Oral glucose load inhibits circulating ghrelin levels to the same extent in normal and obese children

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Summary

Objective The presence of both the GH secretagogue (GHS) receptor and ghrelin in the pancreas indicates an involvement of this hormone in glucose metabolism. Ghrelin secretion is increased by fasting and energy restriction, decreased by food intake, glucose load, insulin and somatostatin in normal adults; however, food intake is not able to inhibit circulating ghrelin levels in children, suggesting that the profile of ghrelin secretion in children is different from that in adults. Moreover, how ghrelin secretion is regulated in childhood as a function of fat mass is still unclear.

Design and subjects We studied the effect of oral glucose load (75 g solution orally) on circulating total ghrelin levels in 14 obese children (group A, four boys and 10 girls, aged 9·3 ± 2·3 years) and 10 lean children (group B, five boys and five girls, aged 9·7 ± 3·8 years).

Measurements In all the sessions, blood samples were collected every 30 min from 0 up to +120 min. GH, insulin and glucose levels were assayed at each time point.

Results Glucose peaks following an oral glucose tolerance test (OGTT) in groups A and B were similar; however, both basal and OGTT-stimulated insulin levels in group A were higher than in group B (P < 0·05). Basal total ghrelin levels in group A (281·3 ± 75·4 pg/ml) were lower (P < 0·0005) than in group B (563·4 ± 81·5 pg/ml). In both groups A and B, the OGTT inhibited total ghrelin levels (P < 0·0005). In terms of absolute values, total ghrelin levels in group A were lower (P < 0·0005) than those in group B at each time point after glucose load. The percentage nadir in total ghrelin levels recorded in group A (–25% at 90 min) was similar to that recorded in group B (–31% at 120 min). Total ghrelin levels were negatively associated with BMI (r = 0·5, P < 0·005) but not with glucose or insulin levels.

Conclusion Ghrelin secretion is reduced in obese children. It is, however, equally sensitive in both obese and lean children to the inhibitory effect of oral glucose load.
Induced hypoglycaemia. An inhibitory input on ghrelin also comes from the activation of gastric somatostatin receptors as indicated, so that the most remarkable inhibition of circulating ghrelin levels is observed under exposure to somatostatin and its analogues. \cite{29,30} Regarding the mechanisms underlying the influence of glucose and insulin on ghrelin secretion, there is still controversy; in particular, it is still a matter of debate whether they play a direct or an indirect inhibitory role.\cite{29,30}

The inhibitory influence of both glucose and food intake has been reported to be a function of either age or fat mass. In fact, glucose load is reported to be unable to inhibit ghrelin secretion in anorexia nervosa while food intake does not modify it in childhood.\cite{28}

Based on the above, we studied the ghrelin response to an oral glucose tolerance test (OGTT) in both obese and lean children to clarify the effects of glucose load on total ghrelin secretion as a function of age and fat mass.

Subjects and methods

Twenty-four prepubertal children were enrolled in this study: 14 obese children (group A, four boys and 10 girls, aged 9.3 ± 2.3 years) and 10 lean children (group B, five boys and five girls, age 9.7 ± 3.8 years). The BMI was above the 90th percentile for group A and from the fifth to the 90th percentile for group B; specifically, the mean BMIs in groups A and B were 26.0 ± 0.6 vs. 17.5 ± 0.6, \(P < 0.0005\). Children in group A as well as those in group B had no endocrine disorder and were receiving no medication at the time of the study. All the children's parents were informed as to the purpose of the study and gave their consent as required by the local ethics committee. Control group subjects were enrolled during a programme of auxological and metabolic screening and their parents gave their consent to the evaluation of glucose tolerance with the OGTT.

The children’s heights were measured by Harpenden’s stadiometer and their weight was measured by using an electronic scale. BMI was calculated as body weight divided by height squared (kg/m\(^2\)). All children in both groups were of normal stature and growing normally. As anticipated, all children were prepubertal.

All subjects underwent the OGTT (75 g glucose solution orally), which was performed in the morning after an overnight fast. Blood samples were taken by an intravenous catheter kept patent by slow infusion of isotonic saline at baseline and then 30, 60 and 120 min after the OGTT. At each time point, circulating ghrelin, glucose and insulin levels were measured.

Human ghrelin (pg/ml) was assayed, after extraction on reverse-phase C18 columns, by a radioimmunometric assay (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 human ghrelin. The sensitivity was 30 pg/tube. Based on our data, the intra-assay coefficient of variation (CV) was 0–3–10.7%.

Serum insulin levels (1 \(\mu\)IU/ml = 7-175 pmol/l) were measured by a chemiluminescent enzyme-labelled immunometric assay (Diagnostic Products). The sensitivity was 2 \(\mu\)IU/ml. Intra- and interassay CVs were 2.5–8.3% and 4.4–8.6%, respectively.

Plasma glucose (mmol/l) was measured by the glucose oxidase method on a Beckman Glucose Analyser (Fullerton, CA, USA).

Data are presented as mean ± SEM of absolute and delta percentage values. The statistical analysis was performed by an analysis of variance (ANOVA) test (to evaluate the ghrelin, insulin and glucose responses to the OGTT) and an unpaired Student's \(t\)-test (to evaluate data at baseline in obese vs. lean children) by using Statview 5 software for Windows (SAS Institute Inc., Cary, NC, USA).

Results

Basal insulin levels in group A were higher than in group B (252.4 ± 68.1 vs. 36.6 ± 4.8 pmol/l; \(P < 0.05\)) (Fig. 1b), whereas basal ghrelin levels were similar in groups A and B (4.4 ± 0.1 vs. 4.6 ± 0.1 mmol/l) (Fig. 1a). In addition, glucose peaks following an OGTT in group A (6.7 ± 0.3 mmol/l) and group B (7.3 ± 0.6 mmol/l; \(P < 0.005\)) (Fig. 1b). Basal total ghrelin levels in group A (281.3 ± 29.5 pg/ml) were lower (\(P < 0.0005\)) than in group B (563.4 ± 81.5 pg/ml) (Fig. 1c). In both groups A and B, OGTT inhibited total ghrelin levels (\(P < 0.0005\)) (Fig. 1c).

In terms of absolute values, after glucose load total ghrelin levels in group A were lower than those in group B at each time point (\(P < 0.0005\)) (Fig. 1c). However, the percentage nadir in total ghrelin levels recorded in group A (~25% at 90 min) was similar to that recorded in group B (~31% at 120 min) (Fig. 2).

Total ghrelin levels were negatively associated with BMI (\(r = 0.5, P < 0.005\)) (Fig. 3). Nevertheless, no correlation between ghrelin and insulin or glucose levels was recorded.

Discussion

The results of this study demonstrate that obese children show reduced secretion of total ghrelin, which, however, displays normal sensitivity to the inhibitory effect of oral glucose load. Total ghrelin levels in the obese are in fact inhibited by the OGTT to the same extent as they are in lean children. Total ghrelin levels are negatively associated with body mass but not with insulin or glucose levels.

It had been clearly demonstrated that ghrelin secretion is negatively associated with BMI and fat mass.\cite{14,17,18,22,27,28} It is in fact, circulating total ghrelin levels are reduced in obesity both in adulthood and in childhood.\cite{14,17,18} Our present data confirm this negative correlation in childhood. A notable exception to this is shown by obese patients with Prader–Willi syndrome; the cause of this peculiarity remains unexplained.\cite{20,21,23}

In agreement with the major link between nutrition, metabolism and ghrelin, the ghrelin circulating levels are increased by fasting and energy restriction but decreased by food intake and overfeeding, at least in adulthood.\cite{14,16–18,21,34–36} Accordingly, these variations in ghrelin secretion under nutritional manipulations predict its clear negative association with insulin secretion.\cite{16,17,22,27,28}

That ghrelin secretion is reduced by both oral and intravenous glucose load has been clearly demonstrated in adults, in whom it is also reduced by insulin-induced hypoglycaemia as well as under the euglycaemic–hyperinsulinaemic clamp.\cite{22,27,28} Thus, either glucose or insulin-induced hypoglycaemia.
insulin are inhibitory inputs on ghrelin secretion although whether they play a direct or an indirect inhibitory role is still a matter of debate.\textsuperscript{26,31}

Previous studies demonstrated that fasting plasma ghrelin levels were high in anorectic patients, suggesting that ghrelin may play an important role in the pathophysiology of anorexia nervosa.\textsuperscript{37} Hyperghrelinaemia in anorectic patients is caused at least partly by increased secretion of active ghrelin, and glucose ingestion suppresses active ghrelin release in these patients.\textsuperscript{18,38} It has been reported that even the inhibitory effect of food intake on ghrelin secretion is not operative in childhood.\textsuperscript{39}

Our present data demonstrate that ghrelin secretion is inhibited by oral glucose load in obese as well as in lean children. Obese children display a ghrelin hyposecretory state in basal conditions but, at least in terms of percentage decrease, hyperglycaemia inhibits circulating levels to the same extent as recorded in lean children. This occurs despite a clear-cut difference in both basal and OGTT-stimulated insulin secretion. Thus, our present data question previous studies showing the refractoriness of ghrelin secretion to the inhibitory effect of food intake in normal lean children\textsuperscript{39} as well as to the inhibitory effect of standard light breakfast (SLB) and glucose load.
in normal adults.\textsuperscript{40,41} The inhibitory effect of oral glucose load on ghrelin secretion in lean and obese adolescents has been reported;\textsuperscript{19} moreover, glucose load and food intake lead to a rapid fall in plasma ghrelin concentration, suggesting that plasma ghrelin reflects an acute feeding state and may also serve as an indicator of short-term energy balance.\textsuperscript{18} Thus, the lack of significant reduction of ghrelin secretion following food intake in children reported by some authors could be related to the small number of subjects studied. Alternatively, a meal would be able to inhibit ghrelin only when sufficient amounts of adsorbed carbohydrates elevate glucose and insulin levels enough.\textsuperscript{42} The low glucose content of the SLB would be another explanation for the lack of ghrelin inhibition in children, although the same SLB was able to inhibit ghrelin secretion in adults.\textsuperscript{40}

The negative association between ghrelin secretion and fat mass, which, in turn, is positively correlated to insulin secretion, would imply that hyperinsulinism has a role in causing the well-known ghrelin hyposecretory state that connotes obesity.\textsuperscript{14,16,17,43} This would question the major role of insulin per se in controlling ghrelin secretion, at least in children. Accordingly, despite hyperinsulinism, ghrelin levels are not reduced at all in obese patients with Prader–Willi syndrome.\textsuperscript{20,21,23} Moreover, children with diabetes type 1 display reduced ghrelin secretion that is not modified by insulin replacement and reduction in glucose levels.\textsuperscript{44} However, it should be emphasized that there is a major drawback of our studies, as well as in the majority of studies addressing ghrelin secretion in physiological and pathological states. In general, total ghrelin is measured, although its acylated form is assumed to be the only active form. However, it has been clearly shown that unacylated ghrelin is also an active peptide exerting remarkable metabolic actions.\textsuperscript{5,11,13,45,46} Thus, a full understanding of the role played by ghrelin at the metabolic level in physiological and pathophysiological states will need an accurate distinction between its circulating acylated and unacylated forms. This knowledge could completely change our understanding of the relationships linking the ghrelin system with insulin secretion and sensitivity, glucose and lipid metabolism.

In conclusion, as in adulthood, obesity in childhood is associated with a significant reduction in total ghrelin secretion. The ghrelin hyposecretory state in obese children is normally sensitive to the feeding effect of ghrelin and leptin.\textsuperscript{40,44} The inhibitory effect of oral glucose load on ghrelin secretion in children would be related to the small number of subjects studied. Alternatively, a meal would be able to inhibit ghrelin only when sufficient amounts of adsorbed carbohydrates elevate glucose and insulin levels enough.\textsuperscript{42} The low glucose content of the SLB would be another explanation for the lack of ghrelin inhibition in children, although the same SLB was able to inhibit ghrelin secretion in adults.\textsuperscript{40}

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In conclusion, as in adulthood, obesity in childhood is associated with a significant reduction in total ghrelin secretion. The ghrelin hyposecretory state in obese children is normally sensitive to the inhibitory effect of glucose load.

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References


