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Intrauterine growth restriction may not suppress bone formation at term, as indicated by circulating concentrations of undercarboxylated osteocalcin and Dickkopf-1

Despina D. Briana[a], *Dimitrios Gourgiotis*[b], *Anestis Georgiadis*[a], *Maria Boutsikou*[a], *Stavroula Baka*[a], *Antonios Marmarinos*[b], *Dimitrios Hassiakos*[a], *Ariadne Malamitsi-Puchner*[a,*]

[a] *Second Department of Obstetrics and Gynecology, Athens University Medical School, Athens 10682, Greece*
[b] *Research Laboratories, 2nd Department of Pediatrics, Athens University Medical School, Athens, Greece*

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ABSTRACT

The objective was to investigate circulating concentrations of bone formation markers (undercarboxylated osteocalcin [Glu-OC], an established marker of bone formation during fetal and early postnatal life], and Dickkopf-1 [DKK-1], a natural inhibitor of osteoblastogenesis during fetal development]) in intrauterine-growth–restricted (IUGR; associated with impaired fetal skeletal development) and appropriate-for-gestational-age (AGA) pregnancies. Circulating concentrations of Glu-OC and DKK-1 were determined by enzyme immunoassay in 40 mothers and their 20 asymmetric IUGR and 20 AGA singleton full-term fetuses and neonates on postnatal day 1 (N1) and 4 (N4). Parametric tests were applied in the statistical analysis. No significant differences in Glu-OC concentrations were observed between IUGR and AGA groups, whereas fetal DKK-1 concentrations were lower in the IUGR group ($P = .028$). In both groups, maternal Glu-OC and DKK-1 concentrations were lower than fetal, N1, and N4 concentrations ($P \leq .012$ in all cases), whereas fetal Glu-OC concentrations were higher than N1 and N4 ones ($P \leq .037$ in all cases). In addition, N1 Glu-OC concentrations were higher than N4 concentrations ($P = .047$). Finally, maternal Glu-OC and DKK-1 concentrations positively correlated with fetal, N1, and N4 ones ($r \geq 0.404$, $P \leq .01$ in all cases). Fetal/neonatal bone formation may not be impaired in full-term asymmetric IUGR infants, as indicated by the similar Glu-OC concentrations in both groups. Fetal DDK-1 concentrations are lower in the IUGR group, representing probably a compensatory mechanism, favoring the formation of mineralized bone. Fetal/neonatal bone turnover is markedly enhanced compared with maternal one and seems to be associated with the latter in both late pregnancy and early postpartum.

Author contribution: Despina D. Briana: had responsibility for patient enrollment, results assessment, and writing the manuscript. Dimitrios Gourgiotis: participated in the analytical framework of the study and performed laboratory determinations. Anestis Georgiadis: participated in the development of the protocol. Maria Boutsikou: performed the statistical analysis of data. Stavroula Baka: participated in the development of the protocol and clinical assessment of the enrolled parturients. Antonios Marmarinos: performed laboratory determinations. Dimitrios Hassiakos: had responsibility for patient enrollment and screening. Ariadne Malamitsi-Puchner: had primary responsibility for protocol development, outcome assessment, and writing the manuscript.

* *Corresponding author*. Tel.: +30 6944443815; fax: +30 2107233330.
E-mail addresses: amalpu@aretaieio.uoa.gr, amalpu@tellas.gr (A. Malamitsi-Puchner).

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1. Introduction

Intrauterine life is associated with a high rate of skeletal growth and intense bone-modeling activity [1]. There is considerable evidence that impaired fetal skeletal development predisposes to late-onset disorders and an accelerated rate of bone loss during later life [2]. However, little is known about the dynamics of fetal bone formation and resorption regarding the normal changes that occur throughout gestation or in clinical situations that result in low bone mass at birth [3]. Intrauterine growth restriction (IUGR) is associated with low bone mineral content at birth and increased risk for osteoporosis development in later life [3-9]. Whether low bone mass in IUGR is the result of compromised intrauterine bone formation, increased bone resorption, or both is not known.

In cord and early neonatal blood, undercarboxylated osteocalcin (Glu-OC) is the major component of OC [10], which is considered a valid marker of bone formation, involved in the regulation of matrix mineralization [11].

Proteins synthesized by the group of wingless (Wnt) genes are key mediators of osteoblastogenesis and govern the formation of the fetal skeleton [12]. Wnt signaling is modulated by several negative regulators. The best studied of these is Dickkopf-1 (DKK-1), a cysteine-rich protein, which disrupts the Wnt cascade, resulting in the inhibition of osteoblast differentiation [13]. Deletion of a single allele of DKK-1 increases bone mass in mice [14]. Increased DKK-1 levels may lead to enhanced osteoblast-dependent osteoclastogenesis [15]. Inhibition of DKK-1 prevents glucocorticoid-induced bone loss [16] and myeloma-related bone disease [17], whereas induction of DKK-1 could be responsible for the formation of the bone metastases in prostate cancer [18]. In addition, circulating DKK-1 levels have been reported to be sensitive enough to reflect its expression in bone microenvironment [16-18].

This study was based on the hypothesis that circulating markers of bone formation may differ between IUGR and appropriate-for-gestational-age (AGA) fetuses and neonates because the former may present with low bone mass and impaired bone mineralization [4-6]. Therefore, we aimed to determine, for the first time to our knowledge, circulating concentrations of Glu-OC and DKK-1 in AGA and asymmetric IUGR pregnancies. The latter were due to pathological conditions occurring after the second trimester, impairing uteroplacental function and leading to fetal growth restriction accompanied by diverting intrauterine Doppler studies [19,20]. Furthermore, we aimed to investigate the association between circulating concentrations of these markers with maternal as well as fetal-neonatal anthropometric and clinical variables.

2. Subjects and methods

The study protocol was approved by the Ethics Committee of our teaching hospital. From July 2009 to December 2009, the first 40 white women consecutively delivering either asymmetric IUGR (n = 20, birth weight ≤5th customized centile) or AGA (n = 20) singleton full-term healthy neonates who met the following criteria were included in the study: participation informed consent, maternal absence of endocrine diseases likely to affect calcium metabolism, absence of bone diseases or medication possibly implicated in bone turnover, lack of substance use during pregnancy (eg, alcohol, cocaine), and negative test results for congenital infections; furthermore, in their offspring, the criteria were lack of symptoms of intrauterine infection; absence of major congenital malformations; lack of muscular, neurologic, or bone disease; as well as normal adaptation to extrauterine life without signs of respiratory distress, lethargy, irritability, or poor feeding. All women were middle class without nutrient restrictions and supplemented with calcium during pregnancy. They were enrolled to the study from the 32nd gestational week onwards following Doppler examinations, whereas their children were enrolled at birth if they fulfilled the inclusion criteria.

The customized centile for each pregnancy was calculated by using a computer program that adjusts for significant determinants of birth weight, such as maternal height and booking weight, ethnic group, parity, gestational age, and sex [21,22] (Gestation Related Optimal Weight computer-generated program, Software version 5.15, and Centile calculator, software v5.12.1, March 2007, www.gestation.net). Gestational age was estimated using the date of the last menstrual period and early antenatal ultrasound. Birth weight was measured with an electronic scale.

The IUGR fetuses were closely observed by Doppler studies every 10 to 15 days from the 32nd gestational week onwards. Three consecutive measurements of the pulsatility index (PI) of the uterine and umbilical and cerebral arteries were performed, and the mean value was recorded. Mean PI values of the uterine and umbilical arteries [23,24] were progressively found to be in the upper physiological limits for the corresponding gestational age in 13 cases (ranging between the 90th and the 95th percentile). In the remaining 7 cases, PI values showed increased impedance to flow, being above the 95th percentile for gestational age. Doppler studies of the middle cerebral arteries [25] showed resistance to be in the lower physiological limits for gestational age. This finding reflects the initiation of blood flow redistribution process to spare vital organs (brain, heart, and adrenals).

Seven mothers with IUGR offspring presented with pregnancy-induced hypertension, 6 with preeclampsia, 2 with hypothyroidism, 1 with iron-deficient anemia, and 1 with gestational diabetes mellitus. Three were heavy smokers (>10 cigarettes per day) during the whole duration of pregnancy.

In the IUGR group, the amniotic fluid was diminished in all cases. For the evaluation of the amniotic fluid, the largest fluid column on the vertical plane was assessed and was defined as diminished if less than 2 cm. Moreover, placental weights were reduced, ranging from 240 to 450 g [26].

In the AGA group, the mothers were healthy and either were nonsmokers or abstained from smoking during pregnancy. The placentas were normal in appearance and weight [26].

One- and 5-minute Apgar scores were at least 8 in all IUGR cases and AGA controls. All neonates were breastfed. The demographic data of the study groups are listed in Table 1.

Blood was collected in pyrogen-free tubes from (1) the mothers during the first stage of labor, or before receiving anesthesia in cases of elective cesarean delivery; (2) the umbilical cords after double clamping, reflecting the fetal state; and (3) the neonates on postpartum day 1 (N1) and 4

Table 1 – Demographic data for AGA and IUGR neonates and their mothers

	AGA	IUGR	P
	Mean (SD)	Mean (SD)	
Birth weight (g)	3280 (333)	2511 (261)	<.001
Birth weight centile	46 (24.7)	2.95 (1.9)	<.001
Gestational age (wk)	39.2 (0.83)	38.5 (1.2)	.36
Sex			0.523
Male	10 (50%)	7 (35%)	
Female	10 (50%)	13 (65%)	
Maternal age (y)	30 (4.8)	32.7 (4.3)	.68
Parity			0.514
Primigravida, 14 (70%)	11 (55%)		
Other, 6 (30%)	9 (45%)		
Mode of delivery			0.191
Vaginal	15 (75%)	10 (50%)	
Cesarean	5 (25%)	10 (50%)	

(N4), characterizing transition and stabilization to extrauterine life, respectively. Serum was separated by centrifugation after clotting and was kept frozen at –80°C until assay.

The determination of plasma Glu-OC concentrations was performed by enzyme immunoassay (Takara Bio, Otsu Shiga, Japan). The minimum detectable concentration and the intra- and interassay coefficients of variation were 0.25 ng/mL, 4.58%, and 5.67%, respectively.

Plasma DKK-1 concentrations were measured by enzyme immunoassay (R&D Systems Abingdon, UK). The minimum detectable concentration and the intra- and interassay coefficients of variation were 4.23 pg/mL, 3.3%, and 4.6%, respectively.

2.1. *Statistical analysis*

Kolmogorov-Smirnov test was used to examine whether data were normally distributed. All data regarding Glu-OC and DKK-1 presented with normal distribution; thus, parametric procedures were applied. Independent-samples *t* test was performed to detect significant alterations regarding birth weight, maternal age, and gestational age, as well as customized centile between AGA and IUGR groups. Pearson χ^2 was used to examine possible alterations regarding dichotomous variables between the 2 groups. Analysis of variance for repeated measurements and regression analysis were applied to examine the effect of sex, maternal age, gestational age, mode of delivery, parity, and birth weight on Glu-OC and DKK-1 concentrations. Spearman or Pearson correlation coefficient—where appropriate—was applied to detect any positive or negative correlations. A $P < .05$ was considered statistically significant. Statistical analysis was performed using SPSS 11.5 (Chicago, IL).

3. Results

Determined mean (95% confidence intervals [CIs]) values of circulating Glu-OC and DKK-1 concentrations in both groups are shown in Figs. 1 and 2, respectively. Tables 2 and 3 show the mean (SD) Glu-OC and DKK-1 concentrations for all categorical variables (parity, sex, mode of delivery) included in Table 1.

No significant differences in Glu-OC concentrations were observed between IUGR cases and AGA controls at all time points. On the other hand, fetal DKK-1 concentrations were lower by 862.57 pg/mL on average in the IUGR group (95% CI, 96.06-1629.08; $P = .028$).

In both groups, maternal Glu-OC and DKK-1 concentrations were lower than fetal, N1, and N4 concentrations ($P \leq .012$, in all cases), whereas fetal Glu-OC concentrations were higher than N1 and N4 ones ($P \leq .037$, in all cases). Furthermore, N1 Glu-OC concentrations were higher than N4 concentrations ($P \leq .047$, in all cases).

In a combined group, maternal Glu-OC concentrations positively correlated with fetal, N1, and N4 concentrations ($r = 0.560$, $P < .001$; $r = 0.618$, $P < .001$; and $r = 0.444$, $P = .004$, respectively); and maternal DKK-1 concentrations positively correlated with N1 and N4 ones ($r = 0.404$, $P = .010$ and $r = 0.405$, $P = .010$, respectively). Moreover, fetal Glu-OC concentrations positively correlated with N4 concentrations ($r = 0.320$, $P = .044$); and N1 Glu-OC concentrations positively correlated with N4 ones ($r = 0.454$, $P = .004$). Similarly, fetal DKK-1 concentrations positively correlated with N1 and N4 concentrations ($r = 0.419$, $P = .007$ and $r = 0.473$, $P = .002$, respectively); and N1 DKK-1 concentrations positively correlated with N4 ones ($r = 0.375$, $P = .017$).

Finally, the effect of maternal age, parity, gestational age, mode of delivery, and sex on Glu-OC and DKK-1 concentrations was not significant in both groups.

4. Discussion

Assessment of bone health in neonates is important because early events in life may predispose to degenerative diseases in

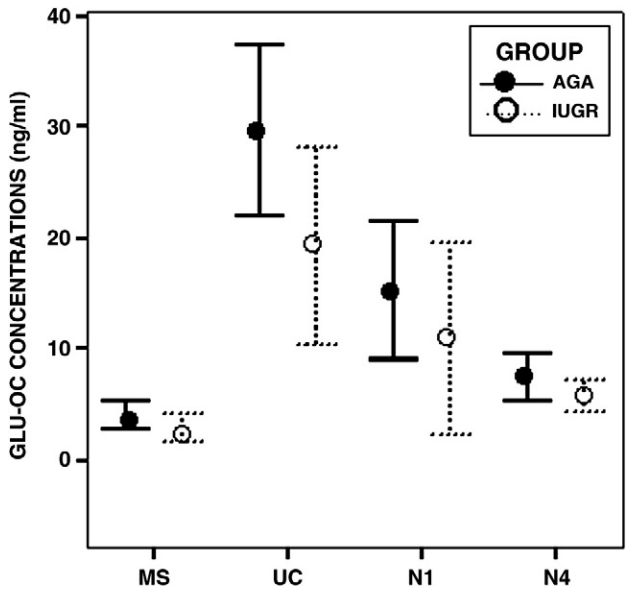

Fig. 1 – Mean (95% CIs) plasma Glu-OC concentrations in maternal, fetal, N1, and N4 samples in AGA and IUGR groups. MS indicates maternal; UC, fetal.

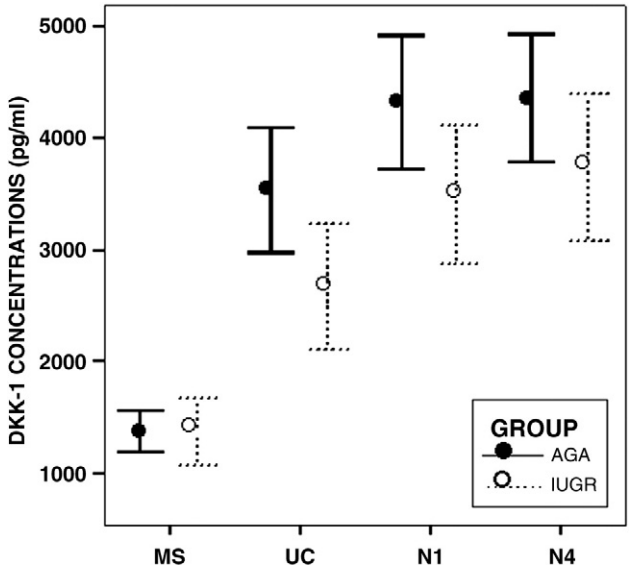

Fig. 2 – Mean (95% CIs) plasma DKK-1 concentrations in maternal, fetal, N1, and N4 samples in AGA and IUGR groups.

adulthood [2]. In this respect, IUGR has been identified as an independent risk factor for adverse fetal bone growth [4-6] and osteoporosis development later in life [7-9]; but limited data exist in the literature regarding the mechanisms involved. Thus, 2 previous studies [5,11] involving small-for-gestational-age infants documented lower fetal/neonatal osteocalcin levels, possibly due to impaired transplacental mineral supply from the mother to the fetus. Indeed, the incidence of neonatal hypocalcemia is reportedly increased in IUGR [27]; but it has been speculated that this may be related to birth asphyxia rather than IUGR per se [28]. Accordingly, placental calcium pump is reported to be activated in asymmetric IUGR, consistent with an increased transplacental calcium transport, which may be secondary to increased fetal calcium demand [29]. The present study, by investigating the dominant component of OC in fetal and neonatal blood (Glu-OC) in well-documented IUGR subjects, did not indicate lower bone formation in IUGRs (in line with a previous report from our group [30]).

In support of the above finding, this study, for the first time to our knowledge, determined DKK-1 concentrations in the perinatal period and demonstrated lower fetal DKK-1 concentrations in the IUGR group. Wnt signaling increases bone mass via a number of mechanisms, including renewal of stem cells, stimulation of preosteoblast replication, induction of osteoblastogenesis, and inhibition of osteoblast and osteocyte apoptosis [31]. Dickkopf-1 interacts with Wnt coreceptors, resulting in the inhibition of osteoblast differentiation and bone formation [32]. Therefore, the downregulation of DKK-1 concentrations in IUGR probably represents a compensatory mechanism, which favors the formation of mineralized bone. It may be speculated that this mechanism could contribute to the normal bone formation in the IUGR fetus and neonate.

In accordance with previous reports [33,34], the documented higher fetal/neonatal Glu-OC concentrations as compared with maternal ones further confirm the markedly enhanced bone turnover observed in fetuses and neonates [35,36]. Furthermore, the gradual decrease in Glu-OC concentrations during the first days of life in the present study may be consistent with the well-known alterations of postnatal calcium homeostasis [37]. In this respect, circulating calcium

Table 2 – Mean (SD) plasma Glu-OC and DKK-1 concentrations in maternal, fetal, N1, and N4 samples in the AGA group for all categorical variables in Table 1

AGA group	Glu-OC MS (ng/mL) Mean (SD)	Glu-OC UC (ng/mL) Mean (SD)	Glu-OC N1 (ng/mL) Mean (SD)	Glu-OC N4 (ng/mL) Mean (SD)
Parity	P = .535	P = .484	P = .625	P = .710
Primigravida	4.39 (2.87)	27.96 (16.57)	14.34 (13.98)	7.21 (3.80)
Other	3.53 (2.57)	33.80 (17.23)	17.62 (12.31)	8.04 (6.06)
Sex	P = .142	P = .031	P = .541	P = .203
Male	3.22 (2.04)	21.94 (10.20)	13.44 (10.98)	6.17 (2.40)
Female	5.04 (3.15)	37.49 (18.42)	17.20 (15.60)	8.74 (5.67)
Mode of delivery	P = .618	P = .347	P = .844	P = .913
Vaginal	4.32 (3.12)	27.65 (17.99)	15.68 (15.01)	7.52 (5.03)
Cesarean	3.58 (1.12)	35.91 (10.13)	14.27 (6.76)	7.26 (2.25)
AGA group	DKK-1 MS (pg/mL) Mean (SD)	DKK-1 UC (pg/mL) Mean (SD)	DKK-1 N1 (pg/mL) Mean (SD)	DKK-1 N4 (pg/mL) Mean (SD)
Parity	P = .875	P = .279	P = .921	P = .598
Primigravida	1370.90 (302.65)	3347.79 (1134.22)	4298.81 (1340.79)	4453.20 (1031.27)
Other	1401.32 (560.41)	3992.16 (1302.88)	4363.14 (1220.78)	4128.11 (1666.66)
Sex	P = .481	P = .770	P = .447	P = .699
Male	1442.19 (294.32)	3460.14 (1356.14)	4541.91 (1589.05)	4465.17 (1070.48)
Female	1317.86 (460.45)	3622.06 (1068.34)	4094.30 (888.93)	4246.17 (1398.62)
Mode of delivery	P = .576	P = .681	P = .598	P = .068
Vaginal	1408.60 (380.68)	3606.90 (1120.78)	4408.19 (1428.68)	4070.41 (1079.67)
Cesarean	1294.30 (414.47)	3343.70 (1509.08)	4047.87 (666.50)	5211.47 (1318.72)

MS indicates maternal; UC, fetal.

Table 3 – Mean (SD) plasma Glu-OC and DKK-1 concentrations in maternal, fetal, N1, and N4 samples in the IUGR group for all categorical variables in Table 1

IUGR group	Glu-OC MS (ng/mL) Mean (SD)	Glu-OC UC (ng/mL) Mean (SD)	Glu-OC N1 (ng/mL) Mean (SD)	Glu-OC N4 (ng/mL) Mean (SD)
Parity	*P* = .140	*P* = .114	*P* = .127	*P* = .046
Primigravida	3.70 (3.21)	25.47 (24.18)	17.01 (23.78)	6.91 (3.40)
Other	1.96 (1.14)	11.76 (5.31)	4.25 (1.27)	4.31 (1.38)
Sex	*P* = .222	*P* = .964	*P* = .481	*P* = .970
Male	3.91 (2.40)	19.03 (30.85)	14.93 (28.03)	5.77 (3.57)
Female	2.39 (2.64)	19.45 (10.38)	8.66 (9.46)	5.72 (2.71)
Mode of delivery	*P* = .291	*P* = .954	*P* = .121	*P* = .797
Vaginal	3.55 (3.39)	19.04 (12.29)	17.79 (25.14)	5.91 (3.00)
Cesarean	2.29 (1.40)	19.56 (25.04)	4.83 (1.54)	5.56 (3.04)
IUGR group	**DKK-1 MS (pg/mL) Mean (SD)**	**DKK-1 UC (pg/mL) Mean (SD)**	**DKK-1 N1 (pg/mL) Mean (SD)**	**DKK-1 N4 (pg/mL) Mean (SD)**
Parity	*P* = .796	*P* = .469	*P* = .802	*P* = .905
Primigravida	1411.88 (695.75)	2496.708 (1004.65)	3431.05 (1226.16)	3783.58 (1565.25)
Other	1334.30 (605.15)	2901.52 (1441.24)	3585.41 (1493.11)	3705.60 (1250.55)
Sex	*P* = .349	*P* = .528	*P* = .922	*P* = .685
Male	1565.26 (327.82)	2438.91 (651.76)	3541.28 (1286.46)	3928.04 (811.45)
Female	1275.58 (751.30)	2807.55 (1424.69)	3478.56 (1385.64)	3651.81 (1651.85)
Mode of delivery	*P* = .970	*P* = .789	*P* = .919	*P* = .355
Vaginal	1371.44 (819.38)	2753.39 (917.93)	3469.43 (1402.89)	3451.09 (1563.78)
Cesarean	1382.49 (441.85)	2603.66 (1484.31)	3531.60 (1301.94)	4045.89 (1213.97)

concentrations drop rapidly after delivery [37]. Consequently, parathyroid hormone (PTH) concentrations increase during the first postnatal week, causing bone catabolism [38]. An older study demonstrated an inverse correlation between OC and PTH levels early postpartum, suggesting that the sudden postnatal decrease in bone formation is associated with the observed upregulation of PTH concentrations [39]. However, the increase in osteoclastic activity stimulated by PTH following delivery should be matched by appropriate new bone formation because bone mineral mass is maintained in the early postnatal phase [40]. Although the mechanism coupling bone resorption with new bone formation is unclear, this (according to a previous study [40] as well as our data) does not seem to take place during the first few days of life.

In addition, the present study revealed a strong positive correlation between circulating maternal and fetal/neonatal Glu-OC and DKK-1 concentrations in both groups. It has been suggested that fetal bone turnover is independent of maternal bone metabolism in late pregnancy [33,34,41]. However, a previous report indicated a positive association between maternal and fetal bone formation markers in preeclampsia [42]. Accordingly, our finding favors the assumption that, in late gestation and early postpartum, an association exists between maternal and fetal/neonatal bone formation in both normal and IUGR pregnancies.

Although this study investigated for the first time Glu-OC and DKK-1 in IUGR fetuses/neonates, the sample size (20 IUGR and 20 AGA pregnancies) is limited; and our data should be considered as preliminary. Nevertheless, the results refer to the same population consecutively reexamined.

In conclusion, the lack of differences in circulating Glu-OC concentrations between IUGR cases and AGA controls possibly indicates that IUGR may not suppress bone formation in full-term fetuses/neonates. Supporting the above observation, fetal DDK-1 concentrations are lower in the IUGR group, probably representing a compensatory mechanism, which favors the formation of mineralized bone. Finally, fetal/neonatal bone turnover is greatly accelerated and seems to be associated with maternal bone metabolism in both late pregnancy and early postpartum. Further studies are required to investigate the impact of intrauterine insults, such as IUGR, on the programming of bone health to ameliorate adult diseases of developmental origins like osteoporosis.

Conflict of Interest

The authors state that they have no conflicts of interest to disclose.

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