Immunoreactive Ghrelin in Human Cord Blood: Relation to Anthropometry, Leptin, and Growth Hormone


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ABSTRACT

Background: Ghrelin is secreted by the stomach, the hypothalamus, and the placenta in humans and has growth hormone–secreting and orexigenic properties. Leptin is secreted mainly by the adipocyte, plays a major role in energy balance, and reflects fat mass in infants as well as adults. Leptin and ghrelin central effects are mediated, at least partly, through the neuropeptide Y/Y1 receptor pathway in the hypothalamus.

Methods: We determined whether ghrelin is present in the fetus and investigated its relationship to leptin, growth hormone, birth weight, and calf and abdominal circumferences in 90 full-term neonates.

Results: Immunoreactive ghrelin was detected in all cord samples (mean ± SD, 187 ± 88 pmol/L; range, 66—594 pmol/L). In contrast to leptin, ghrelin concentrations of boys and girls were not statistically different. In female neonates, ghrelin is inversely correlated with anthropometric measures. In male neonates, ghrelin is positively correlated with leptin and negatively with growth hormone.

Conclusion: The presence of significant ghrelin concentrations in all neonates before the first feeding is intriguing. Unlike the fairly constant concentrations and effects of leptin over the short term, the wide variability of ghrelin concentrations observed in newborns raises the possibility that ghrelin secretion causes short-term changes in feeding behavior. We suggest that ghrelin may play a physiologic role in the initiation of feeding.


Key Words: Ghrelin—Leptin—Growth hormone—Cord blood—Birth weight—Neonate. © 2002 Lippincott Williams & Wilkins, Inc.
MATERIALS AND METHODS

Subjects
We studied 90 healthy, full-term newborns (45 male and 45 female) born at Children’s and Women’s Health Center of British Columbia over a 2-month period. All newborns had both parents and grandparents of European descent according to the information volunteered by the mothers.

Anthropometric characteristics
Birth weight and length were recorded at birth by the attending nurse. Calf circumference (at midcalf) and abdominal circumference (at the umbilicus) were measured within the first 24 hours of life by a single examiner (L. Y.) using a measuring tape. The measure was rounded to the closest millimeter. Intraobserver variation was 1% and 2% for calf and umbilical circumferences, respectively.

Cord blood
Venous cord blood was collected in EDTA tubes and kept at 4°C for 1 to 24 hours before being centrifuged, and the plasma was stored at −80°C. All measurements were performed in the same assay without extraction. Ghrelin concentrations were measured in duplicate using a commercial radioimmunoassay kit (Phoenix Pharmaceuticals, Belmont, CA, U.S.A.). According to the manufacturer, there was no cross-reactivity with the following other human hormones: secretin, vasoactive intestinal polypeptide, prolactin-releasing peptide-31, galanin, GH-releasing factor (GHRF), neuropeptide Y, orexin A, and orexin B. The antibody against ghrelin used in the assay is a rabbit polyclonal antibody against full-length octanoylated human ghrelin. Intraassay and interassay coefficients of variation were 4.5% to 5.3% and 9.0% to 13.0%, respectively. Using this assay, mean ± SD ghrelin concentrations measured in a group of healthy, lean (BMI, 23.3 ± 2.8 kg/m²), adult volunteers (n = 7, three male, four female) were found to decrease from 234 ± 45 pmol/L in the fasting state to 170 ± 54 pmol/L 90 minutes after a nonstandardized breakfast (mean change, 29%; range, 8%-45%; P = 0.016, Wilcoxon test). In our study, EDTA cord blood samples were stored at 4°C for up to 24 hours before being centrifuged, reflecting the 24-hour service of a delivery room. As ghrelin immunoreactivity was recently reported as being unstable (35), we confirmed the stability of ghrelin in the conditions of our study. Mean ± SD ghrelin concentrations measured on blood EDTA samples obtained from adult volunteers (n = 3) and kept 4 and 24 hours at 4°C before centrifugation were 91% ± 21% and 97% ± 23%, respectively, of control values. We also looked at the effect of thawing and refreezing the samples once. Ghrelin concentrations decreased to 97% ± 16% of the values of the corresponding nonthawed samples. Leptin was measured in duplicate by radioimmunoassay (Linco, St.-Charles, MO, U.S.A.) and GH in duplicate by chemiluminescent immunoassay (Beckman Coulter, Fullerton, CA, U.S.A.). Intraassay and interassay coefficients of variation were, respectively, less than 7.5% and less than 8.9% for leptin and less than 2.5% and less than 3.5% for GH (for GH concentrations > 6 ng/mL), according to the specifications of the manufacturer.

Statistical Analysis
Except when otherwise noted, values are expressed as mean ± SD. The significance of the anthropometric and hormonal differences between male and female neonates was assessed by a nonparametric test (Mann-Whitney). Linear regression (Spearman coefficient) was used to express the relation between the variables under study (Analyse-It Software Ltd., Leeds, UK, version 1.62). A P value < 0.05 was considered significant.

Ethical considerations
The study was approved by the Ethics Committee of the University of British Columbia, and written informed consent was obtained from all mothers after the study was explained to them by the investigator (L. Y.).

RESULTS
Table 1 describes the characteristics of the mothers and their neonates. All anthropometric characteristics were similar in male and female newborns. Birth weight correlated with calf circumference (r = 0.84 in male and female neonates; P < 0.0001) and abdominal circumference (r = 0.82 and r = 0.77 in male and female neonates, respectively; P < 0.0001).

Ghrelin and leptin cord plasma concentrations in male and female neonates are shown in Figure 1. Ghrelin was detectable in all samples (187 ± 88 pmol/L; range, 66–594 pmol/L). There was no significant difference between male and female newborns (P = 0.82; Fig. 1). In contrast, as expected, leptin concentrations were higher in female (14.1 ± 11.9 ng/mL) compared with male neonates (7.4 ± 4.4 ng/mL) (P = 0.0002; Fig. 1), and this difference persisted after normalization for birth weight (P < 0.0001). GH concentration was 9.2 ± 12.9 μg/L and was similar in male and female neonates (P = 0.17).

In male neonates, we observed a significant positive correlation between ghrelin and leptin concentrations (r = 0.34; P = 0.022) and a negative correlation between ghrelin and GH concentrations (r = −0.33; P = 0.028).

There was no significant correlation between ghrelin and anthropometric measures. As expected, there was a positive correlation between leptin concentration and birth

### TABLE 1. Characteristics of the mothers and their neonates (n = 45/group)

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (yr)</td>
<td>31.4 ± 4.6</td>
<td>32.7 ± 4.9</td>
</tr>
<tr>
<td>Gestational age (week)</td>
<td>39.4 ± 1.0</td>
<td>39.7 ± 1.1</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>3593 ± 436</td>
<td>3517 ± 367</td>
</tr>
<tr>
<td>Length at birth (cm)</td>
<td>52.5 ± 2.4</td>
<td>51.5 ± 2.3</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>13.0 ± 1.2</td>
<td>13.1 ± 1.1</td>
</tr>
<tr>
<td>Calf circumference (cm)</td>
<td>12.2 ± 1.0</td>
<td>12.1 ± 0.9</td>
</tr>
<tr>
<td>Abdominal circumference (cm)</td>
<td>33.2 ± 2.6</td>
<td>32.9 ± 1.8</td>
</tr>
</tbody>
</table>

Values are mean ± SD.
weight (Fig. 2) and calf and abdominal circumferences ($r = 0.46-0.48; P < 0.002$).

In female neonates, in contrast, there was no significant correlation between ghrelin and leptin or GH concentrations. We observed negative correlations between ghrelin concentration and birth weight ($r = -0.39; P = 0.009$; Fig. 3), calf circumference ($r = -0.33; P = 0.027$), and abdominal circumference ($r = -0.50; P = 0.0005$). As expected, there was a positive correlation between leptin concentration and birth weight (Fig. 3) and calf and abdominal circumferences ($r = 0.45-0.65; P < 0.002$).

**DISCUSSION**

We report for the first time that ghrelin immunoreactivity is detectable in human cord plasma in concentrations similar to those found in fasting and nonfasting adults. The role of ghrelin in the fetus is presently unknown. However, because of its known orexigenic (12), hyperglycemic (36), and GH stimulatory effects (13,14) in adults, ghrelin may promote feeding in the newborn and protect against neonatal hypoglycemia.

The antibody used in the present ghrelin assay (the only commercial assay presently available) is raised against full-length human ghrelin and is directed toward the C-terminal part of the molecule. Although ghrelin has 36% identity with motilin, the antibody used in the present assay has no significant cross-reactivity with motilin (M. Heiman, Eli Lilly, personal communication, November 2001). Our assay recognizes both the active and inactive forms of ghrelin. Studies in the adult rat have shown that both forms of ghrelin are detectable in the plasma (37), raising the possibility that the hormonal concentrations measured with this assay may not fully reflect the physiologic role of the hormone. However, in agreement with the orexigenic role of ghrelin and reports by other investigators (33–35), we observed the expected postprandial decrease in ghrelin concentrations in our group of adult volunteers, suggesting that the ghrelin concentrations measured are indeed of clinical significance.

The source of ghrelin on cord plasma is unknown. It may originate from the maternal compartment, be secreted by the placenta into the fetal circulation, or come directly from fetal tissues. No data are presently available on ghrelin concentrations during pregnancy. In rats, ghrelin mRNA in the stomach, regarded as the main source of ghrelin, is not affected by pregnancy (8). A placental source of ghrelin cannot be ruled out. However, in humans, although ghrelin mRNA is detectable in both first trimester and term placenta by reverse-transcription polymerase chain reaction, the peptide is detected only in the first trimester placenta but not at term by immunocytochemistry (8). In rats, placental ghrelin expression decreases markedly toward term (8). Finally, it is tempting to postulate that ghrelin is secreted in the fetal circulation by the fetal gastrointestinal tract, mainly the stomach. Although no human data are presently available, ghrelin mRNA has been found in relatively high quantities in the stomach, gut, skin, pituitary, and mediobasal hypothalamus as early as on the second postnatal day in the rat, suggesting that it is secreted early in life (38).

Although we confirmed the sexual dimorphism repeatedly observed for leptin concentrations on cord blood, we did not find a significant difference in ghrelin concentrations between male and female neonates. This is consistent with the observation by Gualillo et al., who

![FIG. 1. Ghrelin and leptin concentrations in male and female neonates. *$P = 0.0002$ compared with male neonates.](image1)

![FIG. 2. Relation between birth weight and ghrelin (NS) and leptin ($r = 0.45; P < 0.001$) concentrations in male neonates.](image2)
found similar ghrelin mRNA concentrations in stomach from male and female rats that were not affected by gonadectomy (39).

In contrast, we found a sexual dimorphism in the relation between ghrelin, leptin, GH, and anthropometric measures. These correlations, although statistically significant, are weak, and their relevance to human physiology is presently unknown. In female (but not male) neonates, ghrelin was inversely correlated with anthropometric markers. This is similar to the negative correlation between ghrelin and BMI, body weight, body fat, and fat mass recently reported by Tschop et al. (33) in lean and obese whites and Pima Indians. Whether this sexual dimorphism is also present in adults is presently unknown. In male (but not female) neonates, ghrelin was positively correlated with leptin and, surprisingly, negatively with GH.

One hypothesis to explain this sexual dimorphism is the potentially confounding role of gonadal steroids on most parameters. Testosterone is an important determinant of birth weight (40) and might mask the relation between ghrelin and anthropometric markers in male newborns. Estradiol increases leptin concentrations (41) and might mask the relation between ghrelin and leptin concentrations in girls. Finally, in vitro experiments have shown that GHRF and somatostatin, two major players in the regulation of GH secretion, are differently regulated by testosterone and estradiol. Testosterone increases GHRF and somatostatin tone, whereas estradiol increases GHRF but decreases somatostatin release from the hypothalamus (42).

An important question is whether ghrelin has GH-secreting and orexigenic effects in neonates. We did not find a correlation between ghrelin, a potent GHS, and GH concentrations. One explanation is that GH secretion is regulated by ghrelin locally produced in the hypothalamus and not by stomach ghrelin, which is thought to be the main source of circulating ghrelin. Another possibility is that the expected stimulatory effects of ghrelin on GH secretion are masked by the complex interactions of somatostatin and GHRF on the pituitary. Graphical analysis of ghrelin shows marked variability of concentrations with birth weight (Fig. 2 and 3). This may indicate that, unlike the fairly constant concentrations and effects of leptin over the short term, ghrelin secretion may cause short-term changes in feeding behavior. If this is the case, feeding behavior in newborns with high prefeeding concentrations of ghrelin may be more active.

In conclusion, ghrelin, a potent GH stimulatory and orexigenic agent, is detectable in significant concentrations on human cord plasma. The presence of high immunoreactive ghrelin concentrations in neonates before the first feeding is intriguing. We suggest that ghrelin may play a physiologic role in the initiation of feeding. Further studies will concentrate on the determination of the ratio of active to inactive ghrelin concentrations and on the relation between ghrelin and feeding behavior in the neonate.

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