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GHRELIN SECRETION IN PRETERM NEONATES PROGRESSIVELY INCREASES AND IS REFRACTORY TO THE INHIBITORY EFFECT OF FOOD INTAKE

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Abstract

Context: Ghrelin, a natural GH Secretagogue, is mainly characterized by non-endocrine activities such as orexigenic effect and modulation of the endocrine and metabolic response to variations in energy balance. Ghrelin levels have been reported to be negatively associated to insulin secretion, enhanced in anorexia and reduced in obesity. Ghrelin levels in newborns were shown to be similar to those found in children and adults without any gender-related difference. **Objective:** The aim of this study was to evaluate ghrelin variations in preterm newborns as a function of fasting and feeding.

Methods: To this end, in 31 preterm neonates (13 males and 18 females) categorized as appropriate for gestational age (AGA) total ghrelin levels were measured in cord blood and then on the fourth day of life before and after meals.

Results: Ghrelin levels in cord blood ([median; 25°-75° centile] 184; 122-275 pg/ml) were higher ($p<0.006$) than those measured in their mothers at delivery (167.0; 89-190 pg/ml). In newborns on the fourth day of life ghrelin levels in fasting conditions (451; 348-649 pg/ml) were higher ($p<0.0004$) than those in cord blood. The meal did not at all modify ghrelin levels (476; 302-775 pg/ml), which were unchanged compared to those in fasting condition. Total ghrelin levels in cord blood were not associated with weight and length; conversely, on the fourth day of life ghrelin levels in newborns were negatively correlated to birth weight as well as to the present weight ($p=0.05$, $r=-0.4$). Ghrelin levels were independent of gender, type of delivery and the kind of feeding regimen.

Conclusions: the secretion of total ghrelin increases from delivery to the fourth day of life when it is refractory to the inhibitory effect of food intake, but it is negatively correlated to body weight.

Introduction

Ghrelin is a 28 aminoacid peptide predominantly produced by the stomach that has been discovered as a natural ligand of the orphan GH Secretagogue (GHS) Receptor (GHS-R) type 1a and supposed to play major role in the control of somatotroph function (1). More recently, ghrelin has been mostly considered as a major new orexigenic factor that, coupled with leptin, is likely to signal the metabolic balance and manage the neuroendocrine and metabolic response to starvation (1).

Circulating ghrelin levels are negatively associated to body mass index; ghrelin secretion is increased in anorexia and cachexia, reduced in obesity and normalized by recovery of ideal body weight (1,2). A peculiar exception to the negative association between body mass and ghrelin secretion is Prader-Willi syndrome where obesity is surprisingly associated to absolute or relative ghrelin hypersecretion (3-4).

In humans, circulating ghrelin levels are increased by energy restriction and decreased by food intake (2,5) indicating that the regulation of ghrelin secretion is mostly metabolic. Ghrelin secretion is decreased by an intravenous or oral glucose load as well as during a euglycemic hyperinsulinemic clamp and even after insulin-induced hypoglycemia (6-8). The inhibitory influence of overexposure to insulin on ghrelin secretion agrees with the strong negative association between ghrelin and insulin levels that had been predicted by the negative correlation between ghrelin levels and body mass index (9). Whether insulin and glucose per se play a direct inhibitory role on ghrelin secretion is, however, still unclear (10,11) as well as the influence, if any, of amino acids and lipids (12-14).

In humans ghrelin secretion undergoes marked variations throughout the day with peaks that anticipate food intake suggesting that the latter is triggered by ghrelin discharge (15-16); although these findings have not been confirmed by others (17), there is agreement that morning ghrelin levels positively correlate with 24 h ghrelin secretion (16). On the other hand, whether ghrelin secretion is age- and/or gender-dependent is still a matter of debate (2). In a previous study we found that total ghrelin levels were unchanged from birth to young adulthood (18).

Ghrelin is present in human fetal circulation from 20 weeks to term (19). Regarding newborns, it has been demonstrated that ghrelin levels in small for gestational age subjects (SGA) are higher than in adequate for gestational age (AGA) subjects (19-21). Moreover, lower cord ghrelin levels have been found to be associated with slower weight gain from birth to 3 months of age (22); the clinical consequences, if any, of this picture of ghrelin secretion are unclear. In fact, neither the ghrelin nor the GHS-R1a KO mice are anorectic dwarfs (23,24); this evidence, however, does not definitely rule out a role of ghrelin in the control of somatotroph secretion, energy balance and metabolism.

In order to further clarify the pattern of ghrelin secretion as a function of the nutritional status around birth, we evaluated ghrelin secretion in the first day of life as a function of fasting and fed state in newborns adequate for gestational age.

Subjects and Methods

We studied 31 neonates (13 males and 18 females) categorized as appropriate for gestational age (AGA), born pre-term by caesarean section for maternal indications (15 neonates) or by spontaneous delivery (16 neonates): gestational age: 33.3±1.7 weeks (range 31-35 weeks), weight 1959±393 g, length 44.2±2.5 cm. AGA was defined as a birth weight from 10^{th} to 90^{th} percentile for gestational age (25). None of the babies showed signs of distress at delivery. Birth weight and length were recorded at birth by the attending nurse. The neonates' weight and meals were monitored during the first 4 days of life. In breast fed newborns, infants were weighted before and after breast feeding.

Twelve newborns were completely breast fed and 19 newborns were formula fed. Each feed lasted about 20-30 minutes. Six newborns were fed by NG tube.

Median (25°-75° centile) of total milk intake per day was 14 (9-35) ml in the first day, 56 (35-70) ml for the second day, 96 (64-120) ml in the third day and 135 (80-200) in the fourth day.

Median (25°-75° centile) of calories per meal at the fourth day was 10.7 (6.6-17.8) Kcal.

The content in carbohydrates, lipids and protein and the energy intake calculated for each meal in the different kinds of milk were statistically similar.

Venous cord blood was drawn immediately after birth. Ghrelin levels were evaluated in different conditions: in cord blood and in the mothers at delivery. In the same neonates ghrelin levels were also measured on the fourth day of life fasting (just before a meal i.e. about 3 h after the previous meal) and about 60 minutes after meal.

The study protocol had been approved by the Ethical Committee and informed consent had been obtained from all infants' parents.

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

Hormonal data are expressed as median and 25^{th}-75^{th} centiles, anthropometric parameters as mean ± SD. The statistical analysis was performed using Wilcoxon test and Spearman correlation test as appropriate.

Results

Ghrelin levels in cord blood ([median; 25°-75° centile] 184; 122-275 pg/ml) were higher ($p<0.006$) than those measured in their mothers at the delivery (167.0; 89-190 pg/ml) (figure 1).

In newborns on the fourth day of life ghrelin levels in fasting conditions (451; 348-649 pg/ml) were higher ($p<0.0004$) than those in cord blood (figure 1).

The meal did not modify ghrelin levels at all (476; 302-775 pg/ml), which were unchanged compared to those in fasting condition (figure 2).

Total ghrelin levels in cord blood were not associated with weight and length; they did not correlate with weight even when data were normalized as function of gestational age. Conversely, on the fourth day of life ghrelin levels in newborns negatively correlated to birth weight as well as to the present weight ($p=0.05$, $r=-0.4$ for both) (figure 3).

Ghrelin levels did not correlate with gestational age.

Ghrelin levels proved to be independent of both gender and type of delivery either at birth or on the fourth day of life (table 1).

Ghrelin levels did not vary between breast and formula fed infants.

Cord ghrelin levels positively correlated with ghrelin levels at the 4th day of life ($p=0.005$, $r=0.5$), while did not correlate with ghrelin levels in mothers.

Discussion

The results of the present study demonstrate that preterm newborns adequate for gestational age show an increase in total ghrelin secretion from birth (cord levels) to the fourth day of life which is not modified by a meal. Ghrelin levels are independent of gender and of type of delivery. Circulating total ghrelin levels display negative association to body weight on the fourth day of life but not at birth when, however, they are higher than those in the mothers.

Ghrelin synthesis and secretion from the placenta has been demonstrated (26). In the fetal rat stomach ghrelin cell population progressively increases from the 18^{th} day of pregnancy and ghrelin cell number has been found to be positively associated to stomach growth (27). In humans, ghrelin has been demonstrated in fetal circulation from 20 weeks to term (19). That these ghrelin levels are produced by the fetus is indicated by the lack of difference between

arterial and venous concentration as well as by evidence that ghrelin is almost absent in the placenta during the third trimester (19).

Total ghrelin levels in cord blood at birth have been evaluated both in AGA or SGA (20-22,28-31). Our present data agree with some (22,28,29,31) but not other (20,21,31) previous studies showing that circulating total ghrelin levels in cord blood of AGA newborns are independent of gender, body weight and type of delivery. This is in agreement also with our previous findings in considerable number of newborns in whom ghrelin levels were independent of the two types of delivery (32). Indeed, circulating total ghrelin levels have generally been demonstrated to be higher in SGA than in AGA newborns (20,21,22,29,33). Concerning the correlation between ghrelin and GH/IGF-I axis, no correlation has been reported so often (28-30); in some studies a negative association between ghrelin and IGF-I or IGFBP-3 has been reported (21). These findings agree with the opinion of many authors who believe that ghrelin and somatotroph function are unlikely to be linked by a functional relationship (5). The same concept is likely to apply also to the relationship between ghrelin and leptin; despite the fact that both play relevant actions in the control of appetite and energy expenditure (1,2), these hormones do not seem linked by direct functional feedback (33).

In the present study as well as in two other studies (21,29), progressive increase in circulating total ghrelin levels in the days following birth has been demonstrated. Moreover, our present study firstly demonstrates that feeding does not exert its well known inhibitory effect on circulating total ghrelin levels. It had already been demonstrated that food intake inhibits morning ghrelin secretion in adults but not in prepubertal children (34,35). This ghrelin refractoriness to food intake from birth to childhood would therefore suggest that this hormone is playing a role as an anabolic drive in the phase of life of growth and development. However, it has to be considered that in infants as well as in children ghrelin secretion is sensitive to the inhibitory effect of glucose load despite its refractoriness to feeding (36,37).

On the other hand, it has to be considered that the first days of life are characterized by

physiological weight decrease that reaches a nadir on the fourth day (25). This weight decrease would play a role in stimulating ghrelin secretion and would also account for the refractoriness to the inhibitory effect of feeding, as has been observed also in anorexia nervosa (38). In fact we, as well as other authors, found that soon after birth and namely on the 4^{th} day of life, circulating total ghrelin levels display inverse association with anthropometric variables, including body weight (22,29). Furthermore, it has to be considered that control of growth in newborns is different from later life and is connoted by the immaturity of the neuroendocrine system, in particular in our population consisting of preterm newborns. According to this picture, it has been demonstrated in preterm newborns that circulating concentrations of leptin and fT4 rapidly decrease after birth. This pattern could be a physiological advantage to newborns by limiting body energy expenditure and conserving nutritional reserves for growth and development (39). These data have to be confirmed in a group of full-term newborns.

Furthermore it has to be taken into consideration that in our study, as well as in others performed in neonates, only total ghrelin levels were measured. This is a limitation in the comprehension of ghrelin role in growth and metabolism. In fact it has been demonstrated ghrelin circulates in blood in the acylated form that binds GHS-R1a receptors and possess endocrine activity, and the non-acylated form which is present in human serum in greater quantities than that acylated one and is able to exert some nonendocrine affects, is devoid of any endocrine activity, but exert some non-endocrine actions including metabolic, cardiovascular and antiproliferative effects.

In conclusion, the secretion of total ghrelin increases from delivery to the fourth day of life when it is refractory to the inhibitory effect of food intake. This pattern of ghrelin secretion would be finalized to represent an anabolic drive in newborns.

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Legends

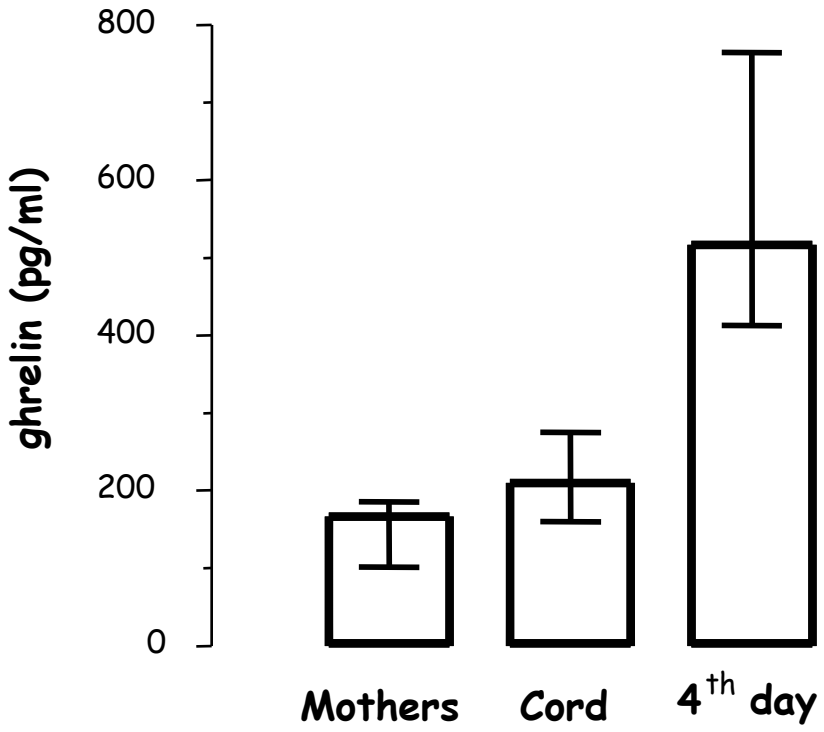
Table 1. Ghrelin levels at each time point in males and females and in neonates born by vaginal or caesarean delivery.

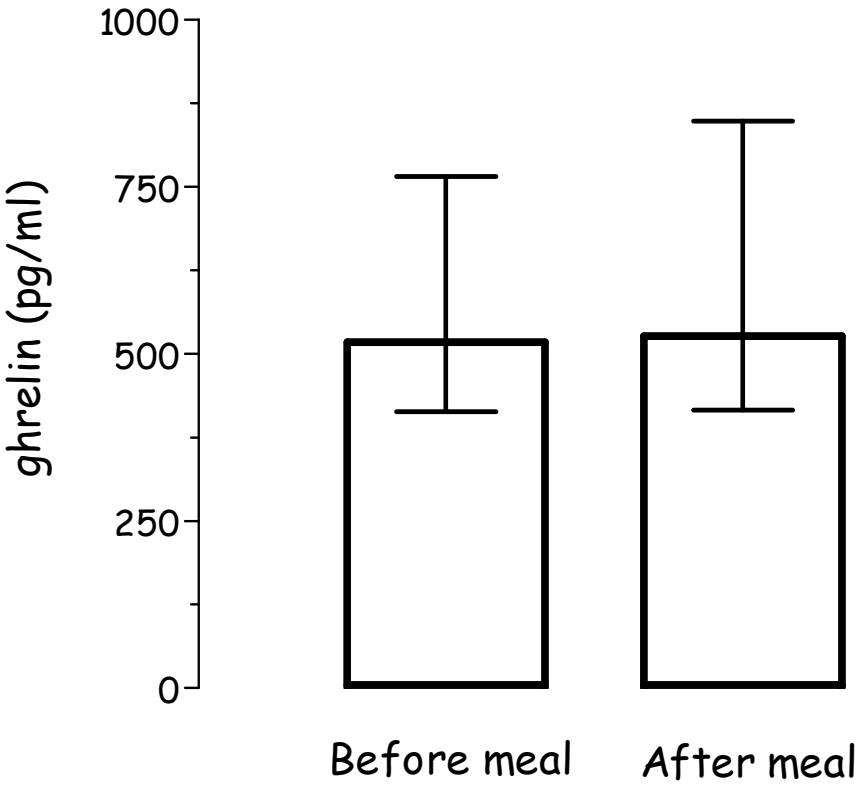
Figure 1. Plasma ghrelin levels in mothers and in newborns in cord blood and fasting on the fourth day of life.

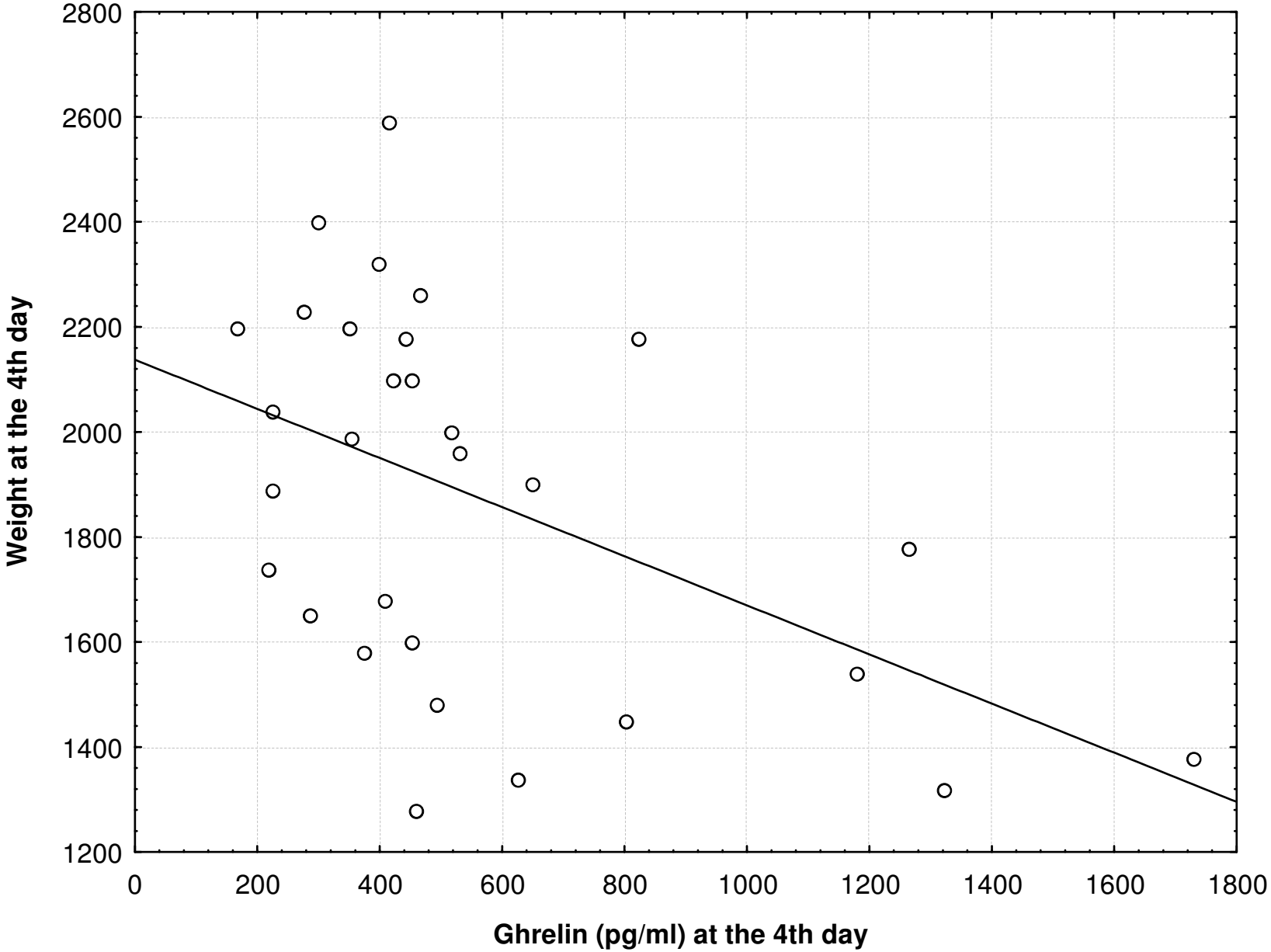
Figure 2. Ghrelin levels on the 4th day of life before and after meal.

Figure 3. Correlation between fasting ghrelin levels and weight at the 4^{th} day of life.

	Cord blood (pg/ml)	**4th day fasting (pg/ml)**	**4th day feeding (pg/ml)**
Males	176, 149-247	494, 374-801	479, 302-741
Females	186, 119-326	414, 275-649	463, 297-833
Vaginal delivery	184, 121-210	420, 300-494	401, 299-526
Caesarean delivery	223, 155-335	541, 353-1264	719, 451-1221


800
600
400
200
0
ghrelin (pg/ml)
Mothers
Cord
4th day


1000
750
500
250
0
ghrelin (pg/ml)
Before meal
After meal


Weight at the 4th day
2800
2600
2400
2200
2000
1800
1600
1400
1200
0
200
400
600
800
1000
1200
1400
1600
1800
Ghrelin (pg/ml) at the 4th day