Perinatal Bone Turnover in Term Human Neonates and the Influence of Maternal Smoking

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ABSTRACT

Bone turnover in neonates appears independently of the comparably low maternal bone turnover, but there is only sparse information on the effect of the *in utero* environment on fetal bone turnover. Postnatally, the resuming growth velocity and alterations in mineral homeostasis affect neonatal bone turnover. This study evaluated the relationship of bone marker concentrations to maternal and fetal auxological variables as well as maternal smoking and assessed the short-term change in bone markers during the first days of life. Serum markers of bone formation [osteocalcin and bone-specific alkaline phosphatase (BALP)] and bone resorption (C-terminal telopeptide of type I collagen) were measured in cord blood and at discharge (median d 3) in 69 healthy term neonates. Concentrations of BALP were significantly lower in neonates of smokers ($n = 16$) compared with nonsmokers ($n = 53$), both at birth ($p = 0.013$) and at discharge ($p = 0.036$). Both cord osteocalcin and BALP were negatively related to maternal weight and maternal body mass index. Maternal smoking and pregnancy weight gain were the predictors of cord BALP ($r^2 = 0.24$; $p < 0.001$), whereas the mode of delivery best predicted cord C-terminal telopeptide of type I collagen levels ($r^2 = 0.19$; $p < 0.001$). C-terminal telopeptide of type I collagen and osteocalcin increased signifi-

cantly ($p < 0.001$) from birth to discharge, whereas BALP levels did not change significantly during the same period. Our results suggest that maternal smoking during pregnancy and maternal obesity may have a negative impact on fetal bone formation. The significant increase of osteocalcin and C-terminal telopeptide of type I collagen may result either from an increase in bone turnover or altered renal clearance. (*Pediatr Res* 53: 817–822, 2003)

Abbreviations

BALP, bone-specific alkaline phosphatase
CTX, cross-linked carboxy-terminal telopeptide of type I collagen (crossLaps)
OC, osteocalcin
ALP, alkaline phosphatase
AGA, appropriate for gestational age
SGA, small for gestational age
BMI, body mass index
BMC, bone mineral content
CV, coefficient of variation
PGH, placental GH

Linear growth in the fetus is high during the second trimester of pregnancy, but drops sharply after the 30th wk of gestation (1). Data obtained from a large cohort of neonates from a Swiss study (2) show that fetal length and weight plateau at approximately 37 wk of gestation, whereas fetal bone mineralization and ossification increase toward the end of pregnancy. This shift from fetal bone growth to maintenance is also evidenced by inverse correlations between gestational age and bone turnover markers in cord blood (3–8) or amniotic fluid (9).

Postnatally, the first 6 to 12 d represent a transitional growth period. This is characterized by large individual variations (2)

in body weight and hydration state that complicate the assessment of the postnatal growth pattern. Because of the large measurement errors during this period, reliable growth data are sparse and inconsistent for the first days of life. However, along with the postnatal resumption in growth velocity and the concurrent alterations in mineral homeostasis (10), one would expect a parallel increase in bone turnover.

Data on physiologic changes in bone metabolism and growth during the early postnatal period are very sparse. Most studies on this topic were performed in preterm infants, to evaluate osteopenia of prematurity, nutritional status, and growth in different cohorts. All these studies revealed an increase in bone turnover after delivery (6, 8, 11–17). However, preterm babies miss the phase of peak bone mineral accretion during the last trimester, and postnatal life in these infants is often complicated by serious illness. Thus, their bone turnover is not

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necessarily comparable to term neonates as it is not likely to reflect physiologic conditions.

There is very little information on maternal or fetal factors influencing bone formation and resorption in neonates. The *in utero* environment may, however, be a particularly critical variable for the growth and bone health of the fetus. A recent study found reduced BMC in neonates of mothers who smoked during pregnancy (18).

The aim of this study was 1) to investigate the relationship between selected bone markers and maternal and fetal auxological and clinical variables, especially maternal smoking, and 2) to assess the postnatal change of these markers during the first days of life in healthy term babies.

METHODS

Subjects

All term (37–42 wk of gestation) babies admitted to the neonatal ward during a 2-mo period were eligible to participate in the study. Newborns of mothers known to suffer from any disease likely to affect bone health or calcium metabolism (including hyperparathyroidism, renal failure, chronic diseases requiring corticosteroid treatment, gestational diabetes, thyroid dysfunction) were excluded from the study. Neonates with an abnormal perinatal or postnatal clinical course, *e.g.* suspected muscular, neurologic, or bone disease, with identifiable genetic syndromes and major congenital malformations were also excluded. All neonates were fed on breast milk, with an energy supplement (Aptamil Primergen; Milupa, Friedrichsdorf, Germany) added if the colostrum supply was inadequate during the early neonatal period. Babies were enrolled in the study only after written informed parental consent was obtained. The study protocol was approved by the local ethics committee.

Design

Age, weight, and height of the mother at delivery, as well as weight gain during pregnancy, were recorded. The number of cigarettes smoked and the timing of smoking during the pregnancy were documented. Cord blood samples were obtained at the time of birth in all term neonates fulfilling the inclusion criteria of the study. The infants' birth weight was measured with an electronic scale and supine length with a neonatal measuring board. Gestational age and Apgar scores were recorded, as well as placental weight, arterial cord blood pH, and the mode of delivery. Weight of the neonate was measured again at discharge from the hospital.

We analyzed serum markers of bone formation (intact OC and BALP as exclusive markers of osteoblasts) and bone resorption (CTX) in cord blood and on the day of discharge from hospital. Discharge blood samples were taken at routine blood sampling for neonatal screening for inborn errors of metabolism and included the measurement of serum creatinine. Each sample of whole blood (1 mL) was centrifuged to obtain serum, which was aliquoted and immediately frozen at -80°C within 1 h after sampling, and stored until the assays were run. This procedure was necessary to avoid the rapid breakdown of

the intact OC molecule at room temperature (19). All samples were analyzed in duplicate concurrently.

Assays

BALP assay. BALP serum levels were measured by a solid-phase, two-site immunoradiometric assay (Tandem-R-Ostase; Hybritech Inc, San Diego, CA, U.S.A.) based on two MABs. The intra- and interassay CVs were 6.4 and 4.6%, respectively.

OC assay. Intact OC was measured by a two-site immunoradiometric assay (Active Human Osteocalcin IRMA; Diagnostic Systems Laboratories, Sinsheim, Germany). Intra- and interassay CVs were 4.6 and 6.0%, respectively.

CTX assay. CTX was measured by ELISA (CrossLaps One Step ELISA; Osteometer Biotech, Herlev, Denmark). This assay uses a polyclonal antiserum raised against an immobilized synthetic peptide with an amino acid sequence (EKAH-DGGR) specific for part of the carboxy-terminal telopeptide of the $\alpha_1(\text{I})$ collagen chain (20). Intra- and interassay CVs were 5.4 and 7.1%, respectively.

Further Validation

Kit controls, serum from a cord blood pool ($n = 5$) and a newborn blood pool ($n = 5$) were tested for accuracy. Mean $\pm$ SD recovery rates for BALP in four different concentrations were $98.2 \pm 3.1\%$, $101.5 \pm 7.6\%$, and $97.2 \pm 8.1\%$ for kit controls, cord pool, and newborn pool, respectively. The corresponding figures for CTX were $97.2 \pm 3.0\%$, $90.6 \pm 9.7\%$, and $86.4 \pm 12.6\%$ and for OC $99.9 \pm 3.7\%$, $96.9 \pm 4.0\%$, and $96.5 \pm 6.8\%$. After addition of different amounts of bone marker to the serum pools, the mean $\pm$ SD recovery rates for BALP were $100.2 \pm 6.1\%$ for the cord pool and $103.4 \pm 8.8\%$ for the newborn pool. The corresponding figures were $94.1 \pm 8.1\%$ and $95.4 \pm 6.9\%$ for CTX, and $101.2 \pm 8.2\%$ and $92.4 \pm 6.0\%$ for OC.

Data Analysis

Wilcoxon's rank test was performed to compare cord blood concentrations with venous concentrations at discharge. The Mann-Whitney *U* test was used to test for differences in quantitative measurements between groups for sex, maternal smoking, mode of delivery, and weight for gestational age. To investigate the influence that maternal age, maternal and neonatal anthropometric measures, placental weight, gestational age, and renal function had on each measure of bone turnover, we performed univariate and multivariate linear regression analyses on pooled data for cord blood and discharge samples, respectively. Dependent variables were tested for their fit to a normal distribution. Cord CTX and OC at both times were log-transformed before regression analysis. Data are expressed as median (range). Changes at $p < 0.05$ were considered significant. Statistical analysis was performed using Statistical Package for Social Sciences (SPSS Inc, Chicago, IL, U.S.A.).

RESULTS

Sixty-nine neonates (38 boys, 31 girls) fulfilled inclusion criteria for the study. Their characteristics, and differences between those born by vaginal delivery ($n = 47$) or cesarean section ($n = 22$), are shown in Table 1. Fifty-four babies were AGA, birth weight between the 10th and 90th percentile for gestational age, 11 were SGA, and four were large for gestational age. The median gestational age at birth was 39.4 wk (range, 37.0 to 41.9 wk). All infants adapted well to extrauterine life conditions with no signs of respiratory distress, lethargy, irritability, or poor feeding. One-minute Apgar scores were between 6 and 10 for AGA infants and between 5 and 9 for SGA infants. The median duration between cord blood and discharge sampling was 3 d (range, 1–7 d). Sixty-three of 69 samples (91.3%) were drawn between d 2 and 4. To facilitate interpretation of data with regard to variables that change with increasing postnatal age, we excluded the six discharge samples not drawn on these days from comparisons of cord and discharge bloods.

There was a significant increase in serum CTX levels (7.65 nM; range, 4.28 to 16.43 nM *versus* 10.22 nM; range, 5.38 to 16.45 nM, cord *versus* discharge sample; $p < 0.001$) and OC levels (16.4 ng/mL; range, 2.6 to 113.1 ng/mL *versus* 28.4 ng/mL; range, 2.5 to 169.5 ng/mL, cord *versus* discharge sample; $p < 0.001$). In contrast, serum BALP levels did not change significantly during the same period (Fig. 1).

Neonates born by cesarean section, all of which were elective or after an uncomplicated pregnancy, did not differ in anthropometric variables from infants delivered via the vaginal route. However, they had significantly ($p < 0.001$) higher CTX levels at birth than neonates born by vaginal delivery. Serum CTX levels negatively correlated with gestational age ($r = -0.27$; $p = 0.027$). In multivariate regression analysis, however, the mode of delivery gave the best prediction ($r^2 = 0.19$; $p < 0.001$) of serum CTX levels at birth. Cord OC was related only to maternal weight ($r = -0.28$; $p = 0.035$; Fig. 2A); its negative relation to maternal BMI was close to statistical significance ($r = -0.26$; $p = 0.055$).

To detect possible alterations in neonatal bone turnover as a result of maternal smoking, we compared mothers who smoked throughout pregnancy with nonsmokers. Mothers who stopped smoking within the first trimester of pregnancy were consid-

Figure 1. Concentrations of bone turnover markers in cord blood (white boxes) and in peripheral venous blood (shaded boxes) drawn at discharge (postnatal d 2–4). Box plots show median, interquartile values, and 5th and 95th percentiles. *** $p < 0.001$.

ered nonsmokers. The smoking group consumed a mean of 10.96 cigarettes/d throughout pregnancy. There were no significant differences between neonates of smoking and nonsmoking mothers in anthropometric variables (Table 2). Serum BALP concentrations were significantly reduced in neonates of smokers ($n = 16$) compared with nonsmokers ($n = 53$), both in cord blood ($p = 0.013$) and at discharge ($p = 0.036$; Fig. 3). In addition, linear regression analysis revealed significant inverse correlations between cord BALP level and maternal weight gain during pregnancy ($r = -0.42$; $p < 0.001$), and maternal weight ($r = -0.35$; $p = 0.008$) and height ($r = -0.28$; $p = 0.03$) at delivery. The negative relation of cord BALP to maternal BMI did not reach statistical significance ($r = -0.26$; $p = 0.057$). Figure 2 shows the relationship of both bone formation markers with maternal anthropometric variables. When multivariate regression analysis was performed, maternal smoking and pregnancy weight gain were the best predictors of BALP level at birth ($r^2 = 0.24$; $p < 0.001$). Because of the small number of smokers, we were not able to calculate dose-related consequences of maternal smoking.

Sex, 1-min Apgar score, neonatal anthropometric variables, neonatal weight gain, arterial cord blood pH, serum creatinine,

Table 1. Characteristics of the study population*

	Vaginal delivery $n = 47$	Cesarean section $n = 22$	Total $n = 69$
SGA/AGA/LGA	9/34/4	2/20/0	11/54/4
Gestational age (wk)	40.2 (37.0–41.9)	38.3 (37.1–40.3)†	39.4 (37.0–41.9)
Male/female	28/19	10/12	38/31
Birth weight (g)	3250 (2430–4300)	3135 (2450–3950)	3230 (2430–4300)
Birth length (cm)	49 (45–54)	49 (46–54)	49 (45–54)
Ponderal index (g/cm^3) $\times 100$	2.7 (2.3–3.5)	2.7 (2.1–3.2)	2.7 (2.1–3.5)
Mothers (smoking/nonsmoking)	10/37	6/16	16/53
1-min APGAR score	9 (5–10)	9 (6–10)	9 (5–10)
Neonatal weight change (g)	–100 (–450 to +560)	–105 (–190 to +110)	–100 (–450 to +560)
Mother's age (y)	29 (16–37)	31 (21–38)	29 (16–38)

* Values are median (range). Differences between data for vaginal and cesarean delivery are not significant, unless otherwise stated.

† $p < 0.001$.

LGA, Large for gestational age.

Figure 2. Relationship of cord OC (A) and cord BALP (B) to maternal weight, and cord BALP to maternal height (C) and pregnancy weight gain (D).

Table 2. Characteristics of the study population according to the maternal smoking status*

	Smokers (n = 16)	Nonsmokers (n = 53)
Maternal weight (kg)	79 (60–108)	74 (56–100)
Placental weight (g)	420 (400–660)	500 (350–790)
Birth weight (g)	3220 (2630–3950)	3250 (2430–4300)
Ponderal index ($\text{g}/\text{cm}^3 \times 100$)	2.6 (2.1–3.3)	2.7 (2.2–3.5)
Neonatal weight change (g)	–60 (–450 to +110)	–110 (–360 to +560)
Creatinine (μM)	47.74 (34.48–70.72)	53.04 (20.33–102.54)

* Values are median (range).

and placental weight were not related to bone marker concentrations. There were no significant differences between SGA, AGA, and large for gestational age babies in any measured variable except birth weight.

DISCUSSION

This study has discovered effects of the *in utero* environment on bone modeling of the fetus. Although fetal bone turnover at late gestation appears to be independent of the comparably

low maternal turnover (4, 21–23), its relationship to maternal factors including anthropometric variables or smoking has not been examined so far using validated methodology. In our study, cord values of BALP and OC were significantly negatively correlated with maternal weight. Cord BALP levels were also inversely correlated with pregnancy weight gain and maternal height. The negative relation of both bone formation markers (BALP and OC) to maternal BMI did not reach statistical significance ($r = -0.26$; $p = 0.057$ and $r = -0.26$; $p = 0.055$, respectively).

Whether maternal obesity or excessive weight gain during pregnancy could result in alterations in fetal mineral supply during pregnancy, or whether BALP may be positively correlated with term neonatal BMC, as seen in preterm infants (17), is unknown. Interestingly, maternal leptin, which is closely related to BMI, has been found to be a negative predictor of PGH (24). PGH plays a crucial role in fetal growth by a yet unidentified modulation of placental substrate supply to the fetus. Thus, a leptin-induced decrease in PGH caused by maternal obesity might result in a low neonatal bone mass. Increased mechanical stress at delivery for neonates of obese mothers or a decrease in fetal movements in late gestation would be further speculative explanations for this finding.

Figure 3. Differences in bone turnover markers between neonates of non-smokers (white boxes) and smokers (shaded boxes). Box plots show median, interquartile values, and 5th and 95th percentiles. The corresponding *p* values for cord and discharge bloods are *p* = 0.23 and *p* = 0.7 for CTX, *p* = 0.04 and *p* = 0.01 for BALP, and *p* = 0.52 and *p* = 0.77 for OC, respectively. **p* < 0.05.

Further studies on larger cohorts are required to investigate whether maternal obesity could suppress fetal bone formation.

Smoking has deleterious effects on bone in adults of all ages (25–27). However, data on the influence of maternal smoking during pregnancy on bone metabolism in the newborn are very sparse. Jones *et al.* (28) have shown a negative long-term effect of maternal smoking during pregnancy on both growth and bone mass in a large cohort of prepubertal children born at term. However, studies examining potential early effects of maternal smoking on newborn BMC are inconclusive (18, 29), especially as differences between groups according to maternal smoking become insignificant after adjusting BMC for infant length (18). Maternal cigarette smoking is well known to retard fetal growth in a dose-dependent relationship on all anthropometric characteristics of the newborn (30, 31). The way in which smoking exerts its negative influence on fetal growth is still not fully understood. Reduced maternal energy intake (32, 33), fetal metabolic alterations (34, 35) an altered IGF system (24, 36, 37), and impaired placental function (38, 39) have been implicated. We detected significantly reduced concentrations of BALP in infants of smoking mothers, which may reflect impaired bone turnover in these newborns. Interestingly, there was no significant difference between the groups in anthropometric variables (Table 2). Thus, the negative impact of maternal smoking on neonatal bone turnover in our cohort appears to be independent of its well-known effect on fetal growth seen in larger cohorts.

Reduced levels of total ALP have previously been found in neonates of chronic smokers (34, 40). This has been attributed to fetal zinc deficiency as a result of placental zinc-trapping, as ALP is a zinc-dependent enzyme (40), and to impaired placental enzymatic activity (34). BALP, however, is the predominant isoenzyme of ALP in healthy term infants (41). It is not a placental enzyme, but an exclusive product of the osteoblast.

Nevertheless, other significant alterations in placental function secondary to maternal smoking (34) may impact on neonatal bone metabolism.

Our results show a significant increase in OC and CTX in term human neonates during the first days of life. There are several potential interpretations of this finding. First, this may be a result of increased bone modeling after the intrauterine deceleration in growth rate and bone turnover during the last weeks of gestation. The increase in these markers is also consistent with the postnatal profile of IGF-1 levels, which increase steadily from the first day of life in term neonates (42–44). A precipitous drop immediately after delivery (cord serum *versus* d 1) reported in IGF-1 (42) and the osteoblast marker OC (45) has been attributed to the influence of the stress at labor (42). In our study, CTX levels were significantly reduced in neonates born by vaginal delivery. There are, however, no studies investigating the underlying mechanisms by which labor could modulate these effects. A second potential explanation for the increase in CTX and OC would be that the adaptation of the newborn to extrauterine life conditions (e.g. increased muscle activity, oral nutrition) induces significant modulations in bone remodeling in addition to the known alterations in postnatal calcium homeostasis. Calcium levels drop rapidly after cessation of the physiologic fetal hypercalcemia. Subsequently, levels of PTH and 1,25-dihydroxy vitamin D increase during the first week postnatally (10). We speculate that these changes may have induced increased bone remodeling activity in our cohort. Finally, the postnatal increase in CTX and OC may also result from altered renal clearance of these small molecules. However, serum creatinine was not related to any bone marker in linear regression analysis and was measured solely at discharge.

The discrepancy in postnatal change between the two measures of bone formation (BALP and OC) in this study may derive from the different functions of these proteins, which are still not fully understood (46). Alternatively, there may be differences in the timing of their release from bone tissue during the formation process.

CONCLUSIONS

We conclude from this study that the *in utero* environment affects bone modeling of the fetus. The markers of bone formation in our study were negatively related to maternal anthropometric measurements, and all markers were unrelated to neonatal measurements. In term babies, there is a significant increase in CTX and OC during the first days of neonatal life, but it remains to be clarified whether this is a result of increased bone modeling, remodeling, or altered renal clearance. BALP appears to be a sensitive tool for the detection of the effects of maternal smoking on bone. Certainly our results suggest that maternal smoking during pregnancy has a negative impact on fetal and neonatal bone turnover. Furthermore, these markers may provide information about whole body turnover of bone and collagen that is complementary to accepted clinical measures of growth and bone mineralization (17), especially in neonates, in whom traditional methods are less applicable. Further studies are required to further explore the relationship

between maternal obesity, maternal smoking, labor, and neonatal bone turnover.

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