CLINICAL STUDY

Effect of oral glucose administration on ghrelin levels in obese children

Leandro Soriano-Guillén, Vicente Barrios, Gabriel Martos, Julie A Chowen, Angel Campos-Barros and Jesús Argente

Department of Pediatric Endocrinology and Laboratory of Research, Universidad Autónoma, Hospital Infantil Universitario Niño Jesús, Avda. Menéndez Pelayo 65, E-28009 Madrid, Spain

(Correspondence should be addressed to J Argente; Email: argentefen@terra.es)

Abstract

Objective: Coexpression of GH secretagogue receptor and ghrelin in the pancreas suggests that this peptide is involved in glucose metabolism. Previous reports in adult humans have demonstrated that plasma ghrelin levels decrease after oral glucose administration. However, no data are available in children. Therefore, the aim of this study was to analyze the response of plasma ghrelin levels in obese children after oral glucose administration.

Subjects and methods: Twenty-eight obese children ranging from Tanner I to Tanner V were studied. All subjects were given 0.75 g/kg (maximum 75 g) glucose solution after overnight fasting. Ghrelin, insulin, glucose and IGF-binding-protein-1 were determined at 0, 30, 60 and 120 min of the oral glucose tolerance test (OGTT).

Results: Basal plasma ghrelin levels were significantly lower than in the respective control groups. These levels decreased significantly during OGTT in obese children, reaching a nadir of $28 \pm 9\%$ at 60 min in parallel with the maximum increase in glucose levels and previous to maximum insulin levels.

Conclusion: The rapid fall in plasma ghrelin concentration in obese children after glucose load suggests a mechanism for the control of appetite after food intake.

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Introduction

Ghrelin is an acylated 28 amino acid peptide produced predominantly by the stomach (1), although a minor proportion of ghrelin synthesis occurs in other sites such as the hypothalamus, pituitary and lung (2). Coexpression of ghrelin and growth hormone receptor within the endocrine pancreas suggests that ghrelin could be implicated in glucose metabolism (2–4). However, conflicting results have been reported concerning the influence of ghrelin on insulin secretion (5–8). On the other hand, evidence suggests that glucose administration decreases ghrelin levels (7, 9), while ghrelin administration induces hyperglycemia (10). It is known that obese children have decreased circulating ghrelin levels (11) and elevated insulin levels (12), but to our knowledge there are no data on ghrelin levels after glucose oral administration in obese children.

In order to investigate the effect of glucose on ghrelin levels, we measured ghrelin levels at fasting and during an oral glucose tolerance test (OGTT) in obese children. The relationship with other parameters involved in

glucose metabolism, such as insulin, glucose and insulin-like growth factor-binding protein-1 (IGFBP-1) levels were also analyzed at each time-point during the OGTT.

Subjects and methods

Subjects

Twenty-eight obese children (17 boys and 11 girls), aged 13.2 ± 3.4 years, were included in this study. These patients had a body mass index (BMI) greater than 2 s.d. above Spanish standards (13). They were at different Tanner stages of pubertal development (Tanner I: 7, Tanner II: 4, Tanner IV: 5 and Tanner V: 12). The obese children had a mean BMI of 5.81 ± 3.37 and had no other known endocrine disorder and were receiving no medication at the time of the study. Ghrelin levels were compared with values in a control population reported previously (14).

All subjects were informed about the purpose of the study and parents or guardians gave consent.

This study was approved by the local human ethics committee.

Methods

All subjects were given 0.75 g/kg (maximum 75 g) glucose solution orally after overnight fasting. Blood was withdrawn from a catheter at 0, 30, 60 and 120 min during the OGTT and kept in chilled tubes containing EDTA (1 mg/ml) plus aprotinin (500 U/ml). The tubes were centrifuged and stored at -80°C until assayed.

Plasma ghrelin levels were measured by a commercial radioimmunoassay (Phoenix Pharmaceutical, Inc., Belmont, CA, USA) using a polyclonal antibody that recognizes octanoylated and non-octanoylated ghrelin and ^{125}I -ghrelin as a tracer molecule. The intra- and interassay coefficients of variation were 5.0% and 11.2% respectively. Assay sensitivity was 12 pg/ml.

IGFBP-1 and insulin levels were measured as reported previously (12). Plasma glucose was measured by the glucose oxidase method on a Beckman Glucose Analyzer (Fullerton, CA, USA).

Statistics

All data are reported as the means $\pm$ S.D. Analysis was performed by ANOVA for repeated measures, followed by Scheffe's F test; $P < 0.05$ was chosen as the level of significance. Multiple regression analysis was performed

to determine the overall interaction of the parameters studied, followed by partial correlation analysis.

Results

A significant decrease in ghrelin levels was found after oral glucose administration, with a maximum decrease of 28% occurring at 60 min after glucose intake. In contrast, insulin and glucose concentrations were increased after glucose intake, with maximum levels found at 60 and 120 min for glucose and insulin respectively. We found no differences in IGFBP-1 levels after glucose administration (Fig. 1).

When the relationship between ghrelin and insulin, glucose and IGFBP-1 was analyzed, a positive correlation between ghrelin and IGFBP-1 levels was found only at 0 min ($r = 0.58$, $P < 0.01$), but at no other time-point. No correlation between ghrelin and glucose or insulin was observed at any time-point. There was no correlation between ghrelin levels and insulin resistance: ($\text{HOMA-R} = \text{glucose (in mg/dl)} \times \text{insulin (in } \mu\text{U/l)} / 405$).

Discussion

We have here demonstrated that although ghrelin levels were significantly decreased in obese children, oral glucose administration decreased circulating ghrelin levels further. This is in agreement with previous

Figure 1 Representation of basal serum levels of (A) ghrelin, (B) insulin, (C) glucose and (D) IGFBP-1 concentrations (●) in obese children and 30 min (○), 60 min (■) and 120 min (□) after glucose administration compared with puberty-matched control values, indicated by the shaded areas which represent the mean $\pm$ 1 S.D. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs control values; # $P < 0.05$ vs basal values.

reports showing that plasma ghrelin levels are significantly decreased after oral glucose ingestion (7, 9).

The maximum decrease in ghrelin levels was reached in parallel with the maximum increase in glucose concentrations and occurred before insulin levels reached their maximum. Inversely, ghrelin administration induced hyperglycemia and this effect was unrelated to the decrease in insulin, as this decrease occurred after the increase in glucose concentrations (10). Indeed, we have previously reported a negative correlation between ghrelin and glucose concentrations throughout normal development with no relationship to basal insulin levels (14). Furthermore, ghrelin levels were decreased in diabetic children and remained low despite insulin therapy (15). Taken together, these results suggest that circulating glucose levels modulate ghrelin concentrations and that ghrelin-producing cells may respond directly to changes in plasma glucose concentrations (7).

Our data have confirmed that the maximum decrease in ghrelin levels is at 60 min after glucose administration (7, 9). The rapid decrease in ghrelin levels observed as early as 30 min after glucose administration is similar to that observed after administration of a meal (7), indicating that this peptide is probably implicated in the rapid control of food intake. However, after oral glucose administration the decrease in ghrelin levels with respect to basal values was only 28% in obese children, while in normal adults it reached approximately 60% (7, 9) and in anorexic patients 50% (9). One possibility is that the glucose-induced decrease in ghrelin levels is less accentuated in obese children because their basal levels are already low (11). Another possible explanation is that these patients are hyperinsulinemic with a certain degree of insulin resistance, suggesting that there may be a modification in their glucose-sensing system.

Although ghrelin and IGFBP-1 had a positive relationship before glucose administration, this correlation was not seen at later time-points. A possible explanation is that IGFBP-1 levels are very low in obese children (12) and remain so in spite of insulin elevation during OGTT, suggesting insulin resistance, as has been previously reported (16). The correlation between IGFBP-1 and ghrelin at baseline could be due to the fact that these two factors are secreted in a parallel pulsatile fashion.

In conclusion, our results showed a link between glucose concentrations and ghrelin levels, suggesting that ghrelin may have an effect over the endocrine pancreas, as well as with the control of food intake.

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