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Ghrelin Secretion in Childhood Is Refractory to the Inhibitory Effect of Feeding

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Ghrelin, a natural GH secretagogue, is predominantly produced by the stomach. Ghrelin has other actions including orexant activity, modulation of energy balance, and modulation of endocrine and nonendocrine functions. Ghrelin secretion is increased by fasting and energy restriction but decreased by food intake, glucose, insulin, and somatostatin. Ghrelin secretion does not seem to be a function of age; in fact, morning ghrelin levels after overnight fasting in prepubertal and pubertal children are similar to those in young adults. To clarify whether children and adults have the same sensitivity to the inhibitory effect of food intake, we studied the ghrelin response to a standardized light breakfast (SLB) in 10 prepubertal lean children whose results were compared with those recorded in 19 normal-weight adults. Basal ghrelin levels in children (median, 224.5; 25th to 75th percentile, 122.0–447.7 pg/ml) and adults (338.0; 238.0–512.0 pg/ml) were similar. SLB inhibited ghrelin levels in adults (263.0; 190.0–399.0 pg/ml). However, no change in ghrelin levels after SLB (206.5; 105.0–274.0 pg/ml) was recorded in children. Thus, food intake inhibits ghrelin secretion in adults but not in children. Ghrelin refractoriness to inhibition by food intake in children would reflect a peculiar functional profile of the ghrelin system in childhood. (*J Clin Endocrinol Metab* 89: 1662–1665, 2004)

GHRELIN IS A 28-amino-acid peptide predominantly produced by the stomach (1–3). In its acylated form, ghrelin displays strong GH-releasing activity mediated by the activation of the GH secretagogue receptor (GHS-R) type 1a (1, 3). GHS-Rs are concentrated in the hypothalamus-pituitary unit but are also distributed in other central and peripheral tissues (3, 4). Indeed, besides a potent GH-releasing effect, ghrelin has several other actions including: 1) stimulation of lactotroph and corticotroph secretion but inhibition of the gonadal axis, 2) orexant activity coupled with control of energy expenditure, 3) influence on behavior and sleep, 4) influence on gastric motility and acid secretion as well as on exocrine pancreatic functions, 5) modulation of insulin secretion and glucose and lipid metabolism, 6) cardiovascular actions, and 7) influence on normal and neoplastic cell proliferation (2, 3, 5, 6).

In adults, circulating ghrelin levels, mostly represented by its unacylated form, are increased by fasting and energy restriction and decreased by food intake, glucose, insulin, and somatostatin; on the other hand, ghrelin secretion seems unaffected by lipids and amino acids, although some influence of amino acids has been recently observed in animals (2, 5, 7–11). Accordingly, circulating ghrelin levels are increased in anorexia and cachexia but reduced in obesity (8, 12, 13). These changes are opposite to those of leptin, and it has been suggested that both ghrelin and leptin are hormones signaling metabolic balance and driving the neuroendocrine and metabolic response to changes in nutritional status (5, 6, 12). In agreement with this assumption, a clear negative association between insulin and ghrelin levels has been also shown (5, 6, 12). As anticipated, ghrelin is under inhibitory control exerted by insulin whose secretion seems, in turn, affected by ghrelin (8–10, 14, 15). The link between ghrelin and insulin suggests that ghrelin modulates the function of the endocrine pancreas where expression of both ghrelin and its GHS-R has been demonstrated (16–18). This hypothesis agrees also with evidence that there is a functional link between ghrelin and somatostatin (19). In fact, ghrelin is under inhibitory control exerted by somatostatin whose synthesis and secretion seem, in turn, to be positively modulated by ghrelin (19, 20).

The orexigenic action of ghrelin and its GH-releasing effect predict that it could exert an anabolic role that would be particularly important in childhood. However, it has been recently demonstrated that morning ghrelin levels after overnight fasting in either prepubertal or pubertal children are not enhanced, being similar to those in young adults despite very different GH profiles (21).

To further clarify the relationship between ghrelin secretion and nutrients as a function of age, we studied the ghrelin response to a standardized light breakfast (SLB) in children in comparison with adults. In fact, we have previously demonstrated that ghrelin secretion is reduced to the same extent by SLB and oral glucose load in adults (22). Our aim was therefore to clarify whether children and adults have the same sensitivity to the inhibitory effect of food intake.

Abbreviations: BMI, Body mass index; CV, coefficient of variation; GHS-R, GH secretagogue receptor; SLB, standardized light breakfast.

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Subjects and Methods

We studied 10 normal-weight prepubertal children [three males and seven females; age (mean ± SD), 8.5 ± 1.3 yr; height, 131.4 ± 3.7 cm; weight, 31.9 ± 3.1 kg; body mass index (BMI), 18.0 ± 0.8 kg/m^2; ponderal excess, 8.6 ± 3.5%) and 19 healthy normal-weight adults (10 males and nine females; age, 28.5 ± 3.4 yr; BMI, 22.4 ± 0.6 kg/m^2). Height was measured by the Harpenden statimeter and weight by using an electronic scale. BMI was calculated as body weight divided by squared height (kg/m^2). All subjects had weights adequate for height and sex.

Ghrelin, insulin, and glucose levels were measured in the morning before and 60 min after SLB (energy intake 300 kCal, 45% carbohydrates, 13% proteins, and 42% lipids at 0 min). In children, GH and IGF-I levels were also evaluated.

The study protocol had been approved by the Ethical Committee, and informed consent had been obtained by all the adult subjects and all children's parents.

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

GH (micrograms per liter) was measured by chemiluminescent enzyme-labeled immunometric assay with Immulite 2000 (Diagnostic Products Corp., Los Angeles, CA). Sensitivity was 0.01 ng/ml. Intra- and interassay CV ranges were 2.9–4.6 and 4.2–6.6%.

IGF-I (1 ng/ml = 0.1307 nmol/liter) was measured by RIA (Diagnostic Systems Laboratories, Inc., Webster, TX) after acid-ethanol extraction. Sensitivity was 0.8 ng/ml. Intra- and interassay CV ranges were 1.5–3.4 and 1.5–8.2%.

Insulin (1 μIU/ml = 7.175 pmol/liter) was measured by chemiluminescent enzyme-labeled immunometric assay (Diagnostic Products). Sensitivity was 2 μIU/ml. Intra- and interassay CV ranges were 2.5–8.3 and 4.4–8.6%.

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

Results

Basal ghrelin levels in children (median, 224.5; 25th to 75th percentile, 122.0–447.7 pg/ml) were similar to those in adults (338.0; 238.0–512.0 pg/ml).

As expected, in children as well as in adults, SLB increased both insulin [peak *vs.* baseline (median and 25th to 75th percentile) was 12.1 and 10.7–19.2 *vs.* 5.3 and 4.4–7.0 mU/liter for children and 48.3 and 41.2–54.3 *vs.* 16.3 and 13.7–18.3 mU/liter for adults; $P < 0.01$ for both] and glucose levels (81.4 and 76.7–85.5 *vs.* 72.1 and 68.9–74.9 mg/dl for children and 82.9 and 78.7–86.7 *vs.* 74.5 and 70.3–77.9 mg/dl for adults; $P < 0.01$ for both).

In adults, 60 min after SLB, ghrelin levels (263.0; 190.0–399.0 pg/ml) were significantly lower ($P < 0.0001$) than at baseline (24% inhibition). In contrast, SLB did not induce any change in ghrelin levels in children (206.5; 105.0–274.0 pg/ml) (Fig. 1, A and B).

No correlation between ghrelin and insulin levels was found at baseline and after SLB in both groups. In children, ghrelin levels at baseline were negatively associated ($r = -0.82$; $P < 0.04$) with IGF-I levels (183.5; 151.0–211.0 ng/ml) but not with GH levels (0.1; 0.1–1.6 μg/liter).

Discussion

The results of the present study demonstrate that food intake inhibits morning ghrelin secretion in adults but not in prepubertal children. Moreover, children show a peculiar lack of negative association between ghrelin and insulin levels either at baseline or after breakfast.

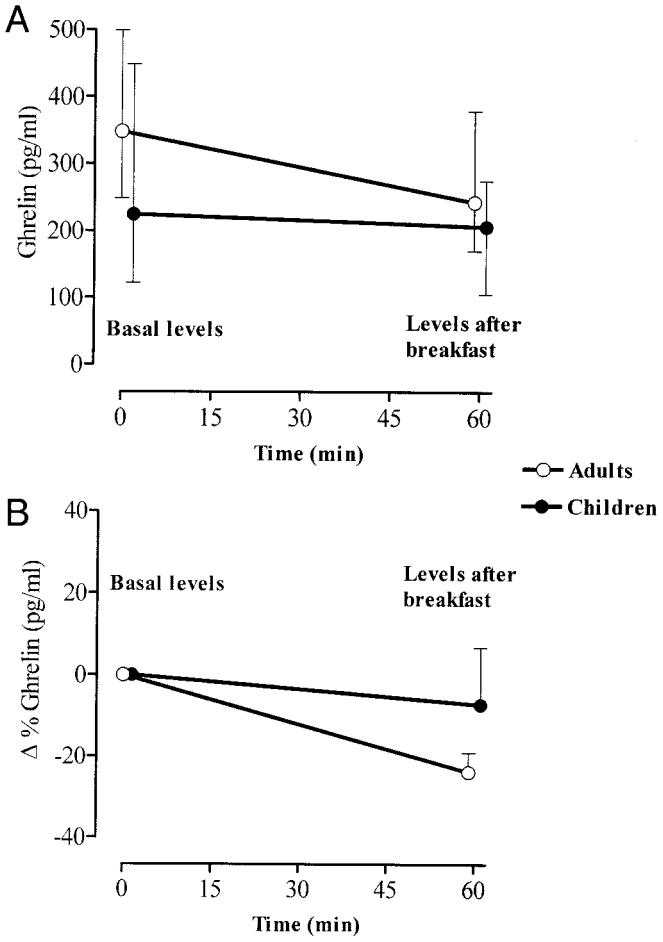

FIG. 1. A, Median and 25th to 75th percentile of ghrelin levels before and after breakfast in adults and in children; B, mean of δ percent variation ± SEM of ghrelin levels.

In addition to potent GH-releasing activity, ghrelin exerts various neuroendocrine and nonendocrine activities reflecting central and peripheral GHS-R distribution (2–6). Particularly, ghrelin coupled with leptin contributes to driving the neuroendocrine and metabolic responses to changes in nutritional status (3, 5). In fact, ghrelin stimulates appetite and food intake and regulates energy balance and the neuroendocrine response to starvation (3, 5). In agreement with this assumption, ghrelin levels are increased in anorexia and cachexia, reduced in obesity, and restored by weight recovery in these conditions (5, 13, 23).

Ghrelin secretion is stimulated by fasting and energy restriction and decreased by food intake and overfeeding (5, 6, 7, 12, 13). Both oral and iv glucose loads inhibit ghrelin secretion in humans as well as in animals (8, 24); thus, glucose load-induced hyperglycemia exerts a negative influence on ghrelin secretion although the mechanisms underlying the inhibitory effect of glucose are still unclear (25).

In agreement with the negative association between ghrelin secretion and body mass, a clear negative association between ghrelin and insulin secretion has been found in

humans as well as in animals (7, 10, 26), suggesting an inhibitory influence of insulin on ghrelin secretion (9, 27). Indeed, during a euglycemic clamp, the steady-state increase in insulin levels is associated with a clear reduction in circulating ghrelin levels (9, 10, 27). The exact mechanisms by which insulin regulates ghrelin secretion are still unknown, although it has been demonstrated that insulin directly modulate ghrelin expression at the gastric level (28).

We have previously shown that morning ghrelin levels show good intraindividual reproducibility (21); in that study, morning ghrelin levels in men and women were similar, but more recently, 24-h ghrelin secretion has been found to be higher in women than in men, suggesting the existence of gender-related dimorphism (29). In normal young adults, it has been shown that circulating ghrelin levels are decreased to the same extent by oral glucose load and light breakfast (22). This inhibitory effect of breakfast shows also good intraindividual reproducibility (22). Because lipids and amino acids do not exert significant inhibitory influence on ghrelin secretion, at least in humans, it is likely that the inhibitory effect of SLB is caused by the glucose intake.

We demonstrate that, differently from in adults, food intake, *i.e.* SLB, does not inhibit circulating total ghrelin levels in prepubertal children; in other words, children seem refractory to the inhibitory influence of food intake on ghrelin secretion. This finding is remarkable taking into account that ghrelin has been proposed as a new anabolic hormone and that childhood is characterized by metabolism that is predominantly anabolic (3, 5).

The explanation of this peculiar functional profile of the ghrelin system in childhood is not obvious. On one hand, one could argue that the insensitivity of ghrelin to the inhibitory effect of food intake in childhood fits well with the hypothesis that the functional profile of the ghrelin system in children is oriented to anabolic purposes. In other words, the lack of any inhibition of ghrelin levels after food ingestion would maintain constant stimulation of appetite and energy intake, thus providing the substrates needed for the anabolic process of growth.

On the other hand, if ghrelin has such an important anabolic role particularly in childhood, it is unclear why circulating ghrelin levels in children are not enhanced with respect to adults. It has been shown that ghrelin secretion does not undergo remarkable age-related changes; in fact, circulating ghrelin levels in newborns, prepubertal and pubertal children, as well as in adults are unchanged despite very different secretory profiles of GH and IGF-I levels (21, 30, 31). In the present study, ghrelin levels in prepubertal children were even lower than in adults, although this difference did not attain statistical significance. Thus, one would reason that food intake reduced ghrelin levels in adults simply because they were relatively elevated in comparison with children. However, to further confuse the definite explanation of our present findings is also evidence that ghrelin hypersecretion in anorectic patients and even ghrelin hyposecretion in obese patients are refractory to the inhibitory effect of food intake (32, 33).

As anticipated, ghrelin secretion is negatively associated with insulin secretion in physiological conditions; a functional feedback link between these hormones is likely to be present (15, 27), and there are also studies suggesting that ghrelin would also modulate insulin sensitivity (34). Even ghrelin hyper- and hyposecretion in anorexia and obesity, respectively, could reflect the opposite changes in insulin secretion that connote these pathophysiological conditions (5, 12, 13). Despite this, in the present study, there was a lack of any negative correlation between ghrelin and insulin levels either before or after food ingestion in children. It might be that insulin does not play a negative influence on ghrelin secretion, thus allowing its refractoriness to the inhibitory effect of food intake in childhood; based on the present findings, this is, however, pure speculation.

Another interesting aspect of this study is that we found no positive correlation between ghrelin and GH or IGF-I levels; actually, the latter were even negatively associated with circulating ghrelin levels in children. These findings agree with others (5, 13, 29) and make it even more unlikely that ghrelin plays a major role on somatotroph function and growth.

In conclusion, our study demonstrates that food intake inhibits ghrelin secretion in adults but not in children. Ghrelin refractoriness to the inhibitory effect of food intake in children would reflect a peculiar functional profile of the ghrelin system in childhood.

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