Umbilical cord ghrelin concentrations in small- and appropriate-for-gestational age newborn infants: relationship to anthropometric markers

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Abstract

Ghrelin is a newly discovered orexigenic peptide originating from the stomach. Circulating ghrelin levels reflect acute and chronic energy balance in humans. However, it is not known whether ghrelin also plays a role in energy homeostasis during fetal life. Forty-one small-for-gestational age (SGA) and 34 appropriate-for-gestational age (AGA) infants were studied in order to determine whether cord blood ghrelin concentrations were different in SGA infants compared with AGA infants and the relationship to anthropometric measurements at delivery. The cord blood ghrelin concentrations of SGA infants (means $\pm$ S.E.M.; 15.20 ± 3.08 ng/ml) were significantly

greater than of AGA infants (2.19 ± 0.24 ng/ml) ($P < 0.0001$). They were negatively correlated with the infants' birth weights ($r = -0.481$, $P < 0.0001$) and with body mass index values ($r = -0.363$, $P < 0.001$). The higher ghrelin concentrations were found in female infants (20.42 ± 4.55 ng/ml) than in males (7.05 ± 2.27 ng/ml) in the SGA group ($P = 0.042$). These data provide the first evidence that cord ghrelin levels of SGA infants are greater than those of AGA infants and it is suggested that ghrelin is also affected by nutritional status in the intrauterine period.

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Introduction

Ghrelin, an acylated peptide recently isolated from rat stomach, is a natural ligand for growth hormone secretagogue receptors and has potent growth hormone (GH)-releasing activity with a dose-dependent manner (Kojima *et al.* 1999). The concentration of ghrelin has been shown to increase with fasting and decrease following feeding in humans and rats (Ariyasu *et al.* 2001, Nakazato *et al.* 2001). Ghrelin has also been shown to stimulate appetite and to induce adiposity, in addition to its effects on GH secretion (Tschöp *et al.* 2000, Wren *et al.* 2000, 2002, Asakawa *et al.* 2001). These data suggest that ghrelin may be an important link between nutrition and growth. Ghrelin levels have been found to be elevated in patients with anorexia nervosa compared with those in healthy controls and it is suggested that plasma ghrelin levels reflect acute and chronic energy balance in humans (Ariyasu *et al.* 2001).

Ghrelin is secreted mainly by the stomach, but also by many other organs, including the pituitary and the placenta (Gualillo *et al.* 2001). The presence of significant ghrelin concentrations in human cord blood has been shown but there is no study evaluating cord blood ghrelin levels in intrauterine growth-restricted infants (Chanoine *et al.* 2002).

The aim of this study was to determine whether cord blood ghrelin concentrations are different in small-for-gestational age (SGA) infants compared with appropriate-for-gestational age (AGA) infants and its relationship to anthropometric measurements at delivery.

Materials and Methods

Seventy-five newborn infants born at Gazi University Medical School Hospital, Ankara, Turkey were enrolled in the study. Infants whose birth weight was below the 10th percentile (SGA group, $n = 41$) were selectively included in the study and defined as SGA. The AGA group, selected at random, consisted of 34 infants whose birth weight was between the 10th and the 90th percentile on the intrauterine growth charts of Lubchenco *et al.* (1963). Because national intrauterine growth curves are not available, the curves described by Lubchenco *et al.* (1963) were used. Demographic features and anthropometric measurements of the infants were recorded. Weight was measured using an electronic scale (Terrallion, Chatou, Cedex, France), length (± 0.1 cm) was measured using a body-length measurer by a pediatric nurse and body mass index (BMI; kg/m^2) was calculated.

Infants with any congenital malformation, those whose mothers had a history of any chronic illness such as diabetes, autoimmune diseases, toxoplasmosis, others, rubella, cytomegalovirus and herpes simplex virus infections, pregnancy-induced hypertension and pre-eclampsia, infants from multiple gestations and those whose mothers had smoked during pregnancy were also excluded from the study. None of the mothers were suffering from malnutrition. Informed consent was obtained and the study was approved by the local ethics committee.

Ghrelin assay

At delivery, the umbilical cord was clamped immediately after the delivery of the infant, and then blood was drawn from the umbilical vein. Blood samples, anticoagulated with EDTA and aprotinin, were kept on ice, centrifuged at 1800 g for 15 min at 4 °C and plasma samples were separated and stored at – 80 °C until assayed.

Ghrelin levels were measured by using a commercial enzyme-linked immunosorbent assay kit (Ghrelin Human EIA Kit, EK-031–30; Phoenix Pharmaceuticals, Inc., Belmont, CA, USA) and samples for quality control were included in each assay. The intra-assay coefficients of variation for the assay were <5% over the sample concentration range.

Statistical analysis

The chi-square test was used for comparing nominal variables between groups and the Mann–Whitney U test was used for numeric variables. Spearman bivariate correlation analysis was applied to calculate the correlation between cord blood ghrelin levels and the anthropometric measurements of the infants. All statistical calculations were performed by using SPSS 10.0 for Windows program. A *P* value of <0.05 was considered statistically significant.

Results

Birth weight, length and BMI of the SGA infants were significantly lower than those of the AGA infants. Gestational age and male/female ratio were similar in both groups. The clinical features of the subjects are shown in Table 1.

Cord blood ghrelin concentrations in SGA infants (means $\pm$ S.E.M.; 15.20 ± 3.08 ng/ml) were significantly greater than in AGA infants (2.19 ± 0.24 ng/ml) ($P < 0.0001$) (Fig. 1). Cord blood ghrelin concentrations were conversely correlated with the infants' birth weight ($r = -0.481$, $P < 0.0001$) and BMI ($r = -0.363$, $P = 0.001$). Although ghrelin levels were similar in both sexes in the AGA infants, ghrelin levels in female infants

Table 1 Clinical features of study infants. Values are means $\pm$ S.D.

	SGA (<i>n</i> =41)	AGA (<i>n</i> =34)	<i>P</i> value
Gestational age (weeks)	38.9 $\pm$ 1.2	38.9 $\pm$ 1.0	0.893
Birth weight (g)	2315 $\pm$ 264	3131 $\pm$ 303	<0.0001
Birth length (cm)	45.8 $\pm$ 2.1	49.6 $\pm$ 1.7	<0.0001
BMI (kg/m ²)	11.2 $\pm$ 1.2	12.8 $\pm$ 0.9	<0.0001
Sex (female/male)	25/16	15/19	0.144
Apgar score (5th min)	9 $\pm$ 1	9 $\pm$ 1	1.000
Delivery route (vaginal/ Caesarean section)	20/21	16/18	0.882
Mothers' age (years)	25.2 $\pm$ 4.7	27.1 $\pm$ 5.4	0.110

Figure 1 Mean (± 2 S.E.M.) cord blood ghrelin concentrations in the SGA and the AGA infants ($P < 0.0001$).

were greater than in male infants in the SGA group ($P = 0.042$) (Fig. 2). Birth weights of female infants (means $\pm$ S.D.; 2268 ± 283 g) were slightly lower than those of males (2388 ± 219 g) in the SGA group ($P = 0.190$). Ghrelin levels in the newborns born by vaginal delivery (means $\pm$ S.E.M.; 17.03 ± 4.79 ng/ml) were similar to those born by Caesarean section (13.46 ± 3.96 ng/ml) in the SGA group ($P = 0.804$) and similar in the AGA group (2.31 ± 0.36 ng/ml for vaginal vs 2.09 ± 0.34 ng/ml for Caesarean section) ($P = 0.654$).

Discussion

We have found that the cord blood concentrations of ghrelin in SGA infants were greater than those in AGA infants. Although ghrelin has been shown to increase in anorexia and decrease in obesity in humans and has been suggested to play a role in the regulation of energy homeostasis, the increased cord ghrelin concentrations in intrauterine growth-restricted infants compared with

Figure 2 Cord blood ghrelin levels in female and male infants in the SGA and AGA groups (* $P < 0.05$). Data are means $\pm$ S.E.M.

healthy newborns has not previously been reported (Ariyasu *et al.* 2001, Tschöp *et al.* 2001, Haqq *et al.* 2003).

Ghrelin has been implicated in both meal-time hunger and the long-term regulation of body weight. The increased levels of circulating ghrelin after weight loss is consistent with a role of ghrelin in the long-term regulation of body weight in humans (Ariyasu *et al.* 2001, Cummings *et al.* 2002, Hansen *et al.* 2002). In addition, continuous administration of ghrelin increases body weight and, besides increasing food intake, exogenous ghrelin decreases the metabolic rate and the catabolism of fat, thereby affecting all aspects of the system of energy regulation in such a way as to increase body weight (Tschöp *et al.* 2000, Nakazato *et al.* 2001). Although ghrelin mRNA transcription has been shown in the placenta in humans, its role in the intrauterine period is not clear. It has been shown that ghrelin administration to pregnant rats caused an increase in the body weight of newborn rats and ghrelin has been suggested to be involved in neonatal growth (Hayashida *et al.* 2002). Therefore, ghrelin may also have a role in energy regulation in fetal life.

Plasma ghrelin levels are found to be extremely high in patients with anorexia nervosa compared with after 12-h fasting in normal subjects, and the levels were negatively correlated with BMI. This observation raises the idea that ghrelin also reflects the chronic feeding state (Ariyasu *et al.* 2001). In other studies, plasma ghrelin concentrations have also been reported to be negatively correlated with BMI in humans (Shiia *et al.* 2002, Haqq *et al.* 2003). Similarly, in our study, we also found that cord ghrelin concentrations correlated with birth weight and BMI which was consistent with the results of previous studies. Birth weight is suggested to be a marker of adiposity in newborns.

Additionally, body weight measurement is the easiest and the most reliable method among anthropometric measurements to indicate the nutritional status of newborns and birth weight is the most used marker indicating fetal neonatal growth. Although birth weight and BMI are indirect markers of adiposity, it has been shown that BMI and weight are correlated with total body fat as derived by total body electrical conductivity which is an accurate, precise and reproducible method for estimating total body fat in infants (deBruin *et al.* 1995). Therefore, we investigated the correlation between cord ghrelin levels and birth weight and BMI of infants as indirect markers of nutritional status. Moreover, markedly increased cord ghrelin concentrations in the SGA infants in the present study may be due to the state of undernutrition of those infants.

Infants in the SGA group in this study were assumed to be intrauterine growth restricted due to unknown etiologies, as infants who had congenital malformations and whose mothers had any condition known to be a cause of intrauterine growth restriction (IUGR) were excluded from the study. Makino *et al.* (2002) have reported a negative correlation between plasma ghrelin concentration and systemic blood pressure in normal pregnant women, and higher ghrelin levels in pregnant women with pregnancy-induced hypertension than normal pregnant women. On the other hand, Tschöp *et al.* (2001) have shown that fasting plasma ghrelin was negatively correlated with fasting insulin and Masaoka *et al.* (2003) have reported significantly increased plasma ghrelin levels in diabetic rats. Therefore, the possible effects of certain pathologic conditions on fetal ghrelin regulation are not clearly understood. As we excluded subjects whose mothers had various pathologic conditions perhaps causing IUGR, we could then omit the possible effects of those conditions on ghrelin levels.

We included SGA infants as a study group and assumed that they were intrauterine growth restricted. Although IUGR denotes a pathophysiologic process resulting in restriction of fetal growth, and SGA refers to a statistical grouping of infants below the 10th percentile, IUGR is often used synonymously with SGA. We did not diagnose IUGR clinically but we defined SGA infants according to their birth weight, which is a more objective criteria than physical findings consistent with IUGR diagnosis clinically. Nevertheless, there would, of course, be a distinction between the SGA and the IUGR classifications in extreme cases.

The neuroendocrine mechanism regulating GH secretion appears to be exquisitely sensitive and responsive to nutritional state in humans. Fasting stimulates GH release in humans and infants with IUGR who characteristically show elevated basal levels of GH (Leger *et al.* 1996, Cance-Rouzaud *et al.* 1998). It has been unclear, however, what factors specifically activate this system in undernutrition. Although we did not measure the GH

levels, which is a weakness of this study, the results suggested that the augmented ghrelin levels might consequently lead to elevated GH levels as ghrelin has a potent GH-releasing activity.

Chanoine *et al.* (2002) showed the presence of immunoreactive ghrelin in cord samples of full-term newborns. They reported that ghrelin was inversely correlated with anthropometric measures and similar ghrelin concentrations were found in female and male infants. Similarly, the cord ghrelin levels were found to conversely correlate with birth weight in our study. Although the ghrelin levels of female infants were similar to those of male healthy newborns, greater values in female than in male infants were found in the SGA group in the present study, in contrast to the results of Chanoine *et al.* (2002). Similarly, there are some studies reporting higher leptin levels in female infants than in male infants (Helland *et al.* 1998, Lönnnerdal & Havel 2000), whereas some reports have suggested no gender difference in cord plasma leptin levels in infants (Schubring *et al.* 1997, Shekhawat *et al.* 1998). Additionally, there has been no study suggesting that ghrelin might be regulated by estrogen. Therefore this finding might be due to the lower birth weights of the female infants in this group.

We have shown that the cord ghrelin levels in infants born by vaginal delivery were not different from infants born by Caesarean section and concluded that delivery mode did not affect the cord ghrelin levels.

Cord ghrelin concentrations measured in this study seem to be higher compared with plasma ghrelin concentrations in children and adults in previous studies (Ariyasu *et al.* 2001, Bellone *et al.* 2002, Haq *et al.* 2003). However, it is necessary to investigate whether ghrelin levels change with age. If plasma ghrelin levels decrease with age, then it will be consistent with decreasing GH levels with age in humans.

The origin of the increased levels of ghrelin in cord blood in the SGA infants is also important. Both fetal and placental ghrelin secretion has been demonstrated so the two sources would be expected. However, Gualillo *et al.* (2001) have shown that ghrelin mRNA in pregnant rats showed a characteristic profile of expression. We did not find any correlation between gestational age and cord ghrelin levels. Additionally, placental size is considered to be lower in SGA pregnancies than in AGA ones. Therefore, we hypothesize that cord ghrelin levels should be fetal in origin rather than placental. Nevertheless, we could not support this hypothesis with the results of this study. It is worthwhile investigating the origin of ghrelin in cord blood.

In summary, cord ghrelin concentrations in the SGA infants were significantly greater than those in the AGA infants, and they were negatively correlated with birth weight and BMI. These findings suggest that ghrelin is involved in energy homeostasis and is affected by nutritional status also in intrauterine life.

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