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Circulating ghrelin levels in the newborn are positively associated with gestational age

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Summary

OBJECTIVE Ghrelin exerts potent GH-releasing activity and stimulates food intake. Circulating ghrelin levels are increased in anorexia and cachexia, reduced in obesity and restored by weight recovery. Newborns are characterized by GH hypersecretion associated with low IGF-I levels reflecting peripheral GH resistance.

STUDY DESIGN The aim of our study was to measure cord ghrelin levels in 117 newborns appropriate for gestational age, born either at term or preterm.

RESULTS Ghrelin levels in cord blood (median; 25th–75th centile: 327·6; 206·0–413·0 pg/ml) were higher ($P < 0·0001$) than those in maternal blood at delivery (133·0; 89·0–173·7 pg/ml), without gender differences. A positive correlation between ghrelin levels in mothers and newborns ($r = 0·26$, $P < 0·01$) was observed. Ghrelin levels in newborns born at term (399·0; 229·0–438·0 pg/ml) were remarkably higher ($P < 0·0001$) than those in born preterm (208·0; 144·5–278·9 pg/ml). A clear positive association was present between ghrelin levels and gestational age. No association between ghrelin and GH, IGF-I, insulin, glucose and leptin levels were found.

CONCLUSIONS Cord ghrelin levels show clear gestational age-related dependency. The lack of any direct relationship between ghrelin and anthropometric or biochemical parameters in adequate for gestational age newborns does not support the hypothesis that ghrelin has major role in foetal GH secretion and growth.

Ghrelin, the endogenous ligand of the growth hormone secretagogue receptor (GHS-R; Kojima *et al*., 2001), exerts potent GH-releasing activity but also has other endocrine and nonendocrine actions among which is a remarkable influence on the control of food intake (Arvat *et al*., 2001; Inui 2001; Muccioli *et al*., 2002). In fact, ghrelin treatment has been shown able to induce appetite, food intake and reduce fat utilization leading to weight gain in both animals and humans (Tschop *et al*., 2000, 2001; Inui 2001; Wren *et al*., 2001a, 2001b; Muccioli *et al*., 2002). Circulating ghrelin levels are increased by fasting and energy restriction while decreased by food intake, glucose and insulin (Inui 2001; Tschop *et al*., 2001; Cummings *et al*., 2002; Ukkola, 2003). Accordingly, circulating ghrelin levels are increased in anorexia and cachexia, reduced in obesity and restored by weight recovery in all these conditions (Muccioli *et al*., 2002; Inui 2001). A considerable amount of evidence suggests that ghrelin coupled with leptin, although with opposing actions, plays a role in managing the neuroendocrine and metabolic adaptation to starvation (Horvath *et al*., 2001; Inui 2001). Ghrelin and leptin are likely signals to the CNS of variations in the nutritional balance; within the CNS, ghrelin and leptin modulate neuropeptide Y/agouti-related protein hypothalamic neurones that express ghrelin and leptin receptors (Inui 2001; Kamegai *et al*., 2001; Muccioli *et al*., 2002; Tschop *et al*., 2002).

The control of growth in newborns is different from later life and involves genetic, nutritional, hormonal and environmental factors (Ogilvy-Stuart *et al*., 1998). In fetuses as well as in newborns, insulin, IGF-I and nutrient availability are the main determinants of growth while GH secretion does not play a major role (Gluckman, 1995; Ogilvy-Stuart *et al*., 1998).

GH levels are high during the fetal and neonatal periods (Wollmann, 2000). High GH levels during fetal life might induce insulin resistance thus protecting fetal brain from hypoglycaemia or, alternatively, GH hypersecretion could provide alternative fuels for metabolism via enhanced lypolysis (Adrian *et al*., 1990; de Zegher *et al*., 1990).

The GH hypersecretory state in the fetal and neonatal periods probably reflect blunted negative feedback action of IGF-I likely due to peripheral GH insensitivity or might reflect age-related variations in the neural control of somatotroph secretion, i.e. hyperactivity of GHRH-secreting neurones and somatostatin

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Newborns	At term	Preterm
Males/females	54/39	13/11
Gestational age (weeks)	38·9 ± 1·2	33·2 ± 2·4
By natural/Caesarean delivery	44/49	12/12
Body weight (g)	3287·6 ± 383·3	2002·7 ± 533·1
Length (cm)	49·7 ± 1·7	44·4 ± 3·5
GH (μg/l), range	15·2 (10·1–21·2)	21·5 (14·9–25·4)
IGF-I (nmol/l), range	8·8 (6·1–12·6)	6·7 (4·4–9·0)
Leptin (ng/ml), range	6·0 (3·6–9·2)	3·5 (1·3–5·7)
Insulin (pmol/l), range	47·4 (36·6–61·7)	35·9 (30·1–43·0)
Glucose (mmol/l), range	4·0 (3·3–5·3)	5·6 (3·6–7·6)

Table 1 Clinical characteristics (mean ± SD) and hormonal parameters (median, 25th–75th centile) of subjects

hypoactivity (Stimano *et al*., 1985; Deiber *et al*., 1989; Wollmann *et al*., 2000); the role, if any, of the ghrelin system in driving GH secretion during fetal life and at birth is still unclear.

Information about ghrelin secretion during fetal life and at birth is scanty. Data available so far suggest that, unlike other hormonal parameters, ghrelin secretion does not undergo relevant age- and gender-related variations and is similar among newborns, prepubertal or pubertal children and young adults (Bellone *et al*., 2003). At birth, no gender-related difference in ghrelin levels has been found while associations among circulating ghrelin levels and anthropometric and biochemical parameters have been reported by some but not by other authors (Chanoine *et al*., 2002; Bellone *et al*., 2003).

The aim of the present study was to analyse the relationships among ghrelin levels, anthropometric and biochemical parameters at birth in a large number of newborns born either at term or preterm.

Subjects and methods

We studied 117 newborns appropriate for gestational age (67 males and 50 females, 93 born at term and 24 born preterm, 56 born from vaginal and 61 from Caesarean deliveries) consecutively born in the Paediatric Departments at Novara and Bolzano. Prematurity was defined as a gestational age less than 37 weeks. Gestational age of term infants was 38·9 ± 1·2 weeks, while that of preterm infants was 33·2 ± 2·4 weeks. All pregnancies were without complications. Vaginal deliveries were uncomplicated, while Caesarean sections were performed because of elective indications such as repeated Caesarean or previous obstetric history of the mothers. After an initial examination in delivery room, all the infants re-examined after stabilization were found to be healthy.

For each newborn we evaluated gestational age, birth weight, length (Table 1) and collected a venous cord blood sample for determination of ghrelin, GH, IGF-I, insulin, glucose and leptin levels. Immediately after delivery a blood sample for the evaluation of ghrelin levels was also drawn from their mothers (mean ± SD age: 32·7 ± 4·7 years).

Human ghrelin (pg/ml) was assayed, after extraction in reverse phase C18 columns, by a radioimmunoassay (RIA; Phoenix Pharmaceuticals, Inc., Belmont CA, USA) using ^{125}I-labelled bioactive ghrelin as a tracer and a rabbit polyclonal antibody *vs.* octanoylated and des-octanoylated h-ghrelin. Sensitivity was 30 pg/tube and intra-assay coefficient of variation (CV) range was 0·3–10·7%.

Leptin (ng/ml) was measured in duplicate by RIA using a commercially available kit (Linco Research Inc., St Charles, MO, USA). Sensitivity was 0·5 ng/ml and intra- and interassay CVs were 3·4–8·3% and 3·0–6·2%, respectively.

IGF-I (nmol/l) were measured by RIA (Diagnostic System Laboratory Inc., Webster, TX, USA) after acid–ethanol extraction. Sensitivity was 0·8 ng/ml and intra- and interassay CV ranges were 1·5–3·4 and 1·5–8·2%, respectively.

Insulin (pmol/l) was measured by chemiluminescent enzyme-labelled immunometric assay with Immulite 2000 (Diagnostic Products Corporation, Los Angeles, CA, USA). Sensitivity was 2 μUI/ml and intra- and interassay CV ranges were 2·5–8·3 and 4·4–8·6%, respectively.

GH (μg/l) was measured by chemiluminescent enzyme-labelled immunometric assay with Immulite 2000 (Diagnostic Products Corporation). Sensitivity was 0·01 ng/ml and intra- and interassay CV ranges were 2·9–4·6 and 4·2–6·6%, respectively.

The study protocol had been approved by the Ethical Committee and informed consent had been obtained by all the adult subjects and either newborns' or infants' parents.

Data are expressed as median and 25th–75th centiles. The statistical analysis was performed using Mann–Whitney *U*-test and Spearman correlation test as appropriate.

To verify the influence of different variables on neonatal ghrelin levels, we used general linear models which enable us to analyse any combination of categorical or continuous predictor variables. We reported multiple R and beta coefficients with 95% Confidence intervals (95% CI) for each independent variable according

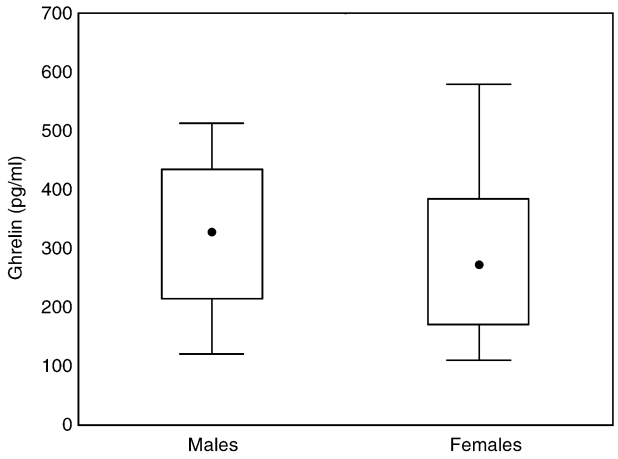

Fig. 1 Cord ghrelin levels in male and female newborns. Points are median, boxes are interquartile range, and whiskers are 10th–90th percentile values.

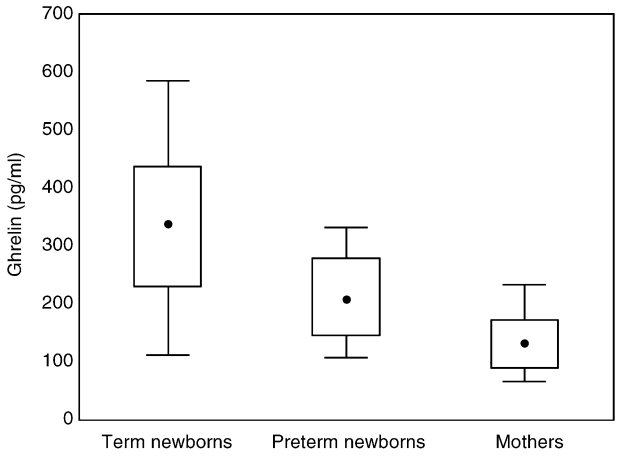

Fig. 2 Plasma ghrelin levels in term and preterm newborns and in their mothers. Points are median, boxes are interquartile range, and whiskers are 10th–90th percentile values.

to the sigma-restricted parameterization. The statistical analysis was performed using Statistica version 6·0 (StatSoft, Inc., Tulsa, OK, USA).

Results

Ghrelin levels in cord blood were 327·6 (range 206·0–413·0) pg/ml and were similar in both sexes (Fig. 1).

Ghrelin levels in maternal blood at delivery (133·0 pg/ml, range 89·0–173·7 pg/ml) were lower ($P < 0·0001$) than those in newborns (Fig. 2): a positive association between ghrelin levels in mothers and newborns ($r = 0·26$, $P < 0·01$) was observed.

Ghrelin levels in neonates born at term (339·0 pg/ml, range 229·0–438·0 pg/ml) were significantly higher ($P < 0·0001$) than in those born preterm (208·0 pg/ml, range 144·5–278·9 pg/ml) (Fig. 2). GH, IGF-I, insulin, glucose and leptin levels were, in turn, similar in both groups.

Ghrelin levels in neonates born by natural delivery were higher than those born by Caesarean section (335·5 pg/ml, range 219·5–434·5 pg/ml *vs.* 282·8 pg/ml, range 161·0–380·0 pg/ml) without reaching statistical significance. Again, the other hormonal parameters were similar in both groups.

In newborns, ghrelin levels were positively associated with gestational age ($r = 0·3$, $P < 0·0009$), birth weight ($r = 0·23$, $P < 0·01$) and length ($r = 0·24$, $P < 0·01$). Considering the influence of birth weight, length and gestational age altogether on neonatal ghrelin levels, a significant correlation was evident only for gestational age (multiple $R = 0·34$, $P < 0·004$; $\beta = 0·33$, 95% CI: 0·05–0·62, $P < 0·02$). On the other hand, no association between ghrelin and GH, IGF-I, insulin, glucose and leptin levels was found. No correlation between ghrelin concentrations in cord blood and maternal weight was found either.

The general linear model analysis showed that both maternal ghrelin levels ($\beta = 0·35$, 95% CI: 0·17–0·52, $P = 0·0002$) and the degree of prematurity ($\beta = 0·36$, 95% CI: 0·18–0·54, $P < 0·0001$) but not the type of delivery significantly contributed to neonatal ghrelin levels (multiple $R = 0·56$, $P < 0·0001$).

Discussion

The results of the present study demonstrate that cord ghrelin levels show clear positive gestational age-related dependency. Ghrelin levels are independent of gender and also of the type of delivery. Unlike in children and adults, ghrelin levels at birth are independent of body weight as well as length and hormonal parameters including GH, IGF-I, insulin and leptin secretion.

These results firstly demonstrate in humans that ghrelin secretion progressively increase during the third trimester of gestation independently of gender. This finding agrees with data in rats showing that ghrelin cells in the fetal stomach progressively increase from pregnancy day 18; in fact, ghrelin cell number in fetal stomach is positively associated with stomach growth (Hayashida *et al*., 2002). Interestingly, ghrelin levels in newborns were higher than those in their mothers indicating feto-placental-derived ghrelin production. Ghrelin expression in rat and human placenta has been demonstrated but peaks in the first half of pregnancy and is almost undetectable at term (Gualillo *et al*., 2001), suggesting that changes in cord ghrelin levels depends mostly on age-related fetal production.

In a preliminary study we found ghrelin levels in neonates born after caesarean section lower than in those born by spontaneous delivery (Bellone *et al*., 2003). This result has not been confirmed

by the present study in which we studied a larger population of subjects considering also preterm newborns.

The peculiar pattern of ghrelin secretion, increasing up to the time of birth would suggest that it is needed for growth purposes. Ghrelin has been discovered as natural ligand of the GHS-R that is distributed at the hypothalamus–pituitary level and mediates strong GH-releasing action, at least after administration of pharmacological ghrelin doses in animals, and even more in humans (Muccioli *et al*., 2002). Moreover, ghrelin coupled with leptin is believed to play major role in driving the central, neuroendocrine and metabolic response to starvation (Inui 2001; Muccioli *et al*., 2002). This action is likely to be the result of activation of GHS-R also at central and peripheral levels (Horvath *et al*., 2001; Inui 2001). In fact, ghrelin system mediates central orexigenic action as well as peripheral modulation of insulin secretion and glucose metabolism (Inui 2001; Ukkola *et al*., 2003).

The neuroendocrine profile of newborns is very similar to that generally observed in malnourished patients. For instance, it includes GH hypersecretion coupled with peripheral GH insensitivity that explains low IGF-I levels that, in turn, exert reduced negative feedback action on somatotroph secretion (Wollmann *et al*., 2000). Although GH is not believed to play a major role in fetal growth, GH hypersecretion would protect from hypoglycaemia and temporarily provide alternative substrates by activating lypolysis (Adrian *et al*., 1990; de Zegher *et al*., 1990). Until now, GH hypersecretion in fetal life and at birth has been supposed to reflect hyperactivity of GHRH-secreting neurones coupled with immaturity of somatostatin activity (Stimano *et al*., 1985; Deiber *et al*., 1989; Wollmann *et al*., 2000). After its discovery, the possibility that ghrelin, as another natural GH secretagogue, might play a role in somatotroph hypersecretion in this period of life, was worthy of consideration. Our present findings show that ghrelin levels are not associated to GH secretion or to IGF-I levels, suggesting that ghrelin is not involved in perinatal GH hypersecretion. This assumption agrees with evidence that ghrelin levels at birth are similar to those in children and adults despite the highest GH secretion occurs in newborns (Bellone *et al*., 2003).

The existence of a causative relationship between ghrelin and GH hyper- and hypo-secretion in anorexia and obesity, respectively, had been hypothesized, but, even in these conditions, a clear functional link between GH and ghrelin secretion has not been demonstrated (Horvath *et al*., 2001; Inui 2001; Otto *et al*., 2001; Tolle *et al*., 2003).

In the present study we found that ghrelin secretion at birth is independent also of insulin and leptin levels as well as of body weight and length. In fact, linear correlation between cord ghrelin levels and body weight and length are functions of gestational age as shown by multiple regression analysis. It should be noted that associations among circulating ghrelin levels and anthropometric and biochemical parameters have been reported by some authors in studies performed in smaller groups of subjects (Chanoine *et al*., 2002). In this context it has to be emphasized that ghrelin knockout mouse does not show any specific alteration (Sun *et al*., 2003).

The lack of direct correlation between ghrelin levels and metabolic parameters as well as with body weight and length suggests that at birth, this new gastric hormone does not play the same metabolic activities exerted in adulthood when associations with body mass index, nutrient intake and insulin secretion are clearly apparent (Inui 2001). However, the lack of association among ghrelin levels and anthropometric or biochemical parameters in the newborn does not rule out the possibility that ghrelin plays important role in the fetal/maternal communication (Gualillo *et al*., 2001).

In conclusion, given the major central role of ghrelin in triggering appetite and food intake (Inui 2001; Muccioli *et al*., 2002), the gestational-dependent increase in ghrelin levels would represent a signal to upregulate the system and activate the seeking of maternal milk after birth. The lack of any direct relationships among ghrelin and anthropometric or biochemical parameters in adequate for gestational age newborns does not support the hypothesis that ghrelin has major role in fetal GH secretion and growth. On the other hand, the lack of any direct correlations between ghrelin and metabolic parameters in newborns adequate for gestational age suggests that the functional link between ghrelin and metabolism is age-related. The possibility that ghrelin plays an important role in the fetal/maternal communication is not ruled out by the present findings but remains to be demonstrated.

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