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Standard light breakfast inhibits circulating ghrelin level to the same extent of oral glucose load in humans, despite different impact on glucose and insulin levels

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ABSTRACT. Ghrelin levels are increased by fasting and energy restriction, decreased by food intake, glucose load and insulin but not by lipids and amino acids. Accordingly, ghrelin levels are elevated in anorexia and cachexia and reduced in obesity. Herein we compared the effects of a standardized light breakfast (SLB) on morning circulating ghrelin levels with those of oral glucose load (OGTT) in normal subjects. Specifically, 8 young adult volunteers [age (mean±SEM): 28.0±2.0 yr; body mass index (BMI): 22.4±0.6 kg/m^2] underwent the following testing sessions: a) OGTT (100 g po at 0 min, about 400 kcal); b) SLB (about 400 kcal, 45% carbohydrates, 13% proteins and 42% lipids at 0 min) on three different days; c) placebo (100 ml water po). In all sessions, at baseline, blood samples were withdrawn twice at 5-min interval to characterize the inter- and intra-individual reproducibility of the variables assayed. After placebo and OGTT, blood samples were withdrawn every 15 min up to +120 min. After SLB, blood samples were taken at 60 min only. Ghrelin, insulin and glucose levels were assayed at each time point in all sessions. Similarly to insulin and glucose levels, at baseline, ghrelin showed remarkable intra-subject reproducibility both in the same sessions and among the different sessions. Placebo did not significantly modify ghrelin, insulin and glucose. OGTT increased (p<0.01) glucose (baseline vs peak: 80.0±3.6 vs 140.5± 6.3 mg/dl) and insulin (20.2±6.2 vs 115.3±10.3 mU/l) levels. SLB increased (p<0.05) both insulin (16.3±1.8 vs 48.3±6.3 mU/l) and glucose (74.5±3.7 vs 82.9±3.1 mg/dl) levels. Notably both the insulin and glucose increases after OGTT were significantly higher (p<0.01) than that induced by SLB. After OGTT, ghrelin levels underwent a significant reduction (baseline vs nadir: 355.7±150.8 vs 243.3±98.8 pg/ml; p<0.05) reaching the nadir at time +60 min. Similarly, ghrelin levels 60 min after SLB (264.8±44.8 pg/ml) were significantly (p<0.01) lower than at baseline (341.4±54.9 pg/ml). No significant differences in the reduction of ghrelin levels after OGTT and SLB were observed. In conclusion, these findings show that light breakfast inhibits ghrelin secretion to the same extent of OGTT in adults despite lower variations in glucose and insulin levels.

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INTRODUCTION

Ghrelin is a 28-amino acid peptide predominantly produced by the stomach (1, 2). In its acylated form, ghrelin displays strong GH-releasing activity mediated by the activation of the GH secretagogue (GHS) receptors (GHS-R) type 1a (1, 3). GHS-R are expressed in the hypothalamus-pituitary unit but also in other central and peripheral tissues (3, 4). Indeed, besides potent GH-releasing effect, ghrelin has other remarkable activities including stimulation of lactotroph and corticotroph secretion, inhibition of gonadal axis, orexigenic effect coupled with control of energy expenditure, cardiovascular actions, modulation of cell proliferation and apoptosis, influence on gastric

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motility and acid secretion, exocrine and endocrine pancreatic function and glucose metabolism, behavior and sleep (2, 5, 6).

Circulating ghrelin levels, mostly represented by its unacylated form, are increased by fasting and energy restriction, decreased by food intake, glucose, insulin and somatostatin but unaffected by lipids and amino acids (5, 7-11). In agreement with the major influence of nutrients on ghrelin secretion, circulating ghrelin levels are increased in lean subjects as well as in patients with anorexia nervosa and cachexia but reduced in obesity (8, 12). These changes are opposite to those of leptin and it has been suggested that both ghrelin and leptin are hormones signaling the metabolic balance and managing the neuroendocrine and metabolic response to changes in the nutritional status (5, 6, 12). Accordingly, a clear negative association between insulin and ghrelin levels has also been shown (5, 6, 12).

Therefore, ghrelin measurement has been suggested as a reliable marker of the nutritional status and the modulation of ghrelin secretion induced by nutrient intake might provide useful information about the neuro-endocrine and metabolic responses to food intake.

The aim of the present study was to compare the effects of standardized light breakfast (SLB) on morning circulating ghrelin levels with those of oral glucose load (OGTT) in order to further clarify the relationship between ghrelin secretion and nutrients.

SUBJECTS AND METHODS

Eight healthy young male volunteers [age (mean±SEM): 28.0±2.0 yr; BMI: 22.4±0.6 kg/m^2] were studied. All subjects gave their written informed consent to participate in the study which had been approved by an independent Ethics Committee.

All subjects underwent the following testing sessions in random order and at least 7 days apart: a) placebo (100 ml water po); b) OGTT (100 g po at 0 min, about 400 kcal); c) SLB (about 400 kcal, 45% carbohydrates, 13% proteins and 42% lipids at 0 min) on three different days. Specifically, SLB was composed of 200 cc of milk (about 130 kcal) with 10 g of sugar (about 40 kcal) and 2 prepacked snacks (about 120 kcal each). All subjects were allowed to consume SLB in no longer than 15 min.

After overnight fasting, the tests began in the morning at 08:30-09:00 h, 30 min after an indwelling catheter had been placed into an antecubital vein of the forearm kept patent by slow infusion of isotonic saline.

In all sessions, at baseline, blood samples were withdrawn twice at 5-min interval in order to characterize the inter- and intra-individual reproducibility of the variables assayed.

Then, in both placebo and OGTT sessions, blood samples were withdrawn every 15 min up to +120 min. Instead, in the SLB sessions, blood samples were taken 60 min after nutrient intake.

In all sessions, all subjects remained seated and no physical exercise, smoking or other food or water ingestion was allowed till the end of the testing sessions.

Ghrelin, insulin and glucose levels were assayed at each time point in all sessions.

Ghrelin levels (pg/ml) 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 h-ghrelin. Sensitivity: 30 pg/tube, intra-assay coefficient of variation (CV) range: 0.3-10.7%.

Insulin levels (mU/l; 1 mU/l=7.175 pmol/l) were measured in duplicate by immunoradiometric assay (INSIK-5, SORIN Biomedica, Saluggia, Italy). The sensitivity of the assay was 2.5±0.3 mU/l. The inter- and intra-assay CVs were 6.2-10.8% and 5.5-10.6%, respectively.

Glucose levels (mg/dl; 1 mg/dl = 0.05551 mmol/l) were measured by gluco-oxidase colorimetric method (GLUCOFIX, by Menarini Diagnostici, Florence, Italy).

All samples from an individual subject were analyzed together. The hormonal responses are expressed as absolute values or as delta percent variations. All the reported baseline levels are the means between two values obtained at 5-min interval.

The statistical analysis was carried out using a non-parametric analysis of variance (ANOVA) (Friedman test), analysis of covariance (ANCOVA), Wilcoxon matched pairs test and Mann-Whitney test, as appropriate.

Results are expressed as mean±SEM.

RESULTS

Ghrelin, as well as insulin and glucose levels, showed remarkable intra-subject reproducibility both in the same sessions (CV: 12.5±2.8%) and among the different sessions (CV: 14.9±1.8%). On the other hand, a low inter-subject reproducibility (CV: 48.7±6.0%) was observed.

At baseline, no significant differences were observed in ghrelin, insulin and glucose levels in the different sessions.

Placebo administration did not significantly modify ghrelin, insulin and glucose (Fig. 1 and 2).

OGTT induced obvious increase (p<0.01) in glucose (baseline vs peak: 80.0±3.6 vs 140.5±6.3 mg/dl; Δ% variation: 39.4±9.7%; median peak time: 30 min) and insulin (20.2±6.2 vs 115.3±10.3 mU/l; Δ% variation: 562.6±161.6%; median peak time: 30 min) levels (Fig. 1 and 2). As expected, SLB increased (p<0.05) both insulin (16.3±1.8 vs 48.3±6.3 mU/l; Δ% variation: 206.6±40.7%) and glucose (74.5±3.7 vs 82.9±3.1 mg/dl; Δ% variation: 12.0±3.4%) levels (Fig. 1 and 2). Notably both the insulin and glucose increase after OGTT was significantly higher (p<0.01) than that induced by SLB (Fig. 1 and 2).

After OGTT administration, ghrelin levels underwent a significant reduction (baseline vs nadir: 355.7±150.8 vs 243.3±98.8 pg/ml; Δ% variation: –29.4±6.0%; p<0.05) reaching the nadir at median time 60 min.

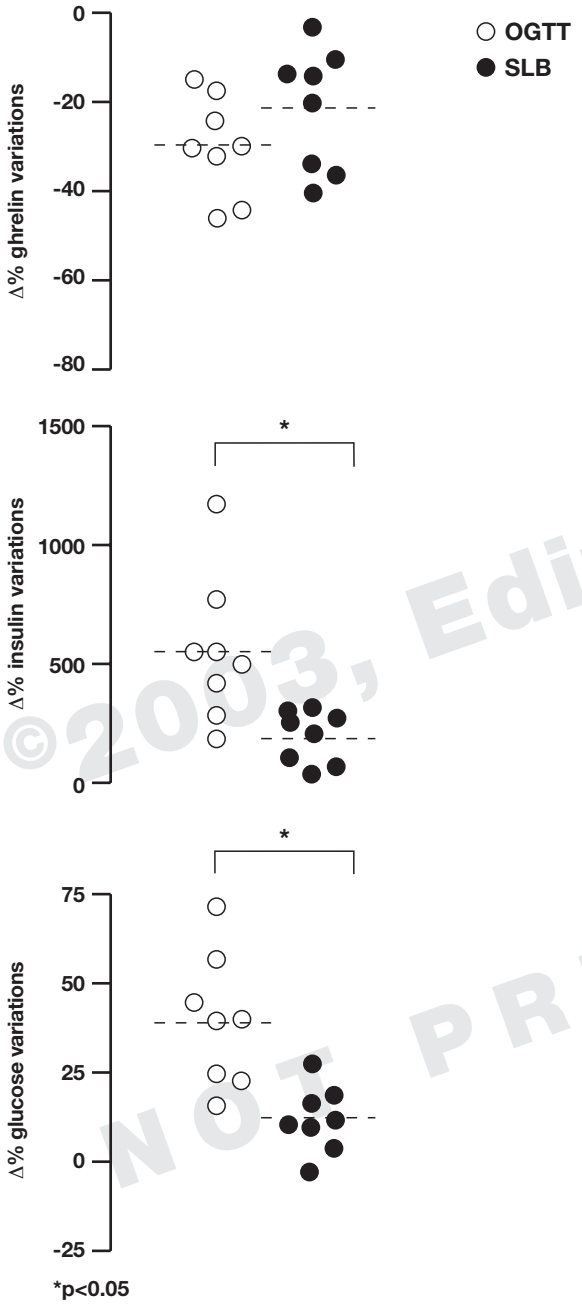

Fig. 1 - *Mean and individual insulin, glucose and ghrelin Δ% variations after standardized light breakfast (SLB) or oral glucose load (OGTT) in 8 normal young subjects.*

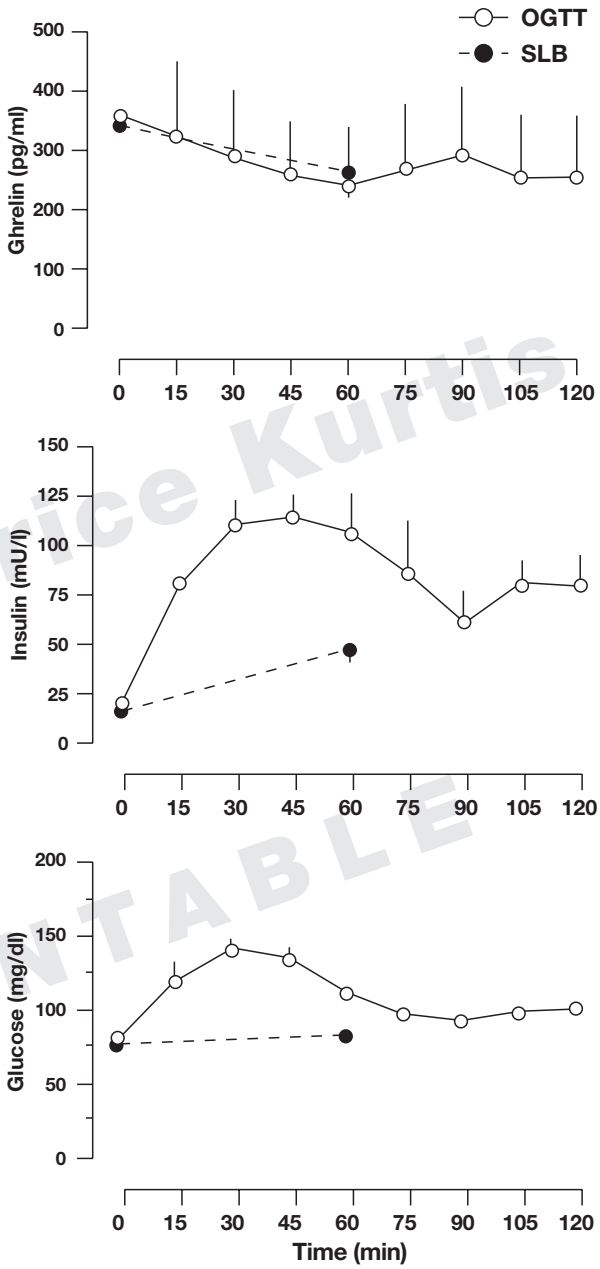

Fig. 2 - *Mean (±SEM) insulin, glucose and ghrelin levels after standardized light breakfast (SLB) or oral glucose load (OGTT) in 8 normal young subjects.*

Similarly, ghrelin levels 60 min after SLB (264.8±44.8 pg/ml) were significantly ($p<0.01$) lower those at baseline (341.4±54.9 pg/ml; Δ% variation: –21.2±5.1%). No significant differences in the reduction of ghrelin levels after OGTT and SLB were observed both in terms of absolute values and after co-varying ghrelin levels with insulin and glucose variations.

DISCUSSION

The results of the present study demonstrate that SLB inhibits circulating ghrelin levels in adults. The

inhibitory effect of SLB on ghrelin secretion at 60 min after its ingestion is of the same magnitude of that observed at the same time after 100 g OGTT although the latter elicits much more remarkable variations in circulating glucose and insulin levels.
Circulating ghrelin levels are mainly represented by its unacylated form despite the endocrine actions being exerted by the acylated form only and mostly reflect gastric secretion (6, 13). Ghrelin secretion occurs in a pulsatile manner without strict correlation with GH levels but with association to food intake episodes and sleep cycles in rats (14). In humans, ghrelin secretion undergoes remarkable variations throughout the day and, as in animals, ghrelin peaks anticipate food intake suggesting that the latter is triggered by ghrelin discharge (6, 12). In particular, although with a high inter-subjects variability, also confirmed by the present study, human plasma ghrelin levels were shown to rise nearly twofold shortly before each meal and fall to trough levels within 1 h after eating, and specifically ghrelin levels after breakfast have been reported to strongly correlate with the 24-h integrated ghrelin secretion (15).
However, although the reliability of a single morning measurement to describe 24-h ghrelin secretion is highly debatable, it must be noticed that morning fasting ghrelin levels show remarkable intra-subject reproducibility as reported by previous findings (15) and also confirmed by the present study.
In agreement with the major influence of nutrition on ghrelin secretion, ghrelin and insulin secretion are negatively associated in humans as well as in animals and an inhibitory influence of insulin on ghrelin secretion has been reported (5, 10, 12). Indeed, both euglycemic and hypoglycemic hyperinsulinemic clamps induce a clear reduction in circulating ghrelin levels (9, 10).
In agreement with the strict relationship between ghrelin secretion and food intake, in humans as well as in animals circulating ghrelin levels undergo a clear decrease after meals (7, 12). Regarding nutrients, glucose has an inhibitory influence on ghrelin secretion as indicated by the clear decrease in circulating ghrelin levels after either oral or iv glucose load both in animals and in humans (8, 9, 16, 17). On the other hand, iv free fatty acid as well as arginine load do not affect circulating ghrelin levels in humans (10). However, ghrelin secretion decreases during low-protein diet and increases during high-fat or high-carbohydrate diet in rats indicating the differential influence of macronutrients (18, 19).
The physiological mechanisms underlying these effects and the explanation of the discrepancies between studies addressing the effects of iv or orally administered nutrients is unknown (18, 19).

We aimed at clarifying the degree, if any, of the inhibitory effect of a SLB on circulating ghrelin levels and to compare its effect with that of an OGTT. We also considered and compared the effects of light breakfast and OGTT on glucose and insulin levels.
Our findings show that SLB induces a gluco-insulinemic response that is significantly lower than that to OGTT. The reasons for these different metabolic effects could be explained by the lower glycidic content in SLB than in OGTT or by the potential different kinetics of absorption after liquid (OGTT) or solid (SLB) glucose ingestion.
Interestingly, despite the different gluco-insulinemic responses, the inhibition of ghrelin secretion induced by SLB is not statistically different from that induced by OGTT. This evidence is remarkable and deserves attention. Possible explanations for these results might be that lower variations of insulin and glucose than those induced by OGTT are effective to significantly modulate ghrelin secretion.
On the other hand, it has to be noticed that SLB also provided some amount of amino acids and lipids that are ineffective after iv administration, at least in humans (10, 11). Thus, one may also argue that the entity of glucose load does not play a major inhibitory role while proteins and lipids would have some inhibitory impact in humans as well but after oral administration only. This latter hypothesis should imply that enteral variables play the most relevant role in influencing ghrelin secretion (10, 18, 19).
Unfortunately, the present study model is not able to provide further information about the exact mechanisms underlying the inhibitory effect of nutrients on ghrelin secretion and further studies are needed to confirm these data and to investigate the potential mechanisms.
In conclusion, this study demonstrates that food intake even such as light breakfast is as effective as OGTT in inhibiting ghrelin secretion and this occurs despite very different gluco-insulinemic responses. Independently of the mechanisms underlying the effect of nutrients on ghrelin secretion, the inhibitory effect of standardized breakfast might theoretically represent an easy dynamic test to investigate ghrelin secretion in different pathophysiological conditions.

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