RAPID COMMUNICATION

Circulating ghrelin levels in newborns are not associated to gender, body weight and hormonal parameters but depend on the type of delivery

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ABSTRACT. Ghrelin, a new gastric-derived hormone, probably plays a major role in managing energy balance and the neuroendocrine response to starvation. Information about the age-related variation in ghrelin secretion is scanty. We measured circulating ghrelin levels in 93 full term newborns adequate for gestational age, in 39 normal children and in 19 lean healthy adults. Our findings demonstrate that ghrelin levels are independent of age and gender from birth to adulthood. Interestingly, ghrelin secretion at birth is not associated to body weight and hormonal parameters such as GH, insulin and leptin levels. On the other hand, ghrelin levels seem dependent on the type of delivery, being lower in newborns after caesarean section with respect to those after normal delivery.
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INTRODUCTION

Ghrelin, the endogenous ligand for the growth hormone secretagogue receptor (GHS-R) (1), exerts potent GH-releasing activity and also possesses other neuroendocrine and non-endocrine activities reflecting central and peripheral GHS-R distribution (2). Ghrelin stimulates food intake, plays a major role in managing the energy balance (2, 3) and the neuroendocrine response to starvation. Circulating ghrelin levels are increased by fasting, decreased by food intake, glucose and insulin (2, 3). In accordance with the major influence of nutrition on ghrelin secretion, circulating ghrelin levels are increased in anorexia and cachexia, reduced in obesity and restored by weight recovery (2, 3). Interestingly, recent data show a functional link between leptin, an adipocytic hormone positively correlated with adiposity, ghrelin and insulin in regulating energy balance and food intake (3).

Newborns are connoted by peculiar metabolic balance and GH hypersecretion associated to low IGF-I levels reflecting peripheral GH resistance (4); this functional profile of the GH/IGF-I axis as well as other neuroendocrine functions at birth is reminiscent of what is generally observed in clinical conditions connoted by anorexia and/or malnutrition. Taking into account the supposed role of ghrelin in managing the neuroendocrine and metabolic response to nutritional impairment, it has been surprisingly reported that ghrelin levels in male newborns are negatively associated to GH and positively to leptin levels (5). Based on the foregoing we aimed at throwing light on the relationships linking ghrelin secretion to gender, body weight, hormonal and metabolic parameters at birth.

SUBJECTS AND METHODS

We studied 93 full-term newborns appropriate for gestational age. Ghrelin, GH, IGF-I, insulin, glucose and leptin levels were measured in cord blood (Table 1). Ghrelin levels were also measured in their mothers immediately after delivery, and in 39 normal-weighted children and in 19 lean healthy adults as comparison (Table 1).

Human ghrelin (pg/ml) was assayed, after extraction in reverse phase C18 columns, by a radioimmunometric assay (RIA) (Phoenix Pharmaceuticals, Inc., Belmont CA) using ^{125}I-labeled bioactive ghrelin as a tracer and a rabbit polyclonal antibody *vs* octanoylated and des-octanoylated h-ghrelin. Sensitivity: 30 pg/tube, intra-assay coefficient of variation (CV) range: 0.3-10.7%.

Key-words: ghrelin, newborns, gender, body weight, delivery.

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

	Newborns	Children	Adults
Males/females	54/39	18/21	10/9
Gestational age (weeks)	38.9±1.2		
Age (years)		9.0±3.2	28.5±3.4
By natural/caesarean delivery	44/49		
Body weight (kg)	3.29±0.39	32.4±11.0	68.9±7.7
GH (ng/ml)	15.2; 9.9-21.1		1.2; 0.1-2.2
IGF-I (ng/ml)	66.2; 46.6-96.0	238.5; 164.8-327.0	207.0; 153.0-209.0
Leptin (ng/ml)	6.0; 3.6-9.2	12.9; 5.0-24.9	5.3; 3.6-8.1
Insulin (mIU/ml)	6.6; 5.1-8.7	7.6; 5.3-10.5	12.7; 11.0-15.2
Glucose (mg/dl)	72.5; 60.0-95.0	75.5; 70.5-84.0	71.0; 63.0-84.0

Leptin was measured in duplicate by RIA using a commercially available kit (Linco Research Inc., St Charles, MO). Sensitivity: 0.5 ng/ml, intra- and interassay coefficients of variation: 3.4-8.3% and 3.0-6.2% respectively.
GH, IGF-I, insulin were assayed as previously described (6). 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.

RESULTS

Ghrelin levels in cord blood [(median; 25th-75th centile) 339.0; 229.0-438.0 pg/ml] were similar to those found in normal children (328.0; 202.0-570.0 pg/ml) as well as to those in adults (338.0; 250.0-502.0 pg/ml) (Fig. 1), despite the highest GH levels being found in newborns. In newborns as well as in children and in adults, ghrelin levels were similar in both sexes.

Ghrelin levels in neonates born by natural delivery were surprisingly higher ($p<0.03$) than in those born by caesarean section (380.0; 300.0-445.0 pg/ml *vs* 310.0; 202.0-393 pg/ml) (Fig. 1). Interestingly, ghrelin levels in maternal blood at the delivery (127.0; 86.0-171.0 pg/ml) were lower ($p<0.0001$) than those in newborns independently of the way of delivery; despite this, ghrelin levels in newborns were positively associated to maternal ghrelin concentrations (Spearman r=0.37, $p<0.002$).
In newborns no association was found between ghrelin levels and birth weight and length as well as IGF-I, GH, insulin and leptin levels. In children ghrelin levels were negatively associated to IGF-I (r=-0.5, $p=0.009$) and leptin (r=-0.4, $p=0.02$) levels, while no association was found to anthropometrical parameters.

DISCUSSION

The results of the present study demonstrate that ghrelin secretion does not undergo relevant age- and gender-related variations. Particularly, newborns show ghrelin levels

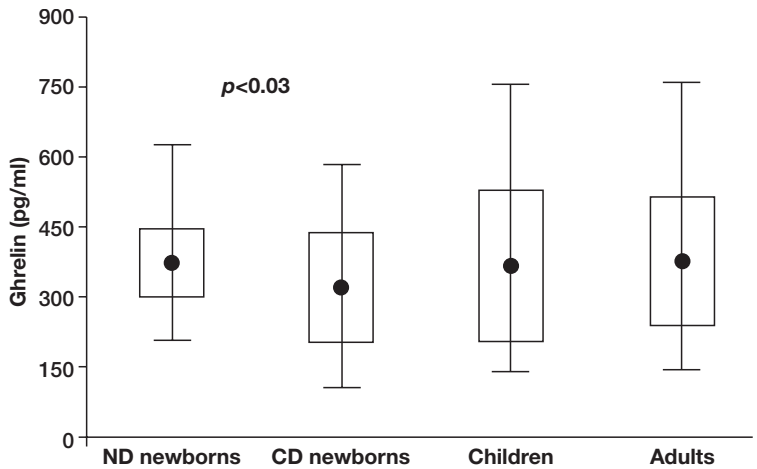

Fig. 1- *Distribution of ghrelin levels in newborns born by natural delivery (ND), caesarean delivery (CD), children and adults. Points are median, boxes are interquartile range, and whiskers are 10th-90th percentile values.*

similar to children and adults; this age-related independency of ghrelin levels is at variance with the age-related variations in GH levels that are higher at birth. Peculiarly, ghrelin secretion at birth, at least in full term appropriate for gestational age newborns, is independent of body weight as well as length and IGF-I, GH, insulin and leptin levels.
Lack of age- and gender-related variations in ghrelin levels have already been reported in a previous study measuring ghrelin secretion in children and adults (6) and is confirmed by the present study in a larger cohort of normal children and adults. This is particularly relevant because newborns are connoted by GH hypersecretion associated with low IGF-I levels reflecting peripheral GH resistance (4). Notably, the present findings bespeak against the possibility that GH hypersecretion at birth is driven by ghrelin whose levels, on the contrary, are not associated to GH levels. We have also found that ghrelin secretion at birth, at least in full term appropriate for gestational age newborns, is independent of body weight as well as length and other hormonal levels including IGF-I, insulin and leptin secretion. On one hand, the lack of any association between ghrelin and IGF-I levels agrees with the hypothesis that ghrelin does not play a major role in regulating the GH/IGF-I axis. In fact, in rats, ghrelin secretion has been reported to be pulsatile and directly related to feeding behaviour but not to GH secretion (7) and, moreover, circulating ghrelin levels have been reported not to be altered in conditions of acromegaly or GH deficiency by some (8) but not by other Authors (9).
On the other hand, the lack of any correlation between ghrelin levels and metabolic parameters as well as with body weight and length suggests that this new gastric hormone does not play at birth the same metabolic roles as in adulthood when clear association with body mass index, nutrients intake and insulin secretion is clearly apparent (3).
Unexpectedly, we have also found that neonates born after caesarean section show lower ghrelin levels than those born by spontaneous delivery. The explanation of this finding is unclear. Interestingly, it has already been shown that fetuses delivered vaginally show a higher activation of the Hypothalamic-Pituitary-Adrenal (HPA) axis than fetuses delivered by caesarean section, as expression of the different intrauterine stress (10). Notably ghrelin has been reported able to stimulate the HPA axis likely acting at the hypothalamic level and also to induce anxiogenesis in different animal models (2, 11). However, although tempting, the hypothesis of a relation between ghrelin levels and the different degrees of stress and of HPA axis activity in these two ways of delivery cannot be supported by present data and needs further investigation.
Finally, evidence that circulating ghrelin levels in mothers are significantly lower than those in newborns supports the evidence of a feto-placental-derived ghrelin production. In fact, ghrelin has been detected both in rat and human placenta showing a pregnancy-related time course of expression (12). Specifically, in humans, ghrelin is mainly expressed in the first half of pregnancy but almost undetectable at term (12). Therefore, present results showing that ghrelin levels from cord blood at term are higher than those in the maternal blood might suggest a fetal ghrelin production. In agreement with this hypothesis it has already been reported that in human foetuses ghrelin is expressed both in the stomach and, even more, at the pancreatic level (13).
In conclusion, the results of the present study demonstrate that ghrelin secretion does not undergo relevant age- and gender-related variations. Notably, this secretory pattern differs from that of GH, further supporting the hypothesis that ghrelin does not play a major role in the physiological control of GH secretion. Accordingly, ghrelin levels in full term appropriate for gestational age newborns do not correlate with IGF-I but also with body weight, length and insulin and leptin levels.

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