CLINICAL STUDY

Ghrelin, insulin sensitivity and postprandial glucose disposal in overweight and obese children

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Abstract

Objective: To explore the changes of ghrelin circulating levels induced by a mixed meal and their relationship with postprandial substrate oxidation rates in overweight and obese children with different levels of insulin sensitivity.

Methods: A group of ten boys (age 9–12 years) with different levels of overweight (standard deviation score of body mass index: 1.6–3.2) was recruited. Body composition was measured by dual-energy X-ray absorptiometry. Insulin sensitivity was assessed by a frequently sampled i.v. glucose tolerance test. Pre-prandial and postprandial (3 h) substrate oxidation was measured by indirect calorimetry. The energy content of the test meal (16% protein, 36% carbohydrate and 48% fat) was 40% of pre-prandial energy expenditure (kJ/day).

Results: Pre-prandial serum concentration of total ghrelin was 701.4 ± 66.9 pg/ml (S.E.M.). The test meal induced a rapid decrease in ghrelin levels and maximal decrease was $27.3 \pm 2.7\%$ below baseline. Meal intake induced a progressive increase of the carbohydrate oxidation rate for 45 min after food ingestion, followed by a slow decrease without returning to pre-prandial values. Postprandial cumulative carbohydrate oxidation was 16.9 ± 0.8 g/3 h. Insulin sensitivity and postprandial maximal decrease of ghrelin concentration showed a significant correlation ($r = 0.803$, $P < 0.01$). Moreover, the postprandial carbohydrate oxidation rate correlated with the area under the curve for both insulin ($r = 0.673$, $P < 0.03$) and ghrelin ($r = -0.661$, $P < 0.04$).

Conclusions: A relevant association between postprandial insulin-mediated glucose metabolism and ghrelin secretion in children with different levels of overweight was found. It is possible that the maintenance of an adequate level of insulin sensitivity and glucose oxidation may affect appetite regulation by favoring a more efficient postprandial ghrelin reduction.

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Introduction

Excess fat accumulation promotes the development of insulin resistance, glucose intolerance and type 2 diabetes mellitus (1). The epidemic of obesity is associated with an impressive increase in the incidence of type 2 diabetes mellitus in young adults and adolescents (2, 3). This alarming emergency is still in progress. Clear evidence exists that the exposure to an 'obesogenic environment' induces the phenotypic expression of the genetic susceptibility to obesity of a large part of the population (4). Unfortunately, interventions in these risk factors are generally fraught with high failure rates (5). These frustrating results have stimulated intensive investigation to identify potentially sensitive targets for intervention among the components of the

complex neuroendocrine system that regulates energy balance and body composition in humans.

Ghrelin and insulin are two hormones which play a relevant role in body weight regulation (6). Ghrelin is the only gastrointestinal peptide that stimulates appetite in humans, whereas insulin plays a pivotal role in the regulation of substrate trafficking, especially of glucose (7–9). A specific action of insulin in the central nervous system has been postulated to modulate food intake and body weight (10, 11). In particular, the injection of insulin into the cerebroventricular fluid promoted a reduction in food intake in experimental animals (10). Ghrelin secretion may be affected by adiposity through insulin and/or glucose metabolism (12, 13). Studies performed in humans demonstrated that i.v. administration of insulin induces a fall in ghrelin

levels (13, 14), although some authors disagree with this (15, 16). This decline in ghrelin concentrations, in turn, is related to insulin sensitivity (S_I). However, these findings were obtained in adults with insulin-clamp studies. It is still unknown whether the same findings are extendable to children and, even more importantly, whether the relationship between S_I and ghrelin suppression is valid also in the more physiological setting of a meal, when many other gut hormones are also involved. Therefore, we hypothesized that ghrelin secretion in postprandial conditions may be affected by the degree of S_I and/or food-induced insulin secretion in children. In particular, we used a mixed meal with a high-fat content, on the basis of the evidence that a fatty meal is preferred by obese children (17) and that a fatty diet has been recognized as a risk factor for obesity (18). Therefore, by measuring ghrelin changes after a high-fat meal, we have the opportunity to explore the role of adiposity and S_I in a high-risk nutritional situation, so common in children and adolescents (19).

Finally, since the thermogenic effect of food is related to S_I and, more specifically, to insulin-stimulated carbohydrate oxidation, we also explored the relationships between substrate oxidation rates and ghrelin concentration after the meal.

The aim of this study was to explore the changes of ghrelin circulating levels induced by a mixed meal in a group of obese children with different levels of adiposity and S_I .

Subjects and methods

Subjects

Ten boys with different levels of overweight participated in the study. The physical characteristics of the boys are given in Table 1. Puberty development was clinically assessed on the basis of Tanner Stages (20). None of the boys had any overt disease other than obesity. Obesity was defined as a body mass index (BMI) above the 95th percentile for age and sex, and normal weight was defined as a BMI lower than the 85th percentile. The BMI percentiles reported by national BMI tables

were used as a reference (21). None of the boys had initiated puberty. None of the boys was taking medication. All boys had normal glucose tolerance, as assessed by an oral glucose tolerance test, performed no more than 1 week before the present test. Informed consent was obtained from children and their parents before taking part in the study. The parents of all children signed the informed consent. The protocol was in accordance with the 1975 Declaration of Helsinki, as revised in 1983, and was approved by the Ethical Committee of the University Hospital of Verona.

Experimental design

The study was cross-sectional and was performed on 2 different days, during which boys were under medical supervision. In the days preceding the tests, no attempt was made to influence the usual diet of each boy (who had free access to food). For 2 days preceding the tests, they did not engage in any strenuous physical activity. Both children and their parents were interviewed to assess and record the physical activity performed by the boys on the day before the tests. Each boy arrived at the Department of Pediatrics at 0830 h on the day of the calorimetric test, after an overnight (10–12 h) fast. A topical anesthetic was applied to one arm and at 0930 h a catheter was inserted in the antecubital vein for blood sampling. After resting 30 min in a comfortable temperature- (24 °C) and humidity-controlled environment, continuous respiratory exchange measurements were taken for 30 min by indirect calorimetry to assess their post-absorptive metabolic rate, i.e. pre-prandial energy expenditure (EE). At 11.00 h each boy received a test meal and consumed it in about 30 min, under medical supervision. Postprandial calorimetric measurements started 30 min after boys finished the test meal. Blood samples for glucose, insulin, ghrelin and leptin measurements were taken before the meal and every 30 min in the 3 h after meal ingestion.

On the second test day, 3 or 4 days apart from the mixed-meal test, S_I was assessed by means of a frequently sampled i.v. glucose tolerance test (IVGTT) (22). Boys arrived at the Department of Pediatrics at 0830 h after an overnight fast. Topical anesthetic was applied to both arms and a 23 g Teflon catheter was inserted into the antecubital vein of each arm, one for i.v. glucose infusion, the other for blood sampling. After an appropriate rest to establish baseline, blood samples were collected at time –10 and 0 min to determine baseline values of glucose, insulin and C-peptide. At time 0 min, an i.v. 25% dextrose infusion was started at a rate designed to deliver 12 g/m² body surface area (BSA) of glucose over approximately 1 min. The time required to infuse the glucose load was recorded in each subject. (The glucose load was enriched with [6,6-²H] glucose for a companion experiment.) Blood samples were drawn from the contralateral arm at 2, 4, 6, 8, 10, 15, 20, 25, 30, 40, 60, 80, 100, 120,

Table 1 Physical characteristics of the obese and overweight children.

	Mean	S.E.M.	Range
Age (years)	11.4	0.5	8.8–12.0
Weight (kg)	69.4	6.5	40.5–96.7
Height (cm)	151.0	4.8	135–175
BMI (kg/m ²)	27.9	1.4	22.2–33.9
BMI SDs	2.4	0.2	1.6–3.2
FM %	43.2	1.6	34.8–51.0
Fat mass (kg)	30.9	3.8	14.1–48.3
S_I BSA	0.19	0.04	0.03–0.42

140, 160, 180, 200 and 220 min to determine glucose, insulin and C-peptide concentrations.

Anthropometry and body composition

Height and weight were measured in post-absorptive conditions and with the subject having an empty bladder. Height was measured to the nearest 0.5 cm on a standardized height board. Weight was determined to the nearest 0.1 kg on a standard physician's beam scale, with the subject dressed only in light underwear and no shoes. BMI was calculated as weight (kg) divided by height squared (m^2). Complete data were obtained from all of the boys at baseline. The standard deviation score (SDS) of the BMI is the deviation from the median of weight and was calculated using the LMS method according to the formula (23): $SDS = ([Measurement/M(t)^{L(t)}] - 1)/(S(t) \cdot L(t))$. The LMS method summarizes the data in terms of three smooth age-specific curves called L, M and S. The M and S curves correspond to the median and coefficient of variation of BMI at each age, whereas the L curve allows for the substantial age (t) dependent skewness in the distribution of BMI. The values of L, M and S are tabulated for a series of ages.

Total body dual-energy X-ray absorptiometry was also performed in all boys to assess body composition using a DPX-L densitometer (Lunar Corp., Madison, WI, USA). Subjects were scanned in light clothing lying flat on their backs. On the day of each test, the DPX-L was calibrated according to the procedures described by the manufacturer. Body fat mass was obtained by multiplying the percentage of body fat by body weight.

Composition of the test meal

Meal composition was identical for each boy, whereas the energy content of the test meal was calculated to be 40% of the 24 h resting EE of each boy, which was extrapolated from the pre-prandial EE.

This choice allows standardization of the energy intake for children with different body weight and body composition. Expressed as a percentage of total

energy value, the test meal contained: 16% proteins, 36% carbohydrate and 48% fat (Table 2). The energy and nutrient content of the meal was calculated using the tables of food composition of the National Institute of Nutrition (24).

Measurements of EE

After 30 min of absolute rest, considered to be an adaptation period during which the procedure was explained to each child as well as to their parents, respiratory exchanges were measured continuously for 30 min on four different occasions during the meal test. During the measurement, the child rested quietly while watching calm cartoons. Special attention was given to prevent extra body movements, which would contribute to artifactually increase EE.

The post-absorptive resting EE measurement was made at 0930 h (pre-prandial baseline). Postprandial calorimetric measurements took place at 1200, 1300 and 1400 h and lasted 30 min each. Respiratory exchange were measured by means of an open circuit indirect calorimeter (Deltatrac; Datex, Inc., Finland) using a transparent ventilated hood system, as previously described (25). EE was calculated from oxygen consumption (VO_2) and carbon dioxide production (VCO_2) using Lusk's formula (26).

Macronutrient oxidation rate

The macronutrient oxidation rate was calculated from VO_2 and VCO_2 using the following formulas: $Fox \text{ (g/min)} = 1.67 \text{ } VO_2 \text{ (l/min)} - 1.67 \text{ } VCO_2 \text{ (l/min)} - 0.307 \text{ Pox}$, and $Gox \text{ (g/min)} = 4.55 \text{ } VCO_2 \text{ (l/min)} - 3.21 \text{ } VO_2 \text{ (l/min)} - 0.459 \text{ Pox}$, where Fox is fat oxidation, Gox is glucose oxidation, and Pox is protein oxidation (27).

Pox was estimated as follows: $Pox \text{ (g/min)} = [EE \text{ (kJ/min)} \times 0.15] / 16.74 \text{ kJ}$. We assumed that Pox covered 15% of EE in both obese and non-obese boys. Postprandial changes in macronutrient oxidation were quantified calculating the areas under the respective 180 min plots.

IVGTT

The analysis of the insulin and glucose curves during the IVGTT followed the general strategy proposed by Bergman and Cobelli (22, 28) with some slight modifications. A complete description of the modeling strategy can be found in the Appendix. Parameters were estimated by implementing this minimal model of glucose metabolism in the SAAM II 1.1.2 software (SAAM Institute, Seattle, WA, USA). Numerical values of the unknown parameters were estimated by using non-linear least squares. Weights were chosen optimally, i.e. equal to the inverse of the variance of the measurement errors, which were assumed to be

Table 2 Energy and composition of the test meal. Data are expressed as means $\pm$ S.E.M.

Mean components	
French fries (g)	94.8 (5.9)
Double cheeseburger (g)	154.3 (9.5)
French fries sauce (g)	12.6 (2.3)
Energy content (kJ)	2881 (178)
Fat (g)	36.7 (2.3)
% of energy intake	48
Carbohydrate (g)	62.0 (3.8)
% of energy intake	36
Protein (g)	29.4 (1.8)
% of energy intake	16

additive, uncorrelated, with zero mean, and a constant coefficient of variation (2.5%). The main outputs of this model are: (i) S_I , expressed as the increase in glucose clearance at steady-state elicited by an increase of 1 pmol/l of insulin (units: ml/min per pmol/l); and (ii) glucose effectiveness (S_G), expressed as an insulin-independent glucose clearance (units: ml/min).

Both S_I and S_G were normalized per m^2 of BSA. The medians of the coefficient of variation of S_I and S_G were 40.3% and 49.2% respectively.

Hormonal assays

Serum ghrelin concentration was determined by commercially available specific RIA kits (Linco Research, Inc., St Charles, MO, USA). Total ghrelin RIA kit sensitivity was 93 pg/ml. Glucose plasma concentration was measured with the glucose oxidase method. Plasma insulin levels were determined by an Insulin Bridge RIA kit (Adaltis, Inc., Montreal, Canada). Sensitivity limit was 0.3 mIU/l.

Statistical analysis

Data are presented as means $\pm$ S.E.M. Spearman correlations were performed in order to show possible correlation between hormone concentrations and metabolic variables. A level of significance of $P < 0.05$ was used for all data analyses. Statistical analyses were performed using SPSS 11.0 software for windows (SPSS Inc., Chicago, IL, USA).

Results

Physical characteristics

Physical characteristics of subjects are shown in Table 1. Boys showed a wide range of BMIs (mean 27.9 ± 4.4 ; range 22.2–33.9) and BMI SDSs (mean 2.4 ± 0.6 ; range 1.6–3.2), from a modest overweight to severe obesity.

Figure 1 Changes of carbohydrate oxidation rate after the ingestion of a mixed meal. Means $\pm$ S.E.M., $n = 10$.

Pre- and postprandial macronutrient oxidation rate

Carbohydrate oxidation rate measured in post-absorptive conditions averaged 41.6 ± 5.8 mg/min. Fat oxidation rate averaged 87.3 ± 8.2 mg/min. During the first 45 min after meal ingestion, carbohydrate oxidation increased rapidly to a maximal mean value of 119.3 ± 8.2 mg/min, and slowly decreased thereafter without returning to pre-prandial values. Fat oxidation rate showed no significant variations after meal ingestion. Postprandial cumulative carbohydrate oxidation was 16.9 ± 0.9 g/3 h and fat oxidation was 14.4 ± 1.0 g/3 h (Fig. 1).

Hormones

Insulin and glucose Pre-prandial serum insulin concentration was 10.2 ± 1.5 mU/l. In the first 60 min after meal ingestion, insulin rose sharply to 65.9 ± 14.0 mU/l ($P < 0.001$) then decreased toward basal levels (Fig. 2). In the 180 min after the test meal, the area under the curve (AUC) of insulin concentration was 8482.5 ± 1174.8 mU/l·min.

The S_I index, measured with minimal model from the IVGTT, averaged 0.192 ± 0.04 ml/min per pmol/l per m^2 BSA.

Serum-glucose concentration averaged 5.8 ± 0.1 mmol/l. In the first hour after the meal, glycemia climbed to 7.4 ± 0.4 mmol/l ($P < 0.001$), while in the following 2 h it decreased to close to baseline.

Ghrelin Pre-prandial serum concentration of total ghrelin was 701.4 ± 66.9 pg/ml. The test meal induced a rapid decrease in ghrelin levels (-7.0% at 30 min, -15.0% at 60 min, $P < 0.005$) (Fig. 3). The difference between basal ghrelin level and the nadir of concentration, expressed as a percentage of basal level (maximal decrease), was $27.3 \pm 2.7\%$. The AUC of the ghrelin concentration after meal intake was 111218 ± 7800 pg/ml·min.

Figure 2 Insulin serum concentration after the ingestion of a mixed meal. Means $\pm$ S.E.M., $n = 10$.

Figure 3 Ghrelin serum concentration after the ingestion of a mixed meal. Means $\pm$ S.E.M., $n = 10$.

Correlation analyses

Nutrient oxidation and adiposity Post-absorptive fat oxidation rate significantly correlates with BMI SDS and fat mass expressed as a percentage of body weight (FM%) (respectively $r = 0.733$, $P < 0.016$ and $r = 0.827$, $P < 0.003$). The correlation between post-absorptive carbohydrate oxidation rate and the indicators of overweight and adiposity was not statistically significant (BMI SDS: $r = -0.309$, $P = 0.38$; FM%: $r = -0.450$, $P = 0.19$).

Postprandial fat oxidation rate significantly correlated with BMI SDS and FM% ($r = 0.770$, $P < 0.009$ and $r = 0.851$, $P < 0.002$ respectively). Postprandial carbohydrate oxidation rate did not show any correlation with FM% and BMI SDS ($r = 0.224$, $P = 0.53$ and $r = 0.235$, $P = 0.416$ respectively).

Hormones and adiposity Both pre-prandial level and AUC of insulin significantly correlated with FM% ($r = 0.655$, $P < 0.04$ and $r = 0.754$, $P < 0.012$ respectively) but only insulin AUC correlated with BMI SDS ($r = 0.754$, $P < 0.012$). The correlations between total ghrelin basal level and FM% and BMI SDS were not statistically significant ($r = -0.277$, $P = 0.43$ and $r = -0.375$, $P = 0.28$ respectively) and no correlation

Figure 4 Correlation between ghrelin AUC and postprandial carbohydrate oxidation. $r = -0.661$, $P < 0.04$.

was found between ghrelin AUC and both FM% and BMI SDS ($r = -0.355$, $P = 0.31$ and $r = -0.456$, $P = 0.18$). Maximal ghrelin postprandial reduction was inversely and significantly correlated with FM% ($r = -0.78$, $P = 0.008$).

Ghrelin and glucose metabolism S_I and postprandial maximal decrease of ghrelin concentration (i.e. the difference between basal ghrelin level and nadir of concentration, expressed as a percentage of basal level) showed a significant correlation ($r = 0.803$, $P < 0.01$). Moreover the postprandial carbohydrate oxidation rate correlated both with insulin AUC ($r = 0.673$, $P < 0.03$) and with ghrelin AUC ($r = -0.661$, $P < 0.04$) (Fig. 4). Insulin AUC and ghrelin AUC were negatively correlated ($r = -0.709$, $P < 0.03$).

Discussion

The three main findings of the present study were: (i) the positive correlation between food-induced increase in carbohydrate oxidation rate and fall in ghrelin levels; (ii) the positive correlation between S_I and food-induced fall in ghrelin; and (iii) the negative correlation between maximal ghrelin postprandial reduction and adiposity. Since carbohydrate oxidation after either a carbohydrate or a mixed meal is controlled primarily by S_I , these data are consistent with a direct link between insulin action and ghrelin suppressibility in children. Complementary findings of our study are that insulin and ghrelin variations after food intake are opposite (Fig. 5) and that the AUC of insulin was inversely correlated with the AUC of ghrelin.

The relevance of these results is increased by the fact that we used a high-fat meal. In fact, a high-fat meal is common in obese children and was demonstrated to be a risk factor of fat gain in both humans and animals

Figure 5 Ghrelin and insulin serum concentration, expressed as percentage of basal level, after the ingestion of a mixed meal. Means $\pm$ S.E.M., $n = 10$.

(17). Moreover, in adults, a high-fat meal was recently demonstrated to promote a lesser postprandial ghrelin reduction than that induced by a low-fat, high-carbohydrate meal (29). In our study, we found a relationship between postprandial ghrelin changes, S_I and carbohydrate oxidation, adjusted for the level of adiposity (FM%), also after a standardized meal with a relatively modest carbohydrate intake. On the other hand, ghrelin suppression also occurs in the complete absence of insulin (30). Ghrelin changes in response to meals may also be elicited by the cephalic phase alone (31). Therefore, it is likely that glucose metabolism, more than insulin *per se*, has a pivotal role in ghrelin postprandial regulation.

Interestingly, the postprandial ghrelin changes we found in obese prepubertal children are similar to those reported in obese adolescents after a mixed liquid formula ingestion (32) and in another group of 7- to 12-year-old children after an oral glucose load (33).

Convincing evidence exists that hyperinsulinemia causes a fast, remarkable fall in ghrelin concentration. In particular, two independent studies, performed with the euglycemic–hyperinsulinemic clamp have recently demonstrated that i.v. administration of insulin was able to reduce ghrelin secretion in non-diabetic non-obese adults, independently from glucose (13, 14). Moreover, i.v. or oral administration of glucose stimulated a reduction of ghrelin concentration detected in blood (12), which may be additional to that induced by insulin (13). Our study conducted in children and after a mixed-meal ingestion confirmed the strong association between postprandial changes in ghrelin and insulin circulating levels. Moreover, the relationships between S_I , glucose metabolism and ghrelin suppressibility by insulin that we found in the children, have the advantage of being assessed after a mixed meal and not in the relatively unphysiological setting of the insulin clamp study, in which potential collinearities between simultaneously measured insulin effects cannot be ruled out (14). Ghrelin pre-prandial circulating levels were negatively associated with BMI or FM%, but not significantly. Possibly due to our sample size, we did not find, as reported by others (14), a significant association between fasting ghrelin and BMI. However, interestingly, maximal ghrelin reduction was significantly associated with adiposity. Therefore, in spite of ghrelin being (at least) relatively lower in children with higher adiposity, its reduction is by far less pronounced after meal ingestion, reinforcing the hypothesis that ghrelin secretion may be already maximally suppressed in obese subjects or that a persistent orexigenic drive, failing to respond to food intake, may predispose to obesity in children (34). Differently from adults (34), children showed a reduction of ghrelin concentration associated with the postprandial increase of insulin concentration also in the obese. Therefore, the level of S_I is likely to affect not only insulin secretion after food ingestion but also postprandial secretory

response of ghrelin. Prolonged exposition to insulin resistance, typical of overweight children, may potentially affect sensitivity of gastric cells to transmembrane glucose flux and/or insulin signaling, with a consequently less efficient reduction in the postprandial phase. It is interesting that in children the relationship between insulin and ghrelin levels was found after the ingestion of a mixed meal containing a relatively small amount of carbohydrate.

In fasting conditions no association between ghrelin circulating levels and carbohydrate oxidation was found, whereas, in the postprandial phase, a significant association between the AUC of ghrelin and carbohydrate oxidation was detected. Since maximal ghrelin reduction is associated with meal-induced insulin secretion expressed as AUC and not with carbohydrate oxidation, it is likely that the relationship between ghrelin and carbohydrate oxidation in the postprandial phase is mediated by S_I , which is strictly related to carbohydrate oxidation rate.

Thus, the most likely scenario in children is one in which food-induced insulin secretion activates a short-term feedback loop, which causes a fall in ghrelin and, presumably, in its related orexigenic effects. The more a child is insulin-resistant, the less ghrelin falls. Concomitantly, insulin resistance brings about a less-pronounced increase in carbohydrate oxidation and, hence, a reduction in the thermogenic effect of food, of which insulin-dependent carbohydrate oxidation is a relevant component (35). Therefore, our data suggest a possible obesogenic loop triggered by insulin resistance, in which unrestrained orexigenic effects of ghrelin and blunted food-induced EE potentially concur to induce a child's weight accrual.

Other factors, such as postprandial changes of free fatty acid levels, may play a significant but still undefined role (14). It is tempting to speculate that this independent relationship between the oxidation rate of a nutrient and ghrelin, for instance an appetite/satiety signal, may reflect the activity of certain (hypothalamic?) nutrient sensors. Further studies are needed to clarify this issue. In addition, ghrelin is one of several hormones involved in the regulation of food intake (leptin, peptide YY, cholecystokinin, 5-hydroxytryptamine, glucagon-like peptide 1, bombesin, amylin, insulin and cortisol) (9). Energy homeostasis in the body is probably promoted by the complex interaction between these hormones. For instance, peptide YY infusion has been shown to reduce fasting ghrelin levels (36) and ghrelin interferes with cortisol secretion, thereby increasing its concentration (37).

The effect of insulin on ghrelin still awaits the identification of a firm molecular basis (38). Indeed, insulin may act directly on the stomach, and/or elicit some hypothalamic circuit capable of stopping ghrelin secretion. While no data are available on the expression of the insulin receptor in the cells (especially the enterochromaffin line) of the gastric mucosa, the effects

of insulin on the central nervous system are well documented, and their lack can cause a syndrome resembling type 2 diabetes in the obese (39).

One drawback of our study was the cross-sectional design that did not allow us to investigate the cause–effect relationship between phenomena. Moreover, although we found a clear relationship between the level of overweight, S_I and a decrease in ghrelin, it would be interesting to make a comparison with normal-weight boys. Unfortunately, we did not get permission from the Ethical Committee to perform this study on normal-weight children. Finally, we only measured total ghrelin and not active ghrelin. It is possible that an analysis of active ghrelin could have augmented the information obtained. However, the measure of total ghrelin offers the chance to compare our results with those obtained in studies on orexigenic and metabolic activity in children (32, 33, 40).

In conclusion, the results of this study suggest a relevant association between insulin-mediated glucose metabolism and the regulation of ghrelin secretion after food ingestion in children with different levels of overweight. This finding suggests that, potentially, the maintenance of an adequate level of S_I may favor a more efficient weight control in children.

Appendix

We applied the minimal model of glucose metabolism introduced by Bergman and Cobelli, with some slight modifications, to exploit the measurements of the size and the timing of the i.v. glucose load. By giving the value 0 to glucose values between 0 and 6 min, the glucose system is well approximated by monocompartmental analysis. Thus: $dG(t)/dt = [gir(t)/V_G] + \{G_{SS}S_G - [S_G + X(t)] \times G(t)\}$, $G(0) = G_b$, where $G(t)$ is the glucose concentration time course; gir is the glucose infusion rate during the administration of the i.v. glucose load; V_G is the volume of the glucose compartment; G_{SS} is glucose concentration when the system returns to a steady-state at the end of the experiment; S_G is the glucose-dependent glucose clearance; and $X(t)$ is the insulin-dependent glucose clearance. At time 0, glucose concentration is the one measured at baseline (G_b). Insulin-dependent glucose clearance is related to the time course of insulin concentration by the following equation: $dX(t)/dt = -p_2 \cdot \{X(t) - (S_I/V_G) \cdot [I(t) - I_{ss}]\}$, $X(0) = 0$, where p_2 is the parameter regulating the fading of insulin action; S_I , or dynamic insulin sensitivity, is the steady-state increase in glucose clearance determined by a 1 pmol/l increase of plasma insulin over the concentration reached by the system when it returns to the steady-state; $I(t)$ is the insulin concentration time course; and I_{ss} (pmol/l) is the insulin concentration reached at steady-state at the end of the perturbation. At time 0, insulin-dependent glucose clearance is 0.

This model was implemented in the SAAM 1.2 software. The unknown parameters estimated by the model were: V_G (ml) = the volume of distribution of plasma glucose; S_G (ml/min) = glucose-dependent glucose clearance, or glucose effectiveness; p_2 (min) = rate constant regulating the fading of dynamic insulin action $X(t)$; and S_I (ml/min per pmol/l) = insulin sensitivity.

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