	Dr. K. Yuen
975-7716	787-1827	788-5710

For questions about your rights as a research participant, you may contact The University of Manitoba Faculty of Medicine Research Ethics Board at (204) 789-3389.

Do not sign this consent unless you have had a chance to ask questions and have received satisfactory answers to all of your questions.

Statement of Consent

I have read this consent form. I have had the opportunity to discuss this research study with either of Drs. Weiler, Seshia, Yuen and/or their study staff. I have had my questions answered by them in language I understand. The risks and benefits have been explained to me. I understand that I will be given a copy of this consent form after signing it. I understand that my participation in this clinical trial is voluntary and that I may choose to withdraw at any time. I freely agree to participate in this research study.

I understand that information regarding my personal identity will be kept confidential, but that confidentiality is not guaranteed. I authorize the inspection of my, and my infant's, medical records by the University of Manitoba and the University of Manitoba Research Ethics Board.

By signing this consent form, I have not waived any of the legal rights that I have as a participant in a research study.

Participant signature:_____ Date:_____

Participant printed name:_____

I, the undersigned, attest that the information in the Participant Information and Consent Form was accurately explained to and apparently understood by the participant or the participant's legally acceptable representative and that consent to participate in this study was freely given by the participant or the participant's legally acceptable representative.

I, the undersigned, have fully explained the relevant details of this research study to the participant named above and believe that the participant has understood and has knowingly given their consent.

Printed Name:_____ Date:_____

Signature:_____

Role in the study:_____

Appendix E

UNIVERSITY
OF MANITOBA

FACULTY OF HUMAN ECOLOGY

Human Nutritional Sciences

Duff Robin Building
Winnipeg, Manitoba
Canada RAT 2N2
(204) 474-6798
(204) 474-7593 FAX
Email:
hweiler@ms.umanitoba.ca

Addendum Term or Preterm Infants

Titles of Related Infant Studies:
B2000: 212 Does earlier introduction of feeds stimulate catch-up growth, bone mineralization and neurodevelopment in preterm infants.
B2000: 204 Growth and bone mineralization in premature infants.

Principal Investigator: Dr. H. Weiler, RD, PhD
Co-Investigators: Dr. M.M. Seshia, MBChB, Dr. C. K. Yuen, MD, MBA

Sponsor: University of Manitoba, Winnipeg, Manitoba, R3T 2N2

Purpose of Infant Studies
These studies are being conducted to study nutritional status, feeding regimens, growth, bone growth and neurodevelopment of infants. These studies only looked at measurements in mothers and infants.

Purpose of This Addendum: We wish to be able to combine the data collected for the above studies in order to create a larger database on infant nutrition and growth. As well, we also wish to learn of the contribution of parental size and bone mass relative to nutrient intake on infant growth and bone mass; thus we would also need to collect data on and measure the father as well. These strategies will help us answer questions about nutrition in general as related to growth of infants.

Mother's Involvement: We are asking for your permission to enter data already acquired for one of the above studies, into a larger database. This will enable us to answer global questions about the role of nutrition in growth, bone growth, and development.

Father's Involvement: Your infant and the infant's mother have already consented for one or both of these studies. We are now asking permission to collect some general information about you; i.e. height, weight, age etc. In addition we would like to measure your bone mass. To do this we would scan you using the same bone scanner we used to measure your infant. The purpose of this scan is to look at the relationship between parents size and bone mass with their infants bone growth and mineralization. This is done more effectively if we scan both parents. The scan is on your whole body (also measures body composition), lower (lumbar) spine, and hip using a low dose x-ray technique. The data will be entered into our database to answer general nutrition questions.

Risks and Discomforts: The physical risks associated with your bone scans are considered to be low. The device uses an x-ray source to measure bone density. However, the amount of radiation is much less than a regular chest x-ray and equivalent to the radiation accumulated during one normal day

Payment for Participation: There will be no financial remuneration for participating in this study with exception of coverage of parking, or reimbursed bus passes. Your scans can be conducted at any of the visits planned through the original study that your family is involved in and will take about 20 to 30 minutes.

Sometimes the research questions we answer suggest a need for continued follow up to look at longer-term outcomes. I agree to the following:

I am willing to be contacted in the future for follow up studies

☐ Yes ☐ No

In the event that I have moved and you are unable to contact me – you may contact:

_____

(Please print contact persons name & phone number)

Statement of Consent

I have read this consent form. I have had the opportunity to discuss this research study with either of Drs. Weiler and Seshia and/or their study staff. I have had my questions answered by them in language I understand. The risks and benefits have been explained to me. I understand that I will be given a copy of this consent form after signing it. I understand that my participation in this study is voluntary and that I may choose to withdraw at any time. I freely agree to participate in this research study. I understand that information regarding my personal identity will be kept confidential, but that confidentiality is not guaranteed. I authorize the inspection of my medical records by the University of Manitoba's Biomedical Research Ethics Board.

By signing this consent form, I have not waived any of the legal rights that I have as a participant in a research study.

Mother's signature: _____ Date: _____

Mother's printed name: _____

☐ I would like my bone scan information sent to my physician, Dr. _____

☐ Do not send my bone scan information to my physician.

Father's signature: _____ Date: _____

Father's printed name: _____

☐ I would like my bone scan information sent to my physician, Dr. _____

☐ Do not send my bone scan information to my physician.

I, the undersigned, attest that the information in the Participant Information and Consent Form was accurately explained to and apparently understood by the participant or the participant's legally acceptable representative and that consent to participate in this study was freely given by the participant or the participant's legally acceptable representative. I, the undersigned, have fully explained the relevant details of this research study to the participant named above and believe that the participant has understood and has knowingly given their consent.

Printed Name: _____ Date: _____

Signature: _____