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The Influence of Adipokines on Fetal Bone Turnover

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Abstract

Background: A relation between adiponectin and bone homeostasis has been illustrated through studying adiponectin secretion and its receptor presentation in bone forming cells. The aim of our study was to investigate the relationship between fetal bone turnover and adipokines.

Methods: In a cross-sectional study performed in Tehran University of medical sciences related hospitals, 77 samples (39 males, 38 females) of umbilical cord blood immediately after delivery were gathered. Clinical characteristics such as gender, weight, length, weight to length ratio were recorded. Measurements of leptin, adiponectin, osteocalcin and crosslaps were done by ELISA methods in biochemistry and hormone laboratory of endocrinology and metabolism research center. The amounts of crosslaps and osteocalcin were expressed as *t*-scores, and then *t*-scores of crosslaps was subtracted from osteocalcin *t*-scores to establish estimation for bone formation, which we named Bone Formation Index.

Results: In Univariate Analysis, after entrance sex, birth weight and birth length as fixed factors, leptin and adiponectin displayed an independent effect on Bone Formation Index.

Conclusion: Our data suggest that both leptin and adiponectin have a remarkable impact on bone turnover in fetus.

Keyword: *Leptin, Adiponectin, Osteocalcin, Crosslaps, Umbilical cord, Bone Turnover*

Introduction

One of the essential elements of healthy fetal growth is Bone metabolism during pregnancy. It has been shown that levels of bone markers like crosslaps (1) (a marker of bone resorption) and osteocalcin (2, 3) (a marker of bone formation) are higher in neonatal circulation than maternal blood. This can be attributed to high bone metabolism rate in newborn (4). During recent years, the role of adipokines like leptin and adiponectin in osteoblastic activation and bone formation has been proposed (5, 6). Leptin is an adipokine mainly produced by adipocytes and has a key role in metabolic regulation (7). Gorrdeledze et al. have demonstrated that collagen synthesis, cell differentiation, bone mineralization and prolong cell survival augment in iliac crest osteoblasts exposed to leptin (5). Others suggest that leptin acts as a regulator of bone mass (8). Adiponectin is 244-amino acid protein (9) secreted exclusively by adipose tissue (10). A relation between adiponectin and bone hemostasis has been illustrated through adiponectin secretion and its receptor presentation in bone forming cells (11). Therefore, a functional role for adiponectin in bone hemostasis can be proposed.

The aim of our study was to investigate the relationship between fetal bone metabolic marker and adipokines and the impact of leptin and adiponectin on fetal bone metabolism.

Material and Methods

This study was a cross sectional survey. Its protocol was approved by the ethic committee and informed written consent was obtained from all participants. Seventy seven samples (39 male, 38 female) of umbilical cord were gathered blood immediately after delivery by trainee technicians in delivery rooms of Tehran University of Medical Sciences hospitals from Sep 2005 to Jan 2006. Under sterile conditions 5cc blood of umbilical vein was obtained after double clamping of the umbilical cord at birth. The samples were centrifuged and the sera were frozen in-80 degree centigrade temperature. By a special ques-

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tionnaire, maternal data about medical history and habits, clinical examinations and pregnancy events were asked. Neonates were examined by delivery room specialist. The anthropometric and clinical characteristics such as gender, weight, length, weight to length ratio were recorded in designed sheets. Ponderal index was calculated as weight in grams divided by the cube of length in centimeters multiplied by 100. The inclusion criteria were, 1- Singleton pregnancy 2- No congenital malformations 3- Absence of critical maternal diseases such as eclampsia, preeclampsia, diabetes mellitus, thyroid, liver, renal and cardiac diseases 4- Apgar score equal or greater than 7 at one minute 5- Mothers without history of cigarette smoking or alcohol consumption.

Leptin measurements were done by ELISA method (DRG Instruments Gmbh, Germany). The sensitivity of the ELISA was 1.0 ng/ml. The intra and inter assay coefficient of variation (CV) were <6% and <9%, respectively. Adiponectin measurements were done by ELISA method (AdipoGen Inc. Seoul, Korea). The sensitivity of it was 100 pg/ml. The intra and inter assay CV were <3.5% and <5%, respectively. Crosslaps was measured by ELISA method using Bioscience kit (Nortic Bioscience Diagnostic A/S, Denmark) with an intra assay CV of 5.4%. Osteocalcin was measured by ELISA method using Bioscience kit (Nortic Bioscience Diagnostic A/S, Denmark). The intra assay CV was 3.4%.

Standard statistical methods were used to calculate means and SD. Associations are given as Pearson correlation coefficient. ANOVA test was used to compare changes between groups. Osteocalcin as a marker of bone formation and crosslaps as a marker of bone resorption were considered. The concentrations of crosslaps and osteocalcin were converted to T scores, and then T scores of crosslaps were subtracted from osteocalcin T scores to establish estimation for bone formation, which we named Bone Formation Index. To calculate the T scores, mean of crosslaps or osteocalcin was subtracted from each sample level and divided by SD. To confirm the independency of the relation between leptin and adiponectin in one side and Bone Formation Index on the other side, the Univariate Analysis of Variance was used. $P< 0.05$ regarded as statistically significant. For statistical purposes SPSS 11.5 (IL, Chicago, USA) was used.

Results

The clinical characteristics of all participants are summarized in Table 1. The neonatal gestational ages range from 34 to 43 wk. Neonatal leptin, adiponectin and bone markers levels are demonstrated in table 2. Correlation between bone metabolic markers with leptin and adiponectin was checked; however none of these markers were correlated with leptin and adiponectin levels. A positive correlation was found between Osteocalcin and crosslaps (P= 0.01, r= 0.29). The amounts of leptin and adiponectin were converted in percentile units with 25 scale intervals. Then these percentiles were computed by levels of osteocalcin and crosslaps through ANOVA test, and no relation was found. Then, T scores for osteocalcin and crosslaps and the Bone Formation Index were calculated. In Univariate Analysis, after entrance sex, birth weight and birth length as fixed factors, leptin and adiponectin displayed an independent effect on Bone Formation Index (Figure 1). P value for leptin was 0.008 and p value for adiponectin was 0.040. Leptin was positively correlated with birth weight ($p < 0.05$ $r = 0.266$).

Table 1: Clinical characteristics of the all newborns

	Males (n=39)	**Females (n=38)**	**All Newborns (n=77)**
Gestational age (wk)	40.10±4.00	41.31±4.70	40.70±4.38
Birth weight (kg)	3.20±0.45	3.21±0.32	3.21±0.39
Birth length (cm)	50.29±2.42	49.84±2.97	50.06±2.70
Body weight/ body length	64.18±7.45	64.68±8.22	64.43±7.80
Ponderal index	2.54 ± 0.30	2.67±0.95	2.61±0.71

Table 2: Levels of serum leptin, adiponectin and bone markers in umbilical cord

	Male **Mean ± SD**	**Female** **Mean ± SD**	**All Newborns** **Mean ± SD**
Leptin (ng/ml)	13.86 ± 8.40	18.35 ± 15.64	16.08 ± 12.63
Adiponectin (µg/ml)	31.47 ± 30.99	27.07 ± 10.12	29.33 ± 23.25
Osteocalcin (ng/ml)	32.74 ± 19.87	25.41 ± 17.63	28.91 ± 18.95
Crosslaps (ng/ml)	0.84 ± 0.26	0.73 ± 0.20	0.79 ± 0.24

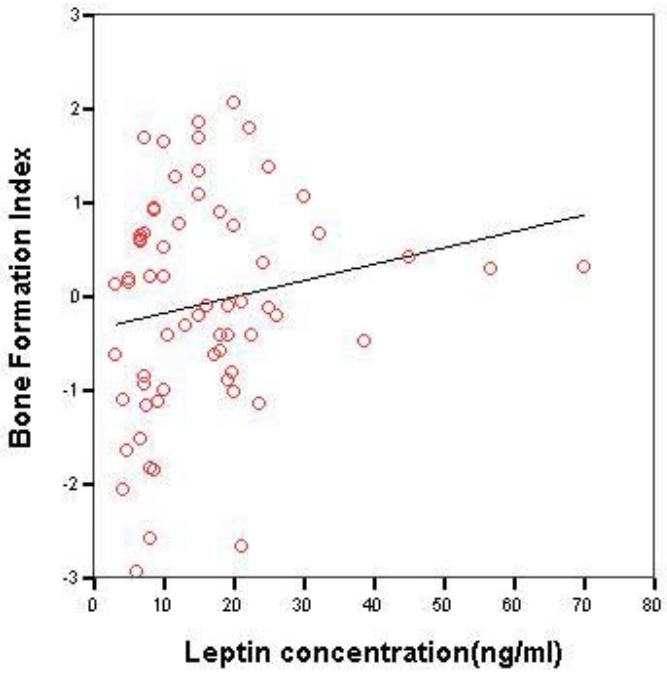

Fig. 1: The relation between umbilical cord leptin concentration and Bone Formation Index

Discussion

There is growing evidence that intrauterine bone formation and development influence sharply the risk of osteoporosis in old age (12). Determination of environmental and fetomaternal factors which contribute in bone metabolism are helpful for osteoporosis prevention or postpone (13). In a recent survey on 143 female and male of 70-75 yr old, the relationship between birth weight and risk of osteoporosis was proposed (14) .On the other hands, positive correlation between leptin and birth weight was reported in several previous surveys (15, 16) Gordeladze et al. demonstrated that iliac crest osteoblasts exposure to leptin increases collagen synthesis, cell differentiation, in vitro mineralization and osteocalcin mRNA expression (5). The positive correlation between neonatal adiponectin levels and birth weight and between leptin concentration and insulin resistance has been shown in some other studies (17-19).

According to recent studies, adiponectin has a positive effect on osteoblast proliferation, osteocalcin and type 1 collagen synthesis (6, 11). Regarding the influence of leptin and adiponectin on bone metabolism, some recent studies focused on the relation between adipokines and bone markers. In a series of surveys, done in adults population, a negative correlation between leptin in one side and osteocalcin and crosslaps

on the other side has been proposed (20-22). However, after adjustment for BMI the correlation was disappeared in majority of studies (21-23). To the best of our knowledge, our study was the first to assess the relationship between adipokines and bone markers in neonates. There wasn't demonstrated any significant correlation between bone markers and adipokines. These results are consistent with the same studies done among adult population (21-23). But, it has been shown that bone markers are much higher in neonatal circulation compared with maternal serum, which indicates higher rate of bone turnover in neonates (4, 24).

Finally, after adjustment for sex, birth weight and birth length a significant relation between Bone Formation Index and leptin and adiponectin was found, which is consistent with recent survey that postulated leptin as a positive effector on neonatal bone mass (12). So, we can come to the conclusion that both leptin and adiponectin have a remarkable impact on bone formation in fetus.

The complete nature of bone metabolism hasn't been fully understood yet, so series of investigations are needed to disclose hidden parts of this process in intrauterine period to prevent osteoporosis in future.

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References

1. De Leo V, Ditto A, la Marca A, Lanzetta D, Massafra C, Morgante G (2000). Bone mineral density and biochemical markers of bone turnover in peri- and postmenopausal women. *Calcif Tissue In*t, 66: 263-67.
2. Peichl P, Griesmacher A, Müller MM, Marteau R, Kumpan W, Bröll H (2000). Serum osteocalcin and urinary crosslaps are suitable markers of bone turnover in response to short-term hormone replacement therapy. *Gynecol Endocrinol*, 14: 374-81.
3. Minisola S, Rosso R, Romagnoli E, D' Erasmo E, Manfredi G, Damiani C, De Antoni F, Mazzuoli G (1997). Serum osteocalcin and bone mineral density at various skeletal sites: a study performed with three different assays. *J Lab Clin Med*, 129: 422-29.
4. Yamaga A, Taga M, Hashimoto S, Ota C (1999). Comparison of bone metabolic markers between maternal and cord blood. *Horm Res*, 51: 277-79.
5. Gordeladze JO, Drevon CA, Syversen U, Reseland JE (2002). Leptin stimulates human osteoblastic cell proliferation, de novo collagen synthesis, and mineralization: Impact on differentiation markers, apoptosis, and osteoclastic signaling. *J Cell Biochem,* 85: 825-36.
6. Luo XH, Guo LJ, Yuan LQ, Xie H, Zhou HD, Wu XP, Liao EY (2005). Adiponectin stimulates human osteoblasts proliferation and differentiation via the MAPK signaling pathway. *Exp Cell Res*, 309: 99-109.
7. Ahima RS, Flier JS (2000). Leptin. *Annu Rev Physiol*, 62:413-37.
8. Cock TA, Auwerx J (2003). Leptin: cutting the fat off the bone. *Lancet*, 362:1572-74.
9. Hu E, Liang P, Spiegelman BM (1996). AdipoQ is a novel adipose-specific gene dysregulated in obesity. *J Biol Chem*, 271: 10697-703.
10. Scherer PE, Williams S, Fogliano M, Baldini G, Lodish HF (1995). A novel serum protein similar to C1q, produced exclusively in adipocytes. *J Biol Chem*, 270: 26746-9.
11. Berner HS, Lyngstadaas SP, Spahr A, Monjo M, Thommesen L, Drevon CA, Syversen U, Reseland JE (2004). Adiponectin and its receptors are expressed in bone-forming cells. *Bone*, 35: 842-49.
12. Javaid MK, Godfrey KM, Taylor P, Robinson SM, Crozier SR, Dennison EM,

Robinson JS, Breier BR, Arden NK, Cooper C. (2005). Umbilical cord leptin predicts neonatal bone mass. *Calcif Tissue Int*, 76: 341-47.

13. Godfrey K, Walker-Bone K, Robinson S, Taylor P, Shore S, Wheeler T, Cooper C (2001). Neonatal bone mass: influence of parental birthweight, maternal smoking, body composition, and activity during pregnancy. *J Bone Miner Res*, 16: 1694-703.
14. Gale CR, Martyn CN, Kellingray S, Eastell R, Cooper C (2001). Intrauterine programming of adult body composition. *J Clin Endocrinol Metab*, 86: 267-72.
15. Pighetti M, Tommaselli GA, D'Elia A, Di Carlo C, Mariano A, Di Carlo A, Nappi C. (2003). Maternal serum and umbilical cord blood leptin concentrations with fetal growth restriction. *Obstet Gynecol,* 102: 535-543.
16. Pardo IM, Geloneze B, Tambascia MA, Pereira JL, Barros Filho AA (2004). Leptin as a marker of sexual dimorphism in newborn infants. *J Pediatr* (Rio J), 80: 305-8.
17. Pardo IM, Geloneze B, Tambascia MA, Barros-Filho AA (2004). Hyperadiponectinemia in newborns: relationship with leptin levels and birth weight. *Obes Res*, 12: 521-524.
18. 18.Kotani Y, Yokota I, Kitamura S, Matsuda J, Naito E, Kuroda Y (2004). Plasma adiponectin levels in newborns are higher than those in adults and positively correlated with birth weight. *Clin Endocrinol* (Oxf), 61: 418-23.
19. Giapros V, Evagelidou E, Challa A, Kiortsis D, Drougia A, Andronikou S. (2007). Serum adiponectin and leptin levels and insulin resistance in children born large for gestational age are affected by the degree of overweight. *Clin Endocrinol (Oxf)*, 66: 353-59
20. Schett G, Kiechl S, Bonora E, Redlich K, Woloszczuk W, Oberhollenzer F, Jocher J, Dorizzi R, Muggeo M, Smolen J, Willeit J (2004). Serum leptin level and the risk of nontraumatic fracture. *Am J Med,* 117: 952-6.
21. Dennison EM, Syddall HE, Fall CH, Javaid MK, Arden NK, Phillips DI, Cooper C. (2004). Plasma leptin concentration and change in bone density among elderly men and women: the Hertfordshire Cohort Study. *Calcif Tissue Int,* 74: 401-6.
22. Blain H, Vuillemin A, Guillemin F, Durant R, Hanesse B, de Talance N, Doucet B, Jeandel C (2002). Serum leptin level is a predictor of bone mineral density in postmenopausal women. *J Clin Endocrinol Metab,* 87: 1030-5.
23. Rauch F, Blum WF, Klein K, Allolio B, Schönau E (1998). Does leptin have an effect on bone in adult women? *Calcif Tissue Int*, 63: 453-55.
24. Alataş O, Colak O, Alataş E, Tekin B, Inal M, Ozalp S (1995). Osteocalcin metabolism in late fetal life: fetal and maternal osteocalcin levels. *Clin Chim Acta,* 239:179-83