

Concentrations of Serum Markers of Type I Collagen Synthesis and Degradation and Serum Osteocalcin in Maternal and Umbilical Circulation

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Abstract. Measurement was made of the serum carboxyl-terminal propeptide of type I procollagen (PICP), carboxyl terminal cross-linked telopeptide of type I collagen (ICTP) and osteocalcin in 17 full-term mother-infant pairs and 17 age-matched nonpregnant women. Serum PICP and ICTP of term women at the time of delivery were significantly higher ($P<0.025$, $P<0.01$, respectively) and serum osteocalcin was significantly lower ($P<0.001$) than in nonpregnant women. The ratio of PICP to ICTP was essentially the same for term and nonpregnant women. Serum PICP, ICTP, and osteocalcin were virtually the same in the umbilical arteries and vein. PICP, ICTP and osteocalcin were much higher in fetal than maternal circulation ($P<0.001$). The fetal levels of these proteins were not correlated with maternal levels, nor with birth weight. Thus, during pregnancy, either osteoclastic or osteoblastic activity would appear to increase slightly, but the balance between bone formation and resorption is maintained. During fetal life, bone turnover may be greatly accelerated and bone metabolism may occur independently of maternal bone metabolism.

Key words: Serum marker, Type I collagen, Osteocalcin, Maternal and fetal circulation

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THE FETUS is a rapidly growing organism. Growth of the fetal body and development of fetal organs are supported by that of its bony structure. Fetal growth during pregnancy can now be evaluated by ultrasound through various measurements of fetal bony structures, but fetal bone metabolism and maternal-fetal interrelations for bone metabolism are not well understood. The impact of gestation on maternal bone also remains essentially unclarified.

Bone is a complex tissue constantly undergoing a process of renewal and remodeling involving many different factors and substances. Theoretically, measurement of the circulating levels of such

substances should provide clarification of the status of bone at any given time. Recently, several promising bone-specific proteins as potential biomarkers for bone turnover were purified and reliable assay systems for these proteins have been developed. These are the carboxyl-terminal propeptide of type I procollagen (PICP), carboxyl terminal cross-linked telopeptide of type I collagen (ICTP), and osteocalcin (bone Gla protein).

PICP reflects the formation rate of type I collagen, the main constituent of the bone matrix [1–3]. ICTP reflects the degradation rate of type I collagen [1, 2, 4]. Osteocalcin is the most abundant noncollagenous protein in bone and may represent a specific index of bone turnover [1, 2]. These proteins are released into the serum at concentrations correlated with the rates of bone formation and resorption [5–8]. Bone metabolism may thus be assessed quantitatively by these biomarkers.

This study was conducted to determine whether pregnancy affects the maternal concentrations of

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these biomarkers, whether fetal concentrations are related to maternal concentrations and whether serum concentrations are related to birth weight in term infants.

Materials and Methods

The procedure for this study was approved by the Ethical Committee of Yamanashi Medical University and informed consent was obtained from 17 full-term Japanese women at the Labor and Delivery unit of the Yamanashi Medical University Hospital after the onset of labor from February to March 1994. They ranged in age from 25–36 yr and in 37–41 weeks of gestation. All subjects were healthy and pregnancies were uncomplicated. Gestational age was determined by history and confirmed by ultrasonographic evaluation during the first trimester. Seventeen nonpregnant female volunteers served as controls. Each was age-matched within 2 yr of the pregnant counterpart.

Blood samples for measurement of PICP, ICTP and osteocalcin were obtained from the mother during the second stage of labor and the infant umbilical vein and artery immediately after delivery by direct aspiration. Samples from nonpregnant women were obtained at 1000–1200 h. Serum was obtained immediately by centrifugation and stored at -40°C until analyzed.

PICP and ICTP were measured in triplicate 100 μl serum samples by radioimmunoassay [3, 4] obtained from Orion Diagnostica (Oulunsalo, Finland). The concentration of intact osteocalcin was determined by immunoradiometric assay [8] obtained from Mitsubishi Yuka (Tokyo, Japan). This system is a two-site immunoradiometric assay with two different monoclonal antibodies to human osteocalcin; one binding to the 12–23 amino acid region of osteocalcin and the second to the 30–49 amino acid region. The minimum detectable amount was 1 ng/ml. The intra- and interassay coefficients of variation were 3.2% and 5.6% for PICP, 3.9% and 6.1% for ICTP, and 2.9% and 7.3% for osteocalcin. To exclude cases of renal insufficiency or hepatic insufficiency, urea nitrogen, creatinine, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in adult samples were assayed.

Statistical analysis was conducted by standard and paired *t*-tests and linear regression analysis as

appropriate. For statistical comparison of means, data were expressed as means \pm SD unless otherwise indicated. Statistical significance was defined as $P < 0.05$.

Results

All the term women had uneventful vaginal delivery between 1200–1900 h on the day of admission. All infants were healthy and weighed 2.1–3.8 kg.

Maternal circulation

Serum urea nitrogen, creatinine, ALT and AST were in the normal range in all term women and nonpregnant controls. Serum PICP and ICTP of term women at the time of delivery were significantly higher and osteocalcin was significantly lower than in nonpregnant age-matched women (Table 1). The ratio of PICP to ICTP (PI/IC) was essentially the same for pregnant and nonpregnant women. Serum osteocalcin could not be detected (less than 1 ng/ml) in 11 (64.7%) maternal samples. No significant correlation could be found between birth weight and maternal PICP, ICTP or osteocalcin (data not shown).

Fetal circulation

Five samples taken from the umbilical artery were excluded owing to hemolysis or insufficient volume for measurement. In all 12 paired samples, serum PICP, ICTP and osteocalcin were almost identical in the umbilical artery and vein. These concentrations for the latter were thus regarded as those of fetal circulation.

PICP, ICTP and osteocalcin were much higher

Table 1. PICP, ICTP and osteocalcin in term and nonpregnant women

		Term women (n=17)	Nonpregnant women (n=17)
PICP	(ng/ml)	100.7 \pm 26.4 ^b	79.5 \pm 15.9
ICTP	(ng/ml)	4.9 \pm 0.9 ^a	3.7 \pm 1.4
PI/IC		20.7 \pm 4.5	24.4 \pm 10.7
Osteocalcin	(ng/ml)	2.3 \pm 0.9 ^a	4.6 \pm 1.3

* Values in 7 detectable samples. ^a $P < 0.01$ vs. nonpregnant women. ^b $P < 0.025$ vs. nonpregnant women.

Table 2. Biomarker levels in maternal and fetal circulation (n=17)

		Maternal circulation	Fetal circulation
PICP	(ng/ml)	100.7 ± 26.4	1280.0 ± 403.1 ^a
ICTP	(ng/ml)	4.9 ± 0.9	102.8 ± 28.4 ^a
Osteocalcin	(ng/ml)	2.3 ± 0.9*	20.5 ± 11.1 ^a

* Values in 7 detectable samples. ^a $P < 0.01$ vs. maternal circulation.

in fetal than maternal circulation (Table 2). The fetal levels of these proteins were not correlated with maternal levels or with birth weight.

No significant sex differences were observed in the levels of PICP, ICTP and osteocalcin in this study (Table 3).

Discussion

Type I collagen is the main constituent of the bone matrix and most type I collagen is found in bone, although the exact distribution of this protein in the skeleton and soft tissue has yet to be determined [1, 10, 11]. During the formation of type I collagen, PICP splits off from the precursor, procollagen, in a 1:1 ratio as newly-formed collagen [4]. PICP is not incorporated into bone but released into extracellular fluid. The resorption of old bone brings about the cleavage of collagen into smaller fragments to be subsequently released into the extracellular fluid. One of these fragments is ICTP, mainly derived from bone [3]. Serum PICP and ICTP are correlated with the rates of bone formation and resorption, respectively, as shown by histomorphometry [5]. The degradation of PICP occurs primarily via hepatic pathways [12]. Judging from its size, ICTP would appear to be cleared by the kidneys [3]. Serum osteocalcin is also influenced by renal filtration [13]. Examination was thus made of hepatic and renal functions in all subjects. This is the first study on serum type I collagen-related biomarkers of bone metabolism in maternal and fetal circulation.

Serum PICP and ICTP in pregnant women were found to be significantly higher than in nonpregnant age-matched women, but the ratios of PICP to ICTP (PI/IC) in pregnant and nonpregnant women were not significantly different. On esti-

Table 3. Serum biomarker levels in male and female fetuses

		Male fetus	Female fetus
Number		9	8
Birth weight	(g)	2982.4 ± 530.6	3062.0 ± 275.1
PICP	(ng/ml)	1124 ± 257	1366 ± 304
ICTP	(ng/ml)	99.6 ± 20.9	112.8 ± 30.4
Osteocalcin	(ng/ml)	18.2 ± 5.7	23.1 ± 14.1

imating these results, the effect of labor on bone turnover, the circadian fluctuation of PICP and ICTP and the influence of high concentrations of these proteins in fetal circulation should be considered. Although both the effect of labor on maternal physiology and the possibility of the influx of fetal PICP and ICTP should not be completely ignored, maternal levels of serum PICP and ICTP presented in this study were essentially the same as those in women either before the onset of labor or at 2 h after delivery, as found in our previous study [14]. Hassager *et al.* presented evidence of a circadian rhythm in serum PICP and ICTP with peak values at night [15]. According to their results, serum PICP and ICTP between 1000 h and 1200 h were higher than those between 1200 h and 1900 h, when serum samples were obtained in the maternal group. Consequently, either the effect of labor or the possible circadian fluctuation of these proteins may be unrelated to the present results. Osteoclastic and osteoblastic activity would therefore appear to increase during pregnancy, but the balance between bone formation and resorption is maintained to preserve the structural integrity of the maternal skeleton.

The impact of pregnancy on maternal bone and mineral metabolism is poorly understood. Whether physiological changes accompanying pregnancy significantly affect maternal bone is unclear. The results of this study may provide some answers to this question.

Serum osteocalcin in pregnancy was low or undetectable. This is in general agreement with the results of other studies [16–18]. The disappearance of serum osteocalcin during pregnancy may be explained in several ways. Although diminished bone turnover is one explanation, as suggested previously [16, 18, 19], the synthesis and degradation of type I collagen presented in this study argues against this. Another and most like-

ly possibility is that the placenta is trapping or destroying osteocalcin [17], this being supported by the finding that a rapid increase in serum osteocalcin occurs after delivery [17, 18].

In the present study, fetal PICP, ICTP and osteocalcin were much higher than maternal or nonpregnant control levels. Even considering possible prematurity in fetal hepatic and renal function, this finding is consistent with the hypothesis that bone turnover, *i.e.*, osteoclastic and osteoblastic activity, is accelerated during fetal life. High serum osteocalcin in fetal circulation is in agreement with earlier reports [20, 21].

The fetal levels of these proteins were not correlated with maternal levels or with birth weights. Fetal bone metabolism thus occurs independently of maternal bone metabolism and may be constant in a mature and healthy fetus. The marked dissociation between high fetal and low maternal PICP, ICTP and osteocalcin indicates little maternal-fetal placental crossover of these proteins.

No significant sex differences could be detected in PICP, ICTP and osteocalcin in this study. Bag-

noli *et al.* reported plasma osteocalcin to be higher in males than females in cord blood and in adult plasma [21]. They consider that sex hormones probably influence the osteocalcin concentration even at birth. Mathur *et al.*, however, report no significant sex difference in the levels of estrogen or any other steroids in umbilical circulation [22].

In summary, either osteoclastic or osteoblastic activity in women would appear to increase during pregnancy, but the balance between bone formation and resorption is maintained. In the fetus, bone turnover may be greatly accelerated and bone metabolism may occur independently of maternal bone metabolism. The serum marker values for type I collagen synthesis and degradation may be constant in a mature and healthy fetus.

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