SHORT REPORT

Plasma levels of active ghrelin until 8 weeks after birth in preterm infants: relationship with anthropometric and biochemical measures

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This study investigated the relationship between plasma levels of ghrelin and postnatal growth in preterm infants. The levels of active ghrelin in cord blood and in plasma in 25 very low birthweight (VLBW) infants were measured. The results indicate that the levels of circulating active ghrelin markedly increases after birth in VLBW infants, and suggest that the increased levels of ghrelin reflects the maturation of ghrelin production in the stomach and an increased physiological need for ghrelin.

Ghrelin is a 28 amino acid peptide secreted by the fundus of the stomach, the hypothalamus and the placenta in rats and humans. Although detectable levels of ghrelin in the cord blood and in the newborn have been reported, the relationship between ghrelin levels and certain anthropometric and biochemical measures, such as the body mass index (BMI) and the levels of leptin and insulin-like growth factor-1 (IGF-1), remains controversial.^{1 2}

Previous studies of ghrelin levels in the cord blood and in newborns measured the total ghrelin level. The levels of active ghrelin, which have been considered not to have any endocrine activity, in the newborn have been measured only recently. One study found a significant correlation between the levels of active ghrelin in the cord blood and birth weight and IGF-1 levels.³ Another study suggested that the levels of active ghrelin in the cord blood did not correlate with birth weight, gestational age, BMI or leptin levels.⁴

No study has evaluated the levels of circulating active ghrelin in very low birthweight (VLBW) infants from immediately after birth to a few months after birth. Therefore, to investigate the relationship between plasma levels of ghrelin and postnatal growth in VLBW infants, we measured the levels of active ghrelin in the cord blood and in the plasma of VLBW infants.

METHOD

The Juntendo University Hospital institutional review board approved the study. Infants were considered eligible for the study if they had a birth weight of less than 1500 g, were born and transported to the neonatal intensive care unit at Juntendo University Hospital between March 2004 and May 2005, and had no major congenital abnormalities, severe asphyxia or infection. We obtained informed consent from the guardians of all the infants prior to enrolment in the study.

A total of 25 VLBW infants (12 boys and 13 girls; mean gestational age (SD) 30.5 (2.5 weeks) and mean birth weight 1073 (266 g)) took part in the study. Of these 12 were classified as appropriate for gestation age (AGA; birth weight between the 10th and the 90th percentile) and 13 were small for gestational age (SGA; birth weight below the 10th percentile). Nine mothers had been given steroids antenatally. One infant was born by vaginal delivery and the others by caesarean

section. All infants started bolus feeding with breast milk or a commercial formula specially designed for premature infants through a nasogastric tube every 3 h, within 24 h after birth. Feeding volumes were increased from 8.0 ml/day to 20.0 ml/day depending on the infant's birth weight and feeding tolerance. At 2 weeks after birth several infants had a small amount of parenteral energy intake.

Cord blood samples were obtained from the umbilical vein at birth, and venous blood samples (approximately 600 µl) were taken at birth and at 2, 4, 6 and 8 weeks after birth. The blood samples were collected immediately before feeding in the morning and stored in tubes containing EDTA2Na (1 mg/ml) and aprotinin (500 U/ml). For the measurement of active ghrelin, centrifuged plasma was acidified with 1 N HCl (1:10 relative to the plasma volume) to stabilise octanoyl modification before storage. Feeding volumes calculated from the ingested volumes of breast milk or formula, body mass index (weight (in g)/length² (in cm) × 10, BMI), and per cent weight gain ((body weight – birth weight)/birth weight × 100) were measured at 2, 4, 6 and 8 weeks after birth.

Plasma levels of active ghrelin were determined with a commercially available two-site sandwich enzyme-linked immunosorbent assay (ELISA) kit (Active Ghrelin ELISA Kit, Mitsubishi Kagaku Iatron, Inc, Tokyo, Japan) using a horseradish peroxidase conjugated antibody that recognises only octanoylated ghrelin. Plasma levels of leptin were measured with a commercially available radioimmunoassay (RIA) kit (Human Leptin RIA Kit, LINCO Research, St Charles, Missouri, USA). Plasma levels of IGF-1 were also measured with a commercially available RIA kit (IGF-1 IRMA, Daiichi Radioisotope Laboratory, Tokyo, Japan).

We used the Mann-Whitney U test to compare differences between the means of AGA and SGA infants, and boys and girls, and analysis of variance and Wilcoxon's signed-rank test to compare the means of different postnatal periods. Correlation between the plasma level of ghrelin and several anthropometric and biochemical measures was determined by Spearman's correlation test.

RESULTS

Plasma levels of active ghrelin in cord blood and immediately after birth in VLBW infants were significantly lower ($p < 0.01$) than that at 2, 4, 6, and 8 weeks of age (fig 1); we found no significant differences between the levels at 2, 4, 6, and 8 weeks of age. There was a significant correlation ($p < 0.01$) between the level of active ghrelin in cord blood and the level immediately after birth. The plasma levels of active ghrelin in AGA and SGA infants in the cord blood and at 2, 4, 6, and

Abbreviations: AGA, appropriate for gestational age; BMI, body mass index; IGF-1, insulin-like growth factor-1; SGA, small for gestational age; VLBW infants, very low birthweight infants

Figure 1 Plasma levels (mean (SD)) of active ghrelin in cord blood and immediately and 2, 4, 6 and 8 weeks after birth in 25 very low birthweight infants. * $p < 0.01$ v cord blood and immediately after birth.

8 weeks of age were not significantly different. There was also no significant difference in the ghrelin levels of boys and girls.

We found no significant correlation between the plasma level of active ghrelin and gestational age, birth weight, BMI, per cent weight gain, feeding volume or plasma levels of leptin and IGF-1 at any time point during the study period.

DISCUSSION

This is the first report of plasma levels of active ghrelin measured serially from birth to 8 weeks of age in VLBW infants. Our findings show that the plasma level of active ghrelin markedly increases at 2 weeks after birth. However, we found no significant correlation between the plasma level of active ghrelin and several anthropometric and biochemical measures; nor did we find any significant difference between the plasma levels of active ghrelin in the VLBW infants who were appropriate for gestational age and those who were small

for gestational age during the study period. Our findings are in accordance with the results of studies measuring total ghrelin in preterm infants at discharge from the neonatal intensive care unit.⁵

Our results indicate that the levels of circulating ghrelin markedly increase after birth in VLBW infants and suggest that the effects of ghrelin on postnatal growth should be further investigated. The increase in plasma level of ghrelin at 2 weeks of age may reflect the maturation of ghrelin production in the stomach and an increased physiological need for ghrelin. As the volume of intake in VLBW infants is influenced by many factors and the feeding amount is not determined by the infant's appetite, it may be difficult to elucidate the effects of circulating ghrelin on food intake and energy balance in the postnatal period.

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