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Ghrelin to obestatin ratio in maternal serum in pregnancies complicated by intrauterine growth restriction

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Summary

Ghrelin, an endogenous for the growth hormone secretagogue receptor, has been shown to participate in fetal growth. Obestatin, encoded by the same gene as ghrelin, is described as a physiological opponent of ghrelin. This study was designed to determine the changes of ghrelin/obestatin ratio in maternal serum in pregnancies with intrauterine growth restriction (IUGR). The authors found that the ghrelin levels in maternal serum were significent lower in IUGR group than in control group (236.34 ± 14.58 pg/ml *vs.* 321.49 ± 18.19 pg/ml, $p = 0.003$). However, the difference of obestatin levels in maternal serum in IUGR group than in control group was not significent (276.25 ± 20.54 pg/ml *vs.* 256.34 ± 21.21 pg/ml, $p = 0.308$). The ratio of ghrelin to obestatin in maternal serum were significent lower in IUGR group than in control group (1.05 ± 0.09 *vs.* 0.82 ± 0.08, $p = 0.03$). Meanwhile, the maternal serum growth hormone (GH) concentration in IUGR group was lower than that in control group (1.08 ± 0.08 pg/ml *vs.* 1.41 ± 0.09 pg/ml, $p = 0.009$), and the maternal serum placental growth hormone (PGH) concentration in IUGR group was lower than that in control group (2.21 ± 1.24 pg/ml *vs.* 2.92 ± 0.27 pg/ml, $p = 0.031$). The ratio of ghrelin to obestatin in maternal serum were positively correlation with GH and PGH concentrations in IUGR group ($r = 0.876$, $p = 0.52$; $r = 0.764$, $p = 0.64$). The findings of this study suggest that the ratio of ghrelin to obestatin in maternal serum were low, and were positively correlated with GH and PGH concentration in IUGR group, which can been considered as evidencees of ghrelin/obestatin balance disorder role in pathogenesis of IUGR.

Key words: Ghrelin; Obestatin; Pregnancy complications; Intrauterine growth restriction.

Introduction

Intrauterine growth restriction (IUGR) is the failure to achieve the genetically predetermined growth potential, which is defined as a birth weight below the 10th percentile for gestational age. IUGR have several consequences, including increased fetal morbidity and mortality, childhood morbidity, and an increased risk of glucose intolerance and cardiovascular disease in adult life [1]. Although the IUGR may be caused by maternal, fetal, placental, and external factors, its etiology is unknown in approximately 60% of cases. Fetal growth is dependent on gentic factors, local growth factors, and placentation, as well as on hormones, oxygen, and nutrient availability. Glucose and lipid are main energy substrate for the growing fetus. Consequently, maternal energy production and hormones are the determinants of fetal growth [2]. During pregnancy, energy production increased with pregnancy-specific hormones increment to meet the demands of the growing fetus and placenta. The endocrine factor playing the most important role in the late gestation fetus is ghrelin, which stimulates fetal development and modulates metabolism.

Ghrelin and obestatin are two peptides isolated from the gastrointestinal tract and encoded by the same preproghrelin gene [3]. They convey to the central nervous system information concerning the nutritional status and/or the energy stores. Ghrelin is a 28 amino-acid peptide, which was initially characterized as the endogenous ligand for the growth hormone (GH) secretagogue receptor (GHS-R) [4]. However, ghrelin also regulates other neuroendocrine and metabolic functions in rodents and humans: it is a potent GH secretagogue, an orexigenic peptide, and a long-term regulator of energy homeostasis [5]. Obestatin is a 23 amino-acid peptide initially described for its anorexigenic effects and its binding to G protein-coupled receptor 39 (GPR39) [6]. The original study reporting the function of obestatin demonstrated that obestatin have opposing effects to ghrelin's actions on energy homeostasis. So the ghrelin/obestatin ratio may be a more informative measure than level of ghrelin alone, and this ratio could prove useful as a biomarker for some disease states. The ghrelin/obestatin ratio is reduced in a number of diseases, including mild to moderate hypertension [7], indicating that ghrelin and obestatin may play a role in the pathogenesis of these diseases. Previous studies have shown that ghrelin can be detected in fetal circulation from 20 to 23 weeks of gestation, and the ghrelin concentration is low in maternal serum of IUGR. However the change of ghrelin/obestatin ratio in maternal serum in pregnancies with IUGR is unknown.

The authors propose that ghrelin and obestatin in maternal serum may influence fetal growth together, and the ghre-

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lin/obestatin balance disorder may lead to IUGR. In this study, the authors sought to investigate the changes of ghrelin/obestatin ratio in maternal serum with IUGR, and the relationship between the ghrelin/obestatin ratio and GH or placental growth hormone (PGH) concentration in maternal serum with IUGR.

Materials and Methods

Study population

In this restrospective case-control study, 72 pregnant women were included. All women were in the third trimester of pregnancy (28~34 weeks of gestation), and were enrolled in the study following written informed consent at Obstetrics and Gynecology Department of Affiliated Hospital of Logistical College of Chinese People's Armed Police Force between January 2007 and December 2013. Ethics approval was granted by the research ethics board of the College.

The IUGR group was composed of 32 women with singleton pregnancies complicated by IUGR, sustained by uteroplacental insufficiency without known maternal or fetal disorders. The ultrasound estimation of fetal weight and fetal growth was appropriate for gestational age in the second trimester. The control group was composed of 44 healthy women with singleton pregnancies, progressing to deliver a healthy term baby, without complications, who received no medications except for prenatal vitamins. Body mass index in two groups range from 18.5 to 25. All women gave birth to live babies.

Gestational age was calculated from the data of the last menstrual period and confirmed by ultrasound in the first trimester. IUGR was defined as an estimation of fetal weight below the 10th percentile for gestational age, and by the presence of ultrasonographic signs (biparietal diameter below the 10th centile and abdominal circumference below the 5th percentile) according to the normograms of Campbell and Thoms. A decrease in the fetal size centile from the first scan after referral to the last scan before delivery was recorded and used to categorize IUGR fetuses.

Ultrasound examinations were performed using ultrasound machines, equipped with transabdominal convex multifrequency probes (2.5-66 MHz) with colour/power Doppler and four-dimensional reconstruction capabilities. For each case, the authors applied 2-D ultrasound scanning in evaluation of fetal biometric parameters: biparietal diameter (BPD), head circumference (HC), abdominal circumference (AC), femur length (FL), as well as fetal morphology, and localization of the placenta. Doppler flow studies in the middle cerebral artery and in the umbilical artery were determined by pulsation index, resistance index, and systolic to diastolic ratio. The wave shape in ductus venosus was also analyzed. The mass of fetus was estimated by ultrasound biometry assessment: BPD, HC, AC, and FL.

Maternal complications such as intrauterine fetal death, fetal genetic anomalies, eclampsia, preeclampsia, abruptio placentae, gestational hypertension, gestational diabetes, or other medical disorders were excluded from this study. As ghrelin and obestatin levels may be influenced by body mass index, pregnant women body mass index ≤ 18.5 and body mass index ≥ 25 were considered exclusion criteria.

Venous blood samples of pregnant women in 28~34 weeks of gestation were collected from the antecubital vein in the morning between 7:00 and 8:00 after an overnight fast, because plasma ghrelin and obestatin levels have been shown to be altered by food intake. The blood sample was immediately transferred into a plastic tubes containing EDTA-2Na (one-mg/ml) and aprotinin (100 μl containing 0.6 trysin inhibitor units per milliliter of blood), and centrifuged at 4°C, 1600×g for 15 minutes. All serum samples were kept at -80°C prior to assay.

Table 1. — *The main clinical characteristics of the study population.*

Parameters	IUGR group (n=32)	Control group (n=44)	*p*-value
Maternal age (years)	28.46±2.74	29.97±2.98	0.553
BMI (kg/m^2)	22.38±1.59	21.62±1.27	0.555
Weight gain (kg)	12.12±1.21	13.01±1.16	0.478
Gestational week	30.12±1.57	29.19±1.63	0.516
Delivery week	39.41±1.02	39.56±1.10	0.871
Birth weight (g)	2190.28±110.45	3241.72±124.31	0.000

Data are mean ± SE.

Hormone assays

The obestatin concentrations were measured after extraction in reverse phase C18 columns with a commercial RIA kit using ^{125}-I-labeled obstatin as tracer and polyclonal antibody raised in rabbits against human obestatin. Intra- and inter-assay coefficient of variation (CV) were less than 5% and 12%, respectively, according to the manufacture. Serum ghrelin concentrations were measured using a commercial RIA kit with ^{125}-I-labeled bioactive ghrelin as a tracer molecule and a rabbit polyclonal antibody against full-length octanoylated human ghrelin. This assay recognizes both acylated and des-acylated forms of ghrelin. The assay protocol was similar to obestatin assay kit except that extraction of plasma was not required according to the manufacture's instructions. The intrassay CV was less than 4%, and interassay CV was less than 8%.

Serum total GH was measured by time-resolved immunofluorometric assay (TR-IFMA) specific to the 22-kD pituitary GH. The detection limited is 0.03 μg/L, and intra- and inter-assay CVs is 2% and 3.2%, respectively. A commercially available solid phase immunoradiometric assay (PGH IRMA, BC1017) was used for determination of serum PGH. In the present setting, both the intra- and inter-assay CVs were < 6%.

Statistical analysis

Clinical data were not normally distributed, and data are presented as mean ± standard deviation. Unpaired *t*-test was used to compare parametric variables. Spearman's correlation coefficient was calculated to investigate the association between ghrelin/obestatin ratio in maternal serum and GH or PGH concentrations in the IUGR group. $p < 0.05$ was considered significant for all statistical analyses. All of the analyses were performed using SPSS (version 10.0).

Results

Subject characteristics

The main clinical and demographic characteristics of the study population are summarized in Table 1. There were no significant differences in age, gestation week, and body mass index between two groups ($p > 0.05$).

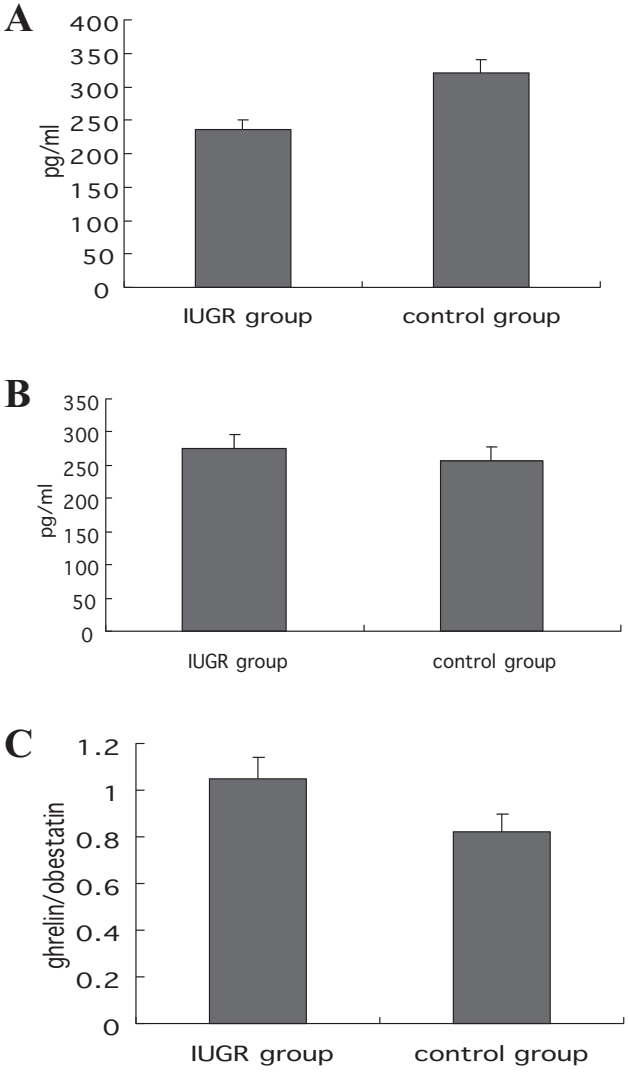

Figure 1. — Ghrelin and obestatin concentrations maternal serum were measured in IUGR and control groups, and ghrelin/obestatin ratios were calculated. (A) Ghrelin concentrations in IUGR and control group. (B) Obestatin concentrations in IUGR and control groups. (C) The ghrelin/obestatin ratios in IUGR and control groups. This figure shows that the ghrelin concentrations sand ghrelin/obestatin ratios were remarkably decreased in IUGR group compared with control group ($p < 0.05$). No significant change of obestatin concentration was observed in IUGR and control groups ($p > 0.05$).

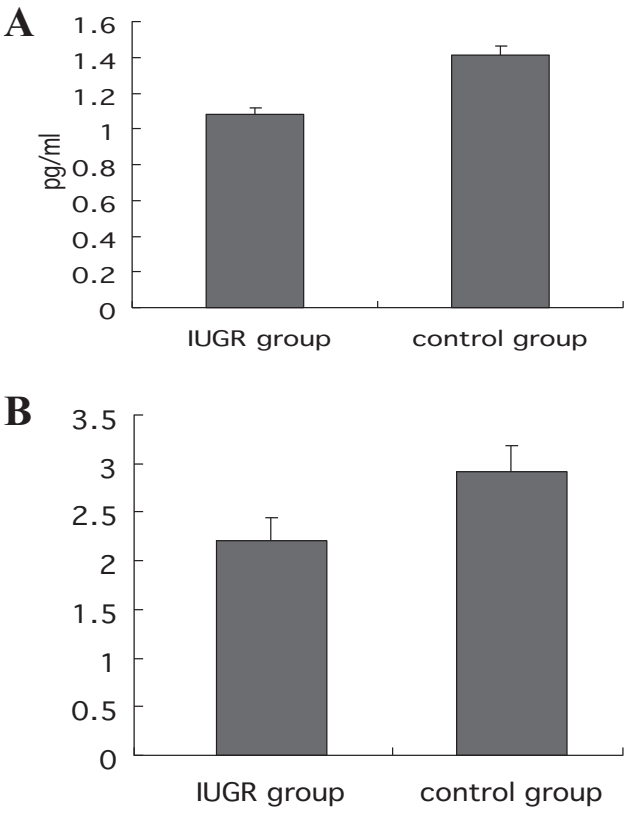

Figure 2. — GH and PGH concentrations in maternal serum were measured in IUGR and control groups. (A) GH concentrations in IUGR and control group. (B) PGH concentrations in IUGR and control groups. This figure shows that the GH and PGH concentrations were remarkably decreased in IUGR group compared with control group ($p < 0.05$).

Differences of ghrelin and obestatin levels in maternal serum

The plasma concentration of ghrelin in maternal serum were 236.34 ± 14.58 pg/ml and 321.49 ± 18.19 pg/ml in IUGR group and control group, respectively. There was a significant difference between the two groups ($p = 0.03$) (Figure 1A). The plasma concentration of obestatin in maternal serum were 276.25 ± 20.54 pg/ml and 256.34 ± 21.21 pg/ml in IUGR group and control group, respectively. The difference between the two groups was not significant ($p = 0.308$) (Figure 1B). however, the ghrelin/obestatin ratio in maternal serum in IUGR group was significantly lower than that in control group (1.05 ± 0.09 *vs.* 0.82 ± 0.08, $p = 0.03$) (Figure 1C).

Differences of GH and PGH concentration in maternal serum

The GH concentrations in maternal serum were 1.08 ± 0.08 pg/ml and 1.41 ± 0.09 pg/ml in IUGR group and control group, respectively. There was a significant difference between the two groups ($p = 0.009$) (Figure 2A). The PGH concentrations in maternal serum were 2.21 ± 0.24 pg/ml and 2.92 ± 0.29 pg/ml in IUGR group and control group, respectively. There was a significant difference between the two groups ($p = 0.031$) (Figure 2B).

Corelations of ghrelin/obestatin ratio with GH and PGH

The ratio of ghrelin to obestatin were positively correlated with GH concentration in maternal serum in IUGR group ($r = 0.876$, $p = 0.52$). The ratio of ghrelin to obestatin

were also positively correlated with PGH concentration in maternal serum in IUGR group (r = 0.764, $p = 0.64$).

Discussion

This study showed that both ghrelin levels and ghrelin/obestatin ratios in maternal serum were significant lower in IUGR group compared with control group, and both GH and PGH concentrations in maternal serum in IUGR group were significant lower compared with control group. The ghrelin/obestatin ratios in maternal serum were positively correlated with GH and PGH concentrations in IUGR group. There were no significant changes in obestatin level in maternal serum between the two groups.

Ghrelin and obestatin are two peptides isolated from the gastrointestinal tract and encoded by the same preproghrelin gene. Ghrelin is a 28 amino-acid peptide which is acylated on the third amino-acid serine by the enzyme Ghrelin-O-AcylTransferase (GOAT) [8]. It was initially characterized as the endogenous ligand for the GHS-R [9]. However, ghrelin also regulates other neuroendocrine and metabolic functions in rodents and humans. Recent studies show that preprandial [10] circulating ghrelin levels are increased in obese individuals, and serum ghrelin levels are correlated with body mass index both in obesity and lean subjects. Central and peripheral ghrelin administrations lead to increased appetite and weight gain [11, 12]. During pregnancy, the high GH levels may be related to ghrelin in the fetus. A novel finding is that both maternal and fetal ghrelin increase with length of gestation at delivery [13]. This finding indicates that ghrelin may be implicated in fetal development. Ghrelin mRNA and ghrelin peptide have also been detected in human placenta in which they are expressed predominantly in cytotrophoblast cells and very sporadically in syncytiotrophoblast cells, and can be detected in cord blood at 30-week gestational age, which also indicates that it may play a role in fetal development [14]. In human placenta, ghrelin is mainly expressed in the first half of pregnancy and is not detectable at term [15]. Involvement of ghrelin in fetal-maternal interaction via autocrine, paracrine, or endocrine mechanisms is discussed. Ghrelin plays important role in the fetal/maternal communication [16]. Fuglsang [17] demonstrated that the placenta contributes to the circulating pool of maternal ghrelin during late gestation, and that maternal ghrelin rapidly and easily crosses to the fetus. The author also demonstrated that exogenous chronic treatment of the mother with ghrelin increases fetal birthweight, whereas mothers immunized against ghrelin deliver fetuses with a lower birthweight. In the present study, the authors found that ghrelin levels in maternal serum were low in IUGR group. Taken together, this data and these findings indicate a role of maternal ghrelin in fetal development.

Obestatin is a 23 amino-acid peptide initially described for its anorexigenic effects and its binding to GPR39. In contrast to ghrelin, obestatin seems to have opposing effects to ghrelin's actions on energy homeostasis and gastrointestinal function [18, 19]. Although there have been some controversies concerning obestatin, many studies have found that obestatin plays a role in the regulation of energy homeostasis [20, 21]. Even less clear is the physiological significance of circulating obestatin levels as indicated in the few studies published so far. Obestatin levels have been demonstrated to be lower in obese subjects when compared with lean subjects, showing a significant increase in the obese patients six months after gastric banding surgery [22]. In mice, acute administration of obestatin (10–100 nmol/kg i.p.) inhibited feeding and similar effects were observed in lean and fatty Zucker rats [23]. In the present study, the authors found that the obestatin levels in maternal serum have no significant changes in IUGR group. These findings seem to indicate that single obestatin have no association with fetal development.

During human pregnancy, the materal pituitary GH production is suppressed and replaced with PGH with advancing gestational age [24]. GH axis switches from the predominance of pituitary GH in the first trimester to the presence of high levels of PGH in the third trimester [25]. Only the placenta secretes PGH and into the maternal circulation. The physiological regulation of PGH has not been clarified. Low glucose levels stimulate PGH secreting in vitro and in vivo [26, 27], and fasting can significant increase PGH levels in pregnant women [28]. Ghrelin infusion in pharmacological doses has been demonstrated to increase primarily the concentration of GH [29]. The present authors observed a decrease in GH and PGF levels of maternal serum in IUGR group, and this implicated that maternal serum GH and PGF can influence fetal growth.

Because obestatin has opposing effect to ghrelin's actions on energy homeostasis, the intricate balance of ghrelin to obestatin in humans energy homeostasis is interesting. The balance between endogenous ghrelin and obestatin appears essential to maintain a homeostatic state of these neuroendocrine systems. Much of the current research is focused on the ghrelin/obestatin ratio in obesity and related metabolic disorders. Guo *et al.* [30] found that obese subjects exhibited a higher plasma preprandial ghrelin/obestatin ratio than in healthy normal-weight controls, whereas another study confirmed that the ghrelin/obestatin ratio was decreased in obese women [31]. There is a decreased tendency of the ghrelin/obestatin ratio in obese children after weight reduction [32]. This suggests that the balance of ghrelin/obestatin might play a role in the regulation of energy homeostasis. A decreased ghrelin/obestatin ratio found in anorexia nervosa (AN) might participate in the restraint in nutriment intake of these patients [33]. In agreement with previous reports [34, 35], plasma obestatin levels were positively correlated with ghrelin in all subjects. Intriguingly, the ghrelin/obestatin ratio was negatively correlated with several metabolic al-

terations [36]. These facts may suggest that the intricate balance of ghrelin/obestatin is important in the regulation of energy homeostasis and body weight control. The interaction between ghrelin/obestatin on fetal development is still under debate. In this study, the ghrelin/obestatin ratios in maternal serum were low in IUGR group, and were positively correlated with maternal serum GH and PGH concentrations in IUGR group. The findings implicated that ghrelin/obestatin balance disorder in maternal serum may influence GH and PGH productions, and may lead to IUGR.

In conclusion, the low ghrelin levels and low ghrelin/obestatin ratio in maternal serum are seen in IUGR group, and the low GH and PGH concentrations are also seen in IUGR group. Meanwhile, the positive correlation between ghrelin/obestatin ratio and two growth hormones could be demonstrated. The ghrelin/obestatin balance disorder in maternal serum impact GH and PGH secretion in IUGR remains to be elucidated.

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