Ghrelin levels are decreased in non-obese prepubertal children born large for gestational age

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Abstract

Background: Ghrelin is the natural ligand of GH secretagogue receptor. It has several metabolic functions including regulation of food intake, energy homeostasis, and body weight. An inverse relationship between fasting plasma ghrelin and insulin concentrations has been shown. Being born large for gestational age (LGA) has an increased risk of developing insulin resistance.

Objective: The aim of this study was to evaluate ghrelin levels in LGA born children who have no obesity at prepubertal ages and the effect of intrauterine and postnatal growth on ghrelin levels.

Patients and methods: Thirty-two (17F, 15M) LGA born non-obese children (mean (±S.E.M.) age 4.4±0.3 years) were evaluated with respect to glucose, insulin, and ghrelin levels. Their data were compared with that of non-obese 45 (19F, 26M) appropriate for gestational age (AGA) children (mean (±S.E.M.) age 4.0±0.1 years).

Results: LGA children, who had similar age and body mass index (BMI) standard deviation score (SDS) as AGA children, had significantly higher insulin (P=0.044) and at a borderline significance higher homeostasis model assessment-insulin resistance levels (P=0.054) than AGA children. Ghrelin level was significantly lower in LGA born than AGA born children (P=0.001) even after controlling for age, sex, and BMI (P=0.006). There were no differences between genders in insulin and ghrelin levels. Multivariate analysis revealed that birth weight was the only significant parameter influencing ghrelin levels (R²=0.13, B = −0.007, P = 0.002).

Conclusions: LGA born non-obese prepubertal children have lower ghrelin levels when compared with age and BMI matched AGA children. Birth weight seems to have the only significant effect on the reduced ghrelin levels.

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Introduction

Intrauterine growth pattern has been shown to be associated with disturbances in glucose metabolism in later life. The fetal programming hypothesis suggests that adverse intrauterine milieu causes structural, hormonal, and metabolic adaptations in the fetus that persist in later life (1). It was shown in several studies that term babies who are born small for gestational age (SGA) may develop insulin resistance and other co-morbidities in adult life (2). Children born large for gestational age (LGA) also have an increased risk of developing obesity, insulin resistance, metabolic syndrome, diabetes, and early cardiovascular disease (3). Maternal gestational diabetes may be the cause of LGA birth in some infants as well as maternal obesity or excessive weight gain during pregnancy; however, there is no identifiable cause for LGA birth in a large proportion of the infants (4). It has been suggested that unidentified hyperglycemia during pregnancy in such children may result in hyperinsulinemia in utero which in turn may cause alterations in metabolic programming in future life (5). We have recently shown that LGA children mostly with no underlying maternal pathology have higher insulin and lower adiponectin levels than normal appropriate for gestational age (AGA) born children in spite of similar body mass index (BMI) at prepubertal ages (6). Both birth weight and postnatal growth pattern of the LGA children seemed to have an effect on the insulin resistance and low adiponectin levels.

Ghrelin, a 28-amino-acid peptide, is predominantly produced in the stomach but its expression has been demonstrated in several other organs including hypothalamus and pituitary gland. It has been shown to be a natural ligand of GH secretagogue receptor and plays a major role in the control of somatotrop function (7). It has also been considered as a major orexigenic factor and is involved in the regulation of feeding behavior and energy homeostasis (8). Ghrelin secretion is

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upregulated in conditions of negative energy balance and downregulated in the setting of positive energy balance. Previous reports demonstrated that ghrelin levels were increased in anorexia and cachexia (9) and significantly decreased in obese children (10). In humans, circulating ghrelin levels are increased before a meal and decreased by food intake (11). In animals, it also increases adiposity by decreasing fat utilization (12). Therefore, ghrelin has a role in short-term regulation of energy homeostasis, and it may also be involved in long-term regulation of energy balance and body weight control (8, 11, 12).

Ghrelin has been implicated in the regulation of glucose homeostasis. It is expressed within endocrine pancreas in human β-cells (13). A number of studies have demonstrated an inverse relationship between fasting plasma ghrelin and insulin concentrations and insulin resistance in adults and children (10, 14, 15). Based on these findings, we hypothesized that ghrelin, which may be involved in long-term regulation of energy balance and body weight control, may be associated with the development of insulin resistance in LGA children.

Thus, we studied primarily ghrelin levels in LGA born children who have no obesity at prepubertal ages by comparing their data with that of normal non-obese AGA children and also the effect of intrauterine and postnatal growth on ghrelin levels in this group of LGA children.

Subjects and methods

The study group consisted of 32 (17F, 15M) prepubertal non-obese LGA born children and the control group consisted of 45 (19F, 26M) AGA born healthy non-obese children. All children were born at term (between 37th and 42nd weeks of gestation). Those with a birth weight above 90th percentile for gestational age were accepted as LGA and those with a birth weight between 10 and 90th percentile for gestational age as AGA (16). Lubchenco curves (16) are used in neonatal practice in our country and the curves are validated for Turkish children. None of the patients had known diseases, congenital malformation or genetic disorders and they were not receiving any medication.

The mean (±S.E.M.) age at investigation was 4.4 ± 0.3 years in the LGA group and 4.0 ± 0.1 years in the AGA group. The age ranges in the LGA group were 2.5–7.9 years in girls and 3.0–7.5 years in boys. The respective values were 3.0–5.5 years and 3.0–6.7 years in the AGA group. All children were prepubertal at investigation (testicular volume <4 ml in boys, no breast budding in girls, and no pubic hair in both sexes).

Medical histories regarding gestational age, weight, and length at birth were taken from the hospital records. All the mothers had undergone a 100 g oral glucose tolerance test performed at 24–28 gestational weeks. Six mothers (four of the LGA and two of the AGA groups) had gestational diabetes, controlled only with diet.

Following physical examination, height, and weight were taken by standard methods. Height measurements were taken using a fixed Harpenden stadiometer with a precision of 0.1 cm and weight was taken in underclothing to the nearest of 0.1 kg.

BMI of the children was calculated as weight (kg)/height (m)². Values of height, weight, and BMI were expressed as SD score (SDS) (17, 18).

Serum samples were drawn for serum insulin, glucose, and ghrelin levels. Venous blood samples for laboratory analysis were collected between 0700 and 0800 after 8–10 h of overnight fasting.

Among other parameters concerning insulin resistance in this cohort of children that have been presented in the previous report (6) only the results of insulin and glucose measurements will be given in conjunction with ghrelin levels in this study.

Sera were stored at −70°C until hormonal assays were done. The samples were run in the same assays. Glucose was analyzed immediately.

Insulin resistance was evaluated by basal insulin levels and homeostasis model assessment-insulin resistance (HOMA-IR), calculated as insulin (µU/ml) × glucose (mmol/l)/22.5 (19).

This study was approved by the Ethical Committee of Istanbul Faculty of Medicine. All parents gave informed consent. The procedure was explained to older children.

Methods

Glucose was measured by standard equipment and methods (Roche Diagnostics using Cobas Integra kits) by hexokinase method. Insulin (µU/ml) was measured by RIA method (DSL-1600 Webster, TX, USA) and the lowest limit of detection was 1.3 µU/ml. All values below this limit were accepted as 1.2 µU/ml. Intra- and interassay coefficients of variance (CV) are 4.5–8.3 and 4.7–12.2%. Serum ghrelin was measured by Linco Research Ghrelin (total) RIA kits (St Charles, MO, USA). Intra- and interassay CV were 4.4–10.0 and 14.7–16.7%, respectively. The sensitivity limit of the assay was 93 pg/ml.

Statistical analyses

An SPSS-12 program was used for statistical analyses. Comparisons were done between the groups by using parametric tests. Skewed data for ghrelin, insulin, and HOMA-IR were transformed to normal distributions by calculating the natural logarithms and were expressed as geometric mean ± S.E.M. All other values are expressed as arithmetic mean ± S.E.M. The relations between variables were analyzed by simple correlation (Pearson’s test) and general linear models. For univariate analysis, children were divided into subgroups according to birth weight status (arbitrarily defined as being in the upper and lower half for the
birth weight in the sample) and overweight status (arbitrarily defined as being in the upper half for the BMI SDS distribution in the sample). In multiple regression analysis, ghrelin was taken as a dependent variable and sex, age, birth weight, recent anthropometric indices, presence or absence of maternal diabetes, glucose and insulin were taken as independent variables and tested. Those independent variables that have an effect on each other were analyzed separately in the model. Significance was granted for \( P < 0.05 \).

**Results**

**Anthropometry**

As seen in Table 1, there was no difference between the gestational ages of the groups. Birth weight, by definition, was significantly higher in LGA children than in AGA children \( (P = 0.0001) \). As seen in Table 1, age at investigation was similar between AGA and LGA children. LGA children were taller \( (P = 0.002) \) and heavier \( (P = 0.004) \) than AGA children but had similar BMI SDS as AGA born children (ranges of BMI SDS were \(-1.2–1.7 \) in LGA children and \(-1.8–1.6 \) in AGA children).

**Laboratory**

As seen in Table 2, there were no significant differences in glucose levels between LGA and AGA born children. LGA children had significantly higher insulin levels \( (P = 0.044) \) and statistically borderline significant HOMA-IR levels \( (P = 0.054) \) than those of AGA children (median (range) values for insulin (\( \mu \)U/ml) were 2.5 \((1.2–26.1) \) in the LGA and 1.7 \((1.2–14.6) \) in the AGA group; and for HOMA-IR 0.52 \((0.21–6.60) \) in the LGA and 0.37 \((0.12–3.4) \) in the AGA group). However, ghrelin level was significantly lower in LGA born than in AGA born children \( (P = 0.001) \), even after controlling for age, sex, and BMI \( (P = 0.006) \). Median (range) values for ghrelin (pg/ml) were 286.9 \((118.9–2282.5) \) in the LGA and 768.8 \((136.9–3654.2) \) in the AGA group.

**Correlation studies**

In the whole group, ghrelin was negatively correlated with birth weight \( (r = -0.36, P = 0.002) \). Ghrelin did not show correlation with chronological age, any present anthropometric and hormonal parameter in either of the groups. The parameters are given in Table 3.

Multivariate analysis revealed that birth weight seemed the only significant parameter influencing ghrelin levels \( \left(R^2 = 0.13, B = -0.007, P = 0.002\right) \).

**Discussion**

In our study, non-obese LGA children had significantly lower ghrelin levels than that of AGA children at prepubertal ages at similar BMI levels. It might be argued that lower ghrelin levels in our study in LGA children might be due to higher insulin levels in this group. Indeed, it has been shown in several studies that ghrelin has a strong inverse relation with insulin in adults and children \( (10, 14, 15) \). However, the difference in ghrelin levels between AGA and LGA born children was more noteworthy than the difference in insulin levels between the respective groups. Moreover, ghrelin did not show a correlation with insulin level. In compliance with this finding, in fact, it was shown in some studies that in prepubertal children there are no correlations between ghrelin and insulin levels either at baseline \((20, 21)\) or after breakfast \((21)\). Conflicting results have also been reported in animal studies regarding the influence of ghrelin on insulin secretion. Some studies reported some stimulatory influence of ghrelin on insulin secretion from isolated rat pancreatic islets \((22)\) and in rats \textit{in vivo} \((23)\). However, it has also been demonstrated that ghrelin

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**Table 2** Hormonal values of the large for gestational age and appropriate for gestational age born children at investigation (mean ± S.E.M).

<table>
<thead>
<tr>
<th></th>
<th>LGA ((n=32))</th>
<th>AGA ((n=45))</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dl)</td>
<td>86.7 ± 1.7</td>
<td>87.3 ± 1.4</td>
<td>0.7</td>
</tr>
<tr>
<td>Ghrelin (pg/ml)</td>
<td>324.9 ± 101.7</td>
<td>702.7 ± 153.5</td>
<td>0.001</td>
</tr>
<tr>
<td>Insulin ((\mu)U/ml)</td>
<td>3.03 ± 1.2</td>
<td>2.09 ± 0.4</td>
<td>0.044</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>0.64 ± 0.3</td>
<td>0.44 ± 0.1</td>
<td>0.554</td>
</tr>
</tbody>
</table>

LGA, large for gestational age; AGA, appropriate for gestational age; HOMA-IR, homeostasis model assessment-insulin resistance. Significant \( P \) values are shown in bold.

\( ^a \)Values are log transformed.
blunts insulin secretion from isolated rat pancreas (24). Moreover, it was shown that ghrelin exerted dose
dependent inhibition of gluocose-stimulated insulin secretion in mice in vivo (25).

The difference in ghrelin levels between LGA and AGA children in our study can not be explained by
differences in the recent anthropometric parameters of the groups. Both groups had similar BMI SDS and we
did not find any relationship with ghrelin levels and anthropometric parameters. Furthermore, BMI values
were in normal ranges in both groups. Ghrelin levels have been shown to have an inverse relation with
BMI (7). However, not in all studies this relation was
evident. Inguez et al. (26) reported that fasting plasma
concentrations of ghrelin at 1 year of age were not
related to weight, height or prior rate of growth from
birth to 1 year of age in infants born either AGA or SGA.
Whatmore et al. (27) demonstrated that ghrelin was not
correlated to BMI in prepubertal children and only
negatively correlated in pubertal children.

Although we did not find any correlation between
serum ghrelin levels and either with anthropometric
parameters or with insulin levels in our study; there was
a significant correlation between birth weight and
ghrelin levels. Although the degree of correlation was
low, birth weight was the only factor that influenced
ghrelin levels in LGA children. This finding implies that
intrauterine milieu results in a large baby also has an
effect on the ghrelin levels in childhood. In fact, it has
been shown in some studies that there is a negative
correlation between cord blood ghrelin concentration
and birth weight and birth length (28). Negative
correlations between cord blood ghrelin concentration
and birth weight were also observed by Farquhar et al.
(29) and by Onal et al. (30). Farquhar et al. (29) reported
that umbilical cord concentrations of ghrelin were
negatively correlated with birth weight SDS but were
not affected by gender. Cord ghrelin concentrations
were inversely related to cord glucose concentrations
but were independent from insulin concentration and
existence of maternal diabetes in SGA and AGA/LGA
neonates. Authors suggested that ghrelin regulation by
ghrelin is already present at birth and raised the
possibility that extreme variations in maternal glucose
metabolism, such as in poorly controlled diabetes, might
affect fetal ghrelin metabolism. It was shown that
plasma ghrelin concentration is suppressed in infants of
insulin-dependent diabetic mothers, suggesting that the
regulation of metabolic hormonal system is probably
operational in fetal and early postnatal life (31). Ghrelin
is present in human fetal circulation from 20th week to
term (32). Ghrelin synthesis and secretion from the
placenta have been demonstrated (33). Nakahara et al.
(34) demonstrated that in rat, maternal ghrelin easily
and rapidly crosses to the fetus and the exogenous
chronic treatment of the mother with ghrelin increases
fetal birth weight, whereas mothers immunized against
ghrelin deliver fetuses with a lower birth weight. These
studies suggest that ghrelin may play a role in fetal
development and energy homeostasis. In a recent study
by Chiesa et al. (35), LGA newborns had similar ghrelin
levels as AGA children but ghrelin had a correlation
with head circumference.

Beyond the fetal period, the potential role of ghrelin in
the neonate remains poorly understood. A simple
hypothesis is that higher ghrelin concentrations would
stimulate appetite and results in higher nutritional
intake by the neonate. Inguez et al. (26) observed a
significantly smaller glucose-induced drop in ghrelin
concentrations in 1 year old infants born SGA who had
experienced catch-up growth compared with those who
had not and proposed that higher postprandial ghrelin
concentrations may have resulted in greater weight
gain early in life. Savino et al. (36) reported a significant
increase in ghrelin concentration throughout the first
year of life despite the negative correlation between
ghrelin concentration and infant weight gain and they
suggested that ghrelin may play a role in the regulation
of growth in this period of life, because of its GH
releasing activity. Lower cord ghrelin levels have been
found to be associated with slower weight gain from
birth to 3 months of age (37). Our finding shows that
the decreased levels of ghrelin in LGA born children in
the neonatal period are sustained up to early childhood.
Thus, reduced levels of ghrelin in LGA children may
play a physiological role in fetal adaptation to
intrauterine environment and in the regulation of
body weight in early childhood. It has been shown that
LGA born neonates, although large at birth, return
to a growth curve within the population standards
postnatally (38). Thus, reduced ghrelin levels in LGA
children may have an effect on this pattern of postnatal
growth in LGA children.

An intrinsic change in production of ghrelin starting
from intrauterine life seems to be a plausible expla-
nation for reduced ghrelin levels in our LGA children in
early childhood. Whether the intrauterine environment

Table 3 Correlations of ghrelin with current anthropometric
and hormonal parameters of all children, large for gestational
age, and appropriate for gestational age groups.

<table>
<thead>
<tr>
<th></th>
<th>All children (n=77)</th>
<th>LGA (n=32)</th>
<th>AGA (n=45)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>P</td>
<td>r</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>−0.36</td>
<td><strong>0.002</strong></td>
<td>−0.29</td>
</tr>
<tr>
<td>Age (year)</td>
<td>−0.15</td>
<td>0.21</td>
<td>−0.03</td>
</tr>
<tr>
<td>Height SDS</td>
<td>−0.20</td>
<td>0.08</td>
<td>−0.2</td>
</tr>
<tr>
<td>Weight SDS</td>
<td>−0.21</td>
<td>0.078</td>
<td>−0.25</td>
</tr>
<tr>
<td>BMI SDS</td>
<td>−0.11</td>
<td>0.35</td>
<td>−0.21</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>−0.03</td>
<td>0.77</td>
<td>0.02</td>
</tr>
<tr>
<td>Insulin (μU/ml)</td>
<td>−0.17</td>
<td>0.14</td>
<td>0.05</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>−0.13</td>
<td>0.24</td>
<td>0.11</td>
</tr>
</tbody>
</table>

LGA, large for gestational age; AGA, appropriate for gestational age; SDS, SD score; BMI, body mass index; HOMA-IR, homeostasis model
assessment-insulin resistance. Significant P values are shown in bold.
that results in a LGA birth also has a permanent effect on ghrelin production in later life can not be explained by available data.

Another issue of relevance may be that of ghrelin isoforms. Although the major active product of ghrelin gene is a 28-amino acid peptide acylated at the serine three position with an octanoyl group, recent developments have shown that the ghrelin gene can generate various bioactive molecules including mainly des-acyl ghrelin and some others, obtained from alternative splicing or from extensive post-translational modifications (39). The factors that regulate the differential expression of ghrelin gene-derived peptides remain largely undetermined. Nevertheless, some studies showed that factors like fasting, feeding, chronic positive energy balance, ingestion of either medium-chain fatty acids or medium-chain triacylglycerols can modulate the ratio of the different ghrelin gene-derived peptides (39–41). Ghrelin isoforms are active and they may have similar or opposite physiological actions to ghrelin (7, 39). We measured total ghrelin concentrations. Recent studies have reported similar changes in total and active ghrelin concentrations in control and anorexic adults (42), suggesting that total ghrelin is a good indicator for active ghrelin. It was also shown that correlation between active ghrelin concentrations and anthropometric or other hormonal parameters were similar to those observed for total ghrelin concentrations (28, 43).

In conclusion, our findings show that in LGA born non-obese prepubertal children ghrelin levels are reduced when compared with age and BMI matched normal children born AGA. Birth weight seems to have the only significant effect on the reduced ghrelin levels. Whether the reduced ghrelin levels are due to an intrinsic change in ghrelin secretion starting from the intrauterine period is not clear and merits further studies.

Declaration of interest
We declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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