Ghrelin Concentration in Cord and Neonatal Blood: Relation to Fetal Growth and Energy Balance

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To investigate the relationship between ghrelin and both fetal and neonatal growth parameters and energy balance, we measured plasma ghrelin concentrations in 54 cord blood samples (male, n = 34; female, n = 20; gestational age, 37.0–41.6 wk; birth weight, 2206–4326 g) and 47 neonatal blood samples (male, n = 27; female, n = 20; postnatal d 3–8). The plasma ghrelin concentrations in cord blood ranged from 110.6–446.1 pmol/liter (median, 206.7 pmol/liter), which were equal to or higher than those in normal weight adults. These values were inversely correlated with birth weight (r = −0.40; P = 0.002), birth length (r = −0.36; P = 0.007), placental weight (r = −0.35; P = 0.01), and IGF-I concentration (r = −0.49; P = 0.0002), but were not significantly correlated with the GH concentration (r = 0.22; P = 0.12). The ghrelin concentrations in small for gestational age newborns were significantly higher than those in appropriate for gestational age newborns (P = 0.0008). The ghrelin concentrations in the vein were significantly higher than those in the artery in 8 cord blood samples (P = 0.01), which suggests that the placenta is an important source of fetal ghrelin. In neonates, the ghrelin concentrations ranged from 133.0–481.7 pmol/liter (median, 268.3 pmol/liter), which were significantly higher than those in cord blood (P < 0.0001). These results suggest that ghrelin may contribute to fetal and neonatal growth.

Subjects and Methods

Subjects

Venous cord blood samples were obtained from 54 full-term newborns (34 males and 20 females; gestational age, 37.0–41.6 wk; birth weight, 2206–4326 g; birth length, 44.0–54.5 cm). Their characteristics are shown in Table 1. Forty-four of the newborns were classified as appropriate for gestational age (AGA), 7 were small for gestational age (SGA), and 3 were large for gestational age (LGA). AGA was defined as a newborn whose birth weight was from −1.5 to +1.5 SD of the mean birth weight in each gestational age. SGA was defined as below −1.5 SD, and LGA was defined as over +1.5 SD. The mean birth weight and SD were calculated according to the Japanese population fetal growth curve published in 1994 by a study group of the Japanese Ministry of Health and Welfare. To estimate the source of ghrelin in the fetal circulation, both arterial and venous cord blood samples were collected from another 8 full-term newborns. Blood samples for ghrelin assay were collected in chilled tubes containing EDTA-2Na (1 mg/ml) and aprotinin (500 U/ml), and plasma was separated at 4 °C immediately after birth. Serum was simultaneously separated for other hormone assays. Neonatal samples were obtained from 47 full-term healthy neonates (27 males and 20 females; postnatal d 3–8), whose characteristics are shown at Table 2. In 27 of these neonates, cord blood had already been collected, and the change in the ghrelin concentration between cord and neonatal blood was compared in this group. Each sample was collected at 0900 h, and plasma was separated immediately.

Plasma and serum samples were kept frozen at −80 °C until analysis. All of the newborns and neonates were healthy, and their mothers had had no remarkable complications during pregnancy. The study protocol was approved by the ethical committee of University of Tokushima School of Medicine, and all parents of the newborns gave their written informed consent before enrollment.

Ghrelin and other hormone assays

Plasma ghrelin concentration was determined by RIA using polyclonal antibodies raised against the carboxyl-terminal fragment ghrelin-[13–28] (15). The value determined by this RIA system gives the total concentration of ghrelin. Serum GH and IGF-I were determined using

Abbreviations: AGA, Appropriate for gestational age; IRI, immunoreactive insulin; LGA, large for gestational age; SGA, small for gestational age.
The partial correlation analysis calculates a correlation to determine which factor was predominantly correlated with additional variables. The partial correlation between ghrelin and IGF-I, while controlling for birth weight, birth length, or placental weight, was still significant (r = –0.43, –0.47, and –0.49; P = 0.008, 0.002, and 0.002, respectively; Figs. 1 and 2). No significant correlation was observed between ghrelin and GH concentrations (r = 0.22; P = 0.12; Fig. 2 and Tables 1 and 3).

As some anthropometrical parameters and IGF-I concentrations were inversely correlated with ghrelin concentrations, we performed some partial correlation analyses to determine which factor was predominantly correlated with them. The partial correlation analysis calculates a correlation between two variables while controlling for the effect of other additional variables. The partial correlation between ghrelin and IGF-I, while controlling for birth weight, birth length, or placental weight, was still significant (r = –0.43, –0.47, and –0.49; P = 0.008, 0.002, and 0.002, respectively; Figs. 1 and 2). No significant correlation was observed between ghrelin and GH concentrations (r = 0.22; P = 0.12; Fig. 2 and Tables 1 and 3).

In 54 cord blood samples, plasma ghrelin concentrations ranged from 110.6–446.1 pmol/liter (median, 206.7 pmol/liter). Ghrelin concentrations were inversely correlated with birth weight (r = –0.40; P = 0.002), birth length (r = –0.36; P = 0.007), placental weight (r = –0.35; P = 0.01), birth weight/birth length ratio (r = –0.38; P = 0.004), Kaup index (r = –0.28; P = 0.04), IGF-I concentration (r = –0.49; P = 0.0002), and IGF-binding protein-3 concentration (r = –0.30; P = 0.03). Ghrelin concentrations in SGA newborns were significantly higher than those in AGA and LGA newborns (P = 0.0008 and P = 0.02, respectively; Figs. 1 and 2). No significant correlation was observed between ghrelin and GH concentrations (r = 0.22; P = 0.12; Fig. 2 and Tables 1 and 3).

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partial correlation between ghrelin and GH while controlling for each anthropometrical parameter or IGF-I confirmed the absence of a significant correlation between GH and ghrelin concentrations.

In the eight newborns whose cord blood samples were collected from both the artery and vein, plasma ghrelin concentrations in the vein (median, 304.9 pmol/liter; range, 218.7–403.8 pmol/liter) were significantly higher than those in the artery (median, 287.5 pmol/liter; range, 181.9–392.4 pmol/liter; P = 0.01; Fig. 3).

In neonates, the plasma ghrelin concentrations ranged from 133.0–481.7 pmol/liter (median, 268.3 pmol/liter), which were significantly higher than those in cord blood (P < 0.0001). The sequential analysis of ghrelin concentrations after birth in 27 newborns confirmed that the ghrelin concentrations significantly increased during the early neonatal period (P < 0.0001; Fig. 4). The ghrelin concentrations in neonates did not significantly correlate with percentage body weight loss from birth to the sampling day (r = −0.13; P = 0.37), calorie intake per body weight on the sampling day (r = 0.04; P = 0.80), or the mean daily body weight gain during the first month of life (r = 0.23; P = 0.13; Table 4). No significant gender difference in ghrelin concentration was observed in either cord or neonatal blood (Tables 1 and 2).

**Discussion**

Our results showed that ghrelin is present in cord and neonatal blood at concentrations 1.5- to 2-fold higher than those found in normal weight adult (6, 9). The existence of ghrelin in cord blood is consistent with previous observations, although the use of nonextracted plasma is different compared with our study (16).

In adults, the plasma ghrelin concentration is mainly determined by the energy balance. Patients with anorexia nervosa and cachexia show elevated levels of ghrelin (6, 17), whereas obese subjects have reduced levels (8, 9). Thus, it is interesting that ghrelin concentrations were inversely correlated with several growth-related anthropometric parameters and the IGF-I concentration in the fetus. This result

**TABLE 3A.** Correlation coefficients between cord blood levels and size at birth

<table>
<thead>
<tr>
<th></th>
<th>Ghrelin</th>
<th>GH</th>
<th>IGF-I</th>
<th>IGF-II</th>
<th>IGFBP-3</th>
<th>Leptin</th>
<th>Insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth weight</td>
<td>−0.29</td>
<td>−0.38</td>
<td>0.29</td>
<td>−0.05</td>
<td>0.22</td>
<td>0.53</td>
<td>0.34</td>
</tr>
<tr>
<td>Birth length</td>
<td>−0.26</td>
<td>−0.41</td>
<td>0.17</td>
<td>−0.23</td>
<td>0.15</td>
<td>0.25</td>
<td>0.24</td>
</tr>
<tr>
<td>Kup index</td>
<td>−0.28</td>
<td>−0.19</td>
<td>0.30</td>
<td>0.19</td>
<td>0.23</td>
<td>0.54</td>
<td>0.27</td>
</tr>
<tr>
<td>Body weight/body length</td>
<td>−0.38</td>
<td>−0.32</td>
<td>0.32</td>
<td>0.05</td>
<td>0.25</td>
<td>0.56</td>
<td>0.33</td>
</tr>
<tr>
<td>Placental weight</td>
<td>−0.35</td>
<td>−0.21</td>
<td>0.33</td>
<td>−0.23</td>
<td>0.12</td>
<td>0.27</td>
<td>0.08</td>
</tr>
</tbody>
</table>

**TABLE 3B.** Correlation coefficients between cord blood levels

<table>
<thead>
<tr>
<th></th>
<th>Median (range)</th>
<th>Ghrelin</th>
<th>GH</th>
<th>IGF-I</th>
<th>IGF-II</th>
<th>IGFBP-3</th>
<th>Leptin</th>
</tr>
</thead>
<tbody>
<tr>
<td>GH (μg/liter)</td>
<td>17.8 (5.7–61.0)</td>
<td>0.22</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IGF-I (μg/liter)</td>
<td>77 (12–160)</td>
<td>−0.49</td>
<td>−0.47</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IGF-II (μg/liter)</td>
<td>310 (181–481)</td>
<td>−0.13</td>
<td>0.08</td>
<td>0.02</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IGFBP-3 (mg/liter)</td>
<td>0.76 (0.45–1.28)</td>
<td>−0.30</td>
<td>−0.40</td>
<td>0.72</td>
<td>0.22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leptin (μg/liter)</td>
<td>3.5 (0.8–13.8)</td>
<td>−0.12</td>
<td>−0.06</td>
<td>0.14</td>
<td>0.30</td>
<td>0.28</td>
<td>0.01</td>
</tr>
<tr>
<td>Insulin (pmol/liter)</td>
<td>11.4 (6.0–100.2)</td>
<td>−0.17</td>
<td>−0.26</td>
<td>0.25</td>
<td>−0.15</td>
<td>0.20</td>
<td>0.01</td>
</tr>
</tbody>
</table>

IGFBP-3, IGF binding protein-3.

a P < 0.005.

b P < 0.05.

c P < 0.0005.

d P < 0.0005.

e P < 0.05.

f P < 0.005.
suggests that the ghrelin concentration might be mainly regulated in a fetal growth-related manner in utero, as if its role was to accelerate fetal growth. In an experimental study, a significant level of GH secretagogue receptor expression was observed in the fetal rat hypothalamus, pituitary, and brainstem (18). However, fetal GH may not contribute to fetal growth itself to a large extent, as patients with Pit-1 gene or GH-1 gene deficiency, which are congenital disorders of pituitary GH synthesis, show nearly normal fetal growth. Fetal growth essentially depends on the energy transport from the mother. A positive correlation between size at birth and cord blood IGF-I concentration has been reported (19, 20). Considering that the ghrelin concentration correlated not with GH but with the IGF-I concentration, and the remarkable elevation of the ghrelin concentration in SGA newborns, negative feedback regulation between fetal growth and the ghrelin concentration may not originate in the fetal GH axis, but, rather, in feto-maternal energy transport, which would affect fetal growth and the IGF-I level.

In the rat, ghrelin mRNA is expressed at a very low level in the fetal stomach (21, 22), whereas significant expression is observed in the placenta (14). In humans, ghrelin mRNA is also expressed in the placenta (14). Therefore, it is possible that some of the ghrelin in the fetal circulation might originate from the placenta, like leptin, and regulate feto-maternal energy transport locally, as ghrelin concentrations in the umbilical vein were significantly higher than those in the artery ($P = 0.01$).

In our previous study the leptin concentration rapidly decreased after birth and remained at a low level during the early neonatal period (11). In contrast, plasma ghrelin concentrations at approximately 5 d after birth were significantly higher than those in cord blood ($P < 0.0001$).

**TABLE 4.** Correlation coefficients between neonatal ghrelin levels and energy balance

<table>
<thead>
<tr>
<th>Ghrelin ($P$)</th>
<th>Birth weight</th>
<th>Body weight</th>
<th>Body length</th>
<th>Kaup index</th>
<th>Mean calorie intake$^a$</th>
<th>Body weight loss$^b$</th>
<th>Body weight gain$^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-0.21 (0.16)</td>
<td>-0.17 (0.26)</td>
<td>-0.22 (0.14)</td>
<td>-0.10 (0.49)</td>
<td>0.04 (0.80)</td>
<td>-0.13 (0.37)</td>
<td>0.23 (0.13)</td>
</tr>
</tbody>
</table>

$^a$ Calorie intake per body weight on sampling day (kilocalories per kilogram).

$^b$ Percentage body weight loss from birth to sampling day (percentage).

$^c$ Mean daily body weight gain during first month of life (grams per day).

and lung from wk 10 of gestation (23, 24). The contributions of these peripheral organs may also be taken into consideration.

In our previous study the leptin concentration rapidly decreased after birth and remained at a low level during the early neonatal period (11). In contrast, plasma ghrelin concentrations at approximately 5 d after birth were significantly higher than those in cord blood. After birth, the energy supply through the placenta is interrupted, and a newborn needs to start taking milk for growth. Thus, in contrast to leptin, which reduces energy intake, it is reasonable for the ghrelin concentration in neonates to increase to stimulate appetite and give a positive energy balance. However, in rat stomach, the expression of ghrelin mRNA in neonates is lower than...
that in adults (21, 22). The origin of the high concentration of ghrelin in neonates may need further investigation, with regard not only to its synthesis and secretion, but also to its degradation and clearance during the neonatal period.

In summary, this study demonstrates the existence of ghrelin in fetal and neonatal blood at rather high concentrations, an inverse correlation between the cord blood ghrelin concentrations and fetal growth-related parameters, and a significant elevation of the ghrelin concentration during the early neonatal period. Further study of ghrelin concentrations in fetuses and neonates with pathological status, such as premature delivery or severe intrauterine growth retardation, may provide useful information about regulation of the ghrelin concentration and its role during the fetal and neonatal periods.

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