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Plenary Lecture

Factors affecting newborn bone mineral content: *in utero* effects on newborn bone mineralization

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Several factors have been found recently to have a significant impact on newborn bone mineral content (BMC) and developing fetal bone. Recently we showed that maternal vitamin D deficiency may affect fetal bone mineralization. Korean winter-born newborn infants had extremely low serum 25-hydroxyvitamin D (25-OHD), high serum cross-linked carboxy-terminal telopeptide of type I collagen (ICTP; a bone resorption marker), and markedly lower (8 %) total body BMC than summer-born newborn infants. Infant total body BMC was positively correlated with cord serum 25-OHD and inversely correlated with ICTP, which was also negatively correlated with vitamin D status. In three separate studies on North American neonates we found markedly lower (8–12 %) BMC in summer newborn infants compared with winter newborn infants, the opposite of the findings for Korean neonates. The major reason for the conflicting BMC results might be the markedly different maternal vitamin D status of the North American and Korean subjects. Recently, we found evidence of decreased bone formation rates in infants who were small-for-gestational age (SGA) compared with infants who were appropriate-for-gestational age; we reported reduced BMC, cord serum osteocalcin (a marker of bone formation) and 1,25-dihydroxyvitamin D (the active metabolite of vitamin D), but no alterations in indices of fetal bone collagen metabolism. In theory, reduced utero–placental blood flow in SGA infants may result in reduced transplacental mineral supply and reduced fetal bone formation. Infants of diabetic mothers (IDM) have low BMC at birth, and infant BMC correlated inversely with poor control of diabetes in the mother, specifically first trimester maternal mean capillary blood glucose concentration, implying that factors early in pregnancy might have an effect on fetal BMC. The low BMC in IDM may be related to the decreased transplacental mineral transfer. Cord serum ICTP concentrations were higher in IDM than in control subjects, implying increased intrauterine bone resorption. BMC is consistently increased with increasing body weight and length in infants. Race and gender differences in BMC appear in early life, but not at birth. Ethanol consumption and smoking by the mother during pregnancy affect fetal skeletal development.

Bone mineral content: Fetal development: Vitamin D: Birth weight: Maternal diabetes

Fetal bone mineralization is affected by season of birth (Namgung *et al*., 1992, 1993, 1994, 1998), low weight relative to gestation (Minton *et al*. 1983; Petersen *et al*. 1989; Pohlandt & Mathers, 1989; Namgung *et al*. 1993) and diabetes in the mother (Mimouni *et al*. 1988). In theory, fetal bone mineralization is determined by placental mineral transfer, fetal bone mineral accretion and fetal bone resorption. Indices of fetal bone turnover appear to be affected by gestation (immaturity) and compromised fetal growth (Mora *et al*. 1997; Harrast & Kalkwarf, 1998; Ogueh

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et al. 1998). Since effects on fetal bone are often probably primarily brought about through the mother, any disturbance in maternal mineral metabolism (i.e. maternal nutrient deficiency or diseases) or conditions that affect placental mineral transfer could be factors affecting fetal bone mineralization. For example, we have shown recently that maternal vitamin D deficiency during pregnancy has a major impact on fetal bone mineralization (Namgung *et al.* 1998). In the present review factors associated with alterations in neonatal bone mineralization are summarized.

Season

Fetal bone metabolism, in theory, can be influenced by season through changes in vitamin D metabolism. Vitamin D is essential for mineral homeostasis and adequate bone mineralization. A lack of vitamin D during pregnancy may adversely affect maternal mineral metabolism, which in turn could affect the fetus, since normal fetal mineral accretion is thought to be derived from the maternal skeleton (Comar, 1956). Maternal vitamin D status may influence placental Ca transfer (Durand *et al.* 1983b); in rats, maternal administration of the synthetic analogue 1-α hydroxycholecalciferol (a highly bioactive form of vitamin D) resulted in increased fetal Ca content (Durand *et al.* 1983a). In the UK higher newborn cord serum parathyroid hormone (PTH) levels have been found in vitamin D-deficient Asian Indians than in white subjects, which is thought to be related to a reduction in placental Ca transport, leading to high fetal PTH levels (Okonofua *et al.* 1987).

Seasonal variations in maternal vitamin D status and their effects on the fetus have been reported. Earlier studies have shown seasonal variations in serum 25-hydroxyvitamin D (25-OHD; the major vitamin D metabolite in blood) concentrations in both pregnant women and their newborn infants, with low values in winter (Kuroda *et al.* 1981; Verity *et al.* 1981). Severe vitamin D deficiency in pregnancy can lead to intrauterine growth retardation (Brooke *et al.* 1980), adverse effects on the appearance of neonatal ossification centres (Specker *et al.* 1992) and congenital rickets (Moncrieff & Fadahunsi, 1974), confirming the potential impact of maternal vitamin D deficiency on fetal bone development.

The effect of maternal vitamin D deficiency on fetal bone mineralization (BMC) and metabolism (bone turnover markers) has not been studied previously. We suggested that seasonally-related changes in maternal vitamin D metabolism may affect fetal bone metabolism (Namgung *et al.* 1992, 1993, 1994, 1998). In vitamin D deficiency bone mineralization is low and bone resorption is thought to be high (Ooms *et al.* 1995; Scharla *et al.* 1996; Peacock *et al.* 1998). Thus, we suggested that BMC would be lower and bone turnover markers higher in winter-born infants than in summer-born infants, possibly related to increased bone turnover. We tested our hypothesis in four separate studies; one in Korean neonates (Namgung *et al.* 1998) and three in American neonates (Namgung *et al.* 1992, 1993, 1994).

In our recent study Korean winter newborn infants had markedly lower ($P = 0.0001$) serum 25-OHD levels (97 % below normal, < 11 ng/ml), significantly higher ($P = 0.0002$) concentrations of cross-linked carboxy-terminal telopeptide
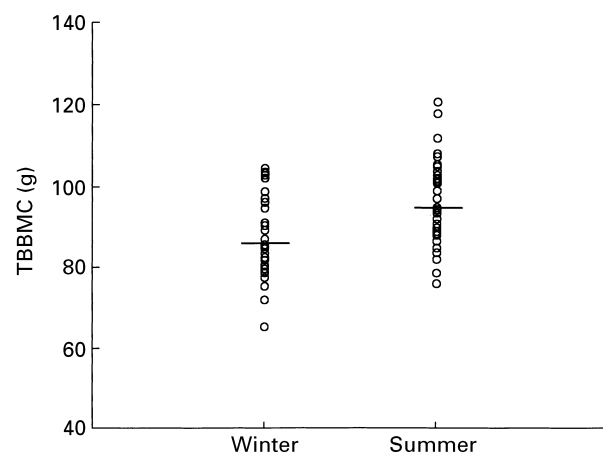
Fig. 1. Total body bone mineral content (TBBMC; g) of Korean newborn infants in winter and summer in a population without routine vitamin D supplementation. Mean value for TBBMC winter-born newborn infants was significantly lower than that for summer-born newborn infants: (*$P = 0.0002$). (Reproduced with permission from Namgung *et al.* 1998.)

of type I collagen (ICTP; a bone resorption marker), and markedly lower (8 %; $P = 0.0002$) total body BMC than summer newborn infants (Fig. 1). Infant total body BMC was positively correlated with cord serum 25-OHD (r 0.24, $P = 0.047$) and negatively correlated with ICTP (r −0.33, $P = 0.008$), which was also negatively correlated with vitamin D status (r −0.39, $P = 0.001$; Fig. 2; Namgung *et al.* 1998). The finding of low total body BMC in Korean winter newborn infants is presumably related to poor materno–fetal vitamin D status, and is consistent with bone mineral alterations (high bone resorption and low BMC) related to seasonally-induced changes in vitamin D status. The seasonal effect (8 % differential) on BMC is markedly greater than the seasonal difference (less than 2 %) in BMC or bone mineral density (BMD) of adults (Aitken *et al.* 1973; Krølner 1983; Bergstralh *et al.* 1990). In Korean winter newborn infants cord serum 1,25-dihydroxyvitamin D (1,25-$(OH)_2$D; the most bioactive form of vitamin D) concentrations were lower than those of summer newborn infants, and was correlated with 25-OHD. Production of 1,25-$(OH)_2$D is substrate (25-OHD)-dependent, and serum 1,25-$(OH)_2$D may be correlated with 25-OHD in vitamin D deficiency (Bouillon *et al.* 1987). Low 1,25-$(OH)_2$D levels in Korean winter newborn infants may reflect decreased synthesis in the fetal kidney associated with limited substrate (25-OHD) availability, and theoretically may be a factor affecting placental Ca transfer and fetal bone mineralization. Cord serum Ca concentration was significantly lower in winter than in summer, but cord serum P, Mg, and PTH concentrations were similar.

However, in three separate studies carried out with North American newborn infants we found markedly lower (8–12 %) BMC in summer newborn infants compared with winter newborn infants from Cincinnati, OH, USA, both for appropriate-for-gestational-age (AGA) infants (Fig. 3) and for small-for-gestational-age (SGA) infants (Fig. 4; Namgung *et al.* 1992, 1993, 1994), which is the opposite of the findings for Korean neonates, and for adults (Aitken
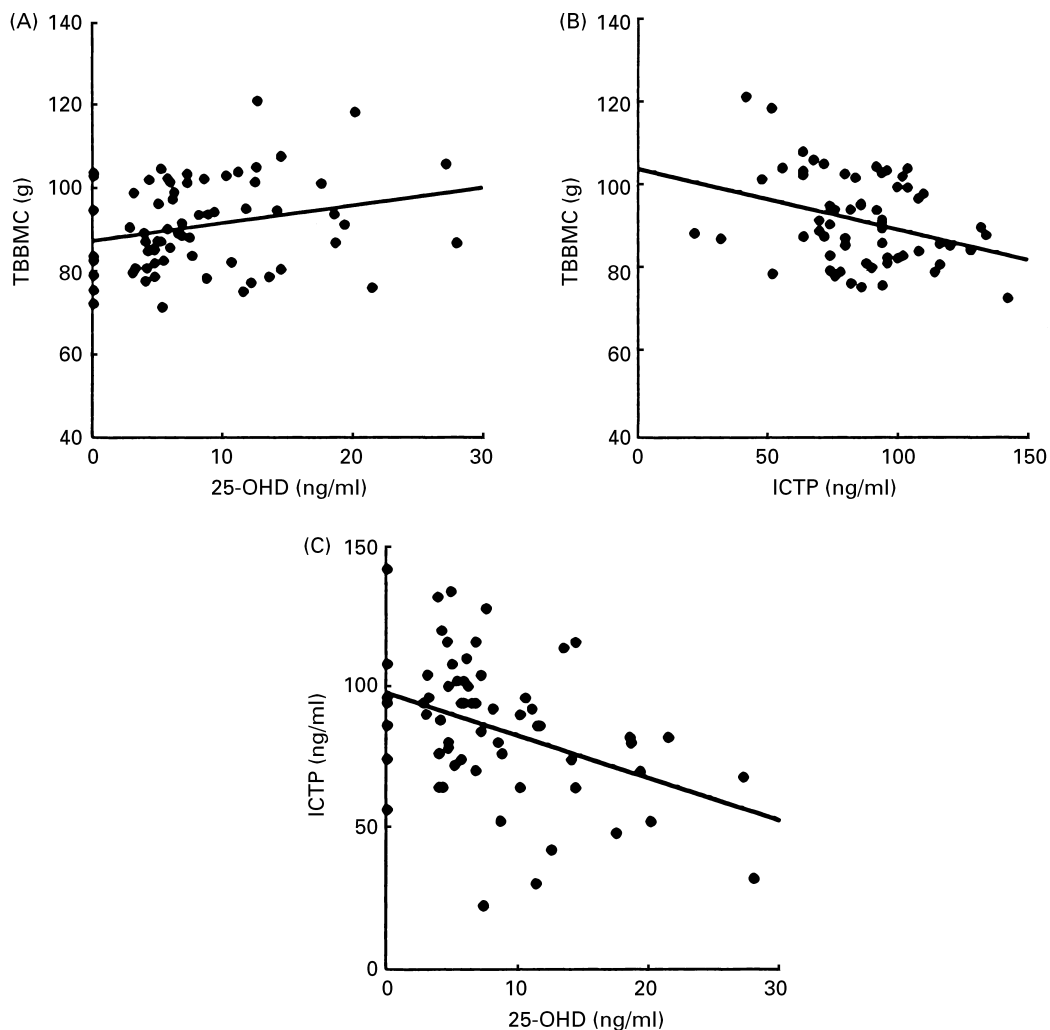
Fig. 2. (A) Relationship between total body bone mineral content (TBBMC) and cord serum 25-hydroxyvitamin D (25-OHD; ng/ml). TBBMC = 87·256 + 0·425 (25-OHD); r 0·24, P = 0·047. (B) Relationship between TBBMC and serum cross-linked carboxy-terminal telopeptide of type I collagen (ICTP; a bone resorption marker; ng/ml). TBBMC = 103·74 − 0·149 (ICTP); r −0·33, P = 0·008. (C) Relationship between serum ICTP and 25-OHD. ICTP = 98·3 − 1·5 (25-OHD); *r* −0·39, P = 0·001. (Reproduced with permission from Namgung *et al.* 1998.)

et al. 1973; Krølner *et al.* 1983; Bergstralh *et al.* 1990). American summer-born newborn infants had higher cord serum osteocalcin (a bone turnover marker) and 1,25-$(OH)_2$D concentrations than American winter-born newborn infants. Osteocalcin is the most abundant non-collagenous bone matrix protein, synthesized by osteoblasts, and reflects osteoblastic activity. Theoretically, the high serum osteocalcin concentrations in summer newborn infants may be an adaptive response to the lower BMC of these infants, possibly secondary to the raised plasma 1,25-$(OH)_2$D concentrations. Cord serum Ca concentration was lower in summer than in winter, but P, Mg, ionized Ca, PTH and 25-OHD concentrations were similar.

Based on these findings in North American and Korean neonates, we suggested that reduced bone mass in North American summer-born newborn infants is related to reduced maternal vitamin D status in the preceding winter, i.e. 6 months before summer birth. We speculated that sunshine deprivation in winter alters vitamin D metabolism

in the mother and fetus during an important period of early fetal skeletal development, resulting in low BMC at birth 6 months later.

The major reason for this discrepancy (conflicting BMC results in American neonates *v.* Korean neonates) might be the markedly different vitamin D status in North America compared with Korea. During the first trimester of pregnancy most women in the USA have not received much prenatal care and are less likely to be taking multivitamin pills that contain vitamin D. Thus, the mother is relatively 'unprotected' (i.e. not given vitamin D supplements), and seasonal changes in vitamin D status can occur. We suggest that these changes in seasonal vitamin D status result in first-trimester bone alterations in winter that are evident 6 months later as reduced bone mass at birth in summer compared with winter. After the first trimester most women receive vitamin D supplements. At the time of delivery in North America there are no differences in serum vitamin D concentrations according to season, presumably because of vitamin D supplementation (Namgung *et al.* 1994). In
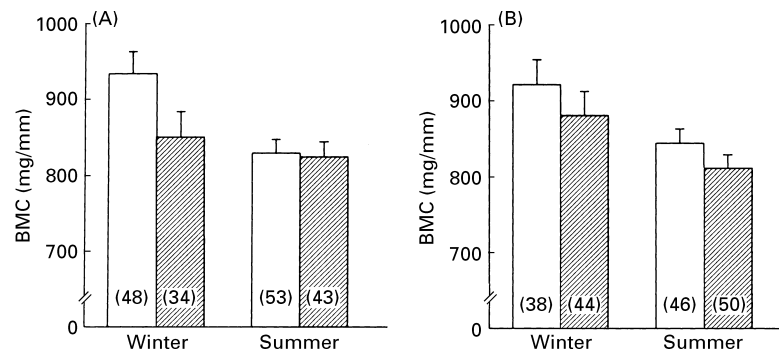
Fig. 3. Bone mineral content (BMC; mg/mm) of newborn infants in winter and summer by (A) race ((□), white; (▨), black) and (B) gender ((□), male; (▨), female) in a population where vitamin D fortification is practiced (Cincinnati, OH, USA). Values are least square means adjusted for birth weight with their standard errors represented by vertical bars for nos. of newborn infants shown in parentheses. Summer-born infants had significantly lower BMC than winter-born infants ($P = 0.009$). Race and gender differences in BMC corresponded to birth weight differences; after adjusting for birth weight, race and gender, differences were $P = 0.09$ and $P = 0.17$ respectively. Seasonal differences in BMC remained significant after adjusting for race and gender effects ($P = 0.002$ and $P = 0.001$ respectively). (Reproduced with permission from Namgung *et al.* 1994.)
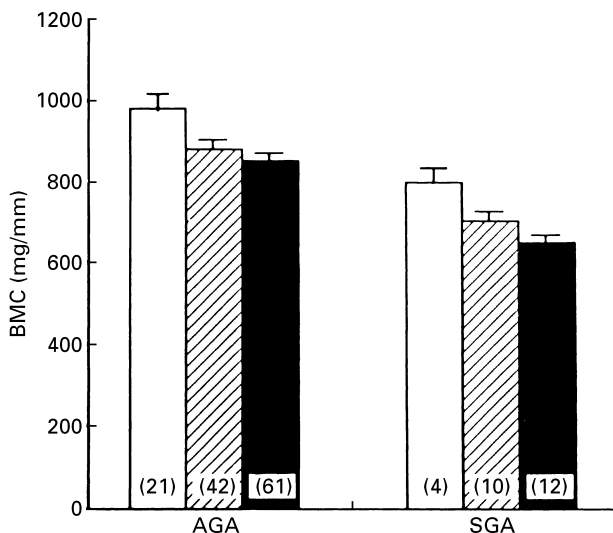
Fig. 4. Bone mineral content (BMC; mg/mm) in newborn infants for small-for-gestational-age (SGA) *v.* appropriate-for-gestational-age (AGA) infants by season of birth ((□), winter; (▨), spring; (■), summer) in Cincinnati, OH, USA. The BMC was significantly lower for SGA than for AGA infants ($P = 0.0001$), and differed by season of birth; spring or summer BMC values were lower than winter values in both SGA and AGA infants ($P = 0.003$). The BMC differences between groups remained significant after adjusting for season ($P = 0.0001$). Values are means with their standard errors represented by vertical bars for nos. of infants shown in parentheses. (Reproduced with permission from Namgung *et al.* 1993.)

contrast, Koreans are often vitamin D deficient throughout pregnancy, and there is little vitamin D supplementation in practice. Korean winter-born neonates had cord serum 25-OHD concentrations in the vitamin D-deficient range (4·3 (SD 3) ng/ml). Of winter-born neonates, 97 % have subnormal vitamin D levels, and thus significant negative effects on fetal bone in winter might be expected. The period of greatest vulnerability of the fetus to altered maternal vitamin D status, if any, is unclear. However, from our studies it could happen either in the early and/or late trimester of pregnancy, depending on the maternal vitamin D status.

In adults the BMC of forearm and BMD of lumbar spine (Aitken *et al.* 1973; Krølner *et al.* 1983; Bergstralh *et al.* 1990) differ according to the season, and are related to seasonal variations in vitamin D status (Dawson-Hughes *et al.* 1991; Sherman *et al.* 1992; Ooms *et al.* 1995). These seasonal differences are, however, much lower (< 2 %) than those (8–12 %) we have found in newborn infants (Namgung *et al.* 1992, 1993, 1994, 1998). Suboptimal vitamin D status in the elderly is associated with high serum PTH concentrations (Orwoll & Meier, 1986; Lukert *et al.* 1987; Dawson-Hughes *et al.* 1991; Ooms *et al.* 1995), osteopenia (Orwoll & Meier, 1986; Fonsca *et al.* 1988), and low BMD of radius or hip (Sherman *et al.* 1992; Ooms *et al.* 1995). In elderly women (> 60 years) in North America (Peacock *et al.* 1998) and in post-menopausal women in Germany (Scharla *et al.* 1996) serum 25-OHD levels were negatively correlated with urinary deoxypyridinoline excretion (an index of bone resorption), and positively related to BMD of the proximal femur, consistent with our findings in Korean neonates. Biochemical and hormonal markers of bone metabolism vary significantly ($P < 0.05$) according to the season, consistent with an acceleration of bone turnover and possible subclinical vitamin D deficiency in winter (Woitge *et al.* 1998). In young women a reduction in serum 25-OHD was associated with decreased intestinal Ca and P absorption during wintertime, but was not associated with the changes in bone turnover markers (serum carboxy-terminal propeptide of type I procollagen (PICP) and urinary deoxypyridinoline; Zittermann *et al.* 1998).

Small-for-gestational age

Low weight relative to gestation (SGA or intrauterine growth retardation) could be a factor influencing fetal bone metabolism, possibly through alterations in placental mineral transfer. In a previous study from our group using *in situ* perfused placentas of intrauterine growth-retarded rat fetuses (modified Wigglesworth model in the rat; Wigglesworth, 1964; Kollee *et al.* 1979), materno–fetal Ca transfer was reduced across the placentas of growth-retarded

v. control fetuses, and the mean total Ca content of growth-retarded fetuses was significantly lower than that of control fetuses (Mughal *et al.* 1989). Presumably the reduced placental blood supply that causes experimental fetal growth retardation also causes reduced placental Ca transport proportional to the reduction in body size. In an earlier study from our group (Minton *et al.* 1983) we found that the BMC of the distal radius (measured using single-photon absorptiometry) was significantly lower ($P < 0.01$) and serum PTH concentrations were higher ($P < 0.01$) for term SGA infants than for term AGA infants at birth. We suggested that the SGA fetus responds to possible *in utero* Ca deficiency by increasing the secretion of PTH.

In a recent study (Namgung *et al.* 1993) we found evidence of decreased bone formation, low BMC and low serum osteocalcin concentrations, and low serum 1,25-$(OH)_2D$ concentrations in term SGA infants compared with term AGA infants. There were no differences in cord serum Ca, P, Mg, 25-OHD and PTH concentrations between SGA and AGA infants; BMC was inversely correlated with cord serum PTH (r −0·228, $P = 0.006$). We suggested that low 1,25-$(OH)_2D$ in SGA infants might be related to a reduction in fetal–placental production of 1,25-$(OH)_2D$, possibly due to reduced utero–placental blood flow in SGA infants, resulting also in low serum osteocalcin (an index of bone formation) and low BMC. Fetal serum PTH values may be relatively increased because of a reduction in placental mineral supply. The finding of low cord serum osteocalcin in SGA *v*. AGA infants supports the concept that decreased fetal bone formation may lead to reduced BMC in SGA infants.

Chunga Vega *et al.* (1996) have recently examined whether low cord serum Zn concentrations in SGA infants are associated with fetal growth retardation and bone mineral alteration, related to low 1,25-$(OH)_2D$. They reported that BMD, measured by dual-energy X-ray absorptiometry, was lower (by 20 %) in term SGA *v*. AGA infants, and linearly related to growth and nutritional measures (weight, length, head circumference, mid-arm circumference and surface area). Cord serum Zn concentrations were in the normal range and similar for SGA and AGA infants. Cord serum PTH, 25-OHD, 1,25-$(OH)_2D$, osteocalcin, total Ca and inorganic P concentrations were not different between groups. They speculated that low BMD in SGA infants could be caused by growth and nutritional deficiencies, possibly related to a reduction in transplacental nutrient substrate supply to the fetus.

We questioned whether alterations in bone collagen type I metabolism could be a cause of low BMC in SGA infants. In theory, conditions that affect fetal growth can lead to changes in bone collagen type I metabolism. Type I collagen, the major collagen product synthesized by bone cells, is the only collagen type found in mineralized bone (Burgeson, 1988). Serum PICP and ICTP are markers of bone collagen type I biosynthesis and degradation respectively (Parfitt *et al.* 1987; Eriksen *et al.* 1993).

Thus, we tested the hypothesis that serum PICP would be low and serum ICTP would be high in term SGA infants compared with term AGA infants, based on the *in vitro* data of a regulatory role of insulin-like growth factor in bone collagen type I metabolism. In comparisons

of SGA infants (Thieriot-Prevost *et al.* 1988; Lassarre *et al.* 1991) and growth-retarded animals with controls (Bernstein *et al.* 1991; Unterman *et al.* 1993), serum insulin-like growth factor-I concentrations were reduced by approximately 30–50 %; insulin-like growth factor-I stimulates type I collagen synthesis and decreases collagen degradation. In SGA *v*. AGA infants serum markers of bone collagen type I synthesis (PICP) and degradation (ICTP) were not different; serum ICTP was correlated with osteocalcin (r 0·596, $P = 0.007$) and with PICP (r 0·663, $P = 0.002$) in SGA infants but not in AGA infants. These findings are not consistent with the theory that altered fetal bone collagen type I formation or breakdown causes the low BMC of SGA infants. Thus, we concluded that the low BMC of SGA *v*. AGA infants may be predominantly related to low mineral supply rather than defective regulation of bone collagen type I metabolism (Namgung *et al.* 1996).

In contrast to our findings in term SGA infants, a recent report from our centre showed that amniotic fluid PICP concentrations were low in SGA infants compared with controls (AGA infants; $P = 0.07$), both PICP and ICTP were inversely associated with gestational age ($P < 0.0001$), and amniotic fluid concentrations of PICP increased exponentially in relation to infant birth weight ($P = 0.008$). These findings are consistent with a high bone turnover early in fetal life, and a reduction in bone formation when fetal growth is compromised (Harrast & Kalkwarf, 1998). These findings are also consistent with the concept of decreased bone formation in SGA infants beginning *in utero*, and add to our finding of low cord serum osteocalcin in term SGA *v*. AGA infants. An alternative explanation is that gestational age (and thus maturity) may affect clearance of bone markers in blood, and thus affect blood concentrations. It is also possible that fetal growth retardation *per se* has an effect on maturation of clearance pathways.

Infants of diabetic mothers

Maternal diabetes could be a factor influencing fetal bone metabolism, through alterations in maternal mineral metabolism or limited mineral availability in a pregnancy where the mother is diabetic. In a diabetic pregnant rat model fetuses of diabetic rats have been reported to have decreased mineralization, as measured by the number of ossification centres (delay in bone maturation) and total ash weight. Ca and P content per fetus, and Ca and P content per unit fetal body weight (Verhaeghe *et al.* 1986, 1988, 1999; Demignon & Robut-Bonneton, 1988; Husain *et al.* 1994).

The pathogenesis of fetal hypomineralization in a pregnancy where the mother is diabetic remains uncertain. Maternal–fetal Ca transport is decreased in perfused placentas from diabetic rats; placental unidirectional materno–fetal flux of Ca is reduced (by 61 % of the control value) in untreated diabetic rats compared with control or insulin-treated diabetic rats. Fetal Ca content was also significantly lower in the untreated diabetic rats compared with the other two groups. Since placental calbindin 9K:cyclophilin mRNA was 11- to 12-fold lower in the untreated diabetic rats, it appears that untreated maternal diabetes mellitus reduces fetal Ca accretion through an effect on the expression of placental transport components

involved in the materno–fetal transfer of Ca (Husain *et al.* 1994).

Using spontaneously-diabetic rats fed on a 10 g Ca/kg diet (a standard rat chow; Trouw, Ghent, Belgium), Verhaeghe *et al.* (1999) recently showed that fetuses of diabetic rats had no mineralization defect (as measured by Ca content per unit body weight, although the mean Ca content was lower in fetuses of diabetic rats *v.* control), caused by disturbed maternal duodenal Ca absorption or transplacental Ca transport. However, they did report a delay in bone maturation. This finding is not consistent with previous findings from the same authors using diabetic BB rats (Verhaeghe *et al.* 1988). The difference between these reports might be related to differences in litter size, since impairment in mineralization was most pronounced in the large litters in the earlier study (Verhaeghe *et al.* 1988).

Decreased BMC has been reported in diabetic patients (Levine *et al.* 1976), and might be related to insulin deficiency, since insulin therapy improves BMC in juvenile diabetes. Insulin has been shown to stimulate proliferation of osteoblast-like cells *in vitro*, and to increase histomorphometric indices of bone formation *in vivo* (Cornish *et al.* 1996). Neonatal hypocalcaemia and neonatal hypomagnesaemia occur frequently in infants born of mothers with insulin-dependent diabetes; the incidence and severity of hypocalcaemia are directly related to the severity of maternal diabetes, and related to the urinary Mg losses in diabetes (Tsang *et al.* 1972).

In an earlier study from our group (Mimouni *et al.* 1988), we suggested that infants of diabetic mothers (IDM) may have decreased BMC at birth, associated with poor control of diabetes during pregnancy. Since elevation of 1,25-$(OH)_2$D concentrations has been reported in IDM (Steichen *et al.* 1981), raised 1,25-$(OH)_2$D may be an adaptive response to the possible poor transplacental mineral supply. BMC was measured at birth (by single-photon absorptiometry) in forty-five infants born of diabetic mothers (class B-RT; 38·2 (1SE 0·2) weeks; body weight percentile 77·4 %), and in fifty-five normal term newborn infants (38·1 (1SE 0·4) weeks; body weight percentile 53·2 %) born of non-diabetic mothers. The birth weight percentiles were significantly higher ($P < 0.01$) in IDM *v.* control infants. BMC at birth was significantly decreased ($P < 0.001$) in IDM compared with control infants. BMC was inversely correlated with first trimester maternal mean blood glucose concentration (r −0·664, $P < 0.01$; Fig. 5), implying that factors early in pregnancy might have an effect on fetal BMC (Mimouni *et al.* 1988). The low BMC in IDM (despite greater body weight) may be related to the decreased mineral transfer across the placenta in early pregnancy, possibly secondary to possible decreased mineral availability in a pregnancy where the mother is diabetic.

In contrast to the earlier findings in IDM, the changes in whole-body BMC in IDM, recently measured using dual-energy X-ray absorptiometry, has been reported as variable. Lapillonne *et al.* (1997b) recently reported that in IDM, the whole-body BMC Z score when adjusted for weight of each infant at the time of dual-energy X-ray absorptiometry measurement (whole-body BMC Z score/W, where W is weight) was positive (1·3 (SD 0·9)) and significantly
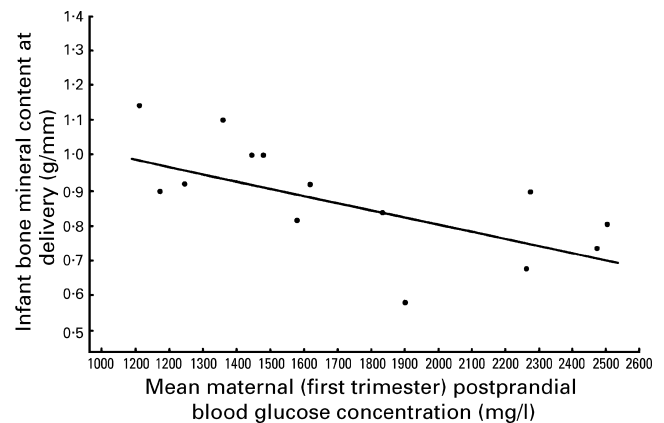
Fig. 5. Neonatal bone mineral content *v.* mean maternal first trimester postprandial blood glucose concentration for infants and their diabetic mothers. There was a significant inverse correlation between bone mineral content and maternal blood glucose concentration (r −0·66, $P < 0.01$). (Reproduced with permission from Mimouni *et al.* 1998.)

different from zero ($P < 0.0001$), but was not influenced either by *in utero* growth (AGA or large-for-gestational age) or by the type of diabetes mellitus of the mother. The reference values for term infants used by Lapillonne *et al.* (1997b) were from different studies (Lapillonne *et al.* 1997a). The difference in these findings might be related to variations in maternal diabetes control, variability in the population studied and lack of an adequate control population. Recent reports on outcomes of pregnancies where the mother was diabetic have documented improved control of the diabetes compared with earlier studies (Mehta *et al.* 1998). The inverse relationship between infant BMC and maternal blood glucose concentration reported by Mimouni *et al.* (1988) supports this conclusion, since if maternal blood glucose control improves with time, infant BMC values in IDM should rise also.

The mechanism for BMC changes in IDM is not clear. A study from our group (Demarini *et al.* 1995) found evidence of increased intrauterine bone resorption in term infants born of mothers with insulin-dependent diabetes (class B-RT). Cord serum ICTP concentrations (a marker of bone resorption) were higher in IDM than in control subjects, supporting the concept of increased osteoclastic activity in IDM. IDM had significantly higher birth weight than the controls. Serum PICP concentration (a marker of bone formation) was not different between groups.

Recently, our centre reported that diabetes (insulin-dependent and gestational) in the mother was not associated with alterations in amniotic fluid PICP and ICTP concentrations; there was no relationship between either of the bone markers in amniotic fluid and maternal diabetes (Harrast & Kalkwarf, 1998). The physiological significance of chemical substances found in amniotic fluid is often unclear, and make comparisons with cord blood studies difficult. A recent report indicates that urinary excretion of N-terminal telopeptide of type I collagen (a bone resorption marker) during the first 48 h in seventeen IDM were comparable with those of control subjects (Mora *et al.*

1997). This finding could also be due to improved metabolic control of diabetes during pregnancy; all mothers in the study had values for blood glycated haemoglobin of < 8 % total haemoglobin. It appears that osteopenia in IDM and factors promoting bone resorption may be ameliorated with improved diabetes management in pregnancy.

Other factors: anthropometric measures, race, gender and maternal ethanol consumption and smoking

BMC is consistently found to increase with increasing body weight and length in infants and children (Li *et al*. 1989; Rupich *et al*. 1996). Race and gender differences in BMC appear in early life, but probably not at birth. In one study a higher total body bone mass was observed in male infants than in female infants aged 1–18 months, even after controlling for weight, length and race (Rupich *et al*. 1996). Race differences appear by 1 year of age, with black infants having greater BMC than white infants (Li *et al*. 1989). No gender or race differences in BMC were observed in term infants at birth (Steichen *et al*. 1976; Namgung *et al*. 1993). Both decreased urinary Ca excretion and increased intestinal Ca absorption have been found in older black children compared with white children, possibly providing a mechanism for race differences (Abrams *et al*. 1995); no data are available in infants.

Maternal ethanol consumption can have deleterious effects on both adult and developing bone. Pregnant rats fed on a liquid diet with ethanol showed low Ca content of maternal bone, low maternal blood ionized Ca concentration and elevated PTH compared with rats fed on diets without ethanol (pair-fed control). Mean fetal body weight and fetal skeletal ossification were reduced in the ethanol-fed rats compared with controls, but no group differences were found in fetal Ca content (Keiver *et al*. 1996). Chronic alcohol consumption in young actively-growing (4-week-old) rats during the age of bone development reduced bone volume and trabecular number in cancellous bone. The rate of bone formation is reduced in alcohol-fed animals, indicating a reduced osteoblastic activity (Sampson *et al*. 1996; Sampson, 1998).

The effects of ethanol on fetal bone development do not appear to be related to alterations in fetal rat serum levels of calciotropic hormones, as shown by the finding of no differences in fetal rat serum levels of PTH and calcitonin between ethanol-fed and control groups (Keiver *et al*. 1997). There might be interactive effects of ethanol intake and maternal nutritional status, since increasing the protein content of the alcohol-containing diet from 180 to 250 g/kg significantly increased ossification of fetal bone in rat (Weinberg *et al*. 1990). Comparable studies in human infants have not been carried out. The effect of alcohol on bone may be related to a more direct effect of alcohol on bone cells (Sampson *et al*. 1998).

Smoking by the mother is detrimental to the developing fetus. In rats there is a dose-related retardation in embryonic growth, and also a reduction in the number of skeletal ossification centres (developmental delay; Seller & Bnait, 1995). There is evidence of a long-term negative association between maternal smoking during pregnancy and both growth and bone mass in children born at term (Jones *et al*.

1999). Smoking by the mother during pregnancy is associated with deficits in growth in 8-year-old children (lower weight and height), and disproportionate deficit in bone mass; children whose mothers smoked during pregnancy had lower size-adjusted bone mass at the lumbar spine and femoral neck but not total body. This association was only present for children born at term. Mothers who smoked during pregnancy also had lower placental weight, and further adjustment for placental weight led to non-significant results for smoking in both growth and bone variables, suggesting that these associations may be mediated through placental size and function.

Tobacco smoking affects the feto–placental unit; carboxyhaemoglobinaemia and chronic hypoxaemia, vasoconstriction of utero–placental circulation and inter-mediary metabolism disturbance are the aetiopathogenetic base of intrauterine fetal growth retardation, i.e. the fetal smoking syndrome (Habek, 1998). Oxidant stress from increased free radicals produced by smoking may have some role in the adverse effects on the skeleton of subjects who smoke, possibly related to alterations in bone resorption (Melhus *et al*. 1999).

In full-term newborn infants of mothers who smoked during pregnancy, smoking had a clear dose-dependent negative effect on all anthropometric measures in infants (Zaren *et al*. 1996). Newborn infants born of non-smoking but exposed mothers (passive smoking) or mothers who were light smokers (less than ten cigarettes per d) showed a significant reduction in fat mass and most anthropometric measurements (birth weight, crown–heel length and skin-folds). Intrauterine growth seems to be negatively influenced not only by active smoking, but also by passive and light active smoking (Luciano *et al*. 1998). The possible effect on fetal bone development has not been examined in this situation.

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